

**Structural and functional characterization of
natural alleles of potato (*Solanum tuberosum* L.)
invertases associated with tuber quality traits**

Inaugural - Dissertation

zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Universität zu Köln

vorgelegt von

Astrid Martina Draffehn

aus Dessau

Köln, 2010



Max Planck Institute for
Plant Breeding Research

Die vorliegende Arbeit wurde am Max-Planck-Institut für Züchtungsforschung in Köln in der Abteilung für Pflanzenzüchtung und Genetik (Prof. Dr. Maarten Koornneef) in der Arbeitsgruppe von PD Dr. Christiane Gebhardt angefertigt.

Berichterstatter/in:

PD Dr. Christiane Gebhardt
Max-Planck-Institut für Züchtungsforschung,
Köln

Prof. Dr. Martin Hülskamp
Institut für Botanik, Universität zu Köln

Tag der mündlichen Prüfung: 30.06.2009

**HIER MEIN GEHEIMNIS. ES IST GANZ EINFACH: MAN SIEHT NUR MIT
DEM HERZEN GUT. DAS WESENTLICHE IST FÜR DIE AUGEN
UNSICHTBAR...**

AUS DER KLEINE PRINZ VON ANTOINE DE SAINT-EXUPÉRY

ABSTRACT

The starch and sugar content of potato tubers are model traits for a candidate gene approach towards understanding the molecular basis of quantitative trait loci (QTL) because carbohydrate metabolism is one of the best studied plant processes at the functional level. Many genes of potato and other plant species involved in carbohydrate metabolism and transport have been cloned and functionally characterized. The starch and sugar content influences the nutritional quality of fresh potatoes and processed products, such as potato chips and French fries. Biochemical and genetic data suggest that genes encoding invertases (β -D-fructofuranosidase) participate in the control of starch and sugar content of the potato tubers. In this context, the focus was on invertases, which are ubiquitous enzymes hydrolyzing sucrose into the reducing sugars glucose and fructose. Under cold storage, reducing sugars accumulate in a genotype dependent manner in tubers (cold sweetening). High reducing sugar levels result in a reduction of potato chips quality e.g. darker chips colour, bitter taste, and a higher acrylamide concentration.

In this PhD work natural alleles of potato invertases, found previously to be significantly associated with tuber starch and sugar content (LI ET AL., 2005, 2008), were characterized at the molecular and biochemical level. Starting point was the PCR based cloning of cDNAs encoded by the five known potato invertase genes *Pain-1* on chromosome III, *invGE* and *invGF* on chromosome IX, and *pCD111* and *pCD141* on chromosome X, which resulted in 64 distinct alleles. Phenetic tree analysis based on amino acid similarity separated the alleles of cell wall and vacuolar invertases into two classes. This was also in line with the genomic organization of the different invertase loci. The genomic structures of the *Pain-1* locus and the gene pair *pCD111/pCD141* were unravelled by isolation and sequence analysis of corresponding BACs. The genes *pCD111* and *pCD141* consist each of six exons and five introns, and are arranged in a direct tandem repeat. The gene *Pain-1* comprises seven exons and six introns without a tandem duplication. Furthermore, structural alterations due to allelic invertase amino acid sequence variation were clarified by 3D-model analysis. Alleles associated with superior potato chips quality showed charge differences in the putative sucrose binding site possibly resulting in a lower conversion of sucrose in reducing sugars, which could influence potato chips quality. To test whether allele specific amino acid variations lead to altered enzyme activities, potato invertases were assayed in the yeast invertase mutant *SUC2*. Measurements of enzyme affinity to sucrose (K_m) and rate of sucrose conversion (v_{max}) did not show differences between vacuolar and cell wall-bound invertase alleles, which could explain the association with chips quality. Expression analysis revealed genotype specific transcription patterns of the alleles from different invertase isoforms.

In conclusion, at this point no definite statement on the functional impact of the characterized invertase alleles on potato chips quality is possible. The results of this study show that invertase regulation takes place at the transcript as well as at the protein level, is genotype specific and depends on allelic sequence variation. This work is a first example of a multi-pronged approach to dissect the natural variation of sugar and starch content in potato at molecular level.

Zusammenfassung

Stärke- und Zuckergehalt von Kartoffelknollen sind ausgezeichnete Beispiele, die molekularen Grundlagen von *quantitative trait loci* (QTLs) mittels Kandidatengen zu identifizieren, da der Kohlenhydratstoffwechsel auf funktionaler Ebene einer der best untersuchten Prozesse in pflanzlichen Organismen ist. So wurden viele Gene aus Kartoffel und anderen Pflanzen, die mit dem Kohlenhydratstoffwechsel und -transport verbundenen sind, kloniert und hinsichtlich ihrer Funktion analysiert. Stärke- und Zuckergehalt beeinflussen unmittelbar die Qualität frischer Kartoffeln und weiter verarbeiteter Kartoffelprodukte wie zum Beispiel Kartoffelchips und Pommes Frites. Biochemische und genetische Daten deuten darauf hin, dass Invertase (β -D-fructofuranosidase) an der Kontrolle des Stärke- und Zuckergehaltes von Kartoffelknollen beteiligt ist. Deshalb wurde der Focus dieser Arbeit auf Invertasen gelegt, die universell verbreitet sind und Saccharose in die reduzierenden Zucker Glukose und Fruktose spalten. Eine durch Kaltlagerung bedingte Erhöhung an reduzierenden Zuckern in der Knolle ist abhängig von der Kartoffelsorte und äußert sich in einer Verschlechterung der Kartoffelchipsqualität, zum Beispiel dunklerer Farbe, bitterer Geschmack und einer höheren Konzentration von Acrylamid.

In dieser Doktorarbeit wurden natürlich vorkommende Allele von Kartoffelinvertasen, welche signifikant mit dem Stärke- und Zuckergehalt von Kartoffelknollen assoziiert sind (LI ET AL., 2005, 2008), hinsichtlich ihrer molekularen und biochemischen Eigenschaften charakterisiert. Die analysierten Gene waren *Pain-1* auf Chromosom III, *invGE* und *invGF* auf Chromosom IX, und *pCD111* und *pCD141* auf Chromosom X. Durch Klonieren von cDNA der fünf bekannten Invertasegene aus Kartoffel mittels PCR, konnten 64 Allele der unterschiedlichen Gene identifiziert werden. Weiterhin zeigte eine *phenetic tree*- Analyse, basierend auf Aminosäureähnlichkeiten der verschiedenen Invertaseisoformen, dass vakuoläre und apoplastische Invertasen in separate Klassen gegliedert werden können. Diese Einteilung wurde durch die unterschiedliche Genstruktur und -organisation der Isoformen, welche durch Isolation und Sequenzierung von entsprechenden BACs gewonnen wurde, bestätigt. So weisen die Gene *pCD111* und *pCD141* jeweils sechs Exons und fünf Introns auf und sind in einer direkten Tandem-Reihung angeordnet. Das Gen *Pain-1* enthält sieben Exons und sechs Introns, ohne ein Tandemduplikat aufzuweisen. Weiterhin wurden die durch allelische Aminosäuren ausgelösten strukturellen Veränderungen der Invertaseallele, mittels 3D-Analyse veranschaulicht. Allele, die nachweislich mit einer guten Kartoffelchipsqualität assoziiert sind, zeichneten sich durch Ladungsveränderungen in der potenziellen Saccharose-Bindedomäne des Enzyms aus. Diese Veränderungen führen möglicherweise zu einer verringerten Saccharosebindung und einer geringeren Konzentration an Glukose und Fruktose, was wiederum die Qualität von Kartoffelchips beeinflusst. Weiterführend wurde überprüft, ob allelspezifische

Aminosäurevariationen zu einer veränderten Enzymaktivität führen können. Hierzu wurden die Enzymaffinität zu Saccharose (K_m) und die Rate der Saccharoseumwandlung (v_{max}) von Invertaseallelen aus Kartoffel mit Hilfe der Hefemutante *SUC2* analysiert. Die Messungen zeigten keine biochemischen Unterschiede zwischen apoplastischen und vakuolären Invertaseallelen, welche die Assoziation mit Kartoffelchipsqualität hätten erklären können.

Zusätzlich wurde mittels Expressionsanalyse veranschaulicht, dass allelische Transkriptionsmuster verschiedener Invertaseisoformen genotypspezifisch sind.

Zusammenfassend ist fest zu stellen, dass an diesem Punkt der Arbeit keine absolute Aussage über den funktionalen Einfluss der beschriebenen Invertaseallele auf Kartoffelchipsqualität möglich ist. Die Ergebnisse dieser Doktorarbeit zeigen, dass Invertaseregulation sowohl auf Transkript- als auch auf Proteinebene erfolgt, genotypspezifisch ist und von der Sequenzvariation der Allele abhängt. Diese Arbeit ist ein erstes Beispiel für einen ebenenübergreifenden Ansatz, die natürlich vorkommende Variation des Stärke- und Zuckergehalts in Kartoffelknollen auf molekularer Ebene zu entschlüsseln.

Table of contents

TABLE OF CONTENTS.....	I
ABBREVIATIONS	IV
FIGURES	VII
TABLES	XI
1 INTRODUCTION.....	1
1.1 The potato	1
1.2 Crosstalk between starch and sugar metabolism and the phenomenon of cold sweetening	2
1.3 Invertases	6
1.4 The genetic map of potato	10
1.5 Objectives of this thesis.....	13
2 MATERIALS AND METHODS.....	15
2.1 Materials	15
2.1.1 Chemicals and Antibiotics	15
2.1.2 Buffers and Culture Media	15
2.1.3 Restriction enzymes, nucleic acid modifying enzymes and kits.....	15
2.1.4 Oligonucleotides.....	16
2.1.5 Plasmids	20
2.1.6 Bacterial strains	21
2.1.7 Yeast strains	21
2.1.8 Plant Material	21
2.2 Methods.....	22
2.2.1 Plant work	22
2.2.2 Bacterial work	22
2.2.3 Yeast work.....	22
2.2.4 Molecular biological methods.....	23
2.2.4.1 Genomic DNA extraction from leaf tissue.....	23
2.2.4.2 Plasmid DNA isolation from bacteria	23
2.2.4.3 Separation of DNA fragments by agarose gel electrophoresis	24
2.2.4.4 Separation of DNA fragments using pulse field gel electrophoresis (PFGE)..	24
2.2.4.5 Purification of PCR products and gel-extracted DNA fragments	25
2.2.4.6 Total RNA extraction from leaf and floral tissue.....	25
2.2.4.7 Total RNA extraction from tuber tissue.....	25
2.2.4.8 cDNA synthesis.....	26
2.2.4.9 Standard Polymerase Chain Reaction (PCR).....	26
2.2.4.10 Molecular cloning of cDNA constructs for yeast <i>SUC2</i> transformation	28

2.2.4.11	Quantitative real-time PCR	29
2.2.4.12	Sequencing	31
2.2.4.13	Pyrosequencing	31
2.2.4.14	Single stranded conformation polymorphism (SSCP) analysis	34
2.2.4.15	BAC DNA library screens.....	35
2.2.4.16	Colony-lift	36
2.2.4.17	Southern blot analysis	37
2.2.5	Three-dimensional modelling of invertase alleles	37
2.2.6	Biochemical methods	38
2.2.6.1	Protein extraction from yeast	38
2.2.6.2	Enzymatic assay of invertase	39
2.2.6.3	Protein separation by SDS-PAGE and Western blot analysis.....	42
3	RESULTS.....	44
3.1	The <i>Pain-1</i> locus on chromosome III.....	44
3.1.1	Structural characterization of the <i>Pain-1</i> locus	44
3.1.1.1	Molecular cloning of <i>Pain-1</i> invertase cDNA alleles from tuber tissue	44
3.1.1.2	Identification of associated <i>Pain-1</i> alleles	54
3.1.1.3	Three-dimensional modelling of <i>Pain-1</i> alleles.....	56
3.1.1.4	Genomic organization of the <i>Pain-1</i> locus.....	61
3.1.2	Functional characterization of the <i>Pain-1</i> gene.....	65
3.1.2.1	Differential expression analysis of <i>Pain-1</i> alleles during cold storage of potato tubers	65
3.1.2.2	Functional complementation of the yeast invertase mutant <i>SUC2</i>	75
3.1.2.3	Biochemical characterization of <i>Pain-1</i> alleles.....	77
3.1.2.4	Western blot analysis	82
3.2	The <i>Invap-b</i> locus on chromosome IX	84
3.2.1	Structural characterization of the genes <i>invGE</i> and <i>invGF</i>	84
3.2.1.1	Identification of associated <i>invGE</i> and <i>invGF</i> alleles	84
3.2.1.2	Molecular cloning of <i>invGE</i> and <i>invGF</i> invertase cDNA alleles.....	85
3.2.1.3	Three-dimensional modelling of <i>invGE</i> and <i>invGF</i> alleles.....	110
3.2.2	Functional characterization of the genes <i>invGE</i> and <i>invGF</i>	119
3.2.2.1	Differential expression analysis of <i>invGE</i> and <i>invGF</i> alleles	119
3.2.2.2	Functional complementation of the yeast invertase mutant <i>SUC2</i>	126
3.2.2.3	Biochemical characterization of <i>invGE</i> and <i>invGF</i> alleles	126
3.2.2.4	Western blot analysis of <i>invGE</i> and <i>invGF</i> invertase proteins.....	130
3.3	The <i>Invap-a</i> locus on chromosome X.....	131
3.3.1	Structural characterization of the genes <i>pCD111</i> and <i>pCD141</i>	131
3.3.1.1	Molecular cloning of <i>pCD111</i> and <i>pCD141</i> invertase cDNA alleles from leaf tissue	131
3.3.1.2	Classification of alleles of the genes <i>pCD111</i> and <i>pCD141</i>	151
3.3.1.3	Genomic organization of the <i>Invap-a</i> locus	152
4	DISCUSSION	156
4.1	The physiological impact of potato invertases on tuber chips quality	156
4.2	Structural characterization of potato invertase alleles.....	158

4.3	Functional characterization of potato invertase alleles	167
4.4	Potato invertase isoforms and corresponding alleles display a large structural and functional variation and are interesting candidate genes in the trait potato chips quality	181
4.5	Future perspectives about the investigation of potato invertases and their natural variation.....	183
5	REFERENCES.....	184
	APPENDIX.....	201
	ACKNOWLEDGEMENTS.....	207
	ERKLÄRUNG.....	209
	LEBENS LAUF	210

Abbreviations

112 A1 NE	yeast expression vector
%	percent
°C	degree celsius
µg	microgram
µl	microliter
3'	three prime end of a DNA fragment
5'	five prime end of a DNA fragment
aa	amino acid
BAC	bacterial artificial chromosome
BNA	Böhm-Nordkartoffel Agrarproduktion GbR, 29574 Ebstorf
Btn	Biotin
bp	base pair(s)
ca.	circa
cDNA	complementary deoxyribonucleic acid
cM	centi Morgan
cm	centimeter
CQA	potato chips quality in autumn after harvest
CQS	potato chips quality in spring after 3-4 months of cold storage
DNA	deoxyribonucleic acid
DNase	desoxyribonuclease
dNTP	deoxynucleosidetriphosphate
<i>E.coli</i>	<i>Escherichia coli</i>
e.g.	exempli gratia (Lat.) for example
EP	electrostatic potential
eQTLs	expression QTLs
EST	expressed sequence tag
<i>et al.</i>	<i>Et alii/et aliae</i> (Lat.) and others
EtBr	ethidium bromide
EtOH	ethanol
F1	first filial generation after cross
FAO	Food and agriculture organization of the United Nations
Fru	fructose
g	gram
Glc	glucose
GUS	β-glucoronidase
h	hour(s)
I	Inosin
InDels	insertions, deletions
INV	invertase
Inv-CW	cell wall-bound invertase
Inv-N	neutral invertase
Inv-V	vacuolar invertase

kb	kilo base pair(s)
kDa	kilo dalton
K_m	Michaelis constant
l	liter
LB medium	Luria Bertani medium
LD	linkage disequilibrium
M	molar
mg	milligram
min	minute(s)
mM	millimolar
mm	millimeter
MPIMP	Max-Planck-Institute for Molecular Plant Physiology/Golm
MPIZ	Max-Planck-Institute for Plant Breeding Research/Köln
mRNA	messenger ribonucleic acid
ng	nanogram
nm	nanometer
NOR	NORIKA GmbH, 18190 Groß Lüsewitz
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PFGE	pulse field gel electrophoresis
pH	negative decimal log. of the H^+ concentration
pmol	picomolar
qRT-PCR	quantitative reverse transcription PCR
QTL	quantitative trait locus/loci
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rpm	rounds per minute
RT	room temperature
S.	Solanum
SAR	SAKA-RAGIS Pflanzenzucht GbR, Zuchtstation Windeby, 24340 Windeby/Eckernförde
SDS	sodium dodecil sulfate
sec	second(s)
SNP	single nucleotide polymorphisms
SSCP	single stranded conformation polymorphism
ssp.	subspecies
SUC	sucrose
TSC	tuber starch content
TSY	tuber starch yield
U	unit(s)
V	volt
v_{max}	maximal velocity
WT	wild type

Abbreviations for nucleic acids

A	Adenine
T	Thymine
C	Cytosine
G	Guanine

Abbreviations for amino acids

A	Alanine
C	Cysteine
D	Aspartate
E	Glutamate
F	Phenylalanine
G	Glycine
H	Histidine
I	Isoleucine
K	Lysine
L	Leucine
M	Methionine
N	Asparagine
P	Proline
Q	Glutamine
R	Arginine
S	Serine
T	Threonine
V	Valine
W	Tryptophane
Y	Tyrosine

Figures

Figure 1.1:	Simplified schematic overview of starch and sugar metabolism.	4
Figure 1.2:	Subcellular location of invertase isoenzymes and phloem unloading pathways.	6
Figure 1.3:	Strategy of the PhD work.	13
Figure 2.2.1:	Simplified scheme of the principle of invertase reaction.	39
Figure 2.2.2:	Simplified scheme of the NADH coupled glucose detection.	39
Figure 2.2.3:	Overview of plate reader output and glucose calculation.	40
Figure 3.1.1:	Amino acid alignment of <i>Pain-1</i> alleles from the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’.	47
Figure 3.1.2:	Amino acid alignment of the <i>Pain-1</i> alleles from the diploid genotypes P18, P40, and P54.	50
Figure 3.1.3:	Amino acid alignment of all cloned <i>Pain-1</i> invertase alleles.	51
Figure 3.1.4:	Amino acid based phenetic tree (Neighbour-joining method) of all cloned <i>Pain-1</i> invertase alleles.	52
Figure 3.1.5:	Nucleotide sequence based phenetic tree (Neighbour-joining method) of all cloned <i>Pain-1</i> invertase alleles.	54
Figure 3.1.6:	Correlation of amino acid regions or exchanges and affected surface areas of <i>Pain-1</i> alleles.	57
Figure 3.1.7:	Structural comparison of ‘Satina’ alleles <i>Pain_SA</i> , <i>Pain_SN</i> , and ‘Diana’ alleles <i>Pain_DA</i> , <i>Pain_DN1</i> , <i>Pain_DN2</i> .	58
Figure 3.1.8:	EP of the alleles <i>Pain_SA</i> , <i>Pain_SN</i> , <i>Pain_DA</i> , <i>Pain_DN1</i> , and <i>Pain_DN2</i> .	59
Figure 3.1.9:	Focusing of the EP of the putative sucrose binding site of the alleles of the cultivars ‘Satina’ and ‘Diana’.	60
Figure 3.1.10:	Southern blot analysis of <i>Sma</i> I digested BC BAC clones.	62
Figure 3.1.11:	Position of the <i>Pain-1</i> gene within the sequenced BAC insert of BC14 (A) and genomic organization of the <i>Pain-1</i> gene (B).	64
Figure 3.1.12:	Pyrosequencing analysis of the alleles <i>Pain_SA</i> and <i>Pain_SN</i> of the cultivar ‘Satina’.	66
Figure 3.1.13:	Overview of the expression pattern during tuber cold storage combining allelic distribution and total invertase transcript analysis of the cultivar ‘Satina’.	67

Figure 3.1.14:	Pyrosequencing analysis of the alleles <i>Pain_DA</i> , <i>Pain_DN1</i> , and <i>Pain_DN2</i> of the cultivar ‘Diana’.	68
Figure 3.1.15:	Overview of the expression pattern during tuber cold storage combining allelic distribution and total invertase transcript analysis of the cultivar ‘Diana’.	69
Figure 3.1.16:	Pyrosequencing analysis of the alleles <i>Pain_TN1</i> and <i>Pain_TN2</i> of the cultivar ‘Theresa’.	70
Figure 3.1.17:	Overview of the expression pattern during tuber cold storage combining allelic distribution and total invertase transcript analysis of the cultivar ‘Theresa’.	71
Figure 3.1.18:	Pyrosequencing analysis of the alleles <i>Pain_P18N1</i> and <i>Pain_P18N2</i> of the genotype P18.	72
Figure 3.1.19:	Overview of the expression pattern during tuber cold storage combining allelic distribution and total invertase transcript analysis of the potato genotype P18.	73
Figure 3.1.20:	Pyrosequencing analysis of the alleles <i>Pain_P40N1</i> and <i>Pain_P40N2</i> of the genotype P40.	74
Figure 3.1.21:	Overview of the expression pattern during tuber cold storage combining allelic distribution and total invertase transcript analysis of the potato genotype P40.	75
Figure 3.1.22:	cDNA alleles used for <i>SUC2</i> complementation and <i>SUC2</i> transformants on solid yeast minimal media.	76
Figure 3.1.23:	Growth of complemented <i>SUC2</i> transformants.	76
Figure 3.1.24:	Ponceau S stained blot membrane.	82
Figure 3.1.25:	Western blot analysis of <i>Pain-1</i> alleles using an antibody against a 58kDa vacuolar invertase of potato.	83
Figure 3.2.1:	Amplification of a 402bp <i>invGF</i> fragment following the approach described by MADDISON ET AL. (1999).	85
Figure 3.2.2:	Full-length amplification of the gene <i>invGF</i> in different genotypes.	86
Figure 3.2.3:	Amino acid alignment of ‘Satina’, ‘Diana’, and ‘Theresa’ <i>invGE</i> alleles.	92
Figure 3.2.4:	Amino acid alignment of P18, P40, and P54 <i>invGE</i> alleles.	95
Figure 3.2.5:	Amino acid alignment of all cloned <i>invGE</i> alleles of all genotypes.	96
Figure 3.2.6:	Amino acid based phenetic tree (Neighbour-joining method) of all cloned <i>invGE</i> invertase alleles.	98

Figure 3.2.7:	Nucleotide sequence based phenetic tree (Neighbour-joining tree) of all cloned <i>invGE</i> invertase alleles.	99
Figure 3.2.8:	Amino acid alignment of ‘Satina’, ‘Diana’, and ‘Theresa’ <i>invGF</i> alleles.	103
Figure 3.2.9:	Amino acid alignment of P18, P40, and P54 <i>invGF</i> alleles.	105
Figure 3.2.10:	Amino acid alignment of all cloned <i>invGF</i> invertase alleles.	106
Figure 3.2.11:	Amino acid based phenetic tree (Neighbour-joining method) of all cloned <i>invGF</i> invertase alleles.	108
Figure 3.2.12:	Nucleotide sequence based phenetic tree (Neighbour-joining tree) of all cloned <i>invGF</i> invertase alleles.	110
Figure 3.2.13:	Correlation of amino acid regions and affected surface areas of <i>invGE</i> alleles.	111
Figure 3.2.14:	Structural comparison of ‘Satina’ alleles <i>E_SA</i> , <i>E_SN3</i> and ‘Theresa’ alleles <i>E_TA</i> , <i>E_TN1</i> .	111
Figure 3.2.15:	Comparative 3-D structures of the associated alleles <i>E_SA</i> and <i>E_TA</i> .	113
Figure 3.2.16:	EP of the ‘Satina’ and ‘Theresa’ alleles.	114
Figure 3.2.17:	Focusing on the EP of the putative sucrose binding site of the ‘Satina’ and ‘Theresa’ alleles.	114
Figure 3.2.18:	Correlation of amino acid regions and affected surface areas of <i>invGF</i> alleles	115
Figure 3.2.19:	Structural comparison of ‘Satina’ alleles <i>F_SN3</i> and <i>F_SN4</i> .	115
Figure 3.2.20:	EP of the alleles <i>F_SN3</i> and <i>F_SN4</i>	116
Figure 3.2.21:	Focusing of the EP of the putative sucrose binding site of the alleles <i>F_SN3</i> and <i>F_SN4</i> .	117
Figure 3.2.22:	Correlation of amino acid regions and affected surface areas of comparative <i>invGE</i> and <i>invGF</i> alleles.	118
Figure 3.2.23:	Structural comparison of <i>invGE</i> allele <i>E_SA</i> and <i>invGF</i> allele <i>F_SN4</i> .	118
Figure 3.2.24:	Pyrosequencing analysis of <i>invGE</i> alleles from the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’.	121
Figure 3.2.25:	Pyrosequencing analysis of <i>invGE</i> alleles from the diploid genotypes P18, P40, and P54.	122

Figure 3.2.26:	Pyrosequencing analysis of <i>invGF</i> alleles from the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’.	124
Figure 3.2.27:	Pyrosequencing analysis of <i>invGF</i> alleles from the diploid genotypes P40 and P54.	125
Figure 3.2.28:	cDNA alleles used for <i>SUC2</i> complementation and <i>SUC2</i> transformants on solid yeast minimal media.	126
Figure 3.3.1:	Amino acid alignment of <i>pCD111</i> alleles from the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’.	135
Figure 3.3.2:	Amino acid alignment of <i>pCD111</i> alleles from the diploid genotypes P40 and P54.	137
Figure 3.3.3:	Amino acid alignment of all cloned <i>pCD111</i> invertase alleles.	138
Figure 3.3.4:	Amino acid based phenetic tree (Neighbour-joining tree) of all cloned <i>pCD111</i> invertase alleles.	139
Figure 3.3.5:	Nucleotide sequence based phenetic tree (Neighbour-joining tree) of all cloned <i>pCD111</i> invertase alleles.	140
Figure 3.3.6:	Amino acid alignment of <i>pCD141</i> alleles from the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’.	143
Figure 3.3.7:	Amino acid alignment of <i>pCD141</i> alleles from the diploid genotypes P18, P40, and P54.	147
Figure 3.3.8:	Amino acid alignment of all cloned <i>pCD141</i> invertase alleles.	148
Figure 3.3.9:	Amino acid based phenetic tree (Neighbour-joining tree) of all cloned <i>pCD141</i> invertase alleles.	150
Figure 3.3.10:	Nucleotide sequence based phenetic tree (Neighbour-joining tree) of all cloned <i>pCD141</i> invertase alleles.	151
Figure 3.3.11:	Genomic organization of the genes <i>pCD111</i> and <i>pCD141</i> .	154
Figure 3.3.12:	Tandem repeat linkage of the genes <i>pCD111</i> and <i>pCD141</i> .	155
Figure 4.1:	Amino acid based phenetic tree of all cloned invertase cDNA alleles.	161
Figure 4.2:	Simplified scheme displaying features of an ‘ideal’ invertase allele acting positively on potato chips quality.	168
Figure 4.3:	Schematic comparison of vacuolar and cell wall-bound preproprotein invertases.	175

Tables

Table 1.1:	Invertase-trait association of tetraploid potato genotypes (Li et al., 2008).	12
Table 2.1.1:	Antibiotics.	15
Table 2.1.2:	Primers used for molecular cloning of PCR products.	16
Table 2.1.3:	Primers used for colony-PCR and for sequencing of constructs and PCR products.	17
Table 2.1.4.:	Primers used for pyrosequencing analysis of the <i>Pain-1</i> locus on chromosome III.	18
Table 2.1.5:	Primers used for pyrosequencing analysis of the gene <i>invGE</i> on chromosome IX.	18
Table 2.1.6:	Primers used for pyrosequencing analysis of the gene <i>invGF</i> on chromosome IX.	19
Table 2.1.7:	Primers used for expression analysis of the gene <i>invGF</i> on chromosome IX.	19
Table 2.1.8:	Primers used for association analysis.	19
Table 2.1.9:	Primers used for quantification of real-time PCR products in SYBR [®] Green based detection assays.	19
Table 2.1.10:	Primers used for BAC analysis and sequencing, colony-lift, and Southern blot analysis.	20
Table 2.1.11:	List of used vectors.	20
Table 2.2.1:	Components of the transformation reaction.	23
Table 2.2.2:	PCR machines.	26
Table 2.2.3:	Standard PCR reaction using <i>Taq</i> -DNA Polymerase	27
Table 2.2.4:	Standard PCR reaction using Roche Fast Start High Fidelity PCR System	27
Table 2.2.5:	Standard PCR reaction using Novagen KOD Hot Start DNA Polymerase	28
Table. 2.2.6:	Overview of generated constructs.	29
Table 2.2.7:	Reaction setup for qRT-PCR	30
Table 2.2.8:	Reaction setup for pyrosequencing	32

Table 2.2.9:	Primers used in the pyrosequencing assay of <i>invGE</i> alleles from the tetraploid cultivars.	33
Table 2.2.10:	Primers used in the pyrosequencing assay of <i>invGE</i> alleles from the diploid genotypes.	33
Table 2.2.11:	Primers used in the pyrosequencing assay of <i>invGF</i> alleles from the tetraploid cultivars.	33
Table 2.2.12:	Primers used in the pyrosequencing assay of <i>invGF</i> alleles from the diploid genotypes.	34
Table 2.2.13:	Reaction setup for invertase assay	39
Table 2.2.14:	Reaction setup for glucose determination.	40
Table 2.2.15:	Anti-invertase antibodies used in Western blot analysis.	43
Table 3.1.1:	Distribution of the associated SSCP fragments <i>Pain1-5c</i> , <i>Pain1-8c</i> and <i>Pain1-9a</i> present in the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’.	44
Table 3.1.2:	Potato chips colour scores for the cultivars ‘Satina’, ‘Diana’, and ‘Theresa’.	45
Table 3.1.3:	Overview of <i>Pain-1</i> alleles.	45
Table 3.1.4:	SNPs present in ‘Satina’, ‘Diana’, and ‘Theresa’ alleles.	46
Table 3.1.5:	SNPs present in P18, P40, and P54 alleles.	48
Table 3.1.6:	Genotype specific nucleotide differences of alleles identical at amino acid level.	53
Table 3.1.7:	Associated SSCP fragments <i>Pain1-5c</i> , <i>Pain1-8c</i> and <i>Pain1-9a</i> present in ‘Satina’, ‘Diana’, and ‘Theresa’.	55
Table 3.1.8:	Comparison and distribution of associated SSCP fragments and SNP 1544.	55
Table 3.1.9:	Positive BAC clones.	61
Table 3.1.10:	Summary of the results for BAC insert sequencing.	62
Table 3.1.11:	BC14 sequence annotation.	63
Table 3.1.12:	Ranges of the exons and introns of the <i>Pain-1</i> gene.	65
Table 3.1.13:	Overview of allele specific SNPs analyzed by pyrosequencing.	68
Table 3.1.14:	<i>Pain-1</i> alleles used for biochemical characterization.	77

Table 3.1.15:	K_m (mM) and v_{max} (mmol/h*mg protein ⁻¹) of <i>Pain-I</i> invertase alleles at 30°C and 4°C.	78
Table 3.1.16:	Overview of statistical significance levels of K_m values from the <i>Pain-I</i> invertase alleles at 30°C.	78
Table 3.1.17:	Overview of statistical significance levels of K_m values from the <i>Pain-I</i> invertase alleles at 4°C.	79
Table 3.1.18:	Overview of statistical significance levels of v_{max} values from the <i>Pain-I</i> invertase alleles at 30°C.	79
Table 3.1.19:	Overview of statistical significance levels of v_{max} values from the <i>Pain-I</i> invertase alleles at 4°C.	80
Table 3.1.20:	SNPs of the nucleotide variants of the allele <i>Pain_SN</i> .	81
Table 3.1.21:	K_m (mM) and v_{max} (mmol/h*mg protein ⁻¹) of <i>Pain-SNI</i> nucleotide variants at 30°C and 4°C.	81
Table 3.1.22:	Overview of statistical significance levels of K_m values from the <i>Pain-SNI</i> nucleotide variants at 30°C and 4°C.	82
Table 3.1.23:	Overview of statistical significance levels of v_{max} values from the <i>Pain-SNI</i> nucleotide variants at 30°C and 4°C.	82
Table 3.2.1:	Distribution of the associated SSCP fragments <i>invGE-6f</i> and <i>invGF-4d</i> present in the genotypes ‘Satina’, ‘Diana’, and ‘Theresa’.	87
Table 3.2.2:	Overview of <i>invGE</i> and <i>invGF</i> alleles.	87
Table 3.2.3:	SNPs present in ‘Satina’, ‘Diana’, and ‘Theresa’ alleles	88
Table 3.2.4:	SNPs present in P18, P40, and P54 alleles.	93
Table 3.2.5:	SNPs present in ‘Satina’, ‘Diana’, and ‘Theresa’ alleles.	100
Table 3.2.6:	SNPs present in P18, P40, and P54 alleles.	104
Table 3.2.7:	Genotype specific nucleotide differences of alleles <i>F_SNI</i> , <i>F_P18N</i> , and <i>F_P54NI</i> .	109
Table 3.2.8:	Allele specific SNPs analyzed by pyrosequencing.	120
Table 3.2.9:	Allele specific SNPs analyzed by pyrosequencing.	122
Table 3.2.10:	Allele specific SNPs analyzed by pyrosequencing.	123
Table 3.2.11:	Allele specific SNPs analyzed by pyrosequencing.	125
Table 3.2.12:	<i>invGE</i> and <i>invGF</i> alleles used for biochemical characterization.	127

Table 3.2.13:	K_m (mM) of <i>invGE</i> invertase alleles.	127
Table 3.2.14:	Statistical significance levels of K_m values from the <i>invGE</i> invertase alleles.	128
Table 3.2.15:	K_m (mM) of <i>invGF</i> invertase alleles.	128
Table 3.2.16:	Statistical significance levels of K_m values from the <i>invGF</i> invertase alleles.	129
Table 3.2.17:	Statistical significance levels of K_m values from the <i>invGE</i> and <i>invGF</i> invertase alleles.	130
Table 3.3.1:	Distribution of the associated SSCP fragment <i>pCD141_3c</i> present in the tetraploid genotypes ‘Satina’, ‘Diana’, and ‘Theresa’.	131
Table 3.3.2:	Overview of <i>pCD111</i> and <i>pCD141</i> alleles.	132
Table 3.3.3:	SNPs present in ‘Satina’, ‘Diana’, and ‘Theresa’ <i>pCD111</i> alleles.	133
Table 3.3.4:	SNPs present in P40 and P54 alleles.	136
Table 3.3.5:	Genotype specific nucleotide differences of alleles identical at amino acid level.	140
Table 3.3.6:	SNPs present in ‘Satina’, ‘Diana’, and ‘Theresa’ <i>pCD141</i> alleles.	141
Table 3.3.7:	SNPs present in P18, P40, and P54 <i>pCD141</i> alleles.	144
Table 3.3.8:	Positive BAC clones for the genes <i>pCD111</i> and <i>pCD141</i> .	152
Table 3.3.9:	BC3 sequence annotation.	153
Table 3.3.10:	Ranges of exons and introns of the genes <i>pCD111</i> and <i>pCD141</i> .	154
Table 4.1:	Overview of isolated invertase cDNA alleles.	158
Table 4.2:	Full-length primers used for gene specific PCR amplification.	159

1 Introduction

1.1 The potato

The potato (*Solanum tuberosum*) originated in the highlands of South America, where it has been cultivated for more than 8,000 years. Spanish explorers introduced the plant into Europe in the 16th century as a botanical curiosity (BRÜCHER, 1975). By the 19th century it was cultivated throughout the continent, providing an abundant and inexpensive food source. Today the potato is the fourth most important food crop in the world following wheat, maize and rice (www.cipotato.org). More than one-third of the global potato output now comes from developing countries, comparing to just 11% in the early 1960s. According to the latest FAO data from 2007 (Food and agriculture organization of the United Nations; <http://faostat.fao.org>), potato production worldwide stands at 322 million tons and covers more than 19 million hectares. China is now the world's largest potato producer with 70 million tons yearly. Although potato production in Europe has fallen since the early 1960s, this decline has been more than offset by the growth in Asia, Africa, and Latin America, thereby explaining the global rise in global potato tonnage. Because of its importance, potato became an object for extensive genetic studies increasing its resistance to pathogens, as well as improving cooking and nutrition qualities like the starch/sugar content and potato chips quality.

The genus *Solanum* contains seven cultivated and 228 wild species. The potato genome occurs in a range of chromosome numbers from $2n=24$ (diploid) in for example. *S. stenotomum*, *S. phureja*, and *S. ajanhuiri*, $3n=36$ (triploid) in *S. caucha*, and *S. juzepczukii*, $4n=48$ in *S. tuberosum* ssp. *tuberosum* and *S. tuberosum* ssp. *andigena*, $5n=60$ (pentaploid) in *S. curtilobum* to $6n=72$ (hexaploid) in *S. demissum* (HAWKES, 1990).

The cultivated potato was named by Carl von Linné from a specimen grown in Europe and the scientific name *Solanum tuberosum* is presently used to include domesticated potatoes from South America, Europe and the USA and derivatives of them in the rest of the world (INGRAM & WILLIAMS, 1991).

The cultivated potato (*S. tuberosum* ssp. *tuberosum*) is far from being an ideal species for genetic analysis due to its tetraploidy with tetrasomic inheritance. In addition, the high heterozygosity, the owing to inbreeding depression after repeated selfing, and multiple allelism generating a multitude of genotypes that are difficult to distinguish by comparing expected with observed segregation ratios complicates molecular analysis. An allele at any given locus

can occur in four allelic states: simplex (present one time at the locus), duplex (present two times), triplex (present three times) and quadruplex (present four times, homozygous state). Therefore, at any given genetic locus, up to four different alleles are possible. Nevertheless, the use of molecular markers enables the selection of favourable genotypes at an early time point in the potato breeding process.

1.2 Crosstalk between starch and sugar metabolism and the phenomenon of cold sweetening

As described above, potato is economically highly important. Being a staple food, potato is not only grown as a vegetable for table use but also processed into French fries and potato chips and used for dried products and starch production. In developed countries, up to 66% of potato in everyday diet is consumed in processed form. Consumers demands for convenient food at home, fast food in restaurants and snacking gave rise to a wide variety of processed products. These include potato chips, French fries, and various other frozen products, dehydrated potato products, as well as chilled-peeled and canned potatoes. Potato chips are thinly sliced potatoes, which are fried at high temperatures in different types of vegetable oil. The biggest problem of potato chips quality is the consumer's preference on fry colour, which is 'light-golden'. Fry colour is strongly dependent upon the reducing sugar concentration in tubers. But also other factors like variety characteristics, maturity at harvest, and ethylene in the storage atmosphere contribute to potato chips quality. A high reducing sugar content in tubers causes major problems for food processing because high frying temperatures lead to the non-enzymatic Maillard reaction between free aldehyde groups of the reducing sugars glucose and fructose and free α -amino groups of amino acids and proteins (SHALLENBERGER ET AL., 1959). With increasing concentration of reducing sugars in the raw tuber, chips and French fries colour changes from light yellow to dark brown and the fried products get a bitter taste (ROE ET AL., 1990). This process also attracts further attention because of the discovery that acrylamide, a potential neurotoxin and group 2A potential carcinogen, is formed from asparagine and reducing sugars via an N-glycoside intermediate in a side step of the Maillard reaction (MOTTRAM ET AL., 2002; CHUDA ET AL., 2003; OHARA-TAKADA ET AL., 2005; MATSUURA-ENDO ET AL., 2006).

The accumulation of reducing sugars in plants at low temperatures is a widespread and well-established phenomenon, often referred to as cold-induced sweetening or cold sweetening (MÜLLER-THURGAU, 1882). In mature potato tubers, which are stored in the cold to prevent tuber sprouting and loss of moisture, a shift in the balance between starch degradation and

glycolysis occurs, which leads to sucrose accumulation. Sucrose is then enzymatically converted into the reducing sugars glucose and fructose, which interfere with potato chips quality.

The sugar and starch metabolism is one of the best studied processes in plants. In higher plants, starch and sucrose are the primary products of photosynthetic carbon fixation. Starch is synthesized as an intermediate deposit in the chloroplasts, and stored in non-photosynthetic organs in amyloplasts. Sucrose is synthesized in the cytosol, transiently stored in the vacuole and exported via the phloem to sink tissues. For long-term storage sucrose can be converted into polymers such as starch, triacyl glycerides, polypeptides, or secondary compounds, enabling plants to cope with environmental biotic or abiotic challenges (STURM ET AL., 1999). In most plants, sucrose is the major form how carbon is transported from source to sink organs because of the non-reducing nature of the disaccharide, in which glucose and fructose are linked ($\alpha 1 \rightarrow \beta 2$); (ARAI ET AL., 1991). Sucrose and the cleavage products glucose and fructose are the central molecules for carbohydrate translocation, metabolism, and sensing in higher plants (ROITSCH & GONZÁLEZ, 2004).

Starch may be degraded either hydrolytically or phosphorolytically (MALONE ET AL., 2006). The products are exported from the amyloplast either as hexose phosphates via the glucose phosphate-phosphate translocator or as free sugars via the glucose and/or maltose transporters (SMITH ET AL., 2005). Once in the cytosol, these metabolites are converted to sucrose via sucrose phosphate synthase (KRAUSE ET AL., 1998). Subsequently, a proportion of the sucrose may be hydrolysed to glucose and fructose by acid invertase (GREINER ET AL., 1999; WINTER & HUBER, 2000).

Several mechanisms have been proposed to explain the sugar accumulation during cold storage in plants (Figure 1.1). An early suggestion is that sweetening occurs because the entry of hexose phosphates into glycolysis is restricted at low temperatures. Therefore, the products of starch breakdown are directed into the pathway of sucrose synthesis (AP REES ET AL., 1981). Other studies implied that cold-induced sweetening requires long-term changes. ISHERWOOD ET AL. (1976) demonstrated that sugar accumulation is delayed several days following transfer to low temperatures. This delay may be associated with changes in gene expression involved in cold acclimation (VAN BUSKIRK & THOMASHOW, 2006).

A precise explanation how low temperatures may stimulate sugar accumulation is still unclear, but several possibilities have been suggested. One is that sugar accumulation requires the induction of starch degrading enzymes. It has been reported that enzyme activities of

endo- and exo-amylase (COCHRANE ET AL., 1991) and starch phosphorylase (CLAASSEN ET AL., 1993) were increased during cold storage leading to increased formation of hexose phosphates. However, this cold induction of starch degrading enzymes was not observed consistently because most studies failed to discriminate between different isoenzymes that might contribute to the overall amylolytic and phosphorylytic activity (MALONE ET AL., 2006). A second possibility is that sugar accumulation requires an increased activity of enzymes involved in sucrose synthesis. DEITING ET AL. (1998) showed a temperature dependence of a novel form of sucrose phosphate synthase accompanied by sugar accumulation. Another possibility suggests that the formation of glucose and fructose might be required to promote sugar accumulation by removing sucrose. DUPLESSIS ET AL. (1996) reviewed the potential of the so-called ‘alternative pathway’ (cyanine resistant respiration) in the starch to sucrose conversion. Low temperatures activate the alternative pathway, which leads to decreased ATP levels and simultaneous increase in sucrose concentration. This sucrose becomes the substrate for vacuolar acid invertase resulting in the accumulation of reducing sugars. However, the physiological sense of soluble sugar accumulation during cold temperatures is presumably frost protection for plant tissues (PRESSEY, 1966, 1969).

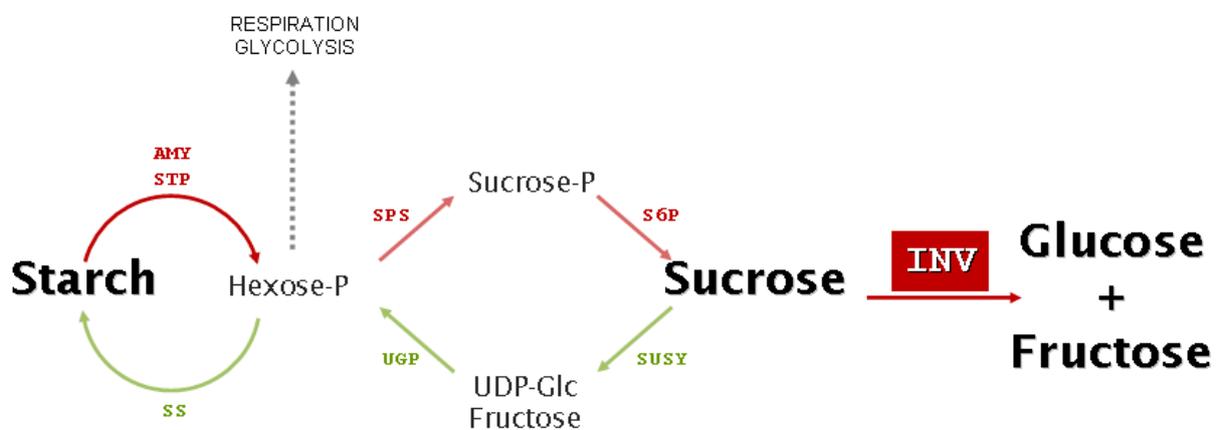


Figure 1.1: Simplified schematic overview of starch and sugar metabolism. Starch is degraded by endo- and exo-amylase (AMY) as well as by starch phosphorylase (STP). Products of these reactions are hexose phosphates (hexose-P), which can be shunted into glycolysis and respiration, or converted in starch by the action of starch synthase (SS). Another possibility is the synthesis of sucrose phosphate (sucrose-P) by sucrose phosphate synthase (SPS). Sucrose-P is then converted by sucrose 6-phosphatase (S6P) to sucrose. Sucrose can be degraded by sucrose synthase (SuSy) leading to the production of UDP-glucose and fructose. UDP-glucose is the substrate of UDP-glucose phosphatase leading to hexose-P. Another possibility of sucrose breakdown is the hydrolysis by invertases leading to the reducing sugars glucose and fructose, which mainly account for potato chips quality.

Cleavage of sucrose is mediated either by sucrose synthase (SuSy, EC 2.4.1.13) or by invertases (EC 3.2.1.26, β -fructosidase, β -fructofuranosidase). The reversible cleavage by sucrose synthase into UDP-glucose and fructose conserves the $\alpha 1 \rightarrow \beta 2$ glycosidic bond energy of sucrose. By contrast, invertases catalyze the irreversible hydrolysis yielding glucose

and fructose. The impact of SuSy and invertases in carbon partitioning are not clearly defined. Depending on the plant organ, tissue, and species, SuSy and invertases fulfil divergent functions and importance in the breakdown of sucrose. It is generally accepted that SuSy acts predominately in growing sink organs, whilst levels in photosynthetic source tissues are low (AP REES, 1984). In the developing potato tuber, the vast majority of sucrose degradation is catalyzed by SuSy, while invertases play only a significant role at the beginning of tuberization (APPELDOORN ET AL., 1997). The crucial role of SuSy in sucrose breakdown in tubers is its importance of sucrose mobilization for the synthesis of storage compounds as well as its predominance in determining the potato tuber sink strength (HEIM ET AL., 1993; SEBKOVA ET AL., 1993; ZRENNER ET AL., 1995).

Although studies suggest the major role of SuSy in sink strength regulation, also the participation of invertases in this process was demonstrated suggesting especially cell wall invertase isoforms as main determinants of sink strength. ROITSCH (1999) und ROITSCH ET AL. (2000) showed that cell wall invertase regulates sink strength especially during the initial stages of sink development. The essential role of cell wall-bound invertase isoforms in regulating phloem unloading and sink strength has been analysed in transgenic carrot plants (STURM & TANG 1999; TANG ET AL. 1999). Using antisense suppression of cell wall-bound invertases under the control of the 35S-CaMV promoter dominantly active in carrot tap roots resulted in lowered carbohydrate content in roots and increased leaf-to-root ratio. STITT & SONNEWALD (1995) demonstrated the induction of sink metabolism in source leaves of transgenic plants by over-expression of a yeast invertase. Another study showed that cell wall-bound invertase isoforms of maize play an important role in sucrose partitioning of the developing maize endosperm (MILLER & CHOUREY, 1992). In the developing seed coat of faba bean seeds, a correlation of the cell wall-bound invertases activity and high levels of hexoses in the cotyledons and in the apoplastic space has been determined (WEBER ET AL., 1995). Additionally, invertases serve as regulators of sucrose movement and utilization beyond developmental processes. As shown elsewhere they play an important role in pathogen defence responses (KOCAL ET AL., 2009) and cold protection (PRESSEY, 1966, 1969). In mature tubers invertase influences the accumulation of the reducing sugars glucose and fructose in response to cold storage. Several studies demonstrated the influence of low temperature on increasing invertase activity (ROREM & SCHWIMMER, 1963; PRESSEY & SHAW, 1966) and invertase transcript up-regulation (ZRENNER ET AL., 1996; ZHOU ET AL., 2004; BAGNARESI ET AL., 2008).

1.3 Invertases

As described above, the accumulation of reducing sugars in mature tubers is due to invertase activity rather than to SuSy induced sucrose cleavage.

Invertases are ubiquitous enzymes in plants and occur in several isoforms. Based on their solubility, subcellular localization, pH optimum, and isoelectric point, three different types of invertase isoenzymes can be distinguished: vacuolar, cell wall-bound, and neutral invertases (Figure 1.2).

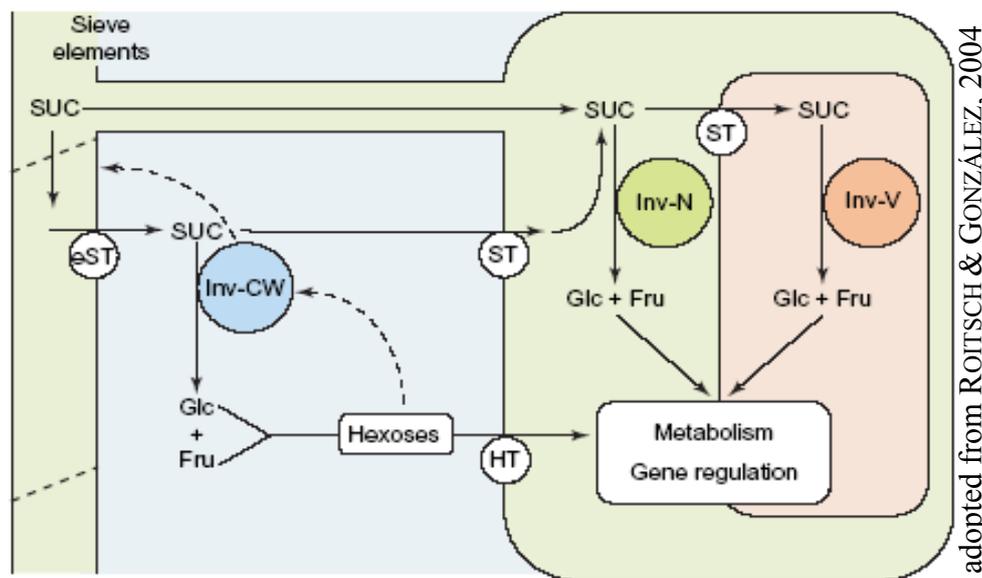


Figure 1.2: Subcellular location of invertase isoenzymes and phloem unloading pathways. Sucrose can be hydrolysed by different invertase isoforms depending on the mechanism of phloem unloading in the sink tissue. In symplastically isolated tissues, sucrose is unloaded from the sieve elements of the phloem into the apoplast by an assumed efflux sucrose transporter (eST). Once arrived in the apoplast, sucrose can be cleaved by a cell wall-bound invertase (Inv-CW). The resulting hexoses will be transported into the sink cell by a hexose transporter (HT). Alternatively, sucrose can be transported into the sink cells by a sucrose transporter (ST). Sucrose can be unloaded in the sink cells through the apoplast or by plasmodesmata. In the cytosol sucrose can be cleaved by neutral invertase (Inv-N) or sucrose synthase (SuSy). Sucrose is also stored in the vacuole for sucrose utilization and remobilization for metabolic processes. In the vacuole sucrose can be hydrolysed by vacuolar invertase (Inv-V). The hexoses glucose and fructose, which resulted from sucrose cleavage, are not only substrates for heterotrophic growth but also function as regulators of gene expression. Invertases also play a role in sink strength regulation maintaining and stimulating the enhancement of the flow of assimilates from source to sink. Abbreviations: SUC=sucrose, Glc=glucose, Fru=fructose.

Vacuolar and cell wall-bound invertases have similar enzymatic and biochemical properties and share two conserved amino acid motifs. Both types of invertases are β -fructofuranosidases, which act in an acidic pH optimum and are able to convert other fructofuranosidases such as stachyose and raffinose. The conversion of alternative substrates is less efficient than sucrose cleavage. Another interesting fact is that vacuolar and cell wall-bound invertases are glycoproteins (STOMMEL & SIMON, 1990). Glycosylation of vacuolar and extracellular glycoproteins is fairly different. Most of the vacuolar glycoproteins described so

far were found to be N-glycosylated with modified N-glycans containing fucose and/or xylose residues, but without terminal glucosamine residues. In contrast, extracellular glycoproteins were found to be N-glycosylated mostly by complex-type N-glycans including large structures with terminal fucose and galactose residues (RAYON ET AL., 1998). Both types of glycoproteins are post-Golgi modified, leading to protein maturation in the vacuole or in the extracellular compartment. It was shown that N-glycosylation in plants plays an important role in the induction of correct folding, the biological activity of the protein, and prevention of proteolytic degradation (reviewed in RAYON ET AL., 1998). FAYE & CHRISPEELS (1989) demonstrated the proteolytic degradation of unglycosylated cell wall-bound invertase of carrot in the secretory pathway or immediately after its arrival in the cell wall. Furthermore, N-linked oligosaccharides may contain targeting information or may be involved in protein recognition (RAYON ET AL., 1998). Glycosylation of acid invertases is required for their transport across either the plasma membrane or the tonoplast, according their localization in the apoplast or the vacuole.

Invertase isoenzymes are encoded by small gene families. The genomic organization and exon/intron structure seems to be conserved between monocotyledons and dicotyledons (reviewed in TYMOWSKA-LALANNE & KREIS, 1998 and ROITSCH & GONZÁLES, 2004). Plant invertases isolated to date have fairly similar structures and consist of six to eight exons. A special feature is the presence of a mini exon of only nine nucleotides that was found in all invertase genes except *InvDC1* of carrot (RAMLOCH-LORENZ ET AL., 1993; LORENZ ET AL., 1995; SIMPSON ET AL., 2000). The mini exon, one of the smallest known, encodes the three amino acids DPN of the highly conserved β -fructosidase motif N-DNP-G/A that spans three exons and is found in invertases from plants, bacteria, and yeasts (reviewed in ROITSCH & GONZÁLES, 2004). BOURNAY ET AL. (1996) observed that under cold stress the mini exon of the *pCD111* potato cell wall invertase was skipped in an alternative splicing event. The functional relevance of the splicing effect was not investigated.

Acid invertases share another conserved peptide domain, which belongs to the active site of the enzyme. Within this domain WECxDF x stands for yaline in vacuolar invertases while it is proline in cell wall-bound invertases (periplasmatic), (TYMOWSKA-LALANNE & KREIS, 1998; STURM, 1999). This conserved single amino acid difference determines the distinctly more acidic pH optimum of cell wall-bound invertases and their substrate specificity (GOETZ & ROITSCH, 1999).

Vacuolar and cell wall-bound invertases are synthesized as preproteins with a leading sequence that is cleaved off during transport and protein maturation. The leader sequence

separates in two fragments, a signal peptide and an N-terminal extension. The signal peptide is required for entry into the endoplasmic reticulum (ER) and consists of a basic region, a hydrophobic core of approximately 20 amino acids, and a polar region. The N-terminal extension is characteristic for all known vacuolar invertases and might be involved in the regulation of enzymatic activity, folding and stability of the polyprotein, or in vacuolar targeting (reviewed in TYMOWSKA-LALANNE & KREIS, 1998). Additionally, vacuolar invertases contain a C-terminal extension, not present in cell wall-bound invertases. This extension might be also involved in vacuolar targeting of the enzyme (BEDNAREK & RAIKHEL, 1992). Mature invertases show a molecular mass in the range of 60kDa.

Vacuolar invertases, also referred to as soluble acid invertases, are characterized by an acidic pH optimum of 5.0-5.5 and are localized in the vacuole (BOLLER & KENDE, 1979). They are crucial in determining the level of sucrose stored in the vacuole and in remobilization of sucrose for metabolic processes. Another well studied function is the regulation of the sugar balance in fruit tissues of tomato and mature potato tubers (OHYAMA et al., 1995; SCHOLES ET AL., 1996; GREINER ET AL., 1998). In potato one locus on chromosome III is known to encode vacuolar invertase. This locus is named *Pain-1*. To date, no information about genomic sequence, exon/intron structure and gene organization is available. Searching for orthologous genes in the syntenic tomato genome resulted in one EST on tomato chromosome III (www.sgn.cornell.edu). The activity of vacuolar invertases is correlated with the hexose/sucrose ratio in cold stored tubers (PRESSEY & SHAW, 1966), which leads to an increased accumulation of glucose and fructose in mature tubers. However, RICHARDSON ET AL. (1990) showed that there is no correlation between invertase activity and the total amount of accumulated sugars. Similarly, while antisense inhibition of invertase results in a decrease in the amount of hexoses and an increase in the amount of sucrose it does not affect the total amount of sugars accumulated (ZRENNER ET AL., 1996). The reducing sugars, increased during tuber cold storage due to cold protection, interfere with the trait potato chips quality that is analyzed in this study. The gene *Pain-1* is known to be differentially expressed during tuber cold storage (ZRENNER ET AL., 1996; ZHOU ET AL., 2004; BAGNARESI ET AL., 2008), showing transcript accumulation under the latter conditions.

Cell wall-bound invertases, also referred to as insoluble acid invertases, extracellular and periplasmic invertases, are characterized by a low pH optimum of 3.5-5.0. They are localized in the apoplast and ionically bound to the cell wall. Cell wall-bound invertases cleave sucrose on its way from sieve elements of the phloem to the apoplast. The cleavage products glucose and fructose are then transported into the sink cells by hexose transporters.

In potato two different loci encoding cell wall-bound invertases are known. The first locus is *Inv_{ap}-b* on chromosome IX, which consists of two invertase genes *invGE* and *invGF* linked in a direct tandem repeat and separated by approximately 2.3kb from each other (MADDISON ET AL., 1999). The locus has a size of approximately 8.6kb. The genes *invGE* and *invGF* exhibit a similar exon/intron structure composed of six exons and five introns. The second locus of cell wall-bound invertases is *Inv_{ap}-a* on chromosome X, which consist of two genes *pCD111* and *pCD141*. Comparative analysis of the potato genetic map revealed that the gene pair *pCD111/pCD141* on chromosome X arose by partial chromosome duplication of the gene pair *invGE/invGF* of chromosome IX, and, therefore, might also be organized in a direct tandem repeat (GEBHARDT ET AL., 2003; FRIDMAN ET AL., 2003).

The third type of invertases are neutral invertases, also known as alkaline or cytoplasmatic invertases because of their pH optimum between 6.8 and 8.0, and their localization in the cytoplasm. Limited information about the physiological function is available due to the labile nature of this invertase isoform and low enzyme activity. In contrast to acidic invertases, neutral invertases are not glycosylated and preferentially or solely hydrolyse sucrose. Therefore, they are no fructofuranosidases, which also convert substrates such as stachyose and raffinose like described for vacuolar and cell wall-bound invertase isoenzymes. Neutral invertases are strongly inhibited by their cleavage products but not by heavy metals. The latter observation suggests marked differences in the catalytic site compared to acidic invertases. Neutral invertases were only found in cyanobacteria and plants suggesting an origin from orthologous prokaryotic genes after endosymbiosis (VARGAS ET AL., 2003). Native polypeptides of neutral invertase are homotetramers composed of subunits with a molecular mass of 54 to 65kDa (ROSS ET AL., 1996), except the enzyme of carrot (LEE & STURM, 1996). In potato no locus for neutral invertase was detected so far and no sequence information is available.

1.4 The genetic map of potato

The potato map is one of the most highly marker-saturated maps among the crop plant species. Initially, two potato maps were constructed concurrently using RFLP markers on different genetic backgrounds (BONIERBALE ET AL., 1988; GEBHARDT ET AL., 1989). These maps were then compared with each other and also aligned to the tomato RFLP map (GEBHARDT ET AL., 1991; TANKSLEY ET AL., 1992). Comparative mapping revealed that the genomes of potato and tomato are co-linear except for paracentric inversions of five chromosome arms (BONIERBALE ET AL., 1988; TANKSLEY ET AL., 1992). Comparison of the potato genetic map with the physical map of the sequenced *Arabidopsis* genome displayed syntenic relationships between circa 40% of the potato genetic map and circa 50% of the physical map of this very distantly related plant species (GEBHARDT ET AL., 2003). With the development of new molecular markers, the potato map was enriched, and at the moment it is based on more than 350 markers, which cover approximately 90% of the potato genome (GEBHARDT & VALKONEN, 2001). Molecular markers originate from natural DNA variation present in a population of individuals of the same species. The molecular basis of the variations are point mutations (SNP, single nucleotide polymorphisms), and insertions, deletions (InDels), or inversions of DNA fragments in one allele versus another.

The existence of highly marker saturated potato maps allows localizing many genes on the twelve potato chromosomes, and markers linked to these genes can be used to perform positive marker-assisted selection. Potato function maps have been constructed for pathogen resistance (GEBHARDT & VALKONEN, 2001), and for tuber traits e.g. tuber starch content (FREYRE & DOUCHES, 1994; SCHÄFER-PREGL ET AL., 1998), chips colour (FREYRE & DOUCHES, 1994), and cold sweetening (MENÉNDEZ ET AL., 2002), and many more traits (reviewed in GEBHARDT ET AL., 2004). Integration of positional information on genetic factors controlling agronomic characteristics results in potato function maps as a basis for innovative approaches improving breeding. Understanding the molecular basis of complex traits and the underlying genes and proteins will enable combination of favourable alleles in improvement programs, leading to ‘precision breeding’.

❖ Quantitative trait loci (QTL)

The starch and sugar content of potato tubers are quantitative traits. These physiological characteristics show continuous or quantitative phenotypic variation because phenotypic expression is controlled by more than one gene. Additionally, environment has a large influence. The precise number of the genes involved in a quantitative trait is usually not

known. The loci where such genes are located in the genome are referred to ‘quantitative trait loci’ (QTL), (GELDERMANN, 1975). The traits can be genetically dissected using linkage maps that are based on molecular markers (reviewed by TANKSLEY, 1993).

Structures and functions of proteins encoded by a QTL are unknown. It is assumed that DNA polymorphisms must exist in the gene(s) responsible for the observed QTL effect. These DNA polymorphisms are the molecular basis for phenotypic selection of superior genotypes by breeding. In contrast to many mutations with drastic effects on the phenotype, the molecular and functional variability present in genes controlling QTL operates under field conditions and, therefore, should not have severe effects on fitness.

QTL maps are the first step toward the identification of the responsible genes, either by positional cloning or by candidate gene approach.

QTLs for tuber starch and sugar content or chips colour have been mapped in potato (DOUCHES & FREYRE, 1994; SCHÄFER-PREGL ET AL., 1998; MENÉNDEZ ET AL., 2002). A number of candidate genes have been identified regarding the fact of co-localisation with QTLs or their position on molecular maps, as well as being functional in the biosynthesis, degradation, or transport of starch and sugars in potato and other plants (CHEN ET AL., 2001, MENÉNDEZ ET AL., 2002). Among others, invertase genes were identified as positional candidates for cold-sweetening QTLs.

❖ Association genetics based on candidate genes

Association genetics based on candidate genes is one approach aiming towards the improvement of crop breeding programs (‘precision breeding’). The candidate gene approach is based on the knowledge of a gene’s function controlling the trait of interest. Additionally, co-localization of a functional candidate gene with a QTL for the trait of interest leads to a positional candidate (PFLIEGER ET AL., 2001).

Association analysis was performed for potato vacuolar invertase and cell wall-bound invertase isoforms due to the fact of being functional candidates in the tuber sugar metabolism as well as their co-localization with QTLs for tuber sugar content (CHEN ET AL., 2001; MENÉNDEZ ET AL., 2002).

The locus *Pain-1* on chromosome III codes for a vacuolar invertase isoform. Association analysis of allelic *Pain-1* fragments revealed strong associations of the SSCP fragment *Pain-1-9a* (8c, 5c) with potato chips quality after cold storage, explaining 10.4% of the phenotypic variance (LI ET AL., 2008). This marker allele also showed association with chips quality without cold storage, tuber starch content and tuber starch yield (Table 1.1). Furthermore, a

negative association of the allelic *Pain-1* fragment *Pain1-5b* with potato chips quality after cold storage was detected, which explains 2.5% of the phenotypic variance. This SSCP fragment is also negatively associated with tuber starch content (Table 1.1).

In addition, association analysis was carried out for the genes *invGE*, *invGF* and *pCD141*, which encode cell wall-bound invertase isoforms. LI ET AL. (2005, 2008) showed that *invGE* and *invGF* allelic fragments, which were in linkage disequilibrium, were associated with better potato chips quality. The SSCP fragments *invGE-6f* and *invGF-4d* explain 2.8% of phenotypic variance of potato chips quality after tuber cold storage (Table 1.1). Additionally, these alleles displayed association with better potato chips quality without cold storage. The genes *invGE* and *invGF* co-localize with a QTL for starch and sugar content of potato tubers *Sug9a* (CHEN ET AL., 2001; MENÉNDEZ ET AL., 2002).

Analysis of allelic fragments of the gene *pCD141* revealed a negative association of one SSCP fragment *pCD141-3c* with potato chips quality without cold storage, after storage in the cold and tuber starch yield (LI ET al., 2008; Table 1.1).

Table 1.1: Invertase-trait association of tetraploid potato genotypes (Li et al., 2008).

Invertase genes	Chr. no.	Marker allele	CQA $q (R^2)$	CQS $q (R^2)$	TSC $q (R^2)$	TSY $q (R^2)$
<i>Pain-1</i>	III	<i>Pain1-5b</i> <i>Pain1-9a (8c, 5c)</i>	ns 0.001 (4.4) ↑	0.048 (2.5) ↓ 0.000 (10.4) ↑	0.002 (5.3) ↓ 0.000 (12.0) ↑	ns 0.003(5.3) ↑
<i>invGE/invGF</i>	IX	<i>invGE-6f</i> (<i>invGF-4d</i>)	0.034 (2.2) ↑	0.040 (2.8) ↑	ns	ns
<i>pCD111/pCD141</i>	X	<i>pCD141-3c</i>	0.008 (3.1) ↓	0.028 (3.1) ↓	0.007 (4.2) ↓	ns

Associations $q > 0.05$ are significant, ns=not significant ($q < 0.05$). The amount of variance (in %) explained by the marker allele is given by the R^2 statistic. Marker fragments shown in parenthesis are in nearly absolute LD, having identical or highly similar distribution in the population and show similar associations. ↑↓: direction of effect. ↑ the marker allele has a positive effect on the trait. ↓ the marker allele has a negative effect on the trait.

Association of one marker allele with more than one trait might be explained by the involvement of the same gene in multiple metabolic pathways interconnecting these complex traits. The pleiotropic effects of individual alleles always have the same direction, either positive (more starch, less reducing sugars, better potato chips quality) or negative (less starch, more reducing sugars, inferior chips quality).

1.5 Objectives of this thesis

Based on the previous studies of QTL detection for potato tuber starch and sugar content as well as association analysis of candidate genes of the starch and sugar metabolism, invertases were found to fulfil criteria being positional and functional candidates. Objective of this study was the identification and characterization of allelic variation of vacuolar and cell wall-bound invertase isoforms contributing to the important agronomic trait potato chips quality. The project aims can be summarized as follows (i) identification of alleles of all three known potato invertase loci *Pain-1*, *Inv_{ap-b}* and *Inv_{ap-a}*; (ii) analysis of putative effects of allele specific amino acid differences using 3D structural comparison; (iii) biochemical analysis of invertase alleles and (iv) allele specific differential expression analysis.

Figure 1.3 shows the aspects dealt with in each of the following chapters.

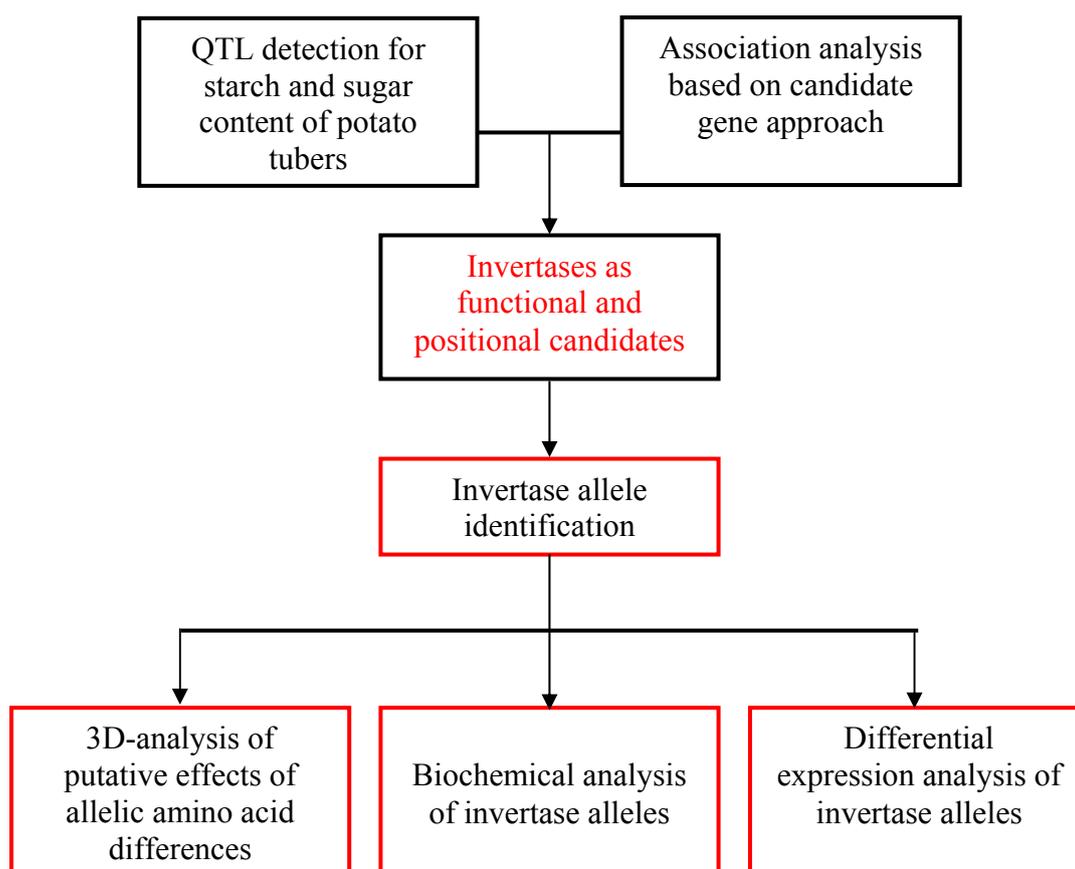


Figure 1.3: Strategy of the PhD work. The black boxes contain aspects, for which information was available. The red boxes contain aims of the study, which were set to elucidate the impact of different alleles of invertase isoforms on the analyzed trait potato chips quality.

The present study pursues to elucidate whether natural allelic variation of functional potato alleles within different genotypes can be identified and to what extent this natural variation

accounts for the observed phenotypic diversity of the selected genotypes regarding the trait chips quality. In consequence, the question arises whether allelic variation manifests at the functional level resulting in phenotypic diversity and whether these functional differences can be characterized, like observed in tomato for the fruit sugar content (FRIDMAN ET AL., 2004). This work will show whether natural variation of potato invertases is responsible for part of the phenotypic variation of tuber starch and sugar content, ultimately of potato chips quality.

2 Materials and Methods

2.1 Materials

2.1.1 Chemicals and Antibiotics

The chemicals and antibiotics (Table 2.1.1) were purchased from the following suppliers: Amersham Pharmacia Biotech (Braunschweig), Carl Roth GmbH (Karlsruhe), Difco Laboratories (Detroit, Michigan, USA), Invitrogen GmbH (Karlsruhe), Merck, Feinchemikalien und Laborbedarf (Darmstadt), Roche (Mannheim), and Sigma-Aldrich Chemie GmbH (Taufkirchen).

Table 2.1.1: Antibiotics.

Antibiotics	Solvent	Final conc. for selection on LB
Ampicillin (Amp)	H ₂ O	100µg/ml
Chloramphenicol (Cam)	Ethanol	12,5mg/l
Tetracyclin (Tet)	H ₂ O	12,5mg/l

2.1.2 Buffers and Culture Media

Stock solutions of the following buffers and culture media were prepared as described by SAMBROOK & RUSSELL (2001): DNA loading buffer, SDS, TAE, TBS, LB, YPD and SC medium. All media and solutions were made with highly purified Milli-Q-water (Millipore, Bedford, USA). Whenever required, the solutions were autoclaved for 20min at 121°C.

2.1.3 Restriction enzymes, nucleic acid modifying enzymes and kits

Restriction enzymes and corresponding buffers were used from New England BioLabs GmbH (Schwalbach/Taunus), MBI Fermentas GmbH (St. Leon-Rot) and Roche (Mannheim).

Nucleic modifying enzymes were used from:

DNA-freeTM Kit (Ambion, Cambridgeshire, UK)

Fast Start High Fidelity PCR System (Roche, Mannheim)

KOD Hot Start DNA Polymerase (Novagen, Darmstadt)

Ribonuclease Inhibitor (Roche, Mannheim)

RNase H (Roche, Mannheim)

SuperscriptTM II reverse transcriptase (Invitrogen, Karlsruhe)

Taq DNA Polymerase (PLUTHERO, 1993)

T4 DNA ligase (Invitrogen, Karlsruhe)

The following commercial reagents and kits were used:

Bio-Safe™ Coomassie G-250 stain (Bio-Rad, Hercules, USA)

ExoSAP-IT® (USB Corporation, Cleveland, USA)

First Strand cDNA Synthesis kit (Invitrogen, Karlsruhe)

High Pure PCR Purification kit (Roche, Mannheim)

LiCrosolv® (Merck KGaA, Darmstadt)

Miniprep® Kit/Midiprep® (Qiagen, Hilden)

P3504 Ponceau S (Sigma-Aldrich Chemie GmbH, Taufkirchen)

pGEM® - T and pGEM® - T Easy Vectors (Promega, Mannheim)

Power SYBR® Green PCR Master Mix (Applied Biosystems, Carlsbad, USA)

Protein Assay (Bio-Rad, Hercules, USA)

PSQ 96 SNP Reagent Kit (Biotage AB, Uppsala, Sweden)

PureLink™ Plant RNA Reagent (Invitrogen, Karlsruhe)

RNAwiz™ (Ambion, Cambridgeshire, UK)

Streptavidin-coated Super Paramagnetic beads (Amersham Biosciences, Sweden)

ToTally RNA™ (Ambion, Cambridgeshire, UK)

2.1.4 Oligonucleotides

Synthetic oligonucleotides were purchased from Invitrogen (Karlsruhe), Sigma-Aldrich Chemie GmbH (Taufkirchen), and Operon Biotechnologies GmbH (Köln). The primers used in this study are listed in the following Tables.

Table 2.1.2: Primers used for molecular cloning of PCR products.

Name	Primer sequence in 5'-3' orientation
ZrPain-F	ATGGCCACGCAGTACC
PainUni-R	GATGAATTACAAGTCTTGCAAGGG
PainNotI_F	CCCCGCGCCGCATGGCCACGCAGTACC
PainNotI_R	ATATGCGGCCGCGATGAATTACAAGTCTGG
PainBamHI_R	CCCCGATCCGATGAATTACAAGTCTTGCAAGGG
PainGeno_For	GGGATTAACATGAGATCGTGTG
PainGeno_Rev	CCTGTACAGATGCCCATCCC
invGE-F-fulgth	ATGGAATTATTTATGAAAAGCTTCTCTTTGGGGGT
invGE-R-fulgth	TTAGTGCATCTTAGGTACATCCATGCTCCAAGC
ENotI_F	GATTGCGCCGCATGGAATTATTTATGAAAAGC
ENotI_R	CCCCGCGCCGCTTAGTGCATCTTAGGTACATCC
invGF-F-fulgth	ATGGAATTATTCATCTAATTCTCGTTGGGCTTTGCCAG
invGF-R-fulgth	TCAATATTGTATCTTAGCTTTGCCATACTCCATGC
FNotI_F	CCCCGCGCCGCATGGATTATTCATCTAATTC
FBamHI_R	CCCCGATCCTCAATATTGTATCTTAGCTTTGCC
CD111fl_F	ATGGAATTGTTTAAAAAAGTCTTCTC
CD111fl_R	TCAATAAGAAGAGTGACCAAATGACCAATTCA
CD141fl_F	ATGGAGATTTTAAAGAAGATCTTCTCTTTGGGTT
CD141fl_R	CTAGTGCAACTTTGCATTAGCCATGCTCCAAGC

Table 2.1.3: Primers used for colony-PCR and for sequencing of constructs and PCR products.

Name	Primer sequence in 5'-3' orientation
T7	GTAATACGACTCACTATAGGGC
SP6P	TATTTAGGTGACACTATAG
Adh1-Prom	CTCACCATATCCGCAATGAC
Adh1-Term	CTTGAGTAACTCTTTCCTGTAGGTC
G112_1	GGTCAGGTTGCTTTCTCAGGTATAG
G112_2	GAGAGTAGTGTGCGTGAATGAAGG
G112_3	CAGAACAGAAATGCAACGCG
G112_4	CCCAGTCACGACGTTGTAAAAC
G112_5	CCAACAAAGAATCTATACTTC
G112_6	CGACGCTCAAGTCAGAGGTGG
G112_7	GCTCGTTACAGTCCGGTGCG
G112_8	CATTGCGGATATGGTGAGACAAC
G112_9	GCGCATCACCAACATTTCTG
G112_10	CTGACAGTTACCAATGCTTAATC
PGo2	GCCTCCCATTACACATTCTC
PGo3	GGTAAAACGGGTATTGCACTTG
PGo3N	GATCCTCTCCTTCTAGACTGGGTC
PGo4	GCATCAAAGACATTTTATGACCCG
EGo2	CTATTACTGTCAACAATGTTTCATAGAAC
EGo3	GTCCTTAAGAATAGCCTTGATG
EGo2N	GCACCAATGTATTATAATGGAG
EGo2_67.11_41	GACTTGCCGTCTTCAAATGC
FGo2	CAATCTCAAATGCTGTAATGTTC
FGo3	GTATGGATCTTACTCGATTTGAG
Pain_SEQ3	GTAACATATCACTAGTAAAATTTG
Pain_SEQ4	CCCTGAGAAACACCTCTTGAC
Pain_SEQ5	CCGGTGACACTGATGATTATGTAC
Pain_SEQ6	CAAGGAGGAAGAACAGTCATAAC
Pain_SEQ7	GGTCCAAAATGCCTCTGC
Pain_SEQ12	GAAAGCTCCATGAAATCTAATGTC
Pain_SEQ11	CTTATCCCGGTACTAATTATTAATC
Pain_SEQ13	GATCTCGCTCGCCTCCCTC
25_SEQ3	GTAACAATTGGTTCAATGGCC
25.12_CheckG	GTATGGATCTTACTCGATTTGAG
A60_For	CCATTGTACCACAAGGGATG
A60_Rev	CCCACATACCCGTACCCG
A60_SEQ3	GAGACCCGACTACTGCTTGG
A60_2F1	CCCATTACACATTCTCCCG
A60_2SEQ4	GGTTCAATTGAGCTACTCCATG
34.1_For	GCATGCGGTTCCGGGTAC
34.1_Rev	CTGCAGCTGAGTCAACATGGAG
40.58_For	GAAGCCTCATTTGAAGTGGAC
40.58_Rev1	GCAACTCCCGGAGCCACTG
40.58_Rev2	CTCATCGAATGTTTTTCACCG
51.81_For	GGGACCATTGGTGTGCTTG
36.1_SEQ3R	CCAGTATGAGGAGATGAATGAAG
36.1_3Nr4-F1	GCTCTTCTTTGGGGTTTAG
37.17_revG	CCTGTAACCTCAACCTTTTCACC
47.17_Rev	GTCCTTGATACGGCATGGC
51.5_T-Rev	CTTCGTTGTTTCTTCGGGTC
35.83_For	CAGAGGCTCCGGGAGTTGC
F_SEQ3	GGACTGATATGTATGCACAAGATG
P54_SEQ3	GCTATTGGATGAAGTGCTGC
CD111_SEQ2	GTAATGATCAAGCCCGATAAC
CD141_SEQ2	GAGGAATCATAGGGGAAAGG
S21_For	CCGAGCCGAGGCTACCTTAAAGAAGC
S21_Rev	CCCCATAACCTTTTAGCACCCGCTACC

Table 2.1.4.: Primers used for pyrosequencing analysis of the *Pain-1* locus on chromosome III.

Name	Primer sequence in 5'-3' orientation
Pyro Pain F	GGGACCATTTGGTGTCTGTTG
Pyro Pain RB	(Btm)GCAAAGCTCTCCACAATTGAG
Pyro PainTher F	GCCTTTTGCCATGGTTCCTG
Pyro PainTherRB	(Btm)GCAGTGGTCGGGTCTCTAAAGTC
Pyro PainP40 RB	(Btm)CTGAGTCAACATGGAGTAGC
Pyro PainP40 F	GGATTGGGGAAACTGATAG
Pyro PainP40 Seq	CAGTGCTTTACGACAAGAAG
Pyro PainTheSeq	CCGGTGACACTGATGATTATG
Pyro PainDiaSq1	GAGCTAACGCCAGTTTACTTC
Pyro PainDiaSq2	CATTTCTAAAGGAGCTGATGG
51.83 PyroF	CAAAATCTTGCGTACCCCAACAAC
51.83 PyroRevBi	(Btm)GTTTCATAAACAAGTGCAATACCCG
51.89 PyroF	CAGCTGCTCTACTAGTGGAGG
51.89 PyroRevBi	(Btm)GAGTCTCAGCTCGGCCATC
51.814 PyroF	CCTCAGAGGCTCCGGG
51.814 PyroRevBi	(Btm)CAATAATCTCATCGAATGTTTTTC
Seq_51.814Pyro	GGGAGITGCTAAACAAGTT

Table 2.1.5: Primers used for pyrosequencing analysis of the gene *invGE* on chromosome IX.

Name	Primer sequence in 5'-3' orientation
invGE-F-fulgth	CAATATCTTTCTCCAAAATCAC
PyroE ParentsRB	(Btm)GCCCCAAACAATATTGCC
PyroE PSNP58Seq	GGGTTTAGAAATTTATTTATTTTG
PyroE PSNP108Se	GGGGTGTGTTGCTTCTCATAATA
PyroE SatF	GCAAGGGAGAAATGTTTGAAG
PyroE SatRBio	(Btm)GTCTCATGGTAGATCTTCTAGCATC
PyroE Sat6fSeq	AAGGAATCTCAGCATCACAG
PyroE Sat4Seq	CGACTATCCAAGGTGGGCT
PyroE Sat3Seq	GCTTGGICCCATTTGGGCTT
PyroE Ther F	GGTTCTCATGTGCTCAGATG
PyroE TherRBio	(Btm)GTGCATCTTAGGTACATCCATG
PyroE Ther1Seq	CTGAGACAATCACAATTGAGAC
PyroE Ther6fSeq	GTAGCTGAGAGTTTTGGTGCTG
PyroE Ther2Seq	GGATATGTAGATGTAGATTTAGTAGAC
PyroE Dia F	CCTTAAGAATAGCCTTGATGTT
PyroE Dia RBio	(Btm)CTCCCTTGCTCAACTTCTTG
PyroE Dia3Seq	CCTGATAACAATTCTATCGATG
PyroE Dia2Seq	CTCGAICITAGTGGTAAACA
PyroE Dia6fSeq	GAATTCAAGCTATTCCGC

Table 2.1.6: Primers used for pyrosequencing analysis of the gene *invGF* on chromosome IX.

Name	Primer sequence in 5'-3' orientation
PyroF_4d_F	GATCCAATGTTTTCTGGCTG
PyroF_4d-RBio	(Btm)GATCCAATGTTTTCTGGCTG
PyroF_Sat_F	GGACTTAATCAATTGGATCAAT
PyroF_Sat_RBio	(Btm)CAGGCTTGATCCAATCACG
PyroF_Sat2Seq	GATCAATTIGGTACITGGTCTG
PyroF_Sat3Seq	GCIAACCAAACCTCAAGTTCAAA
PyroF_SNP58Seq	CCAGTTATCTTAGTTTGCTTTTT
PyroF_SNP96Seq	CAATAATGTTGTTTTTGCTTCTC
PyroF_SNP111Seq	GCTTCTCAIAAAGTTTTTATT
PyroF_P40/54_F	GACTCAAGGGTGCAGATGTACAAG
PyroF_P40/54_RB	(Btm)CCTCGATGTTATACATGTCTTTCC
PyroF_P40Seq	CTGGATTTGTGGATGTTGATTT
PyroF_P54Seq	GCACAACAAAATTACAAGGTTC

Table 2.1.7: Primers used for expression analysis of the gene *invGF* on chromosome IX.

Name	Primer sequence in 5'-3' orientation
F1_F	ATGGATTATTCATCTAATTCTCGTTGGGCTTTGCCAG
F_Expr_Rev	GTTACCAGGTAGAATAGTTGC

Table 2.1.8: Primers used for association analysis.

Name	Primer sequence in 5'-3' orientation
pCD141_3F	ACAAGTTTGGATAAGGCAGAG
pCD141_3R	TAGAGTCTCAATTGTGATTCTCTCC

Table 2.1.9: Primers used for quantification of real-time PCR products in SYBR[®] Green based detection assays.

Name	Primer sequence in 5'-3' orientation
Pyro_Pain_F	GGGACCATTTGGTGTCTGTTG
Pyro_Pain_R	GCAAAGCTCTCCACAATTGAG
EF1 α _F (NICOT ET AL., 2005)	ATTGGAAACGGATATGCTCCA
EF1 α _R	TCCTTACCTGAACGCCTGTCA
L2_For (NICOT ET AL., 2005)	GGCGAAATGGGTCTGTGTTAT
L2_Rev	CATTCTCTCGCCGAAATCG

Table 2.1.10: Primers used for BAC analysis and sequencing, colony-lift, and Southern blot analysis.

Name	Primer sequence in 5'-3' orientation
<i>A) Primers for probes:</i>	
Pain1-5f	CGGAATTGGATTGTGGAATTG
Pain1-5r	TGGCGTTAGCTCAGATAGCTT
Pain_SondF1	CAATACAATCCAGATTCAGCTATTTG
Pain_SondR	CTGCAGCTGAGTCAACATGGAGTAG
CD111S2_For	CCTAGCAAAAATCGAAGAATTATGTG
CD111S1_Rev	CTTGACATTGTCTTATCATTCTTG
pCD141_3F	ACAAGTTTGGATAAGGCAGAG
pCD141_3R	TAGAGTCTCAATTGTGATTCTCTCC
<i>B) Primers for BAC sequencing</i>	
37_F1	GCTGATTGGTTAGGTTGACTG
37_R1	CATACCGTTAATCCAGTTTTTTAG
37_F2 NEW	CAGATTGATCATTTCGGTAGTGG
37_R2	GTAAAGATCAGCCCAACTAGG
37_F3	GCCAAAATATATGACGTAAAGATG
37_R3	CCAATTTCCAGTACCGGG
37_F4	CAAACATCCCTCAAGCCTAAG
37_R4	GGAACAATTGAGATTTGATTAGTC
37_R4N	GACTCACCATTTGGATCTGTTG
37_F5	GAGGAATAAAGAAAAGTCAAAATG
37_F6	CACTTTTCAATGATGATAACGACG
37_F7	CATGAGTTGGTTCCCATTAGAAAC
37_F8	GTTATTCATAGACACAAGAAAAAGC
163_R1	CCTTGAGGCATCGGAACAC
146_F1	CCTATGCATACTACACTTCGATC
146_F4	
146_R3	
146_F5	
239_Seq7	GTCCACTTCAAATGAGGCTTC
P40_BACcheck_R	GTTCATCCAATTTTTTTTGGAG
Seq8	GATGGATTGGGGAAACTGATAG
Seq9	CCGTA AAAATCATCAAATCTATG
Seq10	GTCCATACCTGTACAGATGCC
BAC_CD111_For	GAATTGGTCATTTGGTCACTCTTCTTATTG
BAC_CD111_Rev	GTGTGAAGCATTAACGCCATTGTTGAAAG
BAC_CD141_For	CAACAATGGCGCGGAGAGAATCACAATTG
BAC_CD141_Rev	CTAACATCAACATGGCTAGTAGTAGATTGC

2.1.5 Plasmids

Plasmids used for molecular cloning of cDNA and genomic DNA fragments are listed below.

Table 2.1.11: List of used vectors.

Vector	Supplied/provided by	Resistance
pGEM [®] -T Vector System	Promega	amp ^R
pGEM [®] -T Easy Vector System	Promega	amp ^R
112 A1 NE	Lothar Willmitzer, MPI for Molecular Plant Physiology/Golm	amp ^R

2.1.6 Bacterial strains

For standard cloning electrocompetent or chemical competent cells (Invitrogen, Karlsruhe) of *Escherichia coli* (*E. coli*) strains DH5 α and DH10B were used (HANAHAN, 1983).

MAX Efficiency[®] DH5 α [™] Competent cells:

F- ϕ 80*lacZ* Δ M15 Δ (*lacZYA-argF*) U169 *recA1 endA1 hsdR17* (*r_k*⁻, *m_k*⁺) *phoA supE44* λ -*thi1 gyrA96 relA1*

ElectroMAX[™] DH10B[™] Cells:

F- *mcrA* Δ (*mrr-hsdRMS-mcrBC*) ϕ 80*lacZ* Δ M15 Δ *lacX74 recA1 endA1 araD139* Δ (*ara, leu*)7697 *galU galK* λ - *rpsL nupG*

2.1.7 Yeast strains

In this work the yeast invertase mutant strain *SUC2* (GOZALBO & HOHMANN, 1989), and as a wild type reference the yeast strain *FY 1679* were used. Both strains were obtained from EUROSCARF: European *Saccharomyces Cerevisiae* Archives for Functional Analysis, Frankfurt.

SUC2=YIL162w: BY4741; Mat a; his3D1; leu2D0; met15D0; ura3D0; YIL162w::kanMX4

FY1679: MATa/MAT α ; ura3-52; trp1 Δ 63/TRP1; leu2 Δ 1/LEU2; his3 Δ 200/HIS3; GAL2/GAL2

2.1.8 Plant Material

Potato (*Solanum tuberosum* L.) plants used in this study were obtained from the companies SAKA-RAGIS Pflanzenzucht (SAR), Böhm-Nordkartoffel Agrarproduktion (BNA), and NORIKA (NOR). The genotypes resulted from a number of crosses among different varieties and breeding clones and were selected to represent the variation at the invertase loci, which were the objects of interest in this study. The following tetraploid cultivars were used: ‘Satina’ (SAR), ‘Diana’ (SAR), ‘Theresa’ (BNA), ‘Saturna’ (BNA), and ‘Desireé’.

Furthermore, the diploid potato genotypes P18, P40, and P54 were included because they were used as parents for mapping QTLs and candidate genes for cold sweetening of potato tubers (MENÉNDEZ ET AL., 2002).

2.2 Methods

2.2.1 Plant work

Potato tubers were germinated after the tuber dormancy period (storage at 4°C) of ca. 3 months. In some cases tuber dormancy were broken by the application of a solution containing 10g thio-urea and 2mg giberelline in 1l H₂O. Tubers were cut at the navel and incubated in the solution for 20min at RT. Afterwards the tubers were dried over night, planted in pots containing a mixture of peat and soil, and placed in a warm surrounding.

The plants were either grown in greenhouse (temperature day: 20-24°C; temperature night: 18°C; light: 6-21:00 o'clock) or in Saran-houses under natural occurring conditions through May to September. Leaf and flower material were taken during all stages, whereas tubers were only selected after natural total tuber ripening. For further analysis tubers were stored at 4°C in dark environment.

2.2.2 Bacterial work

E. coli was incubated at 37°C with LB media over night (SAMBROOK & RUSSELL, 2001). Plate incubation was performed with LB media + 1% agar, liquid cultures were incubated on a shaker (200rpm).

Transformation of constructs was performed as described by HANAHAN (1983).

2.2.3 Yeast work

The yeast strains used in this study were grown on media with a suitable carbohydrate source (2%) depending on the genotype of the strain. The invertase mutant strain *SUC2* was grown on YPD media at 30°C overnight. The wild type strain *FY 1679* was either grown on YPD media or selective media (SD) with 2% sucrose as carbohydrate source. Transformed *SUC2* yeast strains were only grown on selective SD media with sucrose as carbohydrate source. Plate incubation was performed with yeast media + 2% agar, liquid cultures were incubated on a shaker (200rpm).

For functional complementation the invertase mutant strain *SUC2* was used. Yeast transformation was performed using a simplified method described by GIETZ & SCHIESTL (1995). The strain was grown in YPD media as a 5ml overnight culture. The next day a 50ml flask with YPD was inoculated with 1ml of the overnight culture and shaken for 3-5h at 30°C. Afterwards the culture was centrifuged at 4000rpm for 5min at RT and the pellet was washed

in 25ml H₂O and centrifuged again. The washed pellet was resuspended in 1ml 100mM LiAc, transferred to a 1,5ml tube and centrifuged at 13,000rpm for 10sec. After discarding the supernatant the pellet was resuspended in 500µl 100mM LiAc.

The transformation reaction contained following components.

Table 2.2.1.: Components of the transformation reaction.

Component	Volume for one transformation
PEG 3000 (50%)	240µl
1M LiAc (pH 8.5)	35µl
ss-herring sperm (2mg/ml, denaturated at 100°C and kept on ice)	50µl
<i>SUC2</i> yeast mutant	50µl

The concentration of the plasmid used for transformation varied between 0.8-1.5µg. 350µl of the transformation reaction were added to the plasmid (in 50µl), mixed and incubated for 30min at 30°C. Afterwards the reaction was incubated for 25-30min at 42°C and centrifuged at 13,000 for 10sec. The pellet was resuspended either in 150µl 1M sorbitol or in 150µl H₂O, plated on selective SD plates and incubated at 30°C between 3 and 5d. Transformants were transferred to fresh selective SD plates and check on their inserts using colony PCR.

2.2.4 Molecular biological methods

If not indicated otherwise, the methods applied in this study were taken from SAMBROOK & RUSSELL (2001) using standard procedures.

2.2.4.1 Genomic DNA extraction from leaf tissue

Young, healthy potato leaves were harvested and immediately frozen in liquid nitrogen. Frozen samples were freeze-dried (Eps1-15, Typ 1815, Christ Gefriertrocknung GmbH, Osterode) and stored in air-tight containers at -20°C. Total genomic DNA was extracted from 0.3-0.4g freeze dried leaf material according to BORMANN ET AL. (2004).

2.2.4.2 Plasmid DNA isolation from bacteria

Small scale and midi scale plasmid isolation from *E. coli* were performed using the column-based Plasmid Isolation Mini or Midi Kit (Qiagen, Hilden) according to the supplier's protocol.

Isolation of BAC DNA has been adapted by customers from the Qiagen® Plasmid Midi Kit protocol. The procedure has been used to isolate 50-200kb BAC DNA of two potato-BAC

libraries BA and BC (BALLVORA ET AL., 2002, 2007). The yield of BAC DNA from a 100ml culture was up to 25µg.

2.2.4.3 Separation of DNA fragments by agarose gel electrophoresis

DNA fragments were mixed with DNA loading buffer and analyzed by agarose gel electrophoresis. The agarose concentration depended on the size of the fragments to be resolved. Electrophoresis was performed at 5V/cm using TAE buffer. Different DNA size markers were used to estimate the size of DNA fragments. The Fermentas ladders 100bp plus and 1kb were used. After electrophoresis, DNA was visualized on a transilluminator under UV light (254nm).

2.2.4.4 Separation of DNA fragments using pulse field gel electrophoresis (PFGE)

Pulse field gel electrophoresis (PFGE) was used to estimate the size of BAC inserts. 20µg of BAC DNA in a 25µl reaction was digested with NotI and incubated for 4h at 37°C. After inactivation of the reaction at 65°C for 15min, 5µl of the reaction were loaded on a 1% agarose gel to check the restriction reaction.

Preparation of the PFGE gel:

A 1% PFGE gel was prepared using Gold Agarose and 0.5x TAE buffer. After loading 20µl of the samples and the Fermentas markers lambda ladder and mid range, the slots were sealed with liquid gel and put in the PFGE chamber.

Running of the PFGE gel:

2.5l of 0.5x TAE buffer was filled in the PFGE chamber and cooled for 20min to 11-14°C.

The machine settings were:

initial time	5sec
final	15sec
run	14h
voltage	5V/cm
incl. angle	120
pump	80

After the run, the PFGE gel was stained in a 2% EtBr solution for 1h. BAC DNA fragments were visualized on a transilluminator under UV light (254nm).

2.2.4.5 Purification of PCR products and gel-extracted DNA fragments

PCR products, gel-extracted PCR products and restriction digested products were either purified using the Roche High Pure PCR Purification Kit or using the Qiagen PCR Purification Kit following the supplier's protocol. PCR products for sequencing were purified using ExoSAP-IT[®].

2.2.4.6 Total RNA extraction from leaf and floral tissue

Total RNA from leaves and inflorescences were extracted using the ToTally RNA Isolation Kit from Ambion following the manufacturer's protocol. Additionally, the RNA was purified from possible genomic DNA contaminations using the DNA-free[™] Kit purchased from Ambion. The concentration and quality of the RNA was determined by measuring the ratios of absorbance $A_{260\text{nm}}/A_{280\text{nm}}$ which should be between 1.8 and 2.0 and $A_{260\text{nm}}/A_{230\text{nm}}$ which should be between 2 and 3 using the Nanodrop ND-1000 spectrophotometer (Peclab, Erlangen). The integrity of the RNA was tested by running a 1% agarose gel which were loaded with 300-500ng of total RNA that was mixed with 5 μ l of DNA loading dye. Total RNA was stored at -80°C .

2.2.4.7 Total RNA extraction from tuber tissue

Total RNA from tubers was extracted using the Plant RNA Isolation Kit from Invitrogen following the manufacturer's protocol. Small amounts of tubers were grinded in liquid nitrogen using mortal and pistil. Larger amounts of tuber tissue were crushed in liquid nitrogen cooled cylinders using the Retsch[®] Schwingmühle MM400 (Retsch GmbH, Haan) following the manufacturer's protocol.

Total RNA from tubers was further purified through additional precipitation steps: a high salt precipitation to remove polysaccharides and a lithium chloride precipitation to remove high molecular weight RNA. The RNA solution was adjusted to a total volume of 1ml by adding RNase free water. 250 μ l of isopropanol and 250 μ l of high salt solution (1.2M sodium citrate, 0.8M sodium chloride) were added, mixed and incubated on ice for 2h. The RNA was recovered by centrifugation at 13,000rpm for 30min at 4°C . The supernatant was removed and the pellet was rinsed with 70% ethanol, and centrifuged at 13,000rpm for 5min at 4°C . After removing the ethanol, the pellet was air-dried and dissolved in an appropriate volume of RNase free water to achieve a minimum concentration of 200ng/ μ l of total RNA.

The precipitation of high molecular weight RNA was done using 0.5 volume of 5M LiCl. The solution was mixed and kept on ice over night in the 4°C room. The RNA was recovered by

centrifugation at 13,000rpm for 30min at 4°C. After removal of the supernatant, the pellet was rinsed with 70% ethanol and centrifuged again at 13000rpm for 5min at 4°C. The ethanol was removed, the pellet air-dried and dissolved with RNase free water to a final volume of 20 to 50µl depending on the pellet size. Additionally, the RNA was purified from possible genomic DNA contaminations using the DNA-free™ Kit purchased from Ambion. The concentration and quality of the RNA was determined by measuring the ratios of absorbance $A_{260\text{nm}}/A_{280\text{nm}}$ which should be between 1.8 and 2.0 and $A_{260\text{nm}}/A_{230\text{nm}}$ which should be between 2 and 3 using the Nanodrop ND-1000 spectrophotometer (Peclab, Erlangen). The integrity of the RNA was tested by running a 1% agarose gel which were loaded with 300-500ng of total RNA that was mixed with 5µl of DNA loading dye. Total RNA was stored at -80°C.

2.2.4.8 cDNA synthesis

For first strand cDNA synthesis 2.0µg of total RNA was used to synthesize cDNA by reverse transcription using 200U of Superscript™ II reverse Transcriptase (Invitrogen, Karlsruhe) per reaction and oligo(dT)₁₆₋₁₈ primers (500ng), (Roche, Mannheim) as priming method. cDNA synthesis was performed according to the manufacturer's protocol and additional treated with RNase H (Roche, Mannheim) for 20min at 37°C.

Synthesized first strand cDNA was either used for molecular cloning (1µl per reaction) or in qRT-PCR (2.5µl of a 1:100 dilution).

2.2.4.9 Standard Polymerase Chain Reaction (PCR)

PCR reactions were performed in different thermal cyclers. The following machines were used.

Table 2.2.2: PCR machines.

Machine	Provided by
MJ Research DNA Engine Tetrad®4 peltier thermal cycler	Biozym, Hess. Oldendorf
Biometra® T1-Thermocycler	Biomedizinische Analytik GmbH, Göttingen
Biometra® T3-Thermocycler	Biomedizinische Analytik GmbH, Göttingen
Labcycler	Sensoquest Biomedizinische Elektronik, Göttingen

For standard reactions and colony-PCR *Thermus aquaticus* DNA Polymerase (*Taq*-DNA Polymerase) was used. *Taq*-DNA Polymerase was prepared described by PLUTHERO (1993). For high accuracy PCR reaction (cloning), the proof-reading Roche Fast Start High Fidelity PCR System and the proof-reading KOD Hot Start DNA Polymerase (Novagen, Darmstadt) were used.

Standard PCR reactions were performed as follows:

Table 2.2.3: Standard PCR reaction using *Taq*-DNA Polymerase

Concentration	Reagent
50-200ng	Template (genomic DNA, plasmid DNA, cDNA, bacterial or yeast colony)
2.5µl	10x PCR buffer (without MgCl ₂)
3mM	MgCl ₂
25mM	dNTP
10-25pmol	Primer 1
10-25pmol	Primer 2
0.5-0.7µl	<i>Taq</i> -DNA Polymerase (0.025-0.05U/µl)
adjust to 25µl	

Before using bacterial colonies for PCR, colonies were picked from the plate, resuspended in 15µl H₂O and denatured at 95°C for 10min. Afterwards 1µl of the denatured colony was used for PCR.

Before using yeast colonies for PCR, fresh grown colonies were picked and resuspended in 15µl 2mM NaOH and denatured at 95°C for 10min. Afterwards 1µl of the denatured colony was used for PCR.

PCR program:	Initial denaturation	94°C for 3min
	Denaturation	94°C for 30sec
	Annealing	55-65°C for 30sec
	Elongation	1min/1kb
	Final extension	72°C for 10min
	Number of cycles	

Table 2.2.4: Standard PCR reaction using Roche Fast Start High Fidelity PCR System

Concentration	Reagent
50-200ng	Template (genomic DNA, plasmid DNA, cDNA)
2.5µl	10x PCR buffer (without MgCl ₂)
3mM	MgCl ₂
25mM	dNTP
10-25pmol	Primer 1
10-25pmol	Primer 2
0.25µl	Fast Start High Fidelity Polymerase (2.5U)
adjust to 25µl	

PCR program:	Initial denaturation and activation	94°C for 2min	}	10x	
	Denaturation	94°C for 15sec			
	Annealing	55-65°C for 30sec			
	Elongation	1min/1kb	}	20x	
	Denaturation	94°C for 15sec			
	Annealing	55-65°C for 30sec			
	Elongation	1min20sec/1kb			
	Final extension	68°C for 10min			
	Number of cycles		30		

Table 2.2.5: Standard PCR reaction using Novagen KOD Hot Start DNA Polymerase

Concentration	Reagent
50-200ng	Template (genomic DNA, plasmid DNA, cDNA)
2.5µl	10x PCR buffer (without MgCl ₂)
1mM	MgSO ₄
25mM	dNTP
10-25pmol	Primer 1
10-25pmol	Primer 2
0.5µl	KOD Hot Start DNA Polymerase(0.5U)
adjust to 25µl	

PCR program:	Initial denaturation and activation	94°C for 2min
	Denaturation	94°C for 15sec
	Annealing	55-65°C for 30sec
	Elongation	1min/1kb
	Number of cycles	

2.2.4.10 Molecular cloning of cDNA constructs for yeast *SUC2* transformation

Unless otherwise described, cloning strategies performed in this study included directional cloning of PCR products into pGEM[®]-T Vector System or pGEM[®]-T Easy Vector System (Promega, Mannheim). All reactions were performed following the manufacturer's protocol. All constructs were verified by sequencing. Alleles were defined as sequences, which were found twice in two independent PCRs. In some cases where sequences varied a lot, the allelic consensus sequence was used for allele definition.

Production of the final expression vector constructs for yeast transformation was performed using the 112 A1 NE yeast expression vector (Appendix A 2) kindly provided by Lothar Willmitzer's Group, MPI for Molecular Plant Physiology/Golm. 112 A1 NE inserts are driven under the control of the constitutive strongly expressed *Adh1* promoter. The vector, originally

an *E. coli* vector, also includes the *Adh1* terminator, an ampicillin resistance for *E. coli* selection, and tryptophane for yeast selection. pGEM[®]-T constructs bearing invertase alleles were amplified using proof-reading high fidelity *Taq*-Polymerase (Roche, Mannheim) to insert specific cloning sites. Alleles of the loci *Pain-1* and *invGF* were amplified with primers inserting *NotI* and *BamHI* restriction sites for sticky end ligation into the vector 112 A1 NE. *Pain-1* and *invGF* PCR products as well as the vector were digested with the corresponding restriction enzymes, followed by ligation of the linearized vector with the digested PCR products. Alleles of the *invGE* locus were amplified with primers inserting only *NotI* restriction sites because a *BamHI* restriction site is included in the gene. *invGE* PCR products as well as the vector were digested with *NotI* restriction enzyme, followed by blunt end ligation of the linearized vector with the digested PCR product. 112 A1 NE constructs were verified by sequencing.

All constructs generated in this study are indicated in the Table below.

Table 2.2.6: Overview of generated constructs.

Gene	Vector	Primer pairs used (Table 2.1.2)
Initial cloning of invertase alleles:		
<i>Pain-1</i>	pGEM [®] -T/ T Easy	ZrPain-F/ PainUni-R
<i>invGE</i>	pGEM [®] -T/ T Easy	invGE-F-fulgth/ invGE-R-fulgth
<i>invGF</i>	pGEM [®] -T/ T Easy	invGF-F-fulgth/ invGF-R-fulgth
<i>pCD111</i>	pGEM [®] -T/ T Easy	CD111fl_F/ CD111fl_R
<i>pCD141</i>	pGEM [®] -T/ T Easy	CD141fl_F/ CD141fl_R
Cloning of constructs for yeast transformation:		
<i>Pain-1</i>	112 A1 NE	PainNotI_R/ PainBamHI_R
<i>invGE</i>	112 A1 NE	ENotI_F/ ENotI_R
<i>invGF</i>	112 A1 NE	FNotI_F/ FBamHI_R

The presence of the transformed plasmid was tested via colony-PCR using a primer that was located in the backbone of the vector and a second primer localized within the insert sequence (Table 2.1.3, primer pair T7/SP6P).

2.2.4.11 Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was performed using a Mastercycler[®] ep *realplex* (Eppendorf, Hamburg). Primers were tested for undesired primer dimer formation by melting curve analysis (55°C to 95°C with a heating rate of 0,1°Cs⁻¹ and continuous fluorescence measurement) using the Power SYBR[®] Green PCR Master Mix (Applied Biosystems.). For standard curves tuber cDNA of the 4th week of cold storage were diluted 1:10, 1:100 and 1:1000. Total invertase transcripts were quantified using the primer pair Pyro_Pain_F/Pyro_Pain_R in a PCR with a 100 fold template dilution. The quantification of

2.2.4.12 Sequencing

DNA sequencing was performed by the MPIZ DNA core facility on Applied Biosystems (Weiterstadt) Abi Prism 377, 3100 and 3730 sequencers using BigDye-terminator v3.1. chemistry. Premixed reagents were purchased from Applied Biosystems. Sequences were analysed with the software Chromas Pro version 1.33 (share-it, Cologne) or the Lasergene software.

2.2.4.13 Pyrosequencing

Pyrosequencing is a robust and quantitative method of DNA sequencing based on real-time detection of pyrophosphate, which is released as a result of nucleotide incorporation in a the ‘sequencing by synthesis’ reaction (RONAGHI ET AL., 1996). ‘Sequencing by synthesis’ involves taking a single strand of the DNA to be sequenced and then synthesizing its complementary strand enzymatically. The pyrosequencing method is based on detecting the activity of DNA polymerase with another chemiluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step. The template DNA is immobilized, and solutions of A, C, G, and T nucleotides are added and removed after the reaction, sequentially. Light is produced only when the nucleotide solution complements the first unpaired base of the template. The sequence of solutions, which produce chemiluminescent signals, allows the determination of the sequence of the template. The templates for pyrosequencing can be made both by solid phase template preparation (Streptavidin coated magnetic beads) and enzymatic template preparation (Apyrase+Exonuclease). In this study, Streptavidin coated magnetic beads were used.

PCR conditions:

The primers used for pyrosequencing analysis are listed in the Tables 2.4.1 for the *Pain1* locus on chromosome III, 2.4.2 for the *invGE* locus on chromosome IX and 2.4.3 for the *invGF* locus on chromosome IX.

PCR setup and conditions are listed in Table 2.10.

Solid phase template preparation for single-stranded DNA template:**Table 2.2.8: Reaction setup for pyrosequencing**

Volume	Reagent
20µl	PCR product
15µ	H ₂ O (LiCroSolv Merck)
40µl	Binding buffer (10mM Tris-HCl, 2M NaCl, 1mM EDTA, 0.1% Tween 20; pH 7.6)
5µl	Streptavidin-coated Super Paramagnetic beads

The reaction mixture was incubated for 5min on a shaker at RT. The Streptavidin-template complex was captured using the PSQ96 Sample Prep tool (Biotage AB, Uppsala, Sweden) and single-stranded template was generated by washing in 70% EtOH for 3sec, in 0.2M NaOH for 5sec followed by washing in washing buffer (10mM Tris acetate pH 7.6) for 10sec. 1µl of the sequencing primer (0.25mM) was annealed to the immobilized template in 39µl annealing buffer (20mM Tris acetate, 2mM Mg acetate, pH 7.6) and heated at 80°C for 2min followed by slow cooling to room temperature.

Pyrosequencing:

The sequencing reaction was performed automatically with the PSQ 96 system (Biotage AB, Uppsala, Sweden) at 28°C using a SNP reagent kit according to the manufacturer's protocol. The ssDNA template is hybridized to a sequencing primer and incubated with the enzymes DNA polymerase, ATP sulfurylase, luciferase and apyrase, and with the substrates adenosine 5' phosphosulfate (APS) and luciferin. The four enzyme mixture, the substrates and the four separate deoxynucleotide triphosphates were loaded into the reagent cartridge (PSQ 96 SNP Reagent Kit, Biotage AB, Uppsala, Sweden).

1. The addition of one of the four deoxynucleotide triphosphates initiates the second step. DNA polymerase incorporates the correct, complementary dNTPs onto the template. This incorporation releases pyrophosphate (PPi) stoichiometrically.
2. ATP sulfurylase quantitatively converts PPi to ATP in the presence of adenosine 5' phosphosulfate. This ATP acts as fuel to the luciferase-mediated conversion of luciferin to oxyluciferin that generates visible light in amounts that are proportional to the amount of ATP. The light produced in the luciferase-catalyzed reaction is detected by a camera and analyzed in a program.
3. Unincorporated nucleotides and ATP are degraded by the apyrase, and the reaction can restart with another nucleotide.

The following Tables contain information about primers used in the pyrosequencing assay for detection of more than one SNP to dissect the corresponding alleles due to the complexity of the approach. Tables for the dissection of two alleles are not shown because the setup is described in the corresponding result section.

Table 2.2.9: Primers used in the pyrosequencing assay of *invGE* alleles from the tetraploid cultivars.

Genotype	Allele	SNP position	Allele specific SNP	Sequencing primer
‘Satina’	<i>E_SA</i>	SNP 1237	<i>E_SA/E_SN1/E_SN2/E_SN3</i> T/G/G/G	PyroE_Sat6fSeq
	<i>E_SN1</i>	SNP 1366	A/G/A/A	PyroE_Sat4Seq
	<i>E_SN2</i>	SNP 1379	C/C/T/C	PyroE_Sat3Seq
	<i>E_SN3</i>	SNP 1216	A/A/A/G	PyroE_Dia_F
‘Diana’	<i>E_DA</i>	SNP 1086	<i>E_DA/E_DN1/E_DN2</i> A/T/T	PyroE_Dia6fSeq
	<i>E_DN1</i>	SNP 1117	T/C/T	PyroE_Dia2Seq
	<i>E_DN2</i>	SNP 924	T/T/C	PyroE_Dia3Seq
‘Theresa’	<i>E_TA</i>	SNP 1615	<i>E_TA/E_TN1/E_TN2/E_TN3</i> A/T/T/T	PyroE_Ther6fSeq
	<i>E_TN1</i>	SNP 1720	C/A/C/C	PyroE_Ther1Seq
	<i>E_TN2</i>	SNP 1553	T/T/C/T	PyroE_Ther2Seq
	<i>E_TN3</i>	SNP 1473	T/T/T/G	PyroE_Ther_F

For PCR fragment amplification the following primers were used (Table 2.1.5): ‘Satina’: PyroE_SatF/PyroE_SatRBio; ‘Diana’: PyroE_Dia_F/PyroE_Dia_RBio; ‘Theresa’: PyroE_Ther_F/PyroE_TherRBio.

Table 2.2.10: Primers used in the pyrosequencing assay of *invGE* alleles from the diploid genotypes.

Genotype	Allele	SNP position	Allele specific SNP	Sequencing primer
P18	<i>E_P18N1</i>	SNP 108	A	PyroE_PSNP108Se
	<i>E_P18N2</i>		T	
P40	<i>E_P40N1</i>	SNP 58	C	PyroE_PSNP58Seq
	<i>E_P40N2</i>		T	
P54	<i>E_P54N1</i>	SNP 58	C	PyroE_PSNP58Seq
	<i>E_P54N2</i>		T	

For PCR fragment amplification the following primers were used (Table 2.1.5): P18, P40, P54: *invGE*-F-fulgth/PyroE_ParentsRB.

Table 2.2.11: Primers used in the pyrosequencing assay of *invGF* alleles from the tetraploid cultivars.

Genotype	Allele	SNP position	Allele specific SNP	Sequencing primer
			<i>F_SN1/F_SN2/F_SN3/F_SN4</i>	
‘Satina’	<i>F_SN1</i>	SNP 111	T/C/C/C	PyroF_SNP111Seq
	<i>F_SN2</i>	SNP 459	C/T/C/C	PyroF_Sat2Seq
	<i>F_SN3</i>	SNP 378	A/A/G/A	PyroF_Sat3Seq
	<i>F_SN4</i>	SNP 96	C/C/C/T	PyroF_SNP96Seq
‘Diana’	<i>F_DN1</i>	SNP 96	T	PyroF_SNP96Seq
	<i>F_DN2</i>		C	
‘Theresa’	<i>F_TN1</i>	SNP 96	T	PyroF_SNP96Seq
	<i>F_TN2</i>		C	

For PCR fragment amplification the following primers were used (Table 2.1.6): ‘Satina’: PyroF_Sat_F/PyroF_Sat_RBio and PyroF_4d_F/PyroF_4d-RBio for the alleles *F_SN1* and *F_SN4*; ‘Diana’ and ‘Theresa’: PyroF_4d_F/PyroF_4d-RBio.

Table 2.2.12: Primers used in the pyrosequencing assay of *invGF* alleles from the diploid genotypes.

Genotype	Allele	SNP position	Allele specific SNP	Sequencing primer
P40	<i>F_P40N1</i>	SNP 1534	G	PyroF_P40Seq
	<i>F_P40N2</i>		A	
P54	<i>F_P54N1</i>	SNP 1446	T	PyroF_P54Seq
	<i>F_P54N2</i>		C	

For PCR fragment amplification the following primers were used (Table 2.1.6): P40 and P54: PyroF_P40/54_F/PyroF_P40/54_RB.

2.2.4.14 Single stranded conformation polymorphism (SSCP) analysis

SSCP is the electrophoretic separation of single-stranded nucleic acids based on subtle differences in sequence (often a single base pair), which results in a different secondary structure and a measurable difference in mobility through a gel.

Background:

The mobility of double-stranded DNA in gel electrophoresis is dependent on strand size and length but is relatively independent of the particular nucleotide sequence. The mobility of single strands, however, is noticeably affected by very small changes in sequence, possibly one changed nucleotide out of several hundred. Small changes are noticeable because of the relatively unstable nature of single-stranded DNA; in the absence of a complementary strand, the single strand may experience intrastrand base pairing, resulting in loops and folds that give the single strand a unique 3D structure, regardless of its length. A single nucleotide change could dramatically affect the strand's mobility through a gel by altering the intrastrand base pairing and its resulting 3D-conformation (MELCHER, 2003).

Single-strand conformation polymorphism analysis takes advantage of this quality of single-stranded DNA. First announced in 1989 as a new means of detecting DNA polymorphisms, or sequence variations, SSCP analysis offers an inexpensive, convenient, and sensitive method for determining genetic variation (SUNNUCKS ET AL., 2000).

Like restriction fragment length polymorphisms (RFLPs), SSCPs are allelic variants of inherited, genetic traits that can be used as genetic markers. Unlike RFLP analysis, however, SSCP analysis can detect DNA polymorphisms and mutations at multiple places in DNA fragments (ORITA ET AL., 1989). As a mutation scanning technique, though, SSCP is more often used to analyze the polymorphisms at single loci, especially when used for medical diagnoses (SUNNUCKS ET AL., 2000).

The SSCP analysis of the invertase genes *invGE* and *invGF* was published by LI ET AL. (2005). The invertase genes *Pain-1* and *pCD141* were analyzed also by LI ET AL. (2008). Primers for *pCD141* (pCD141_3F/pCD141_3R) were generated in the course of this work (Table 2.1.8).

SSCP procedure was performed as described in the cited publications.

2.2.4.15 BAC DNA library screens

BAC library screens were performed using two different libraries (supplied by LION Bioscience AG, Heidelberg). The first library, so called 'BA' library (BALLVORA ET AL., 2002), was constructed from partially *HindIII* digested high-molecular weight genomic DNA of the potato genotype P6/210 in the binary vector pCLD04541. P6/210 is a F1 hybrid of the parental clones P41 (H79.1506/1) and P40 (H80.696/4), (RITTER ET AL., 1991). The BAC library consists of approximately 100.000 clones with an average insert size of 70kb. With the size of a haploid potato genome being approximately 109 base pairs, the genome coverage was 6-7 times per haploid genome.

The second BAC library, so called 'BC' library (BALLVORA ET AL., 2007), was constructed from partially *EcoRI* digested genomic P6/210 DNA in the binary vector pBeloBAC11. The average insertion size was 80kb, corresponding to an, on average, 8-fold coverage of the potato genome.

Library screens were performed using a set of four filters carrying $\approx 100,000$ clones.

Preparation of radioactively-labeled probes and DNA hybridisation:

200ng of gel-purified or ExoSAP-IT[®] purified PCR products were diluted up to a final volume of 12 μ l and 2.5 μ l buffer A1 (0.2mM dTTP, 0.2mM dCTP, 0.2mM dGTP; Invitrogen, Karlsruhe), 2.5 μ l DNaseI DNA Polymerase (Invitrogen, Karlsruhe) and 3 μ l α 32 pdATP were added. The reaction was incubated at 16°C for 1h. The probe was purified on a Sephadex-G50 column (Amersham Biosciences, Sweden) and heated at 95°C for 5min.

Pre-hybridisation and hybridisation were carried out in hybridisation solution (20x SSPE [3M NaCl, 0.2M NaH₂PO₄, 20mM EDTA pH 7.0], 100x Denhardtts, 10% SDS) in glass tubes (30cm x 4cm) at 65°C under continuous rotation in a hybridisation oven (Bachofer, Reutlingen). Pre-hybridisation was performed at least for 1h before hybridisation or overnight. Probe hybridisation was carried out overnight.

After hybridisation the filter was washed accordingly:

1. 50ml 2x SSPE + 0.1% SDS at 65°C for 10-15min
2. 50ml 1x SSPE + 0.1% SDS at 65°C for 10min
3. 50ml 0.2x SSPE + 0.1% SDS at 65°C for 10min

The filter was wrapped in thin plastic foil (Saran wrap) and exposed overnight either to a phosphorimager screen (Molecular Dynamics) in a cassette at RT or to film (Kodak[®] X-Omat AR Film, XAR 5, 35x43cm (Sigma-Aldrich Chemie GmbH, Steinheim) in a cassette at -70°C.

The *Pain-1* locus of BC library clones BC14 and BC17 was custom sequenced by GATC Biotech, Konstanz using primer walking as sequencing method. Full-length BAC insert sequencing was performed for the *Pain-1* clones BC2 (insert size: 75kb), BC14 (insert size: 75kb), and BC15 (insert size: 97kb), and for the BC clone BC3 (insert size: 130kb) for the genes *pCD111* and *pCD141*. Full-length BAC insert sequencing was carried out by MWG Biotech AG, Ebersberg using the 454 sequencing technique on the GS FLX system.

2.2.4.16 Colony-lift

The colony-lift is an easy method to confirm hybridisation positive BAC clones. For this purpose single colonies from hybridisation positive BAC clones were grown on selective LB plates (BA BACs: Tet; BC BACs: Cam) at 37°C overnight. To transfer the DNA, Amersham Hybond[™] – N⁺ membrane (GE Healthcare, Buckinghamshire, UK) was placed on the plate and then stripped off again. The membrane was washed accordingly:

1. once 10% SDS for 3min
2. once denaturation solution (1.5M NaCl; 0.5M NaOH) for 5min
3. twice neutralisation solution (1.5M NaCl; 0.5M Tris-HCl pH 8.0) for 5min

After washing, the membrane was air-dried (ca. 15min) and then cross linked with a UV crosslinker (Stratagene) by applying 120,000J x cm⁻² of energy. The membrane was wrapped in thin plastic foil (Saran wrap) and stored in the refrigerator at 4°C.

Preparation of radioactively-labelled probes and DNA hybridisation:

Previously described in 2.2.4.15.

2.2.4.17 Southern blot analysis

Southern analysis was performed according to SAMBROOK ET AL. (1998). 20ng of purified BAC DNA were digested to completion with *HindIII*, *SmaI* and *BamHI* for 4h.

DNA separation and blotting onto membranes:

Digested BAC DNA was subsequently mixed with loading buffer, loaded on a 1% agarose gel and separated for 5h at 70V via electrophoresis. After the run, the gel was stained in a 2% EtBr solution for 15min at RT and DNA was visualized on a transilluminator under UV light (254nm). The gel was destained in H₂O for 20min at RT.

DNA was transferred on Amersham HybondTM – N⁺ membrane for 18h at RT using 0.4M NaOH buffer. After the transfer procedure DNA was cross linked to the filter with a UV crosslinker (Stratagene) by applying 120,000J x cm⁻² of energy. The blot was wrapped in thin plastic foil (Saran wrap) and stored in the refrigerator at 4°C.

Preparation of radioactively-labelled probes and DNA hybridisation:

Previously described in 2.2.4.15.

2.2.5 Three-dimensional modelling of invertase alleles

3D-modelling was performed by Pawel Durek, MPIMP/Golm. The modelling of the allelic invertase molecular structure was based on the invertase 3D and crystal structure of cyanobacteria (ALBERTO ET AL., 2004). The models are comparative, superimposing two allelic sequences. Differences of the alleles are highlighted. The modelling was applied for associated and non associated *Pain-1* alleles of the tetraploid potato cultivars ‘Satina’ and ‘Diana’. The models include the putative sucrose binding site with the substrate sucrose. In addition to the structural visualization of amino acid exchanges, also the electrostatic potential (EP) of the molecules was mapped at pH 4.7 mimicking the vacuolar and apoplastic conditions.

All models were predicted by homology modelling applying the automated structure prediction server 3djigsaw (BATES ET AL., 2001). Subsequently, the models were prepared for continuum electrostatics calculation utilizing the PDB2PQR package (Version: 1.3.0) (DOLINSKY ET AL., 2004). For the calculation, the AMBER force field and the protonation states at pH 4.7 were used (LI H. ET AL., 2005), to reflect the proper vacuolar and apoplastic acidity. The isoelectric surfaces were computed by the adaptive Poisson-Boltzmann Solver (APBS) (BAKER ET AL., 2001) utilizing standard parameters at the temperature of 298K. The

comparison of the isoelectric surfaces revealed no significant changes upon lowering the temperature parameter. The isoelectric surfaces were visualized by PyMol (DELANO, 2002).

The allelic models were superimposed by Swiss-PDB Viewer (GUEX ET AL., 1997). Differences between structures were marked by color-code as judged by the RMSs between the structures and visualized by PyMol. In Figures deep blue colour indicates highly similar protein regions, whilst red characterizes high structural differences.

2.2.6 Biochemical methods

2.2.6.1 Protein extraction from yeast

50ml of yeast cultures were grown for 72h at 30°C on a shaker (200rpm). Cells were harvested $OD_{600} \approx 2.3$ by centrifugation at 4,000rpm, for 10min, at 4°C. The pellet was washed with 25ml cold dest. H₂O and centrifuged again (4,000rpm, for 10min, at 4°C). The pellet was kept on ice and resuspended in 4ml cold protein extraction buffer (25mM Tris phosphate pH 6.7; 10mM glycerol, 0.1mM DTT, 1mM EDTA, 2% Protease Inhibitor Cocktail for general use (Sigma, St. Louis, USA). After adding half of the solution volume of acid washed glass beads (425-600 micron, Sigma-Aldrich Chemie GmbH, Taufkirchen), the tubes were vortexed for 10min in time intervals of 2min, keeping the solutions on ice in between. After this procedure a centrifugation followed at 6,000rpm for 10min at 4°C. The supernatant were loaded on PD-10 columns (GE Healthcare, Buckinghamshire, UK) equilibrated with protein extraction buffer without protease inhibitors. Proteins were eluted from the column by adding 2ml of protein extraction buffer without protease inhibitors and kept on ice.

Determination of the protein concentration using the Bradford assay:

The protein concentration was determined using the Bradford assay (BRADFORD, 1976) with Bradford dye reagent (Protein assay, Bio-Rad, Hercules, USA). 2-10µl of the protein extracts were added to 198-190µl H₂O and 800µl of Bradford dye reagent. The mixture was incubated for 10-15min at RT and extinction was measured at 595nm against a blank (200µl H₂O + 800µl dye) and bovine serum albumin as a standard.

2.2.6.2 Enzymatic assay of invertase

The enzymatic invertase assay was performed using a modified protocol of ZRENNER ET AL. (1995). The principle of the enzymatic reaction is the following:



Figure 2.2.1: Simplified scheme of the principle of invertase reaction.

For a 100 μ l invertase assay reaction 20 μ g of total yeast protein extracts were used. The reaction components were the following:

Table 2.2.13: Reaction setup for invertase assay.

Concentration	Component
20 μ g	total yeast protein extract
20mM	NaOAc (pH 4.7)
2.5-120mM	sucrose
fill up to 100 μ l with H ₂ O	

The reaction was incubated for 1h at 30°C for invertase alleles from the genes *Pain-1*, *invGE*, and *invGF*. Additionally, invertase alleles from the *Pain-1* locus were incubated for 1:30h at 4°C. After incubation 10 μ l of 1M NaHPO₄ (pH 7.2) were added and the reaction was stopped by heating at 95°C for 10min. Blanks had the same reaction mixture, but were heat inactivated without incubation.

It was checked that the assay was linear with time for at least 90min, and was linearly dependent on the amount of protein in the crude extract added up to 50 μ g.

The quantitative, enzymatic determination of glucose was performed using the enzymes hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PDH). The principle of the reaction is the following:

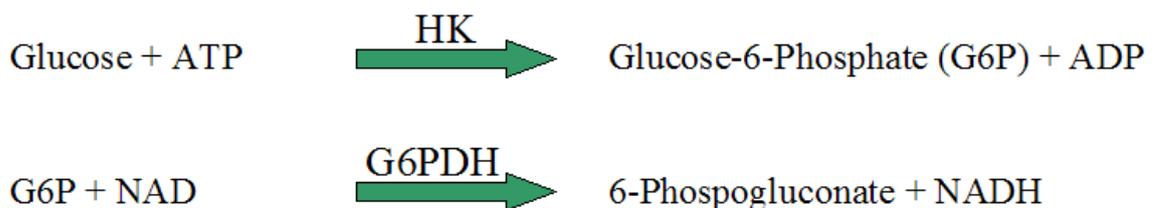


Figure 2.2.2: Simplified scheme of the NADH coupled glucose detection.

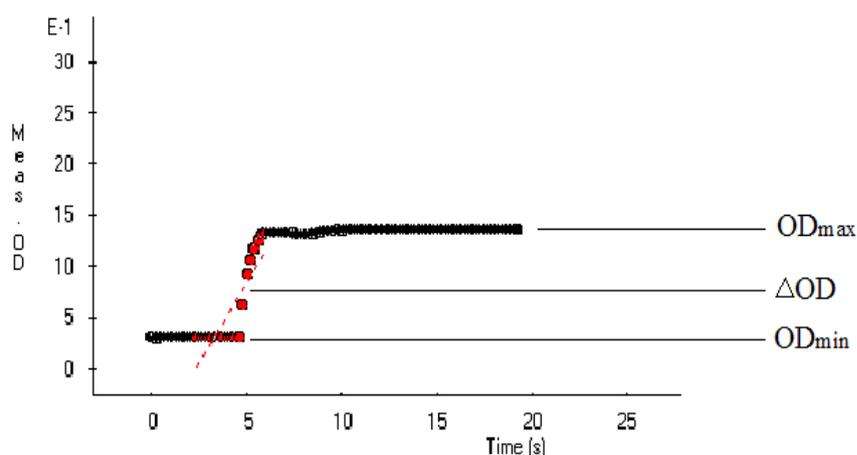
Glucose is phosphorylated by adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase (HK). Glucose-6-phosphate (G6P) is then oxidized to 6-phospho-gluconate in the presence of the oxidized nicotinamide adenine dinucleotide (NAD) in a reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PDH). During this oxidation, an equimolar amount of NAD is reduced to NADH. The consequent increase in absorbance at 340nm is directly proportional to glucose concentration.

The components of a 300 μ l reaction were the following:

Table 2.2.14: Reaction setup for glucose determination.

Stock concentration/storage	Final concentration	Volume/reaction
1M Imidazol pH 6,9 HCl / 4°C	100mM	30 μ l
1M MgCl ₂ / RT	5mM	1.5 μ l
100mM NADP ⁺ / -20°C	2mM	6 μ l
100mM ATP / -20°C	1mM	3 μ l
G6PDH from yeast (400U/ml) / 4°C	2U	5 μ l
Enzymatic invertase reaction / -20°C		10 μ l
fill up to 294 μ l with H ₂ O		

G6PDH was directly resuspended in the reaction components after spinning out of ammonium sulphate. HK (300U/ml) was also spun out of ammonium sulphate and resuspended in 6 μ l of the reaction components without G6PDH. The reaction was started by adding HK to the assay and absorbance was measured at 340nm. For absorbance measurements, a plate reader was used and OD_{min} and OD_{max} values were determined resulting in Δ OD used for calculation



$$\text{nmol Glc/mg protein/h} = \Delta\text{OD}/5.3/\text{A}*\text{B}*C/\text{D}/\text{E}$$

Figure 2.2.3: Overview of plate reader output and glucose calculation. 5.3=millimolar extinction coefficient of NAD⁺, NADH, NADP⁺ or NADPH at 340nm using the 96 well plate. A=volume of invertase reaction for glucose detection, B=total volume of invertase reaction, C=total volume of glucose detection reaction, D=protein concentration used in invertase assay, E=incubation time of invertase assay.

Calculated glucose concentrations were blotted against all tested sucrose concentrations and tested for Michaelis-Menten kinetics. Transforming the measured values by Lineweaver-Burk plot, led to the determination of the Michaelis constant (K_m) and the maximal velocity (v_{max}) of invertase reaction. The K_m value describes the affinity of an enzyme to its substrate. K_m is inversely proportional to the substrate affinity, and is for a one-substrate reaction independent of the enzyme concentration. The enzymatic substrate conversion increases with increasing substrate concentration until the enzyme is saturated. This characteristic is described by the maximum velocity (v_{max}). In contrast to K_m , v_{max} depends on the enzyme concentration. Both biochemical characteristics vary with pH, with temperature and with the structure of the substrate.

Calculation of the significance values for the biochemical parameters

The significance values for differences between the K_m and v_{max} values measured for the *Pain-1* alleles at 30°C and 4°C, and for the alleles of the genes *invGE* and *invGF* at 30°C were calculated by Benjamin Stich, MPIZ/Köln.

In a first step, K_m and v_{max} estimates were calculated for each of the two biological replicates and the three technical replicates. These estimates were then used in a mixed-model context to examine:

- (i) the presence of differences between the alleles in each of the four experiments (*Pain-1* assay at 30°C and 4°C, *invGE* assay at 30°C, *invGF* assay at 30°C):

$$Y = \mu + \text{allele} + \text{allele:biolrep} + \text{error},$$

where μ was the general mean, allele an effect for the allele (without wild type reference *FY 1679*), and allele:biolrep was an effect for the biological replicate nested within the allele effect. Allele was regarded as fixed and allele:biolrep as random.

- (ii) the influence of the temperature regime (*Pain-1* assay at 30°C and 4°C):

$$Y = \mu + \text{tempreg} + \text{allele} + \text{tempreg*allele} + \text{tempreg:allele:biolrep} + \text{error},$$

where tempreg was the effect for the temperature regime, and tempreg*allele the interaction between the temperature regime and the allele. Tempreg and tempreg*allele were regarded as fixed.

- (iii) the influence of the location of the proteins (vacuolar vs. cell wall-bound):

$$Y = \mu + \text{loc} + \text{loc:allele:biolrep} + \text{error},$$

where loc was the effect for the location of the protein, which was regarded as fixed.

(iv) the presence of differences between all examined alleles:

$$Y = \mu + \text{allele} + \text{allele:biolrep} + \text{error}.$$

For fixed effects, a Wald-F-test was performed.

2.2.6.3 Protein separation by SDS-PAGE and Western blot analysis

Protein separation by SDS-PAGE:

The protein extracts were prepared for SDS-PAGE (LAEMMLI, 1970) by adding SDS sample buffer and by heating the samples at 95°C for 10min to denature the proteins.

The extracts were applied onto precast 4-12% Bis-Tris gradient gels (NuPAGE[®], Invitrogen, Carlsbad, USA) and were separated in MOPS running buffer (Invitrogen, Carlsbad, USA) at 200V in XCell SureLock[™] Mini-Cells (Invitrogen, Karlsruhe). The following molecular weight markers were used:

1. Spectra[™] Multicolor Broad Range Protein Ladder (10-260kDa), (MBI Fermentas GmbH (St. Leon-Rot)
2. MagicMark[™] XP Western Protein Standard (Invitrogen, Carlsbad, USA)

After the run the gels were stained with Coomassie Brilliant Blue (Bio-Safe[™] Coomassie G-250 stain, Bio-Rad, Hercules, USA) to detect the proteins. Gels for blotting were not stained.

Western blot analysis:

The protein extracts were blotted on Amersham Hybond-P PVDF Membrane (GE Healthcare, Buckinghamshire, UK) by wet electrotransfer using a blotting module (XCell[™] II Blot Module, Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. After blotting, the protein transfer onto the membrane was visualized with P3504 Ponceau S (Sigma-Aldrich Chemie GmbH, Steinheim) and after destaining and TBS (50mM Tris-HCl pH 7.5, 150mM NaCl) washing, the membrane was incubated in blocking solution (TBS + 3% non-fat dried milk powder) for 1h at RT and continuously shaking. For primary antibody reaction, the membrane was incubated with polyclonal anti-invertase antibodies overnight at RT on a shaker.

Table 2.2.15: Anti-invertase antibodies used in Western blot analysis.

Antibody	Concentration	Supplied/provided by
25kDa vacuolar invertase carrot isoform I	1:400	Arnd Sturm(former member of the Friedrich Miescher Institut, Basel, Switzerland)
43kDa vacuolar invertase carrot isoform I	1:500	
vacuolar invertase carrot isoform I	1:1000	Heather A. Ross (Scottish Crop Research Institute, Dundee, Scotland)
vacuolar invertase carrot isoform II	1:1000	
58kDa vacuolar invertase potato	1:200	

All antibodies were used in TBS + 1.5% non-fat dried milk powder.

As a secondary antibody the ECLTM Anti-rabbit IgG Horseradish Peroxidase linked whole antibody (from donkey, GE Healthcare, Buckinghamshire, UK) in a concentration 1:10000 in TBS without milk powder was used and incubated for 1h at RT on a shaker. For visualisation, the Amersham ECL Plus Western Blotting Detection Reagents (GE Healthcare, Buckinghamshire, UK) was used according to the manufacturer's protocol.

3 Results

3.1 The *Pain-1* locus on chromosome III

The *Pain-1* locus encodes a soluble acid invertase, which is located in the vacuole. The pH optimum of the corresponding enzyme ranges between 4.5 and 5.0 (TYMOWSKA-LALANNE & KREIS, 1998). cDNAs of several potato soluble acid invertases have been cloned and proteins were partially characterized at functional level (ZHOU ET AL., 2004; accession L29099, ZRENNER ET AL., 1996; accession X70368, ZHANG ET AL., unpublished; accession AY341425, MATSUURA-ENDO ET AL., 2006 unpublished; accession DQ478950).

3.1.1 Structural characterization of the *Pain-1* locus

3.1.1.1 Molecular cloning of *Pain-1* invertase cDNA alleles from tuber tissue

Soluble acid invertase transcripts are strongly induced during tuber cold storage (ZRENNER ET AL., 1996; BAGNARESI ET AL., 2008). Therefore, molecular cloning of *Pain-1* alleles was performed using potato tubers stored for four weeks at 4°C in the dark.

Using the full-length *Pain-1* specific primers ZrPain-F/PainUni-R (chapter 2, Table 2.1.2), cDNA invertase alleles were cloned and sequenced from the three tetraploid potato cultivars ‘Satina’, ‘Diana’, and ‘Theresa’, and from three diploid potato genotypes P18, P40, and P54. The tetraploid genotypes are representatives for the associated SSCP fragments of the *Pain-1* gene (LI ET AL., 2008; Table 3.1.1) and, therefore, were chosen in this study. Phenotypic characterization of potato chips quality from the cultivars showed variability within the trait (Table 3.1.2). The diploid potato genotypes were included because they were used as parents for mapping QTLs and candidate genes for cold sweetening of potato tubers (MENÉNDEZ ET AL., 2002).

Table 3.1.1: Distribution of the associated SSCP fragments *Pain1-5c*, *Pain1-8c* and *Pain1-9a* present in the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’.

Genotype	<i>Pain1-5c</i>	<i>Pain1-8c</i>	<i>Pain1-9a</i>
‘Satina’	0	1	1
‘Diana’	1	1	1
‘Theresa’	0	0	0

0=SSCP fragment is absent, 1=SSCP fragment is present.

Table 3.1.2: Potato chips colour scores for the cultivars ‘Satina’, ‘Diana’, and ‘Theresa’.

Genotype	CQA	CHS
‘Satina’	3.33	0.83
‘Diana’	7.83	4.67
‘Theresa’	6.83	3.00

Potato chips quality was assessed by visually scoring the chips colour after deep frying of 1.2-2.0mm tuber slices in oil at 160-180°C for 2-3min (PUTZ, 1989), using a 1-9 colour scale. 1=very dark chips colour, very bad chips quality; 9=very light yellow chips colour, very good chips quality. CQA refers to the chips quality after harvest in autumn; CQS refers to the chips quality in spring after tuber cold storage for 3-4 months.

Out of 80 cloned cDNA sequences per genotype, a sequence was defined as an allele when it was found twice in two independent PCRs. Additionally, the consensus sequence of all alleles found in one genotype was used for allele definition when variable sequence polymorphisms occurred.

Pain-1 invertase alleles obtained from each genotype are listed in Table 3.1.3.

Table 3.1.3: Overview of *Pain-1* alleles.

Genotype	Full-length clones	<i>Pain-1</i> alleles
‘Satina’	9	<i>Pain_SA</i>
		<i>Pain_SN</i>
‘Diana’	16	<i>Pain_DA</i>
		<i>Pain_DN1</i>
		<i>Pain_DN2</i>
‘Theresa’	8	<i>Pain_TN1</i>
		<i>Pain_TN2</i>
P18	7	<i>Pain_P18N1</i>
		<i>Pain_P18N2</i>
P40	8	<i>Pain_P40N1</i>
		<i>Pain_P40N2</i>
P54	6	<i>Pain_P54N</i>

The full-length clone number refers to the number of fully sequenced clones from each genotype used for allele definition. Allele names refer to the identification of associated *Pain-1* alleles, described in section 3.1.1.2. The ‘A’ in the allele name stands for ‘association with better potato chips quality’, and refers to clones containing SNP 1544. The ‘N’ in the allele name means ‘not associated’ with better potato chips quality.

From all six genotypes analyzed in this study, 12 *Pain-1* alleles were identified.

3.1.1.1.1 cDNA alleles of the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’¹

From nine full-length cDNA clones of the genotype ‘Satina’ two different alleles *Pain_SA* and *Pain_SN* were defined. Cloning and sequencing of ‘Diana’ cDNA resulted in 16 full-length clones, from which three different *Pain-1* alleles *Pain_DA*, *Pain_DN1*, and *Pain_DN2* were identified. For the cultivar ‘Theresa’ eight full-length clones were obtained. Two different alleles *Pain_TN1* and *Pain_TN2* were defined.

¹ Alleles of each genotype were defined by alignment of all fully sequenced cDNA clones: ‘Satina’ (Appendix A 3.1.1), ‘Diana’ (Appendix A 3.1.2), ‘Theresa’ (Appendix A 3.1.3). The nucleotide sequence for one allele was defined by aligning all obtained nucleotide sequences for this allele and using the consensus sequence when SNPs occurred: *Pain_SA* (Appendix A 3.1.4), *Pain_SN* (Appendix A 3.1.5), *Pain_DA* (Appendix A 3.1.6), *Pain_DN1* (Appendix A 3.1.7), *Pain_DN2* (Appendix A 3.1.8), *Pain_TN1* (Appendix A 3.1.9), *Pain_TN2* (Appendix A 3.1.10).

Comparing all seven alleles at the nucleotide level (Appendix A 3.1.11), 26 single nucleotide polymorphisms (SNPs) were detected. Ten of them resulted in an amino acid exchange (Table 3.1.4; Figure 3.1.1).

Table 3.1.4: SNPs present in ‘Satina’, ‘Diana’, and ‘Theresa’ alleles.

Position of cDNA SNP	<i>Pain_SA</i>	<i>Pain_SN</i>	<i>Pain_DA</i>	<i>Pain_DN1</i>	<i>Pain_DN2</i>	<i>Pain_TN1</i>	<i>Pain_TN2</i>	aa
32	C	T	C	T	C	T	C	C/T P11L
75	C	T	C	T	C	T	C	s.
174	G	A	G	A	G	A	G	s.
213	A	G	G	G	G	G	G	s.
280	G	G	A	G	G	G	G	A/G I94V
528	T	A	T	A	T	A	T	s.
552	C	T	C	T	T	T	T	s.
612	A	A	A	A	G	A	G	s.
718	A	G	A	G	G	G	G	A/G I240V
741	T	C	C	C	C	C	C	s.
777	T	T	T	T	C	T	C	s.
1068	C	C	C	C	G	C	G	C/G N356K
1212	G	G	G	G	G	G	A	s.
1316	C	C	T	C	C	C	C	T/C V439A
1143	C	G	G	G	G	G	G	s.
1544	A	C	A	C	C	C	C	A/C K515T
1574	A	A	A	A	T	A	T	A/T Y525F
1596	T	C	T	C	T	C	T	s.
1629	T	C	T	C	T	C	T	s.
1661	A	A	A	A	G	A	G	A/G Q554R
1689	G	G	A	G	G	G	G	s.
1749	T	T	C	T	T	T	T	s.
1830	C	C	C	C	C	C	T	s.
1843	G	T	G	T	G	T	G	T/G S615A
1857	C	C	T	C	C	C	C	s.
1895	G	A	G	A	G	A	G	A/G Q632R

SNP position numbering refers to cDNA sequence where ‘1’ represents the adenine of the start codon ATG and the polymorphism is described. aa=amino acid exchanges ; s.=synonymous nucleotide exchange that does not lead to an amino acid exchange. Non synonymous nucleotide exchanges lead to an amino acid exchange and are orange coloured. SNP 1544 (blue) was used to assign the associated SSCP fragments to a cloned allele (section 3.1.1.2) as well as for pyrosequencing analysis (section 3.1.2.1).

The two alleles of ‘Satina’ had a total of 14 SNPs, of which five caused an amino acid exchange. In the three ‘Diana’ allelic sequences 21 SNPs occurred, from which ten resulted in an amino acid substitution. The two ‘Theresa’ alleles differed in 15 SNPs. Six of them led to amino acid exchanges.

The amino acid alignment highlights the differences of all seven *Pain-1* alleles from the three tetraploid potato cultivars (Figure 3.1.1). The comparison of these deduced protein sequences revealed ten variable amino acid positions in the different genotypes.

```

Pain_DN1 : MATQYHSSYDLENSASHYTFLLPDQDPSGHRKSLKIIISGIFLSSFLLLSVAFFPILNNSQSPDLQSNRSRSPAPPSRGVSGQVSDK : 83
Pain_TN1 : MATQYHSSYDLENSASHYTFLLPDQDPSGHRKSLKIIISGIFLSSFLLLSVAFFPILNNSQSPDLQSNRSRSPAPPSRGVSGQVSDK : 83
Pain_SN : MATQYHSSYDLENSASHYTFLLPDQDPSGHRKSLKIIISGIFLSSFLLLSVAFFPILNNSQSPDLQSNRSRSPAPPSRGVSGQVSDK : 83
Pain_DN2 : MATQYHSSYDLENSASHYTFLLPDQDPSGHRKSLKIIISGIFLSSFLLLSVAFFPILNNSQSPDLQSNRSRSPAPPSRGVSGQVSDK : 83
Pain_TN2 : MATQYHSSYDLENSASHYTFLLPDQDPSGHRKSLKIIISGIFLSSFLLLSVAFFPILNNSQSPDLQSNRSRSPAPPSRGVSGQVSDK : 83
Pain_SA : MATQYHSSYDLENSASHYTFLLPDQDPSGHRKSLKIIISGIFLSSFLLLSVAFFPILNNSQSPDLQSNRSRSPAPPSRGVSGQVSDK : 83
Pain_DA : MATQYHSSYDLENSASHYTFLLPDQDPSGHRKSLKIIISGIFLSSFLLLSVAFFPILNNSQSPDLQSNRSRSPAPPSRGVSGQVSDK : 83

* 20 * 40 * 60 * 80
Pain_DN1 : TFRDVVNASHVSYAWSNAMLSWQRRTAYHFQPPQKNWMDPNGLYHKGWYHLFYQYNPDSAIWGNITWGHAVSKDLIHWLWYLPF : 166
Pain_TN1 : TFRDVVNASHVSYAWSNAMLSWQRRTAYHFQPPQKNWMDPNGLYHKGWYHLFYQYNPDSAIWGNITWGHAVSKDLIHWLWYLPF : 166
Pain_SN : TFRDVVNASHVSYAWSNAMLSWQRRTAYHFQPPQKNWMDPNGLYHKGWYHLFYQYNPDSAIWGNITWGHAVSKDLIHWLWYLPF : 166
Pain_DN2 : TFRDVVNASHVSYAWSNAMLSWQRRTAYHFQPPQKNWMDPNGLYHKGWYHLFYQYNPDSAIWGNITWGHAVSKDLIHWLWYLPF : 166
Pain_TN2 : TFRDVVNASHVSYAWSNAMLSWQRRTAYHFQPPQKNWMDPNGLYHKGWYHLFYQYNPDSAIWGNITWGHAVSKDLIHWLWYLPF : 166
Pain_SA : TFRDVVNASHVSYAWSNAMLSWQRRTAYHFQPPQKNWMDPNGLYHKGWYHLFYQYNPDSAIWGNITWGHAVSKDLIHWLWYLPF : 166
Pain_DA : TFRDVVNASHVSYAWSNAMLSWQRRTAYHFQPPQKNWMDPNGLYHKGWYHLFYQYNPDSAIWGNITWGHAVSKDLIHWLWYLPF : 166

* 100 * 120 * 140 * 160
Pain_DN1 : AMVPDQWYDINGVWTGSATILPDGQIMMLYTGTDYVQVQNLAYPTNLSDDLDDWVYKGNPVLVPPPGIGIKDFRDPPTTA : 249
Pain_TN1 : AMVPDQWYDINGVWTGSATILPDGQIMMLYTGTDYVQVQNLAYPTNLSDDLDDWVYKGNPVLVPPPGIGIKDFRDPPTTA : 249
Pain_SN : AMVPDQWYDINGVWTGSATILPDGQIMMLYTGTDYVQVQNLAYPTNLSDDLDDWVYKGNPVLVPPPGIGIKDFRDPPTTA : 249
Pain_DN2 : AMVPDQWYDINGVWTGSATILPDGQIMMLYTGTDYVQVQNLAYPTNLSDDLDDWVYKGNPVLVPPPGIGIKDFRDPPTTA : 249
Pain_TN2 : AMVPDQWYDINGVWTGSATILPDGQIMMLYTGTDYVQVQNLAYPTNLSDDLDDWVYKGNPVLVPPPGIGIKDFRDPPTTA : 249
Pain_SA : AMVPDQWYDINGVWTGSATILPDGQIMMLYTGTDYVQVQNLAYPTNLSDDLDDWVYKGNPVLVPPPGIGIKDFRDPPTTA : 249
Pain_DA : AMVPDQWYDINGVWTGSATILPDGQIMMLYTGTDYVQVQNLAYPTNLSDDLDDWVYKGNPVLVPPPGIGIKDFRDPPTTA : 249

* 180 * 200 * 220 * 240
Pain_DN1 : WTGPQNGQWLLTIGSKIGKTGIALVYETSNFTSFKLLDEVLHAVPGTGMWECVDFYFPVSTTEKTNGLDTSYNGPGVKHVLKASL : 332
Pain_TN1 : WTGPQNGQWLLTIGSKIGKTGIALVYETSNFTSFKLLDEVLHAVPGTGMWECVDFYFPVSTTEKTNGLDTSYNGPGVKHVLKASL : 332
Pain_SN : WTGPQNGQWLLTIGSKIGKTGIALVYETSNFTSFKLLDEVLHAVPGTGMWECVDFYFPVSTTEKTNGLDTSYNGPGVKHVLKASL : 332
Pain_DN2 : WTGPQNGQWLLTIGSKIGKTGIALVYETSNFTSFKLLDEVLHAVPGTGMWECVDFYFPVSTTEKTNGLDTSYNGPGVKHVLKASL : 332
Pain_TN2 : WTGPQNGQWLLTIGSKIGKTGIALVYETSNFTSFKLLDEVLHAVPGTGMWECVDFYFPVSTTEKTNGLDTSYNGPGVKHVLKASL : 332
Pain_SA : WTGPQNGQWLLTIGSKIGKTGIALVYETSNFTSFKLLDEVLHAVPGTGMWECVDFYFPVSTTEKTNGLDTSYNGPGVKHVLKASL : 332
Pain_DA : WTGPQNGQWLLTIGSKIGKTGIALVYETSNFTSFKLLDEVLHAVPGTGMWECVDFYFPVSTTEKTNGLDTSYNGPGVKHVLKASL : 332

* 260 * 280 * 300 * 320 *
Pain_DN1 : DDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKLGYGKYASKTFYDPPKQRRVLWGWIGETDSEADLQKGWASVQSIPT : 415
Pain_TN1 : DDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKLGYGKYASKTFYDPPKQRRVLWGWIGETDSEADLQKGWASVQSIPT : 415
Pain_SN : DDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKLGYGKYASKTFYDPPKQRRVLWGWIGETDSEADLQKGWASVQSIPT : 415
Pain_DN2 : DDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKLGYGKYASKTFYDPPKQRRVLWGWIGETDSEADLQKGWASVQSIPT : 415
Pain_TN2 : DDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKLGYGKYASKTFYDPPKQRRVLWGWIGETDSEADLQKGWASVQSIPT : 415
Pain_SA : DDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKLGYGKYASKTFYDPPKQRRVLWGWIGETDSEADLQKGWASVQSIPT : 415
Pain_DA : DDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKLGYGKYASKTFYDPPKQRRVLWGWIGETDSEADLQKGWASVQSIPT : 415

* 340 * 360 * 380 * 400 *
Pain_DN1 : VLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIEASFEVDKVALQGIIEADHVGFSCSTSGGAA : 498
Pain_TN1 : VLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIEASFEVDKVALQGIIEADHVGFSCSTSGGAA : 498
Pain_SN : VLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIEASFEVDKVALQGIIEADHVGFSCSTSGGAA : 498
Pain_DN2 : VLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIEASFEVDKVALQGIIEADHVGFSCSTSGGAA : 498
Pain_TN2 : VLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIEASFEVDKVALQGIIEADHVGFSCSTSGGAA : 498
Pain_SA : VLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIEASFEVDKVALQGIIEADHVGFSCSTSGGAA : 498
Pain_DA : VLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIEASFEVDKVALQGIIEADHVGFSCSTSGGAA : 498

* 420 * 440 * 460 * 480 * 5
Pain_DN1 : SRGILGPPFGVVVIADQTLSELTPVYFYIISKGADGRAETHFCADQTRSSEAPGVAKQVYGSSVPVLDGEEKHSMRLLVDHSIVES : 581
Pain_TN1 : SRGILGPPFGVVVIADQTLSELTPVYFYIISKGADGRAETHFCADQTRSSEAPGVAKQVYGSSVPVLDGEEKHSMRLLVDHSIVES : 581
Pain_SN : SRGILGPPFGVVVIADQTLSELTPVYFYIISKGADGRAETHFCADQTRSSEAPGVAKQVYGSSVPVLDGEEKHSMRLLVDHSIVES : 581
Pain_DN2 : SRGILGPPFGVVVIADQTLSELTPVYFYIISKGADGRAETHFCADQTRSSEAPGVAKQVYGSSVPVLDGEEKHSMRLLVDHSIVES : 581
Pain_TN2 : SRGILGPPFGVVVIADQTLSELTPVYFYIISKGADGRAETHFCADQTRSSEAPGVAKQVYGSSVPVLDGEEKHSMRLLVDHSIVES : 581
Pain_SA : SRGILGPPFGVVVIADQTLSELTPVYFYIISKGADGRAETHFCADQTRSSEAPGVAKQVYGSSVPVLDGEEKHSMRLLVDHSIVES : 581
Pain_DA : SRGILGPPFGVVVIADQTLSELTPVYFYIISKGADGRAETHFCADQTRSSEAPGVAKQVYGSSVPVLDGEEKHSMRLLVDHSIVES : 581

* 600 * 620 *
Pain_DN1 : FAQGGRTVITSRIYPTKAVNGAARLFVFNNAATGSSVTASVKIWSLESANIQSFPQLDL : 639
Pain_TN1 : FAQGGRTVITSRIYPTKAVNGAARLFVFNNAATGSSVTASVKIWSLESANIQSFPQLDL : 639
Pain_SN : FAQGGRTVITSRIYPTKAVNGAARLFVFNNAATGSSVTASVKIWSLESANIQSFPQLDL : 639
Pain_DN2 : FAQGGRTVITSRIYPTKAVNGAARLFVFNNAATGASVTASVKIWSLESANIRSFPLQDL : 639
Pain_TN2 : FAQGGRTVITSRIYPTKAVNGAARLFVFNNAATGASVTASVKIWSLESANIRSFPLQDL : 639
Pain_SA : FAQGGRTVITSRIYPTKAVNGAARLFVFNNAATGASVTASVKIWSLESANIRSFPLQDL : 639
Pain_DA : FAQGGRTVITSRIYPTKAVNGAARLFVFNNAATGASVTASVKIWSLESANIRSFPLQDL : 639

```

Figure 3.1.1: Amino acid alignment of *Pain-1* alleles from the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’. Amino acid exchanges are highlighted in colour.

Comparison of allelic cDNA sequences of the *Pain-1* gene from the three tetraploid cultivars revealed that different genotypes harbour amino acid sequence identical alleles. ‘Satina’, ‘Diana’, and ‘Theresa’ share one identical allele ($Pain_{SN}=Pain_{DNI}=Pain_{TNI}$). Furthermore, ‘Diana’, and ‘Theresa’ contain another allele identical at amino acid level ($Pain_{DN2}=Pain_{TN2}$).

3.1.1.1.2 cDNA alleles of the diploid potato genotypes P18, P40, and P54²

Cloning and sequencing of P18 cDNA resulted in seven full-length clones, from which two different alleles $Pain_{P18N1}$ and $Pain_{P18N2}$ were identified. In the case of P40 two alleles $Pain_{P40N1}$ and $Pain_{P40N2}$ out of eight full-length clones were found. From the genotype P54 six full-length clones were obtained. All six clones exhibited the same amino acid sequence. The P54 allele was named $Pain_{P54N}$.

The nucleotide sequence comparison (Appendix A 3.1.20) of the alleles described above detected 47 SNPs, from which 20 resulted in amino acid exchanges (Table 3.1.5; Figure 3.1.2).

Table 3.1.5: SNPs present in P18, P40, and P54 alleles.

Position of cDNA SNP	$Pain_{P18N1}$	$Pain_{P18N2}$	$Pain_{P40N1}$	$Pain_{P40N2}$	$Pain_{P54N}$	aa
18	T	T	C	C	T	s.
21	C	C	A	A	C	s.
32	C	T	C	C	T	C/T P11L
74	C	C	A	A	C	C/A P25H
75	C	C	C	C	T	s.
93	G	G	A	A	G	s.
97	C	C	A	C	C	A/C I33L
130	T	T	C	C	T	T/C F44L
145	G	G	T	T	G	G/T V49L
149	C	C	T	T	C	C/T A50V
174	G	A	G	G	A	s.
189	G	G	A	A	G	s.
196	T	T	G	G	T	T/G S66A
200	G	G	A	G	G	A/G H67R

² Alleles of each genotype were defined by alignment of all fully sequenced cDNA clones: P18 (Appendix A 3.1.12), P40 (Appendix A 3.1.13), P54 (Appendix A 3.1.14). The nucleotide sequence for one allele was defined by aligning all obtained nucleotide sequences for this allele and using the consensus sequence when SNPs occurred: $Pain_{P18N1}$ (Appendix A 3.1.15), $Pain_{P18N2}$ (Appendix A 3.1.16), $Pain_{P40N1}$ (Appendix A 3.1.17), $Pain_{P40N2}$ (Appendix A 3.1.18), $Pain_{P54N}$ (Appendix A 3.1.19).

Position of cDNA SNP	<i>Pain_P18N1</i>	<i>Pain_P18N2</i>	<i>Pain_P40N1</i>	<i>Pain_P40N2</i>	<i>Pain_P54N</i>	aa
280	A	G	G	G	G	A/G I94V
369	T	T	T	C	T	s.
528	T	A	T	T	A	s.
534	T	T	T	G	T	s.
552	C	T	T	T	T	s.
591	C	C	T	T	C	s.
612	A	A	G	G	A	s.
637	A	A	G	G	A	T/A T213A
718	A	G	G	G	G	A/G I240V
723	G	A	G	G	G	s.
834	C	C	C	A	C	s.
852	G	G	A	G	G	s.
927	T	T	T	C	T	s.
1050	C	T	C	C	C	s.
1161	A	A	G	A	A	s.
1267	G	G	A	G	G	A/G R423G
1316	T	C	C	C	C	T/C V439A
1340	T	T	C	C	T	T/C V447A
1359	A	A	A	G	A	s.
1522	G	G	G	A	G	G/A V508I
1544	A	C	C	C	C	A/C K515T
1596	T	C	T	T	C	s.
1603	G	G	C	G	G	C/G Q535E
1629	T	C	T	T	C	s.
1640	A	A	T	T	A	A/T E547V
1683	C	C	C	G	C	s.
1689	A	G	G	G	G	s.
1698	G	A	G	G	A	s.
1749	C	T	T	T	T	s.
1776	G	G	A	G	G	s.
1843	G	T	G	G	T	G/T A615S
1857	T	C	C	C	C	s.
1895	G	A	G	G	A	G/A R632Q

SNP position numbering refers to cDNA sequence where '1' represents the adenine of the start codon ATG and the polymorphism is described. aa=amino acid exchanges ; s.=synonymous nucleotide exchange that does not lead to an amino acid exchange. Non synonymous nucleotide exchanges lead to an amino acid exchange and are orange coloured. SNP 1544 (blue) was used to assign the associated SSCP fragments to a cloned allele (section 3.1.1.2) as well as for pyrosequencing analysis (section 3.1.2.1).

From 17 SNPs of the two P18 alleles, seven caused an amino acid exchange. In the two P40 allelic sequences 14 SNPs occurred, from which five resulted in an amino acid substitution.

The amino acid alignment of all five *Pain-1* alleles of the three diploid genotypes showed the variability of positions where amino acids differed (Figure 3.1.2).

```

*           20           *           40           *           60           *           80
Pain_P18N2 : MATQYHSSYDLENSASHYTFLPDQPDSDGHRKSLKIISGIFLSSLLLSVAFFPILNNQSPDLQSNRSRSPAPPSRGVSGQVGS : 81
Pain_P54N : MATQYHSSYDLENSASHYTFLPDQPDSDGHRKSLKIISGIFLSSLLLSVAFFPILNNQSPDLQSNRSRSPAPPSRGVSGQVGS : 81
Pain_P18N1 : MATQYHSSYDLENSASHYTFLPDQPDSDGHRKSLKIISGIFLSSLLLSVAFFPILNNQSPDLQSNRSRSPAPPSRGVSGQVGS : 81
Pain_P40N1 : MATQYHSSYDLENSASHYTFLPDQHSDGHRKSLKIISGIFLSSLLLSLVFFPILNNQSPDLQSNRHSRSPAPPSRGVSGQVGS : 81
Pain_P40N2 : MATQYHSSYDLENSASHYTFLPDQHSDGHRKSLKIISGIFLSSLLLSLVFFPILNNQSPDLQSNRHSRSPAPPSRGVSGQVGS : 81

*           100          *           120          *           140          *           160
Pain_P18N2 : DKTFRDVTNASHVSYAWSNAMLSWQRTAYHFQPKNWMNDPNGPLYHKGWYHLFYQYNPDSAIWGNITWGHAVSKDLIHHL : 162
Pain_P54N : DKTFRDVTNASHVSYAWSNAMLSWQRTAYHFQPKNWMNDPNGPLYHKGWYHLFYQYNPDSAIWGNITWGHAVSKDLIHHL : 162
Pain_P18N1 : DKTFRDVTNASHVSYAWSNAMLSWQRTAYHFQPKNWMNDPNGPLYHKGWYHLFYQYNPDSAIWGNITWGHAVSKDLIHHL : 162
Pain_P40N1 : DKTFRDVTNASHVSYAWSNAMLSWQRTAYHFQPKNWMNDPNGPLYHKGWYHLFYQYNPDSAIWGNITWGHAVSKDLIHHL : 162
Pain_P40N2 : DKTFRDVTNASHVSYAWSNAMLSWQRTAYHFQPKNWMNDPNGPLYHKGWYHLFYQYNPDSAIWGNITWGHAVSKDLIHHL : 162

*           180          *           200          *           220          *           240
Pain_P18N2 : YLPFAMVPDQWYDINGVWTGSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSDDLDDWVKYKGNPVLVPPPGIGVKDF : 243
Pain_P54N : YLPFAMVPDQWYDINGVWTGSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSDDLDDWVKYKGNPVLVPPPGIGVKDF : 243
Pain_P18N1 : YLPFAMVPDQWYDINGVWTGSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSDDLDDWVKYKGNPVLVPPPGIGVKDF : 243
Pain_P40N1 : YLPFAMVPDQWYDINGVWTGSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSDDLDDWVKYKGNPVLVPPPGIGVKDF : 243
Pain_P40N2 : YLPFAMVPDQWYDINGVWTGSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSDDLDDWVKYKGNPVLVPPPGIGVKDF : 243

*           260          *           280          *           300          *           320
Pain_P18N2 : RDPTTAWTGPQNGQWLLTIGSKIGKTGIALVYETSNTSFKLLDEVLHAVPGTGMWECVDFYFPVSTTEKTNGLDTSYNGPGV : 324
Pain_P54N : RDPTTAWTGPQNGQWLLTIGSKIGKTGIALVYETSNTSFKLLDEVLHAVPGTGMWECVDFYFPVSTTEKTNGLDTSYNGPGV : 324
Pain_P18N1 : RDPTTAWTGPQNGQWLLTIGSKIGKTGIALVYETSNTSFKLLDEVLHAVPGTGMWECVDFYFPVSTTEKTNGLDTSYNGPGV : 324
Pain_P40N1 : RDPTTAWTGPQNGQWLLTIGSKIGKTGIALVYETSNTSFKLLDEVLHAVPGTGMWECVDFYFPVSTTEKTNGLDTSYNGPGV : 324
Pain_P40N2 : RDPTTAWTGPQNGQWLLTIGSKIGKTGIALVYETSNTSFKLLDEVLHAVPGTGMWECVDFYFPVSTTEKTNGLDTSYNGPGV : 324

*           340          *           360          *           380          *           400
Pain_P18N2 : KHVLFKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKLDDYGYKYYASKTFYDPPKQRRVLWGWIGETDSESADLQKG : 405
Pain_P54N : KHVLFKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKLDDYGYKYYASKTFYDPPKQRRVLWGWIGETDSESADLQKG : 405
Pain_P18N1 : KHVLFKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKLDDYGYKYYASKTFYDPPKQRRVLWGWIGETDSESADLQKG : 405
Pain_P40N1 : KHVLFKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKLDDYGYKYYASKTFYDPPKQRRVLWGWIGETDSESADLQKG : 405
Pain_P40N2 : KHVLFKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKLDDYGYKYYASKTFYDPPKQRRVLWGWIGETDSESADLQKG : 405

*           420          *           440          *           460          *           480
Pain_P18N2 : WASVQSIPTVLYDKKTCGTHLLQWPVEEIESLRAGDPPIVKQANLQPGSIELLHVDSAAELDIEASFVVDKVALQGIIEADH : 486
Pain_P54N : WASVQSIPTVLYDKKTCGTHLLQWPVEEIESLRAGDPPIVKQANLQPGSIELLHVDSAAELDIEASFVVDKVALQGIIEADH : 486
Pain_P18N1 : WASVQSIPTVLYDKKTCGTHLLQWPVEEIESLRVGDPIVKQANLQPGSIELLHVDSAAELDIEASFVVDKVALQGIIEADH : 486
Pain_P40N1 : WASVQSIPTVLYDKKTCGTHLLQWPVEEIESLRAGDPPIVKQANLQPGSIELLHVDSAAELDIEASFVVDKVALQGIIEADH : 486
Pain_P40N2 : WASVQSIPTVLYDKKTCGTHLLQWPVEEIESLRAGDPPIVKQANLQPGSIELLHVDSAAELDIEASFVVDKVALQGIIEADH : 486

*           500          *           520          *           540          *           560
Pain_P18N2 : VGFSCSTSGGAASRGILGPFVGVVIADQTLSELTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYVYSSVPLDGEK : 567
Pain_P54N : VGFSCSTSGGAASRGILGPFVGVVIADQTLSELTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYVYSSVPLDGEK : 567
Pain_P18N1 : VGFSCSTSGGAASRGILGPFVGVVIADQTLSELTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYVYSSVPLDGEK : 567
Pain_P40N1 : VGFSCSTSGGAASRGILGPFVGVVIADQTLSELTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYVYSSVPLDGEK : 567
Pain_P40N2 : VGFSCSTSGGAASRGILGPFVGVVIADQTLSELTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYVYSSVPLDGEK : 567

*           580          *           600          *           620          *
Pain_P18N2 : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVVTASVKIWSLESANIRSFPLQDL : 639
Pain_P54N : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVVTASVKIWSLESANIRSFPLQDL : 639
Pain_P18N1 : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVVTASVKIWSLESANIRSFPLQDL : 639
Pain_P40N1 : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVVTASVKIWSLESANIRSFPLQDL : 639
Pain_P40N2 : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVVTASVKIWSLESANIRSFPLQDL : 639

```

Figure 3.1.2: Amino acid alignment of the *Pain-1* alleles from the diploid genotypes P18, P40, and P54. Amino acid exchanges are highlighted in colour.

The amino acid alignment of the five *Pain-1* alleles of the diploid genotypes showed that P18 and P54 contain an allele identical at amino acid level (*Pain_P18N2*=*Pain_P54N*).

3.1.1.1.3 Amino acid alignment of all *Pain-1* invertase alleles of the analyzed potato genotypes

Multiple alignment of allelic *Pain-1* deduced protein sequences revealed partially overlapping amino acid polymorphisms between the tetraploid and diploid genotypes. Several amino acid exchanges occurred in different genotypes at the same position. Intriguingly, the two P40

alleles *Pain_P40N1* and *Pain_P40N2* showed less similarity to the alleles retrieved from the other genotypes (Figure 3.1.3; Figure 3.1.4).

```

*          20          *          40          *          60          *          80
Pain_DA   : MATQYHSSYD*ENSASHYTFLLPDQ*PDSGHRKSLKIIISGIFLSS*FLLLSVAFFPILNNQSPDLQSN*SRSPAPPSRGVSOQVGS : 81
Pain_P18N1 : MATQYHSSYD*ENSASHYTFLLPDQ*PDSGHRKSLKIIISGIFLSS*FLLLSVAFFPILNNQSPDLQSN*SRSPAPPSRGVSOQVGS : 81
Pain_SA   : MATQYHSSYD*ENSASHYTFLLPDQ*PDSGHRKSLKIIISGIFLSS*FLLLSVAFFPILNNQSPDLQSN*SRSPAPPSRGVSOQVGS : 81
Pain_DN2  : MATQYHSSYD*ENSASHYTFLLPDQ*PDSGHRKSLKIIISGIFLSS*FLLLSVAFFPILNNQSPDLQSN*SRSPAPPSRGVSOQVGS : 81
Pain_TN2  : MATQYHSSYD*ENSASHYTFLLPDQ*PDSGHRKSLKIIISGIFLSS*FLLLSVAFFPILNNQSPDLQSN*SRSPAPPSRGVSOQVGS : 81
Pain_SN   : MATQYHSSYD*ENSASHYTFLLPDQ*PDSGHRKSLKIIISGIFLSS*FLLLSVAFFPILNNQSPDLQSN*SRSPAPPSRGVSOQVGS : 81
Pain_P54N : MATQYHSSYD*ENSASHYTFLLPDQ*PDSGHRKSLKIIISGIFLSS*FLLLSVAFFPILNNQSPDLQSN*SRSPAPPSRGVSOQVGS : 81
Pain_P18N2 : MATQYHSSYD*ENSASHYTFLLPDQ*PDSGHRKSLKIIISGIFLSS*FLLLSVAFFPILNNQSPDLQSN*SRSPAPPSRGVSOQVGS : 81
Pain_TN1  : MATQYHSSYD*ENSASHYTFLLPDQ*PDSGHRKSLKIIISGIFLSS*FLLLSVAFFPILNNQSPDLQSN*SRSPAPPSRGVSOQVGS : 81
Pain_DN1  : MATQYHSSYD*ENSASHYTFLLPDQ*PDSGHRKSLKIIISGIFLSS*FLLLSVAFFPILNNQSPDLQSN*SRSPAPPSRGVSOQVGS : 81
Pain_P40N1 : MATQYHSSYD*ENSASHYTFLLPDQ*HDSGHRKSLKIIISGIFLSS*FLLLSLVFFPILNNQSPDLQSN*H*SRSPAPPSRGVSOQVGS : 81
Pain_P40N2 : MATQYHSSYD*ENSASHYTFLLPDQ*HDSGHRKSLKIIISGIFLSS*FLLLSLVFFPILNNQSPDLQSN*H*SRSPAPPSRGVSOQVGS : 81

```

```

*          100         *          120         *          140         *          160
Pain_DA   : DKTFRD*VNVNASH*ISYAWSNAMLSWQRTAYHFQ*PKNNWMD*PNGLYHKGWYHLFYQYNPDS*AIWGNITWGHAVSKDLIHWL : 162
Pain_P18N1 : DKTFRD*VNVNASH*ISYAWSNAMLSWQRTAYHFQ*PKNNWMD*PNGLYHKGWYHLFYQYNPDS*AIWGNITWGHAVSKDLIHWL : 162
Pain_SA   : DKTFRD*VNVNASH*ISYAWSNAMLSWQRTAYHFQ*PKNNWMD*PNGLYHKGWYHLFYQYNPDS*AIWGNITWGHAVSKDLIHWL : 162
Pain_DN2  : DKTFRD*VNVNASH*ISYAWSNAMLSWQRTAYHFQ*PKNNWMD*PNGLYHKGWYHLFYQYNPDS*AIWGNITWGHAVSKDLIHWL : 162
Pain_TN2  : DKTFRD*VNVNASH*ISYAWSNAMLSWQRTAYHFQ*PKNNWMD*PNGLYHKGWYHLFYQYNPDS*AIWGNITWGHAVSKDLIHWL : 162
Pain_SN   : DKTFRD*VNVNASH*ISYAWSNAMLSWQRTAYHFQ*PKNNWMD*PNGLYHKGWYHLFYQYNPDS*AIWGNITWGHAVSKDLIHWL : 162
Pain_P54N : DKTFRD*VNVNASH*ISYAWSNAMLSWQRTAYHFQ*PKNNWMD*PNGLYHKGWYHLFYQYNPDS*AIWGNITWGHAVSKDLIHWL : 162
Pain_P18N2 : DKTFRD*VNVNASH*ISYAWSNAMLSWQRTAYHFQ*PKNNWMD*PNGLYHKGWYHLFYQYNPDS*AIWGNITWGHAVSKDLIHWL : 162
Pain_TN1  : DKTFRD*VNVNASH*ISYAWSNAMLSWQRTAYHFQ*PKNNWMD*PNGLYHKGWYHLFYQYNPDS*AIWGNITWGHAVSKDLIHWL : 162
Pain_DN1  : DKTFRD*VNVNASH*ISYAWSNAMLSWQRTAYHFQ*PKNNWMD*PNGLYHKGWYHLFYQYNPDS*AIWGNITWGHAVSKDLIHWL : 162
Pain_P40N1 : DKTFRD*VNVNASH*ISYAWSNAMLSWQRTAYHFQ*PKNNWMD*PNGLYHKGWYHLFYQYNPDS*AIWGNITWGHAVSKDLIHWL : 162
Pain_P40N2 : DKTFRD*VNVNASH*ISYAWSNAMLSWQRTAYHFQ*PKNNWMD*PNGLYHKGWYHLFYQYNPDS*AIWGNITWGHAVSKDLIHWL : 162

```

```

*          180         *          200         *          220         *          240
Pain_DA   : YLFFAMV*PDQWYDINGV*WVTGSATILPDGQ*IMMLYTG*DTDDYV*VQVQNLAY*PTNLS*SDP*LLLD*WVKYKGNP*VLPV*PP*PGIG*IKDF : 243
Pain_P18N1 : YLFFAMV*PDQWYDINGV*WVTGSATILPDGQ*IMMLYTG*DTDDYV*VQVQNLAY*PTNLS*SDP*LLLD*WVKYKGNP*VLPV*PP*PGIG*IKDF : 243
Pain_SA   : YLFFAMV*PDQWYDINGV*WVTGSATILPDGQ*IMMLYTG*DTDDYV*VQVQNLAY*PTNLS*SDP*LLLD*WVKYKGNP*VLPV*PP*PGIG*IKDF : 243
Pain_DN2  : YLFFAMV*PDQWYDINGV*WVTGSATILPDGQ*IMMLYTG*DTDDYV*VQVQNLAY*PTNLS*SDP*LLLD*WVKYKGNP*VLPV*PP*PGIG*IKDF : 243
Pain_TN2  : YLFFAMV*PDQWYDINGV*WVTGSATILPDGQ*IMMLYTG*DTDDYV*VQVQNLAY*PTNLS*SDP*LLLD*WVKYKGNP*VLPV*PP*PGIG*IKDF : 243
Pain_SN   : YLFFAMV*PDQWYDINGV*WVTGSATILPDGQ*IMMLYTG*DTDDYV*VQVQNLAY*PTNLS*SDP*LLLD*WVKYKGNP*VLPV*PP*PGIG*IKDF : 243
Pain_P54N : YLFFAMV*PDQWYDINGV*WVTGSATILPDGQ*IMMLYTG*DTDDYV*VQVQNLAY*PTNLS*SDP*LLLD*WVKYKGNP*VLPV*PP*PGIG*IKDF : 243
Pain_P18N2 : YLFFAMV*PDQWYDINGV*WVTGSATILPDGQ*IMMLYTG*DTDDYV*VQVQNLAY*PTNLS*SDP*LLLD*WVKYKGNP*VLPV*PP*PGIG*IKDF : 243
Pain_TN1  : YLFFAMV*PDQWYDINGV*WVTGSATILPDGQ*IMMLYTG*DTDDYV*VQVQNLAY*PTNLS*SDP*LLLD*WVKYKGNP*VLPV*PP*PGIG*IKDF : 243
Pain_DN1  : YLFFAMV*PDQWYDINGV*WVTGSATILPDGQ*IMMLYTG*DTDDYV*VQVQNLAY*PTNLS*SDP*LLLD*WVKYKGNP*VLPV*PP*PGIG*IKDF : 243
Pain_P40N1 : YLFFAMV*PDQWYDINGV*WVTGSATILPDGQ*IMMLYTG*DTDDYV*VQVQNLAY*PTNLS*SDP*LLLD*WVKYKGNP*VLPV*PP*PGIG*IKDF : 243
Pain_P40N2 : YLFFAMV*PDQWYDINGV*WVTGSATILPDGQ*IMMLYTG*DTDDYV*VQVQNLAY*PTNLS*SDP*LLLD*WVKYKGNP*VLPV*PP*PGIG*IKDF : 243

```

```

*          260         *          280         *          300         *          320
Pain_DA   : RDPTT*AWTGPQNGQ*WLLTIGSKIGKTG*IALVYETS*NF*TSFKLL*DEVLH*AVPGTGMW*ECVDFY*PV*ST*EKT*NGLD*TSYNG*PGV : 324
Pain_P18N1 : RDPTT*AWTGPQNGQ*WLLTIGSKIGKTG*IALVYETS*NF*TSFKLL*DEVLH*AVPGTGMW*ECVDFY*PV*ST*EKT*NGLD*TSYNG*PGV : 324
Pain_SA   : RDPTT*AWTGPQNGQ*WLLTIGSKIGKTG*IALVYETS*NF*TSFKLL*DEVLH*AVPGTGMW*ECVDFY*PV*ST*EKT*NGLD*TSYNG*PGV : 324
Pain_DN2  : RDPTT*AWTGPQNGQ*WLLTIGSKIGKTG*IALVYETS*NF*TSFKLL*DEVLH*AVPGTGMW*ECVDFY*PV*ST*EKT*NGLD*TSYNG*PGV : 324
Pain_TN2  : RDPTT*AWTGPQNGQ*WLLTIGSKIGKTG*IALVYETS*NF*TSFKLL*DEVLH*AVPGTGMW*ECVDFY*PV*ST*EKT*NGLD*TSYNG*PGV : 324
Pain_SN   : RDPTT*AWTGPQNGQ*WLLTIGSKIGKTG*IALVYETS*NF*TSFKLL*DEVLH*AVPGTGMW*ECVDFY*PV*ST*EKT*NGLD*TSYNG*PGV : 324
Pain_P54N : RDPTT*AWTGPQNGQ*WLLTIGSKIGKTG*IALVYETS*NF*TSFKLL*DEVLH*AVPGTGMW*ECVDFY*PV*ST*EKT*NGLD*TSYNG*PGV : 324
Pain_P18N2 : RDPTT*AWTGPQNGQ*WLLTIGSKIGKTG*IALVYETS*NF*TSFKLL*DEVLH*AVPGTGMW*ECVDFY*PV*ST*EKT*NGLD*TSYNG*PGV : 324
Pain_TN1  : RDPTT*AWTGPQNGQ*WLLTIGSKIGKTG*IALVYETS*NF*TSFKLL*DEVLH*AVPGTGMW*ECVDFY*PV*ST*EKT*NGLD*TSYNG*PGV : 324
Pain_DN1  : RDPTT*AWTGPQNGQ*WLLTIGSKIGKTG*IALVYETS*NF*TSFKLL*DEVLH*AVPGTGMW*ECVDFY*PV*ST*EKT*NGLD*TSYNG*PGV : 324
Pain_P40N1 : RDPTT*AWTGPQNGQ*WLLTIGSKIGKTG*IALVYETS*NF*TSFKLL*DEVLH*AVPGTGMW*ECVDFY*PV*ST*EKT*NGLD*TSYNG*PGV : 324
Pain_P40N2 : RDPTT*AWTGPQNGQ*WLLTIGSKIGKTG*IALVYETS*NF*TSFKLL*DEVLH*AVPGTGMW*ECVDFY*PV*ST*EKT*NGLD*TSYNG*PGV : 324

```

```

*          340         *          360         *          380         *          400
Pain_DA   : KHVLK*ASLDDNKQDHYAIGTYDLTKNKW*TPD*PELDCGIGLKLDY*GKY*YASKTFYD*PKK*QRRV*LV*GWIGETDS*ESADLQKG : 405
Pain_P18N1 : KHVLK*ASLDDNKQDHYAIGTYDLTKNKW*TPD*PELDCGIGLKLDY*GKY*YASKTFYD*PKK*QRRV*LV*GWIGETDS*ESADLQKG : 405
Pain_SA   : KHVLK*ASLDDNKQDHYAIGTYDLTKNKW*TPD*PELDCGIGLKLDY*GKY*YASKTFYD*PKK*QRRV*LV*GWIGETDS*ESADLQKG : 405
Pain_DN2  : KHVLK*ASLDDNKQDHYAIGTYDLTKNKW*TPD*PELDCGIGLKLDY*GKY*YASKTFYD*PKK*QRRV*LV*GWIGETDS*ESADLQKG : 405
Pain_TN2  : KHVLK*ASLDDNKQDHYAIGTYDLTKNKW*TPD*PELDCGIGLKLDY*GKY*YASKTFYD*PKK*QRRV*LV*GWIGETDS*ESADLQKG : 405
Pain_SN   : KHVLK*ASLDDNKQDHYAIGTYDLTKNKW*TPD*PELDCGIGLKLDY*GKY*YASKTFYD*PKK*QRRV*LV*GWIGETDS*ESADLQKG : 405
Pain_P54N : KHVLK*ASLDDNKQDHYAIGTYDLTKNKW*TPD*PELDCGIGLKLDY*GKY*YASKTFYD*PKK*QRRV*LV*GWIGETDS*ESADLQKG : 405
Pain_P18N2 : KHVLK*ASLDDNKQDHYAIGTYDLTKNKW*TPD*PELDCGIGLKLDY*GKY*YASKTFYD*PKK*QRRV*LV*GWIGETDS*ESADLQKG : 405
Pain_TN1  : KHVLK*ASLDDNKQDHYAIGTYDLTKNKW*TPD*PELDCGIGLKLDY*GKY*YASKTFYD*PKK*QRRV*LV*GWIGETDS*ESADLQKG : 405
Pain_DN1  : KHVLK*ASLDDNKQDHYAIGTYDLTKNKW*TPD*PELDCGIGLKLDY*GKY*YASKTFYD*PKK*QRRV*LV*GWIGETDS*ESADLQKG : 405
Pain_P40N1 : KHVLK*ASLDDNKQDHYAIGTYDLTKNKW*TPD*PELDCGIGLKLDY*GKY*YASKTFYD*PKK*QRRV*LV*GWIGETDS*ESADLQKG : 405
Pain_P40N2 : KHVLK*ASLDDNKQDHYAIGTYDLTKNKW*TPD*PELDCGIGLKLDY*GKY*YASKTFYD*PKK*QRRV*LV*GWIGETDS*ESADLQKG : 405

```

```

*          420         *          440         *          460         *          480
Pain_DA   : WASVQ*SI*PRTVLYDKK*TG*THLLQWPVEEIESLR*AGDPI*VKQV*NLQ*PQGSIELLHV*DSAAELDIEASFEV*DKVALQGI*EADH : 486
Pain_P18N1 : WASVQ*SI*PRTVLYDKK*TG*THLLQWPVEEIESLR*AGDPI*VKQV*NLQ*PQGSIELLHV*DSAAELDIEASFEV*DKVALQGI*EADH : 486
Pain_SA   : WASVQ*SI*PRTVLYDKK*TG*THLLQWPVEEIESLR*AGDPI*VKQV*NLQ*PQGSIELLHV*DSAAELDIEASFEV*DKVALQGI*EADH : 486
Pain_DN2  : WASVQ*SI*PRTVLYDKK*TG*THLLQWPVEEIESLR*AGDPI*VKQV*NLQ*PQGSIELLHV*DSAAELDIEASFEV*DKVALQGI*EADH : 486
Pain_TN2  : WASVQ*SI*PRTVLYDKK*TG*THLLQWPVEEIESLR*AGDPI*VKQV*NLQ*PQGSIELLHV*DSAAELDIEASFEV*DKVALQGI*EADH : 486
Pain_SN   : WASVQ*SI*PRTVLYDKK*TG*THLLQWPVEEIESLR*AGDPI*VKQV*NLQ*PQGSIELLHV*DSAAELDIEASFEV*DKVALQGI*EADH : 486
Pain_P54N : WASVQ*SI*PRTVLYDKK*TG*THLLQWPVEEIESLR*AGDPI*VKQV*NLQ*PQGSIELLHV*DSAAELDIEASFEV*DKVALQGI*EADH : 486
Pain_P18N2 : WASVQ*SI*PRTVLYDKK*TG*THLLQWPVEEIESLR*AGDPI*VKQV*NLQ*PQGSIELLHV*DSAAELDIEASFEV*DKVALQGI*EADH : 486
Pain_TN1  : WASVQ*SI*PRTVLYDKK*TG*THLLQWPVEEIESLR*AGDPI*VKQV*NLQ*PQGSIELLHV*DSAAELDIEASFEV*DKVALQGI*EADH : 486
Pain_DN1  : WASVQ*SI*PRTVLYDKK*TG*THLLQWPVEEIESLR*AGDPI*VKQV*NLQ*PQGSIELLHV*DSAAELDIEASFEV*DKVALQGI*EADH : 486
Pain_P40N1 : WASVQ*SI*PRTVLYDKK*TG*THLLQWPVEEIESLR*AGDPI*VKQV*NLQ*PQGSIELLHV*DSAAELDIEASFEV*DKVALQGI*EADH : 486
Pain_P40N2 : WASVQ*SI*PRTVLYDKK*TG*THLLQWPVEEIESLR*AGDPI*VKQV*NLQ*PQGSIELLHV*DSAAELDIEASFEV*DKVALQGI*EADH : 486

```

```

*           500           *           520           *           540           *           560
Pain_DA    : VGFSCSTSGGAASRGILGPFVVIADQKLSLTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYGSSVPVLDGEK : 567
Pain_P18N1 : VGFSCSTSGGAASRGILGPFVVIADQKLSLTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYGSSVPVLDGEK : 567
Pain_SA    : VGFSCSTSGGAASRGILGPFVVIADQKLSLTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYGSSVPVLDGEK : 567
Pain_DN2   : VGFSCSTSGGAASRGILGPFVVIADQKLSLTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYGSSVPVLDGEK : 567
Pain_TN2   : VGFSCSTSGGAASRGILGPFVVIADQKLSLTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYGSSVPVLDGEK : 567
Pain_SN    : VGFSCSTSGGAASRGILGPFVVIADQKLSLTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYGSSVPVLDGEK : 567
Pain_P54N  : VGFSCSTSGGAASRGILGPFVVIADQKLSLTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYGSSVPVLDGEK : 567
Pain_P18N2 : VGFSCSTSGGAASRGILGPFVVIADQKLSLTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYGSSVPVLDGEK : 567
Pain_TN1   : VGFSCSTSGGAASRGILGPFVVIADQKLSLTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYGSSVPVLDGEK : 567
Pain_DN1   : VGFSCSTSGGAASRGILGPFVVIADQKLSLTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYGSSVPVLDGEK : 567
Pain_P40N1 : VGFSCSTSGGAASRGILGPFVVIADQKLSLTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYGSSVPVLDGEK : 567
Pain_P40N2 : VGFSCSTSGGAASRGILGPFVVIADQKLSLTPVYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYGSSVPVLDGEK : 567

*           580           *           600           *           620           *
Pain_DA    : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL : 639
Pain_P18N1 : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL : 639
Pain_SA    : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL : 639
Pain_DN2   : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL : 639
Pain_TN2   : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL : 639
Pain_SN    : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL : 639
Pain_P54N  : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL : 639
Pain_P18N2 : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL : 639
Pain_TN1   : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL : 639
Pain_DN1   : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL : 639
Pain_P40N1 : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL : 639
Pain_P40N2 : HSMRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL : 639

```

Figure 3.1.3: Amino acid alignment of all cloned *Pain-1* invertase alleles. Amino acid exchanges are highlighted in colour.

Protein sequence comparison of the deduced *Pain-1* alleles from all analyzed genotypes showed that amino acids differed at 23 positions, of which five were genotype specific and occurred only once.

3.1.1.1.4 Phenetic trees of all *Pain-1* invertase alleles of the analyzed potato genotypes

In addition to the multiple amino acid alignment (section 3.1.1.1.3), the phenetic tree analysis was applied to group the invertase alleles according to similarity at amino acid as well as at nucleotide level. Using the neighbour-joining method, the allelic classification visualized that *Pain-1* alleles from all analyzed potato genotypes grouped in two clades and four to five subclades (Figure 3.1.4, Figure 3.1.5).

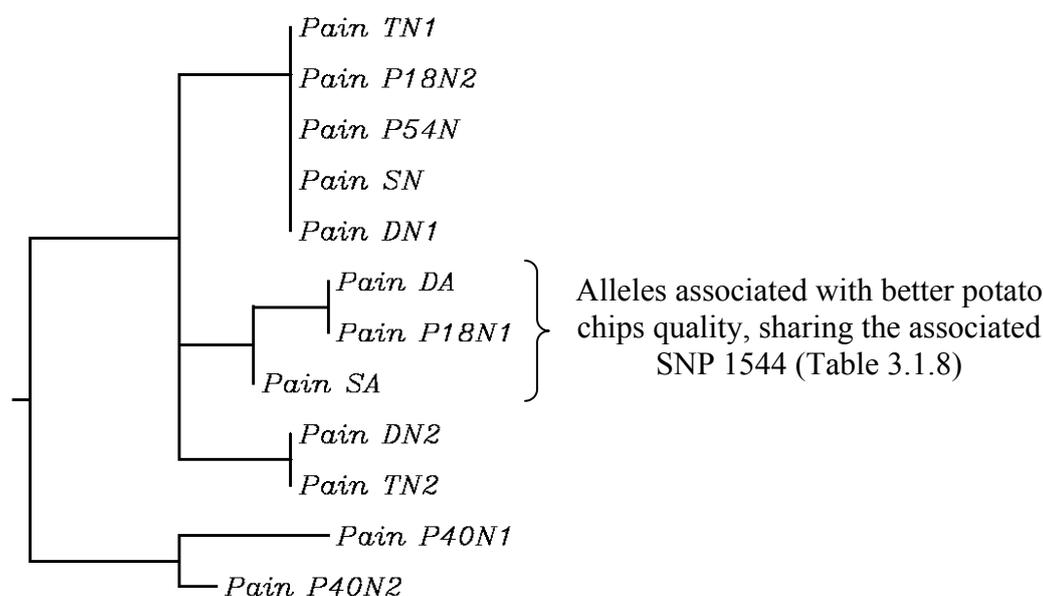


Figure 3.1.4: Amino acid based phenetic tree (Neighbour-joining method) of all cloned *Pain-1* invertase alleles.

The first clade includes alleles from the genotypes ‘Satina’, ‘Diana’, ‘Theresa’, P18, and P54. The first subclade of the first clade consists of five alleles from five different genotypes. The alleles *Pain_SN*, *Pain_DN1*, *Pain_TN1*, *Pain_P18N2*, and *Pain_P54N* have the same amino acid sequence, although polymorphisms in the corresponding nucleotide sequences occurred (Figure 3.1.5).

The second subclade consists of the alleles *Pain_SA* and *Pain_DA*, which are associated with better potato chips quality. The two alleles are not identical at the amino acid level but possess cDNA SNP 1544 that is associated with better potato chips quality (section 3.1.1.2). The allele *Pain_P18N1* of the diploid genotype P18 has an identical amino acid sequence to *Pain_DA*.

The third subclade includes the two alleles *Pain_DN2* and *Pain_TN2*, which have the same amino acid sequence.

The second main clade consists of the two outlying P40 alleles *Pain_P40N1* and *Pain_P40N2*. The two alleles show the highest diversity compared to the other cloned alleles.

Cloning and sequencing of *Pain-1* alleles showed that tetraploid and diploid genotypes contain alleles identical in their amino acid sequence but different at nucleotide level. The following Table 3.1.6 summarizes the nucleotide comparison of amino acid identical alleles of different genotypes. The allelic nucleotide sequence was defined based on the consensus sequence of multiple alignment of full-length clones obtained from each genotype (Table 3.1.3). Although SNPs are present at positions in the corresponding cDNA, the nucleotide polymorphisms resulted in one and the same amino acid sequence.

Table 3.1.6: Genotype specific nucleotide differences of alleles identical at amino acid level (Appendix A 3.1.21).

A) Comparison of the alleles *Pain_SN*, *Pain_DN1*, *Pain_TN1*, *Pain_P18N2*, and *Pain_P54N*.

Position of cDNA SNP	<i>Pain_SN</i>	<i>Pain_DN1</i>	<i>Pain_TN1</i>	<i>Pain_P18N2</i>	<i>Pain_P54N</i>
75	T	T	T	C	T
486	A	G	G	G	G
723	G	G	G	A	G
1050	C	C	C	T	C
1236	T	C	T	T	T

B) Alleles of the alleles *Pain_DA* and *Pain_P18N1*.

Position of cDNA SNP	<i>Pain_DA</i>	<i>Pain_P18N1</i>
816	G	A
1698	A	G

C) Comparison of the alleles *Pain_DN2* and *Pain_TN2*.

Position of cDNA SNP	<i>Pain_DN2</i>	<i>Pain_TN2</i>
1830	C	T

The nucleotide polymorphisms between all *Pain-1* (Appendix A 3.1.21) were visualized using the phenetic tree analysis (Figure 3.1.5).

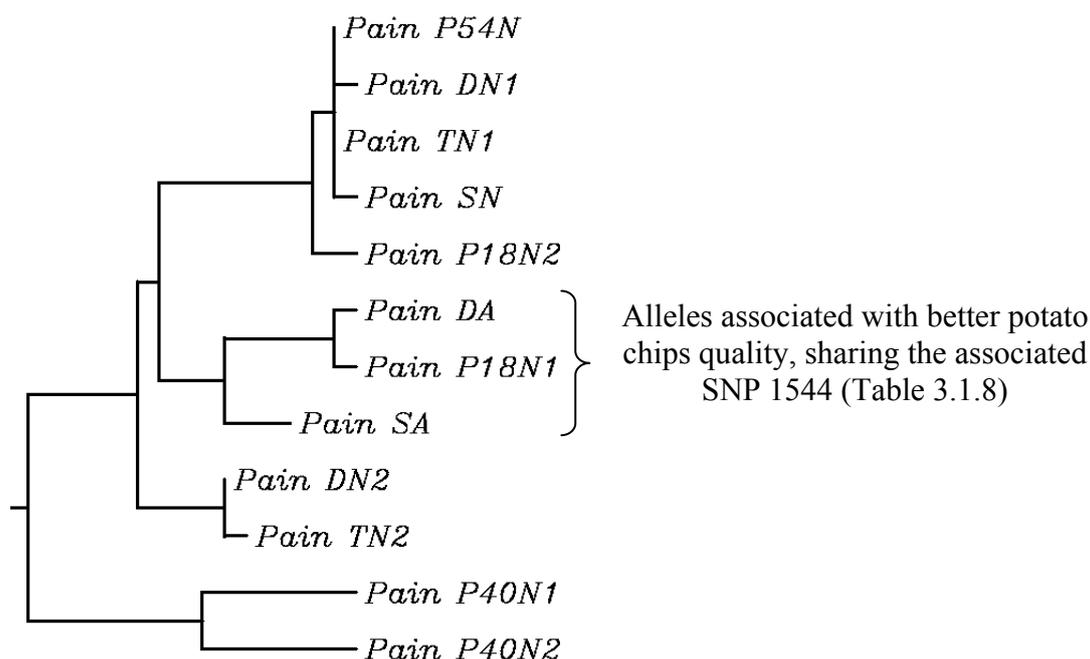


Figure 3.1.5: Nucleotide sequence based phenetic tree (Neighbour-joining method) of all cloned *Pain-1* invertase alleles.

Allele specific SNPs were detected in at least two sequences and used for comparing the allelic nucleotide polymorphisms. The two phenetic trees are very similar, just displaying more subclades due to a higher number of nucleotide polymorphisms compared to the amino acid exchanges (Figure 3.1.4).

3.1.1.2 Identification of associated *Pain-1* alleles

The *Pain-1* locus maps to potato chromosome III (CHEN ET AL., 2001) in a region associated with tuber quality traits where a QTL for potato tuber sugar content, *Sug3a*, was identified (MENÉNDEZ ET AL., 2002). In the latter study, the *Pain-1* gene was not mapped directly in the mapping population used, but was considered as a candidate gene for the QTL *Sug3a*.

Single-strand conformation polymorphism (SSCP) analysis revealed an association of *Pain-1* SSCP fragments with starch and sugar content of potato tubers. SSCP fragments found to be associated with better potato chips quality were named *Pain1-5c*, *Pain1-8c* and *Pain1-9a* (LI ET AL., 2008). The occurrence of these fragments in the genotypes was highly correlated indicating that the three fragments are in nearly absolute LD, and suggesting that these allelic fragments correspond to the same associated *Pain-1* invertase allele. The associated SSCP fragments are present in the cultivars ‘Satina’, ‘Diana’, and absent in ‘Theresa’ (Table 3.1.7).

Table 3.1.7: Associated SSCP fragments *Pain1-5c*, *Pain1-8c* and *Pain1-9a* present in ‘Satina’, ‘Diana’, and ‘Theresa’.

Genotype	<i>Pain1-5c</i>	<i>Pain1-8c</i>	<i>Pain1-9a</i>
‘Satina’	0	1	1
‘Diana’	1	1	1
‘Theresa’	0	0	0

0=SSCP fragment is absent, 1=SSCP fragment is present.

To assign a cloned invertase allele (section 3.1.1.1) to the corresponding associated SSCP fragments, two groups, each consisting of 15 potato genotypes that were scored either positive (present) or negative (absent) for the associated SSCP fragments, were used to identify the corresponding SNPs.

Based on multiple nucleotide alignment (Appendix A 3.1.22) of the three cultivars ‘Satina’, ‘Diana’, and ‘Theresa’, the cDNA SNP 1544 was selected present in ‘Satina’ and ‘Diana’, and absent in ‘Theresa’ referring to the SSCP fragment distribution (Table 3.1.7). The distribution of SNP 1544, which causes an amino acid exchange of threonine (T) in the non associated to lysine (K) in the associated alleles is shown in Table 3.1.8.

Table 3.1.8: Comparison and distribution of associated SSCP fragments and SNP 1544.**A) Genotypes scored negative for associated SSCP fragments**

Genotype	SSCP			SNP 1544
	<i>Pain1-5c</i>	<i>Pain1-8c</i>	<i>Pain1-9a</i>	
‘Leyla’ (St01)	0	0	0	C
‘Marabel’ (St02)	0	0	0	C
‘Solara’ (St03)	0	0	0	C
‘Vitará’ (St04)	0	0	0	C
‘Ponto’ (St06)	0	0	0	C
‘Marlen’ (St08)	0	0	0	C
‘Eldena’ (St09)	0	0	0	C
‘Theresa’ (St10)	0	0	0	C
‘Goldika’ (St11)	0	0	0	C
‘Saturna’ (St12)	0	0	0	C
‘Karlana’ (St14)	0	2	0	C
‘Kolibri’ (St15)	0	2	0	C
‘Terra’ (St18)	0	0	0	C
‘Solist’ (St19)	0	2	0	C
‘Melina’ (St20)	0	2	0	C

B) Genotypes scored positive for associated SSCP fragments

Genotype	SSCP			SNP 1544
	<i>Pain1-5c</i>	<i>Pain1-8c</i>	<i>Pain1-9a</i>	
‘Milva’ (St05)	1	0	1	C/A
‘Tomensa’ (St07)	1	0	1	C/A
‘Fasan’ (St16)	1	2	1	C
‘Apart’ (St24)	0	1	1	C/A
‘Diana’ (St28)	1	1	1	C/A
‘Orlando’ (St31)	1	0	1	C/A
‘Satina’ (St33)	0	1	1	C/A
B05	1	1	1	C/A
B07	1	0	1	C/A
B16	1	1	1	C
B17	1	1	1	C/A
B30	1	0	1	C
B32	1	1	1	C/A
B38	1	1	1	C/A

St=potato cultivars defined as standards, B=BNA breeding clones, 0=SSCP fragment is absent, 1=SSCP fragment is present, 2=SSCP fragment could not be analyzed properly, SNP 1544=analyzed SNP for association with better potato chips quality, C=cytosine, A=adenine.

All genotypes, which scored negative for the associated SSCP fragments (Table 3.1.8 A), contain the nucleotide C at cDNA SNP position 1544. Genotypes, which scored positive for associated SSCP fragments (Table 3.1.8 B), show a C/A-polymorphism nearly equally distributed as the corresponding SSCP fragments. Three out of 14 analyzed genotypes did not display the C/A-polymorphism.

For further analysis and molecular description of the cloned *Pain-1* alleles, the term ‘associated with better potato chips quality’ is used for alleles of phenotypically characterized genotypes displaying the C/A-polymorphism at cDNA position 1544.

This polymorphism results in an amino acid substitution at position 515 of the corresponding protein sequence, exchanging threonine (T) in the non associated invertase alleles to lysine (K) in the associated *Pain-1* alleles. To study the effects of the amino acid differences of cloned *Pain-1* alleles, analysis was continued with 3D-modelling (section 3.1.1.3) and functional characterization (section 3.1.2).

3.1.1.3 Three-dimensional modelling of *Pain-1* alleles

3D-modelling was performed by Pawel Durek, MPIMP/Golm. The modelling of the allelic invertase molecular structure was based on the invertase 3D and crystal structure of cyanobacteria (ALBERTO ET AL., 2004). The models are comparative, superimposing two allelic sequences. Differences of the alleles are highlighted. The modelling was applied for associated and non associated *Pain-1* alleles of the tetraploid potato cultivars ‘Satina’ and ‘Diana’. The models include the putative sucrose binding site with the substrate sucrose. In

addition to the structural visualization of amino acid exchanges, also the electrostatic potential (EP) of the molecules was mapped at pH 4.7 mimicking the vacuolar conditions.

3.1.1.3.1 Structural modelling of alleles of the cultivars ‘Satina’ and ‘Diana’

In the first analysis of allelic molecular structures, the two ‘Satina’ alleles *Pain_SA* and *Pain_SN*, and the three ‘Diana’ alleles *Pain_DA*, *Pain_DN1*, and *Pain_DN2* were used. The models compare the allelic sequences with each other, meaning that one sequence superimposes the other and vice versa. Based on multiple alignment (Figure 3.1.1) of the allelic protein sequences, regions, which are affected directly or indirectly by amino acid exchanges, were identified (Figure 3.1.6; Figure 3.1.7).

Amino acid region	Affected surface area
225-236	1
512-518	2
479-500	3
529-531	4

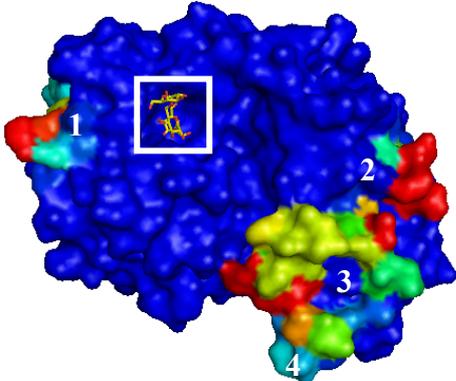


Figure 3.1.6: Correlation of amino acid regions or exchanges and affected surface areas of *Pain-1* alleles. Amino acids are numbered based on the multiple sequence alignment (Figure 3.1.1). Areas are numbered from 1-4. Red: strong structural differences; blue: no structural differences; all colours in between represent a transition of structural differences from red (strong) to blue (weak). The putative sucrose binding site with the substrate sucrose is framed.

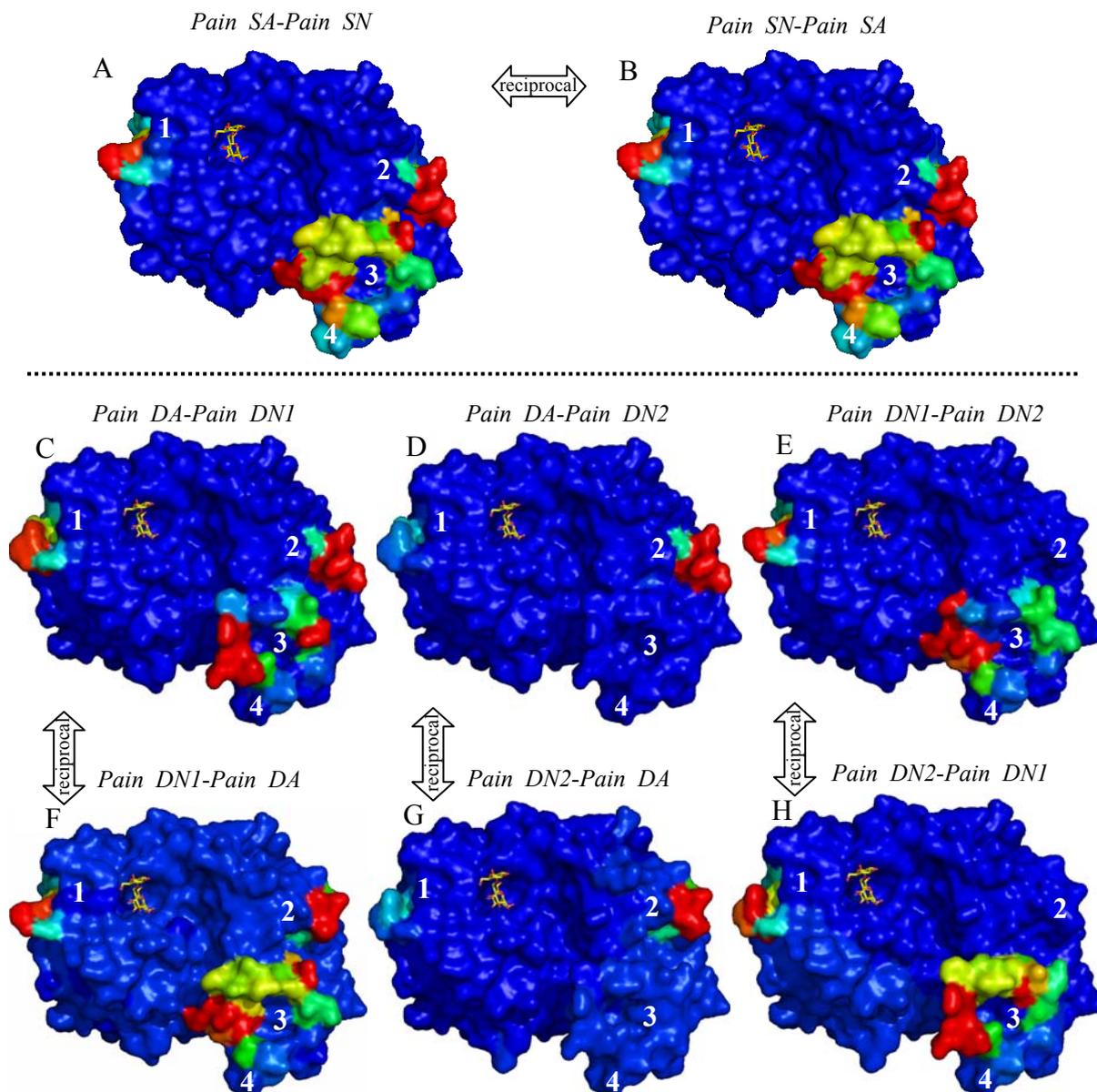


Figure 3.1.7: Structural comparison of ‘Satina’ alleles *Pain_SA*, *Pain_SN*, and ‘Diana’ alleles *Pain_DA*, *Pain_DN1*, *Pain_DN2*. **A:** *Pain_SA* superimposes *Pain_SN*; **B:** *Pain_SN* superimposes *Pain_SA*; **C:** *Pain_DA* superimposes *Pain_DN1*; **D:** *Pain_DA* superimposes *Pain_DN2*; **E:** *Pain_DN1* superimposes *Pain_DN2*; **F:** *Pain_DN1* superimposes *Pain_DA*; **G:** *Pain_DN2* superimposes *Pain_DA*; **H:** *Pain_DN2* superimposes *Pain_DN1*. Red: strong structural differences; blue: no structural differences; all colours in between represent a transition of structural differences from red (strong) to blue (weak).

The models of the invertase molecules of the alleles *Pain_SA* and *Pain_SN* and *Pain_DA*, *Pain_DN1*, and *Pain_DN2* showed structural differences on the enzyme’s surface. All analyzed molecules possess similar affected surface areas (area definition: Figure 3.1.6). Surface area 1 differs in all represented models, but is less distinctive between the alleles *Pain_DA* and *Pain_DN2* (Figure 3.1.7 D, G). Region 1, defined by the amino acids 225-236, manifests sterical differences although amino acids in the corresponding region do not differ between the alleles. The same observations were made for the surface areas 3 and 4, defined by amino acids 479-500 and 529-531 respectively, where the alleles display identical amino

acid sequences. These areas are affected indirectly by other amino acid substitutions leading to a variable folding of the protein. Accountable amino acids are subject of ongoing investigations.

Region 2 is defined by amino acids 512-518 showing a lysine (K) in the associated alleles *Pain_SA* and *Pain_DA*, and a threonine (T) in the alleles *Pain_SN*, *Pain_DNI*, and *Pain_DN2* at position 515. The corresponding nucleotide SNP 1544 was found to be associated with better potato chips quality (section 3.1.1.2). The structural modification 2 is absent in the models E and H (Figure 3.1.7) because of the absence of SNP 1544 in the superimposed alleles *Pain_DNI* and *Pain_DN2*.

The structural effects caused by allelic sequences resulted in invertase folding variations of the *Pain-I* molecules. The function of all identified variable surface areas is unknown.

3.1.1.3.2 Modelling the electrostatic potential (EP) of alleles of the cultivars ‘Satina’ and ‘Diana’

The mapping of the EP of the *Pain-I* alleles *Pain_SA*, *Pain_SN*, *Pain_DA*, *Pain_DNI*, and *Pain_DN2* revealed charge differences of the molecules at pH 4.7 (Figure 3.1.8).

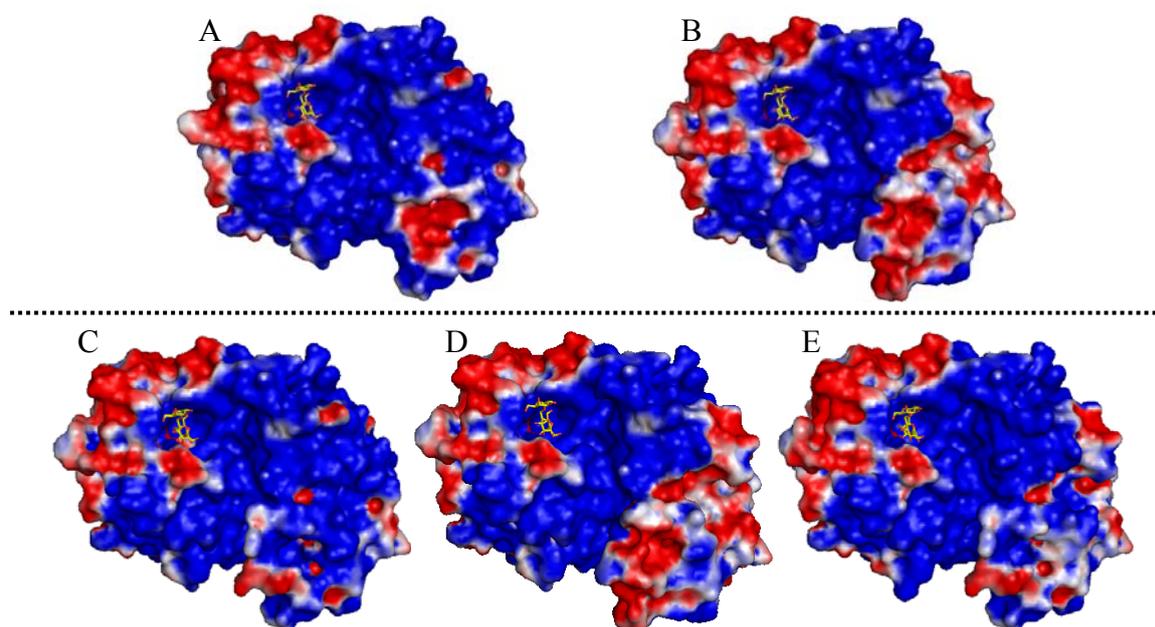


Figure 3.1.8: EP of the alleles *Pain_SA*, *Pain_SN*, *Pain_DA*, *Pain_DNI*, and *Pain_DN2*. A: *Pain_SA*. B: *Pain_SN*. C: *Pain_DA*; D: *Pain_DNI*; E: *Pain_DN2*. Red: negatively charged; blue: positively charged; white: neutrally charged.

The EP modelling of the two alleles from the cultivar ‘Satina’ showed that *Pain_SA* is more positively charged than *Pain_SN* (Figure 3.1.8 A, B). The three ‘Diana’ alleles also exhibit charge differences to each other. The allele *Pain_DNI* (Figure 3.1.8 D) possesses an extended

negative charge in comparison to the other two alleles (C, E). The EP of the allele *Pain_DA* (C) showed more positive charges in regions where allele *Pain_DN2* was more neutral.

Focusing on the EP of the putative sucrose binding site

The putative sucrose binding site (Figure 3.1.6) is positively charged matching the partial negative charge of the substrate sucrose due to the hydroxyl groups.

Zooming into the putative sucrose binding domain revealed charge differences between the molecules *Pain_SA* (A), *Pain_SN* (B), *Pain_DA* (C), *Pain_DN1* (D), and *Pain_DN2* (E); (Figure 3.1.9).

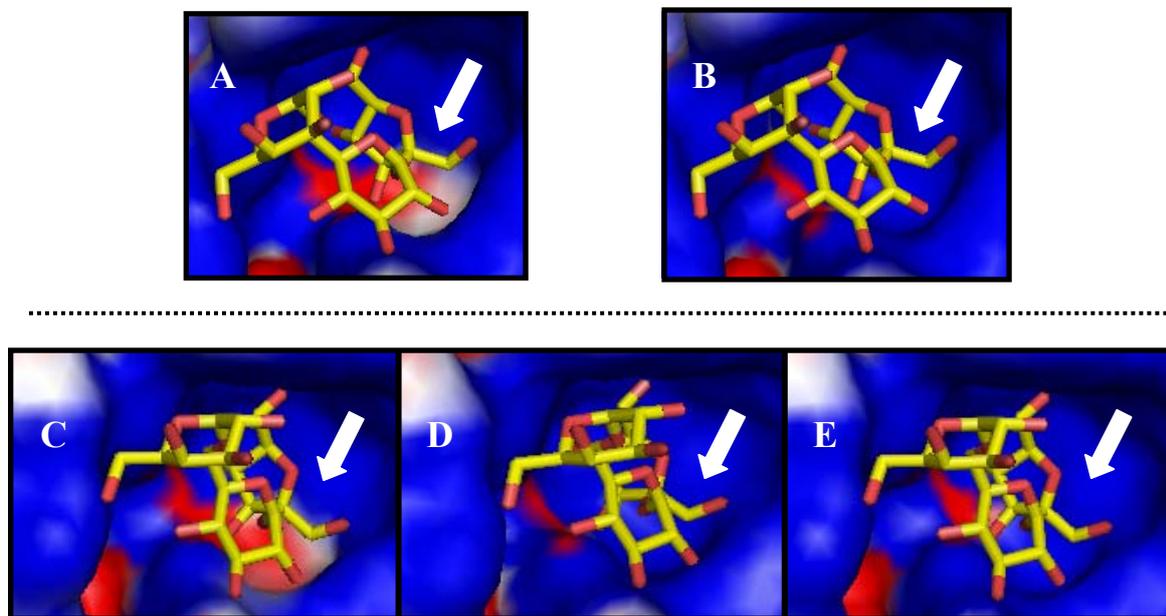


Figure 3.1.9: Focusing of the EP of the putative sucrose binding site of the alleles of the cultivars ‘Satina’ and ‘Diana’. A: *Pain_SA*; B: *Pain_SN*; C: *Pain_DA*, D: *Pain_DN1*, E: *Pain_DN2*. Red: negatively charged; blue: positively charged; white: neutrally charged.

Close-up views of the putative sucrose binding domain revealed strong charge changes between the molecules *Pain_SA* (A) and *Pain_SN* (B) (Figure 3.1.9). The site of the associated allele *Pain_SA* is characterized by a neutral to negative charge composition compared to the positive charge of the allele *Pain_SN*. The EP of the putative sucrose binding site of the three ‘Diana’ alleles also showed apparent changes of charge. The EP of the associated allele *Pain_DA* (C) switched from a positive to a neutral/negative charge. The charge of the alleles *Pain_DN1* (D), and *Pain_DN2* (E) differed not visibly.

3.1.1.3.3 Overview of 3D-modelling analysis of *Pain-1* alleles

3D-modelling is a tool to gain first insights in the possible consequences of different invertase alleles. The models nicely show that amino acid exchanges manifest on the surface of the enzyme. However, no effect on the structure of the putative sucrose binding domain was

detected in any of the modelled molecules. The characterization of the EP showed differences in charge among the analyzed proteins. A dramatic charge difference of the putative sucrose binding site was observed in the associated alleles *Pain_SA* and *Pain_DA* (Figure 3.1.9 A, C). The EP switched from positive to neutral. The EP changes could not be correlated to amino acid exchanges nearby the sucrose binding site. The causative amino acids were not yet analyzed, but are subject of ongoing investigations in the research project.

3.1.1.4 Genomic organization of the *Pain-1* locus

❖ BAC library screens

High density BAC library screens with two different PCR generated probes were performed to determine the genomic sequence of the *Pain-1* locus. Using the primers Pain1-5f/Pain1-5r (LI ET AL., 2005; chapter 2, Table 2.1.10A), Probe 1 was generated, which consists of exon and intron based sequences of *Pain-1*. Probe 2 only consists of exon based sequences and was amplified with the primers Pain_SondF1/Pain_SondR (chapter 2, Table 2.1.10A).

The screening of two different BAC libraries (BA and BC, BALLVORA ET AL., 2002, 2007) constructed with genomic DNA of the same diploid genotype resulted in 21 positive BAC clones (Table 3.1.9).

Table 3.1.9: Positive BAC clones.

Library	BAC clones	
BA BAC library	BA1: Plate 32N19	
	BA2: Plate 128A5	
	BA3: Plate 213B14	
BC BAC library	BC1: Plate 14O7	BC10: Plate 47C9
	BC2: Plate 21K3	BC11: Plate 57A16
	BC3: Plate 27G10	BC12: Plate 57P6
	BC4: Plate 32J7	BC13: Plate 68K22
	BC5: Plate 34G11	BC14: Plate 149O15
	BC6: Plate 34O24	BC15: Plate 216L1
	BC7: Plate 35N9	BC16: Plate 223N21
	BC8: Plate 35E21	BC17: Plate 239N10
	BC9: Plate 45P17	BC18: Plate 244C12

BACs are numbered and their position in the *E. coli* microtiter plates is listed.

Following the hybridisation, clones from the BC library for sequencing were selected based on the results of PCR, colony-lift, and Southern blot. Out of 18 hybridization positive *Pain-1* BC BAC clones, eight clones were found positive for full-length PCR amplification using

Pain-1 specific primers. All analyzed clones were colony-lift positive and eight BACs were Southern blot positive (Table 3.1.10; Figure 3.1.10).

Table 3.1.10: Summary of the results for BAC insert sequencing.

BC clones	Filter hybridisation	PCR	Colony-lift	Southern blot
BC1: Plate 14O7	yes	no	yes	no
BC2: Plate 21K3	yes	no	yes	yes
BC3: Plate 27G10	yes	no	yes	yes
BC4: Plate 32J7	yes	no	yes	no
BC5: Plate 34G11	yes	yes	yes	yes
BC6: Plate 34O24	yes	no	yes	no
BC7: Plate 35N9	yes	no	yes	no
BC8: Plate 35E21	yes	no	yes	yes
BC9: Plate 45P17	yes	yes	not analyzed	not analyzed
BC10: Plate 47C9	yes	no	yes	no
BC11: Plate 57A16	yes	no	yes	not analyzed
BC12: Plate 57P6	yes	no	yes	no
BC13: Plate 68K22	yes	no	yes	no
BC14: Plate 149O15	yes	no	yes	no
BC15: Plate 216L1	yes	no	yes	no
BC16: Plate 223N21	yes	yes	yes	yes
BC17: Plate 239N10	yes	no	yes	yes
BC18: Plate 244C12	yes	yes	yes	yes

For PCR reaction Probe 1 (*Pain1-5f/Pain1-5r*, chapter 2, Table 2.1.10A), Probe 2 (*Pain_SondF1/Pain_SondR*, chapter 2, Table 2.1.10A), or *Pain-1* full-length primers (*ZrPain-F/PainUni-R*, chapter 2, Table 2.1.2) were used. Colony-lift and Southern blot hybridisation were performed using Probe 1.

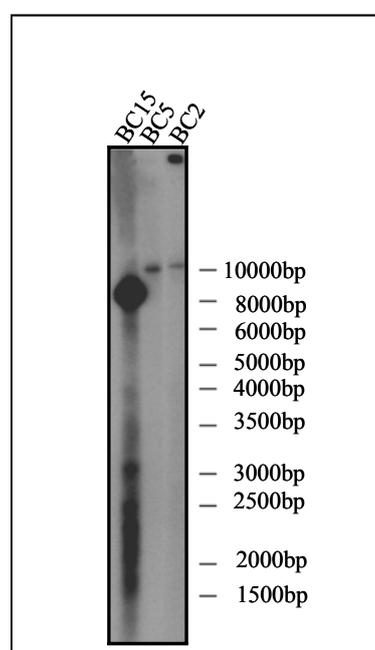


Figure 3.1.10: Southern blot analysis of *SmaI* digested BC BAC clones. The pattern of this first line was only represented by the BAC BC15. The restriction pattern of BC5 was also observed for the clones BC12 and BC14. The BC2 pattern was also detected for the BACs BC8 and BC13.

The *Pain-1* locus of BC library clones BC14 and BC17 was custom sequenced by GATC Biotech, Konstanz using primer walking as sequencing method. Full-length BAC insert sequencing was performed for the clones BC2 (insert size: 75kb), BC14 (insert size: 75kb), and BC15 (insert size: 97kb), which showed differential restriction patterns in Southern blot

analysis (Figure 3.1.10) suggesting different *Pain-1* alleles or gene loci. Full-length BAC insert sequencing was carried out by MWG Biotech AG, Ebersberg using the 454 sequencing technique on the GS FLX system. The BAC clones BC14 and BC15 as well as BC17 harboured the same genomic invertase allele. BAC clone BC2 was found invertase negative, meaning that no invertase sequence was detected.

The genomic sequences of the *Pain-1* gene received by different sequencing technologies differed in one to six nucleotides in the second intron of the gene. The sequences resulted from primer walking showed more nucleotide differences than sequences obtained by 454 sequencing.

❖ BAC Annotation

Full-length sequencing of the BAC inserts revealed sequence and structural information of *Pain-1* and of genes flanking the *Pain-1* locus. Since the BAC clones BC14 and BC15 contained the same genomic *Pain-1* invertase sequence, only the annotation of BC14 is shown (Table 3.1.11).

Table 3.1.11: BC14 sequence annotation.

Strand	Apollo name	Position on the BAC insert (bp)	Description
+	gene 10	9,298-13,989	putative retrotransposon protein
+	gene 20	15,377-16,656	unknown
+	gene 30	31,121-33,084	putative transposon protein
+	gene 40	33,625-35,078	putative transposase
+	gene 50	74,626-78,901	putative retroelement polyprotein
-	gene 60	6,138-5,488	putative reverse transcriptase
-	gene 70	61,166-57,216	invertase <i>Pain-1</i>, beta-fructofuranosidase (glucoside hydrolase family 32)

BAC annotation was carried out using the software Apollo Genome Annotation and Curation Tool, version 1.9.8. The invertase gene was named 'gene 70' in the Apollo BAC sequence characterization. The invertase gene is written in bold.

The screened BAC libraries BA and BC harbour genomic DNA of the same diploid genotype P6/210, which is a hybrid derived from the cross of the parental genotypes P40 x P41 (LEISTER ET AL., 1996). The genotype P40 was also selected in this study for invertase allele characterization. The sequence alignment of the two P40 cDNA alleles with the protein sequence of the gene 70 (*Pain-1*) from BAC BC14, which is identical to the *Pain-1* genomic sequences of the BACs BC 15 and BC17, showed no sequence identity (Appendix A 3.1.23). Therefore, the detected BAC allele for the gene *Pain-1* originates from the other parental genotype P41.

❖ Genomic structure of the *Pain-1* gene

The exon and intron organization of the *Pain-1* gene was determined using multiple sequence alignment of *Pain-1* cDNA alleles and the genomic *Pain-1* sequence of BAC BC14 (Appendix A 3.1.24³).

The *Pain-1* gene consists of seven exons and six introns and has a length of 3951bp (Figure 3.1.11).

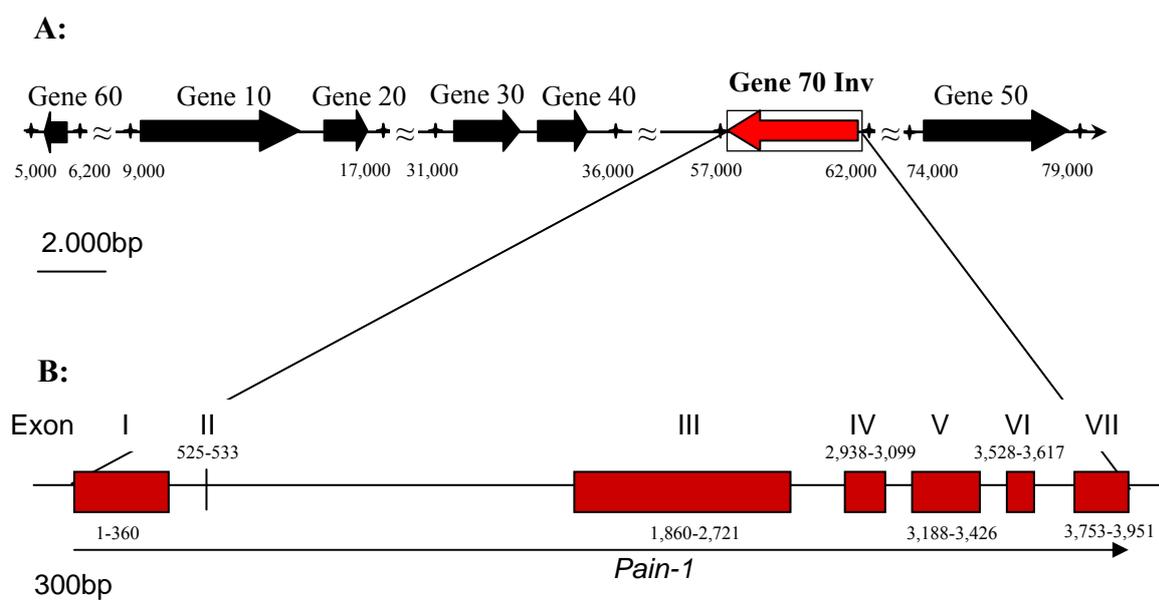


Figure 3.1.11: Position of the *Pain-1* gene within the sequenced BAC insert of BC14 (A) and genomic organization of the *Pain-1* gene (B). A: The direction of the annotated genes (Table 3.1.11) is indicated by arrows. \blacktriangleright : 5'-3' orientation (+ strand); \blacktriangleleft : 3'-5' orientation (- strand). The lengths are given in base pairs. B: Exons are drawn in red and numbered from I to VII, the length is given in base pairs. The arrow demonstrates the length of the gene without promoter and terminator sequences.

It is known from potato invertase loci encoding insoluble acid isoforms that two genes are linked in direct tandem repeat (MADDISON ET AL., 1999). The genes organized in this way are either separated by 2,3kb (MADDISON ET AL., 1999) or by 8kb (chapter 3.3, Figure 3.3.12). The insert of BAC BC14, which has a length of 75kb contained no second invertase gene. Within the BAC insert *Pain-1* gene surrounding sequences should allow to detect a probable tandem repeat organization if present (Figure 3.1.10 A). This possibility was excluded for the *Pain-1* locus.

The range of *Pain-1* exons and introns are summarized in Table 3.1.12.

³ The alignment program Multalin version 5.4.1 (<http://bioinfo.genetoul.fr>) used revealed problems in the comparison of the genomic *Pain-1* sequence with the *Pain-1* cDNA sequence. The mini-exon II was not aligned properly, one additional nucleotide occurred.

Table 3.1.12: Ranges of the exons and introns of the *Pain-1* gene.

Exon number	Range (bp)	Intron number	Range (bp)
I	1-360	I	361-524
II	525-533	II	534-1859
III	1,860-2,721	III	2,722-2,937
IV	2,938-3,099	IV	3,100-3,187
V	3,188-3,426	V	3,427-3,527
VI	3,528-3,617	VI	3,618-3,752
VII	3,753-3,951		

Plant invertases isolated to date have fairly similar structures and consist of six to eight exons (reviewed in TYMOWSKA-LALANNE & KREIS, 1998). The genes contain one extremely small exon (exon II), which only codes for the core tripeptide DPN of the conserved β -fructosidase motif NDPNG (TYMOWSKA-LALANNE & KREIS, 1998).

3.1.2 Functional characterization of the *Pain-1* gene

3.1.2.1 Differential expression analysis of *Pain-1* alleles during cold storage of potato tubers

Plant invertases are influenced by a variety of intra- and extracellular factors. It has been shown that invertases are regulated by temperature. ROREM & SCHWIMMER (1963) first demonstrated that temperature affects the level of invertase activity. Invertase activity is always detectable in potato tubers, but the highest level is measured when tubers have been stored at low temperature (PRESSEY, 1966). ZHOU ET AL. (1994) detected soluble acid invertase transcripts in potato tubers stored at 1°C, but not in those stored at 10°C. Strongly induced transcript accumulation of soluble acid invertase was also shown for tuber samples stored at 4°C after 7-10 days (ZRENNER ET AL., 1996; BAGNARESI ET AL., 2008).

Expression analysis was performed with two biological replicates of potato tubers stored for 1, 2, 3, and 4 weeks at 4°C in the dark as well as with potato tuber samples frozen in liquid nitrogen directly after harvest and kept at -80°C.

Two different strategies were used to define the allelic expression pattern. The first strategy was based on pyrosequencing analysis to separate the different *Pain-1* alleles within a genotype. The pyrosequencing analysis was performed using cDNA from tubers stored for 0-4 weeks at 4°C in the dark, and tuber genomic DNA to determine the allele dosage. Comparison of both samples revealed specific expression patterns not just for each genotype but also between different alleles of the same genotype. In the following Figures the relationship between the presence of alleles in the genome and their transcription levels are illustrated. Accordingly, both values are shown together in terms of the relative expression level.

Additionally, plasmids harbouring one allele of complementary SNPs of corresponding genotypes were mixed in different ratios to monitor the accuracy of pyrosequencing analysis. Plasmid based measurements, working as positive controls, showed that SNP depending variations of $\pm 5\%$ occurred. Values of cDNA and genomic dosages of the analysed alleles as determined by pyrosequencing were corrected for the observed SNP specific variations.

qRT-PCR was carried out as second strategy to measure the total amount of invertase transcript levels during the applied cold storage period of four weeks.

3.1.2.2.1 Expression pattern of *Pain-1* alleles in tubers of the tetraploid cultivar ‘Satina’

The cultivar ‘Satina’ is tetraploid. Potentially four different *Pain-1* alleles can occur. Cloning and sequencing of ‘Satina’ cDNA resulted in two different alleles *Pain_SA* and *Pain_SN*.

In a pyrosequencing assay the alleles were analyzed on the basis of the A/C polymorphism of the associated SNP at cDNA position 1544 (Table 3.1.4). This SNP allows the comparison of the expression of *Pain_SA*, which exhibits the allele specific nucleotide A against the allelic fraction containing nucleotide C. The nucleotide C fraction can contain up to three different alleles, due to the ploidy state of the cultivated potatoes but is only represented by *Pain_SN*.

The pyrosequencing analysis was performed using cDNA from cold stored tubers and tuber genomic DNA to measure the dosages of the alleles (Figure 3.1.12).

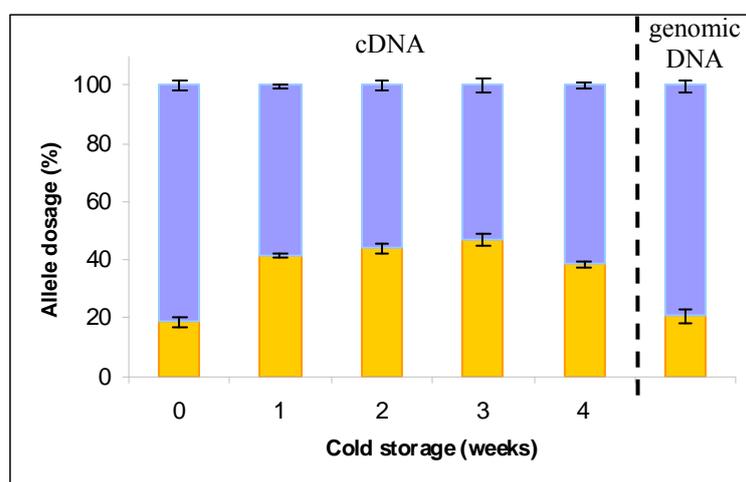


Figure 3.1.12: Pyrosequencing analysis of the alleles *Pain_SA* and *Pain_SN* of the cultivar ‘Satina’.

■ : *Pain_SA*; ■ : *Pain_SN*. For allele discrimination the primers Pyro_Pain_F/Pyro_Pain_RB (chapter 2, Table 2.1.4) were used. The primer Pyro_Pain_F was also used as sequencing primer. In addition to pyrosequencing analysis of cDNA samples, the genomic dosage of the alleles was determined using genomic DNA of tubers. Percentages of the expression level of the cold stored samples are related to the genomic dosage of the alleles. 100% is defined as the sum of alternative peaks measuring allele specific SNPs. Standard deviations are derived from two biological replicates done in technical triplicates.

From ‘Satina’ two *Pain-1* alleles were identified. Pyrosequencing analysis of genomic tuber DNA revealed that the associated allele *Pain_SA* represents 25% of the potential four *Pain-1* alleles in the genotype ‘Satina’ (Figure 3.1.11). If the alleles were transcribed according to the genomic allele dosage, *Pain_SA* is expected to contribute 25% of the total transcripts and *Pain_SN* 75%. Comparison of allele dosage and allele transcription revealed that the alleles *Pain_SA* and *Pain_SN* differed in their abundance during tuber cold storage. The expression level of *Pain_SA* increased during tuber cold storage relative to *Pain_SN*. Maximal expression level was at $\approx 43\%$ in the 3rd week of cold storage.

Transferring the information about allelic distribution from pyrosequencing analysis to the total amount of invertase transcript levels from qRT-PCR resulted in detailed expression pattern during tuber cold storage (Figure 3.1.13).

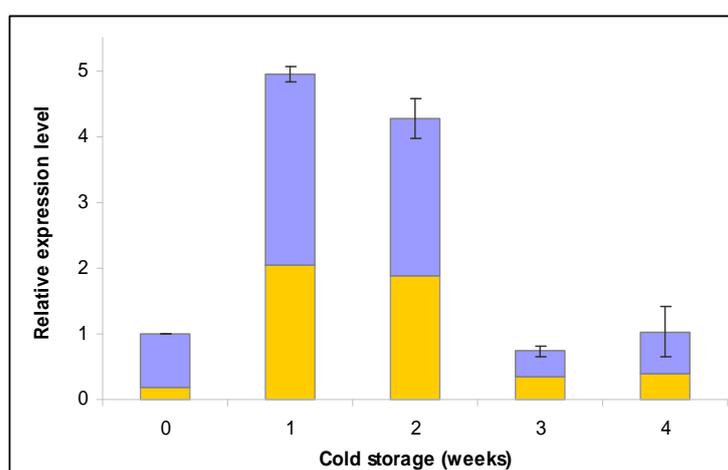


Figure 3.1.13: Overview of the expression pattern during tuber cold storage combining allelic distribution and total invertase transcript analysis of the cultivar ‘Satina’. ■: *Pain_SA*; ■: *Pain_SN*. The quantification of total invertase transcript was performed using the primers Pyro_Pain_F/Pyro_Pain_R (chapter 2, Table 2.1.9) in qRT-PCR analysis. Total transcript data were normalized with the reference gene *EF1a*. The relative expression level was calculated by defining transcript data of 0 weeks of cold storage as ‘1’. All other transcript data were related to this time point. Allelic distribution was calculated by relating the pyrosequencing data to the total transcript data. Standard deviations derived from two biological replicates done in technical duplicates. Standard deviations represent the variation within the replicates of the total transcripts, standard deviations for allele specific expression are shown in Figure 3.1.12.

qRT-PCR analysis showed an approximately four fold induction of total invertase transcripts after one week of tuber cold storage at 4°C (Figure 3.1.13). Transcript up-regulation declined in the 3rd and 4th week of cold storage to levels similar to samples not stored in the cold.

Expression analysis of the associated ‘Satina’ allele *Pain_SA*, which was found in one genomic dosage revealed an up-regulation up to 43% during tuber cold storage. Total invertase transcripts were induced up to four fold during the applied cold storage period. In

consequence tuber cold storage leads to a relative induction of the *Pain_SA* expression in contrast to the remaining allelic fraction in the tetraploid genotype ‘Satina’.

3.1.2.2.2 Expression pattern of *Pain-1* alleles in tubers of the tetraploid cultivar ‘Diana’

Referring to the cultivar tetraploidy, ‘Diana’ can consist of four possible *Pain-1* alleles. In this study three different alleles *Pain_DA*, *Pain_DN1*, and *Pain_DN2* were found. The expression of the three alleles was analyzed by tracing allele specific SNPs (Table 3.1.4; Table 3.1.13), which were used in pyrosequencing assays (Figure 3.1.14).

Table 3.1.13: Overview of allele specific SNPs analyzed by pyrosequencing.

Allele	SNP position	Allele specific SNP
<i>Pain_DA</i>	SNP 1544	<i>Pain_DA</i> / <i>Pain_DN1</i> / <i>Pain_DN2</i> A /C/C
<i>Pain_DN1</i>	SNP 1596	T/ C /T
<i>Pain_DN2</i>	SNP 1574	A/A/ T

SNP positions refer to cDNA sequence where ‘1’ represents the adenine of the start codon ATG. Primers used for pyrosequencing are listed in chapter 2, Table 2.1.4 (Pyro_Pain_F/Pyro_Pain_RB; sequencing primer Pyro_Pain_F). Allele specific SNPs are highlighted in bold capitals.

Pyrosequencing analysis was performed using cDNA from cold stored tubers and tuber genomic DNA to measure the dosages of the alleles (Figure 3.1.13).

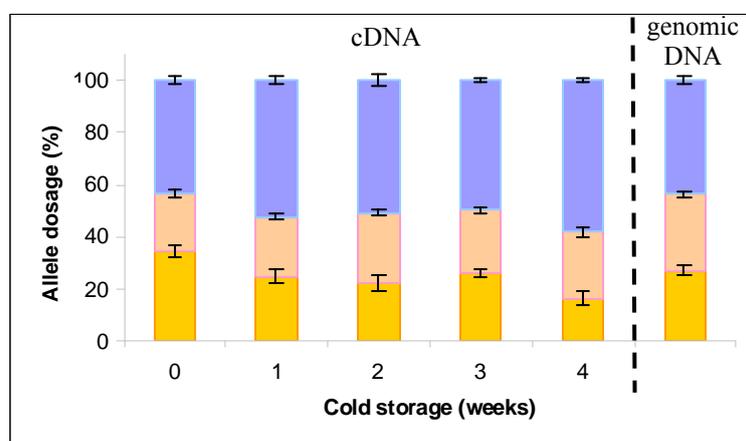


Figure 3.1.14: Pyrosequencing analysis of the alleles *Pain_DA*, *Pain_DN1*, and *Pain_DN2* of the cultivar ‘Diana’. ■: *Pain_DA*; ■: *Pain_DN1*; ■: *Pain_DN2*. In addition to pyrosequencing analysis of cDNA samples, the genomic dosage of the alleles was determined using genomic DNA of tubers. Percentages of the expression level of the cold stored samples are related to the genomic dosage of the alleles. 100% is defined as the sum of alternative peaks measuring allele specific SNPs. Standard deviations are derived from two biological replicates done in technical triplicates.

Pyrosequencing analysis of genomic DNA showed that the ‘Diana’ alleles *Pain_DA* and *Pain_DN2* are present in simplex (25%), whilst *Pain_DN1* occurs in duplex (50%).

The allele *Pain_DN1* displays at SNP position 1596 the nucleotide C but it might be possible that this fraction, representing half of the allelic entity, contains more than one allele because

of tetraploidy. If the alleles were transcribed according to their allele dosage, *Pain_DA* and *Pain_DN2* are expected to contribute 25% of the total transcripts and *Pain_DN1* 50%.

The expression of the associated allele *Pain_DA* changed in tubers during cold treatment from 38% before storage to a level of 18% in tubers stored in the cold for four weeks. *Pain_DN1* was the most prevalent allele of the cultivar ‘Diana’ and was expressed from approximately 40% (0 weeks) up to 60% (4 weeks). The expression level of the third allele *Pain_DN2* did not show strong variation during tuber cold storage. The level of expression remained stable between 20 and 25%, and, therefore, similar to the genomic dosage.

Relating the pyrosequencing data to the total amounts of invertase transcripts obtained by qRT-PCR resulted in detailed information about invertase expression during tuber cold storage (Figure 3.1.15).

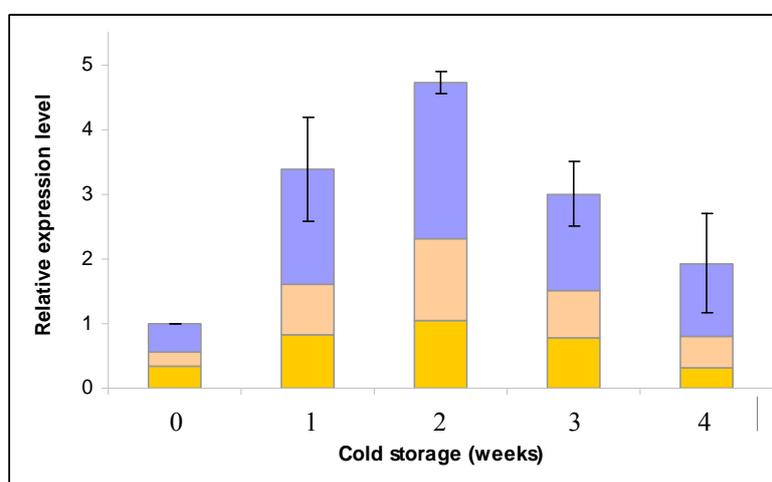


Figure 3.1.15: Overview of the expression pattern during tuber cold storage combining allelic distribution and total invertase transcript analysis of the cultivar ‘Diana’. ■: *Pain_DA*; ■: *Pain_DN1*; ■: *Pain_DN2*.

The quantification of total invertase transcript was performed using the primers Pyro_Pain_F/Pyro_Pain_R (chapter 2, Table 2.1.9) in qRT-PCR analysis. Total transcript data were normalized with the reference gene *EF1α* (section 2, Table 2.1.9). The relative expression level was calculated by defining transcript data of 0 weeks of cold storage as ‘1’. All other transcript data were related to this time point. Allelic distribution was calculated by relating the pyrosequencing data to the total transcript data. Standard deviations derived from two biological replicates done in technical duplicates. Standard deviations represent the variation within the replicates of the total transcripts, standard deviations for allele specific expression are shown in Figure 3.1.14.

qRT-PCR analysis showed an approximately 2.5 fold induction of total invertase transcript after one week of tuber cold storage (Figure 3.1.15). The transcript level increased in the 2nd week of cold storage up to about four fold compared to the control samples (no cold storage). In the 3rd and 4th week of storage the relative expression level declined but remained higher compared to levels in tubers not stored in the cold.

The expression of ‘Diana’ alleles during cold storage differed not strongly compared to their genomic dosages. Total invertase transcripts were induced up to three fold after the 1st and 2nd week of tuber cold storage.

3.1.2.2.3 Expression pattern of *Pain-1* alleles in tubers of the tetraploid cultivar ‘Theresa’

From the tetraploid cultivar ‘Theresa’ two different alleles *Pain_TN1* and *Pain_TN2* were obtained by cDNA cloning and sequencing. These two alleles were analyzed by means of the discriminative SNP at cDNA position 612 by pyrosequencing analysis (Figure 3.1.16). *Pain_TN1* has nucleotide A at SNP position 612, whilst *Pain_TN2* has nucleotide G (Table 3.1.4).

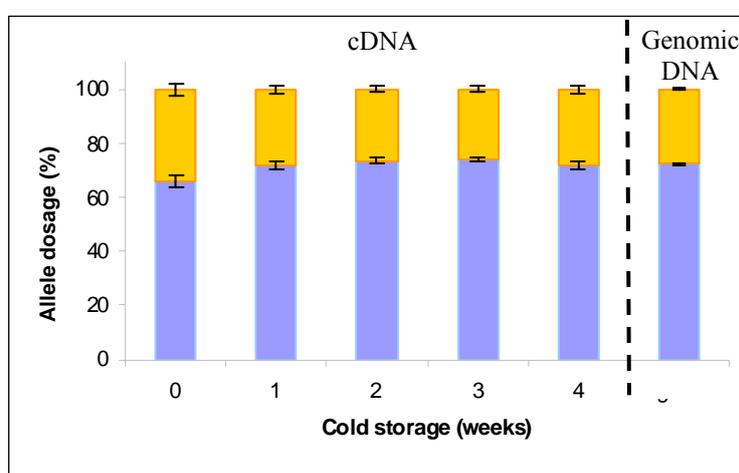


Figure 3.1.16: Pyrosequencing analysis of the alleles *Pain_TN1* and *Pain_TN2* of the cultivar ‘Theresa’. ■: *Pain_TN2*; ■: *Pain_TN1*. For allele discrimination the primers Pyro_PainTher_F/Pyro_PainTherRB (chapter 2, Table 2.1.4) were used. The primer Pyro_PainTheSeq was used as sequencing primer. In addition to pyrosequencing analysis of cDNA samples, the genomic dosage of the alleles was determined using genomic DNA of tubers. Percentages of the expression level of the cold stored samples are related to the genomic dosage of the alleles. 100% is defined as the sum of alternative peaks measuring allele specific SNPs. Standard deviations are derived from two biological replicates done in technical triplicates.

Pyrosequencing analysis of genomic DNA showed that the allele *Pain_TN2* is present in simplex (25%) and the allele *Pain_TN1* in triplex (75%). If the alleles were transcribed according to the allele dosage, *Pain_TN1* is expected to contribute 75% of the total transcripts, and *Pain_TN2* 25%. Both alleles showed little changes in their presence in cDNA samples and their expression remained at similar levels during tuber cold treatment.

The expression level of *Pain_TN2* changed marginal during tuber cold storage compared with samples not stored at 4°C. *Pain_TN1* was the prevalent allele during tuber cold storage.

Referring to ‘Theresa’s’ tetraploidy, it is possible that the triplex fraction represented by *Pain_TN1* consists of more than one allele.

Pyrosequencing analysis gave information of allele expression during tuber cold storage. Transferring this allelic pattern to the total amount of invertase transcripts led to a detailed overview of *Pain-1* allele expression (Figure 3.1.17).

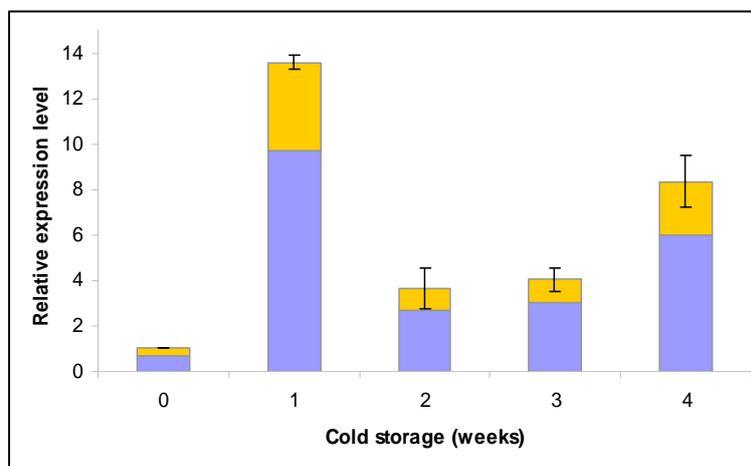


Figure 3.1.17: Overview of the expression pattern during tuber cold storage combining allelic distribution and total invertase transcript analysis of the cultivar ‘Theresa’. ■: *Pain_TN2*; ■: *Pain_TN1*. The quantification of total invertase transcript was performed using the primers Pyro_Pain_F/Pyro_Pain_R (chapter 2, Table 2.1.9) in qRT-PCR analysis. Total transcript data were normalized with the reference gene *EF1α* (chapter 2, Table 2.1.9). The relative expression level was calculated by defining transcript data of 0 weeks of cold storage as ‘1’. All other transcript data were related to this time point. Allelic distribution was calculated by relating the pyrosequencing data to the total transcript data. Standard deviations derived from three biological replicates done in technical duplicates. Standard deviations represent the variation within the replicates of the total transcripts, standard deviations for allele specific expression are shown in Figure 3.1.16.

qRT-PCR analysis showed an about 12 fold induction of total invertase transcript after the 1st week of tuber cold storage (Figure 3.1.17). Transcript up-regulation declined in the 2nd and 3rd week of cold storage to levels remaining higher than in control tubers (no cold storage). In the 4th week of cold treatment invertase transcripts increased again up to seven fold compared to control samples.

Expression analysis of both alleles of the cultivar ‘Theresa’ revealed no relevant changes of allele distribution in cold stored tubers. An intense up-regulation of total invertase transcripts was observed after the 1st week of tuber cold storage. The cultivar ‘Theresa’ is the only genotype analyzed, where total invertase transcripts increased again after four weeks at 4°C storage.

3.1.2.2.4 Expression pattern of *Pain-1* alleles in tubers of the diploid genotype P18

The differentiation between the two P18 alleles *Pain_P18N1* and *Pain_P18N2* relied on the C/A polymorphism of SNP at cDNA position 1544 using pyrosequencing analysis (Figure 3.1.18). The allele *Pain_P18N1* exhibits at SNP 1544 the nucleotide A, whilst allele *Pain_P18N2* consists of the nucleotide C (Table 3.1.5).

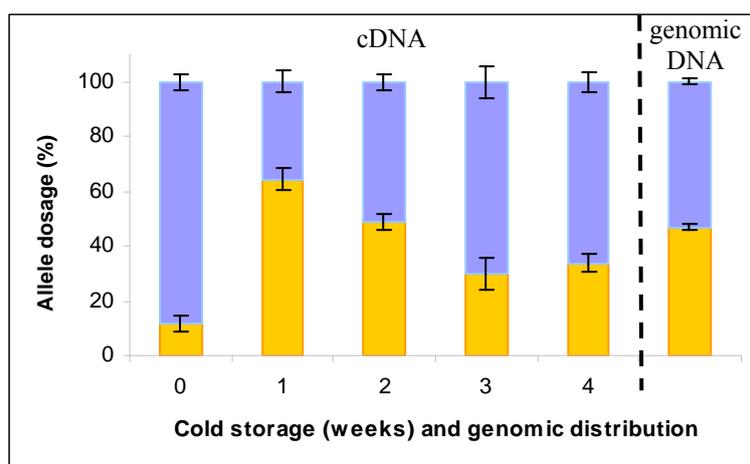


Figure 3.1.18: Pyrosequencing analysis of the alleles *Pain_P18N1* and *Pain_P18N2* of the genotype P18. ■: *Pain_P18N1*; ■: *Pain_P18N2*. For allele discrimination the primers Pyro Pyro_Pain_F/Pyro_Pain_RB (chapter 2, Table 2.1.4) were used. The primer Pyro_Pain_F was also used as sequencing primer. In addition to pyrosequencing analysis of cDNA samples, the genomic dosage of the alleles was determined using genomic DNA of tubers. Percentages of the expression level of the cold stored samples are related to the genomic dosage of the alleles. 100% is defined as the sum of alternative peaks measuring allele specific SNPs. Standard deviations are derived from two biological replicates done in technical triplicates.

Pyrosequencing analysis of genomic DNA showed that both P18 alleles are present in duplex (50%) as expected for a heterozygous diploid genotype. Pyrosequencing analysis of cDNA samples showed a strong increase of allele *Pain_P18N1* after one week of tuber cold storage (Figure 3.1.18). Expression level was 50% higher than in tubers that were not stored in the cold. *Pain_P18N1* up-regulation declined in the 2nd, 3rd, and 4th week of cold storage to level ranging from about 30 to 50% of total expression.

Analyzing the relative expression level of P18 allele transcripts by transferring pyrosequencing data to qRT-PCR analysis showed that expression pattern changed during tuber cold storage (Figure 3.1.19).

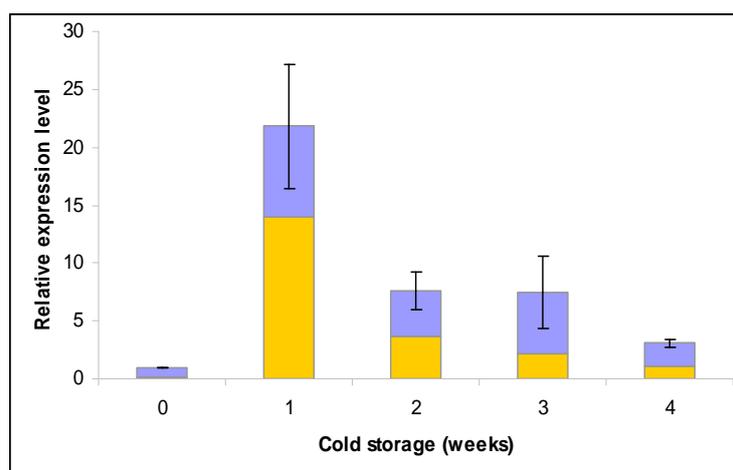


Figure 3.1.19: Overview of the expression pattern during tuber cold storage combining allelic distribution and total invertase transcript analysis of the potato genotype P18. ■: *Pain_P18N1*; ■: *Pain_P18N2*. The quantification of total invertase transcript was performed using the primers Pyro_Pain_F/Pyro_Pain_R (chapter 2, Table 2.1.9) in qRT-PCR analysis. Total transcript data were normalized with the reference gene *EF1 α* (chapter 2, Table 2.1.9). The relative expression level was calculated by defining transcript data of 0 weeks of cold storage as ‘1’. All other transcript data were related to this time point. Allelic distribution was calculated by relating the pyrosequencing data to the total transcript data. Standard deviations derived from two biological replicates done in technical duplicates. Standard deviations represent the variation within the replicates of the total transcripts, standard deviations for allele specific expression are shown in Figure 3.1.18.

qRT-PCR analysis demonstrated that total invertase transcripts were strongly induced after one week of tuber cold storage at 4°C (Figure 3.1.19). Transcript up-regulation occurred up to 20 fold compared to control tubers (0 weeks). The relative expression level showed an about eight fold induction in the 2nd and 3rd week and decreased toward the end of cold storage.

Expression analysis of P18 invertase transcripts revealed an up-regulation of allele *Pain_P18N1* up to about 50% as well as an intense induction of total transcripts after one week of tuber cold storage. The diploid genotype P18 showed the highest accumulation of invertase transcripts (up to \approx 20 fold) due to cold storage compared to control samples of all tested potato genotypes. Comparing the expression levels of the alleles *Pain_P18N1* and *Pain_DA*, which are identical at amino acid level, showed that expression of *Pain_DA* in contrast to *Pain_P18N1* was not effected by tuber cold storage (Figure 3.1.14). *Pain_DA* was found to be associated with better potato chips quality.

3.1.2.2.5 Expression pattern of *Pain-1* alleles in tubers of the diploid genotype P40

Pyrosequencing analysis of the P40 allele specific SNP at cDNA position 1267 were used to separate the alleles *Pain_P40N1* and *Pain_P40N2* (Figure 3.1.20). *Pain_P40N1* is characterized by adenine at SNP position 1267, whilst *Pain_P40N2* shows guanine (Table 3.1.5).

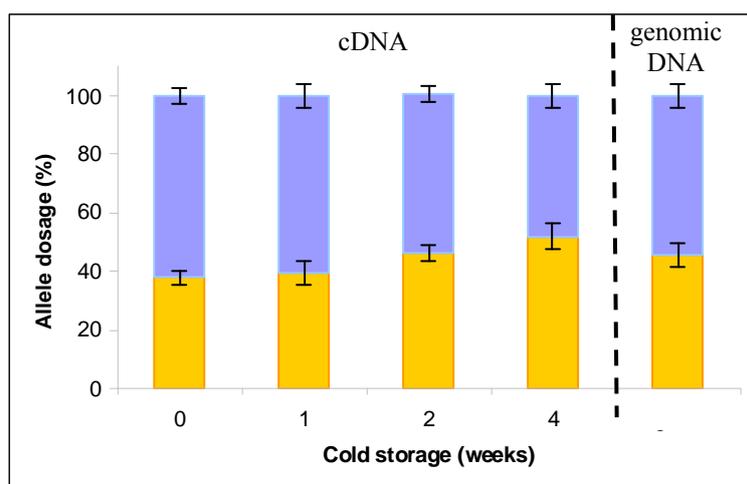


Figure 3.1.20: Pyrosequencing analysis of the alleles *Pain_P40N1* and *Pain_P40N2* of the genotype P40. ■ : *Pain_P40N1*; ■ : *Pain_P40N2*. For allele discrimination the primers Pyro_PainP40_F/Pyro_PainP40_RB were used. The primer Pyro_PainP40_Seq was used as sequencing primer (chapter 2, Table 2.1.4). In addition to pyrosequencing analysis of cDNA samples, the genomic dosage of the alleles was determined using genomic DNA of tubers. Percentages of the expression level of the cold stored samples are related to the genomic dosage of the alleles. 100% is defined as the sum of alternative peaks measuring allele specific SNPs. Standard deviations are derived from two biological replicates done in technical triplicates.

Pyrosequencing analysis of genomic DNA showed that both P 40 alleles are present in duplex (50%) as expected for a heterozygous diploid genotype. Pyrosequencing analysis of cDNA samples revealed a slight but continuous increase of allele *Pain_P40N1* during tuber cold storage (Figure 3.1.20). Expression levels ranged from about 38% in control samples up to 50% in tubers stored in the cold for four weeks. Tuber tissues stored for three weeks were not analyzed in pyrosequencing assay due to sample limitation.

Relating the pyrosequencing data to the total amounts of invertase transcripts obtained by qRT-PCR resulted in detailed information about invertase expression during tuber cold storage (Figure 3.1.21).

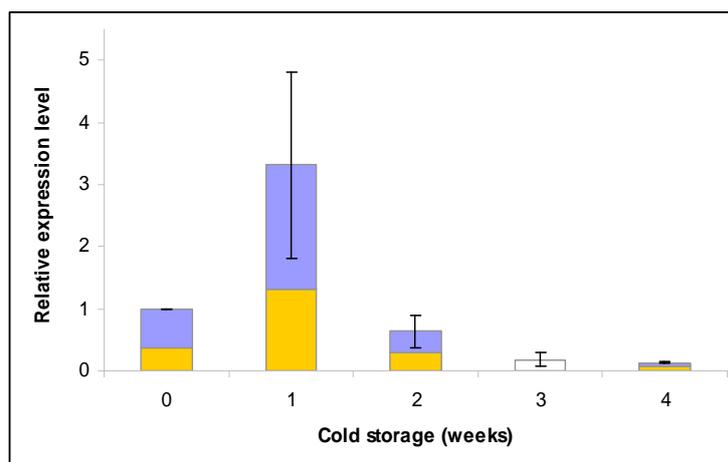


Figure 3.1.21: Overview of the expression pattern during tuber cold storage combining allelic distribution and total invertase transcript analysis of the potato genotype P40. ■: *Pain_P40N1*; ■: *Pain_P40N2*. The quantification of total invertase transcript was performed using the primers Pyro_Pain_F/Pyro_Pain_R (chapter 2, Table 2.1.9) in qRT-PCR analysis. Total transcript data were normalized with the reference gene *EF1 α* (chapter 2, Table 2.1.9). The relative expression level was calculated by defining transcript data of 0 weeks of cold storage as ‘1’. All other transcript data were related to this time point. Allelic distribution was calculated by relating the pyrosequencing data to the total transcript data. The 3rd week of tuber cold storage was not analyzed by pyrosequencing due to samples limitations. The bar of total invertase transcripts, therefore, is left empty. Standard deviations derived from two biological replicates done in technical duplicates. Standard deviations represent the variation within the replicates of the total transcripts, standard deviations for allele specific expression are shown in Figure 3.1.20.

Monitoring the relative expression level of total invertase transcripts showed an about 2.5 fold induction after one week of tuber cold storage at 4°C (Figure 3.1.21). Transcript up-regulation declined in the 2nd, 3rd, and 4th week of cold storage to levels lower than in control tubers that were not stored in the cold.

Expression analysis of both P40 alleles revealed slight changes of allele distribution in cold stored tubers. Allele *Pain_P40N1* expression increased during cold storage from about 38% to 50%. Up-regulation of total invertase transcripts was observed after one week of cold treatment up to 2.5 fold. The relative expression level of total transcripts declined dramatically from the 2nd to the 4th week of tuber cold storage.

3.1.2.2 Functional complementation of the yeast invertase mutant *SUC2*

Plant invertases are functional in heterologous systems (FRIDMAN ET AL., 2004). Therefore, the model organism yeast (*Saccharomyces cerevisiae*) was used to analyze potato invertase alleles. The yeast invertase mutant *SUC2* lacks invertase activity and uses glucose as carbohydrate source. Transforming *SUC2* with *Pain-1* cDNA alleles resulted in yeast transformants that were able to grow on sucrose as sole carbohydrate source, indicating functional complementation of the *SUC2* mutation.

The *Pain-1* cDNA alleles listed below were used for complementation of *SUC2* (Figure 3.1.22) and subsequent analysis of invertase activity (section 3.1.2.3).

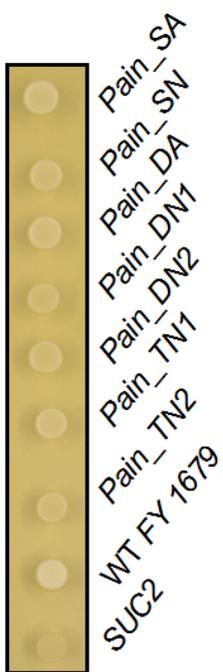
Genotype	Allele name	<i>SUC2</i> complementation
‘Satina’	<i>Pain_SA</i>	 <p>Representative yeast transformants complemented with invertase alleles of the cultivars ‘Satina’, ‘Diana’, and ‘Theresa’ were spotted on solid yeast minimal broth with 2% sucrose as carbon source. The wild type <i>FY 1479</i> was plated as positive control, whilst the invertase mutant <i>SUC2</i> was the negative control. Growth of all complemented yeast transformants was quantified (Figure 3.1.23).</p>
	<i>Pain_SN</i>	
‘Diana’	<i>Pain_DA</i>	
	<i>Pain_DN1</i>	
	<i>Pain_DN2</i>	
‘Theresa’	<i>Pain_TN1</i>	
	<i>Pain_TN2</i>	
P18	<i>Pain_P18N1</i>	
	<i>Pain_P18N2</i>	
P40	<i>Pain_P40N1</i>	
	<i>Pain_P40N2</i>	
P54	<i>Pain_P54N</i>	

Figure 3.1.22: cDNA alleles used for *SUC2* complementation and *SUC2* transformants on solid yeast minimal media.

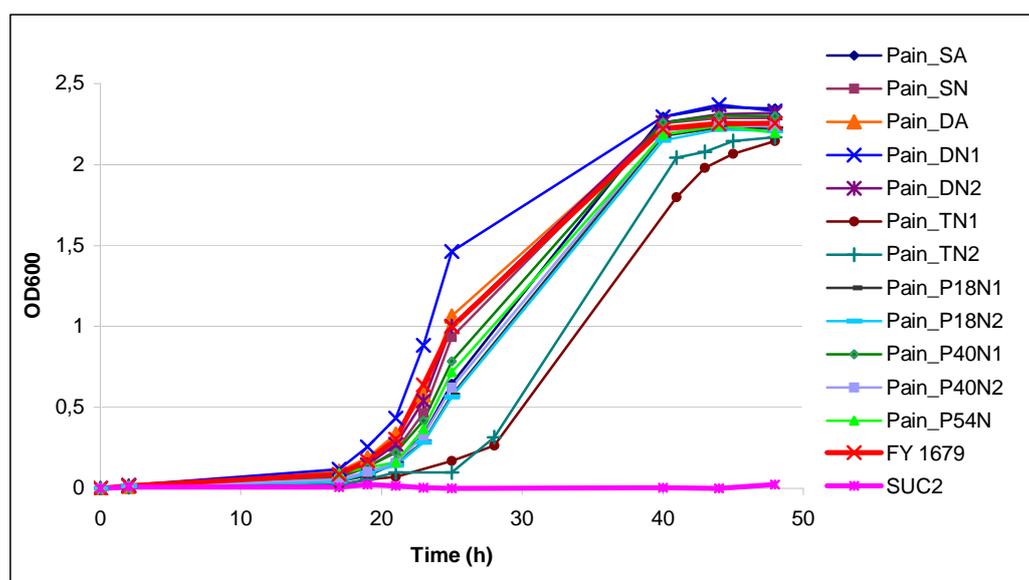


Figure 3.1.23: Growth of complemented *SUC2* transformants. Yeast reference strain *FY 1679* (red), yeast invertase mutant strain *SUC2* (pink), and *Pain-1* transformants were grown in yeast minimal media with 2% sucrose as the sole carbon source. OD600 is plotted against growth time in hours. OD600 values represent the means of two replicates. Standard deviations were less than 20% of mean.

All *SUC2* invertase transformants exhibited substantial growth on sucrose. ‘Satina’ and ‘Diana’ alleles and alleles of the diploid genotypes P18, P40, and P54 complemented the invertase deficiency of the yeast strain better than the alleles of ‘Theresa’. However, it was not possible to correlate the altered complementation efficiency to quantitative invertase protein differences using immunoblot studies (section 3.1.2.4; Figure 3.1.25).

3.1.2.3 Biochemical characterization of *Pain-1* alleles

Putative 3D-models of allelic *Pain-1* molecules (3.1.1.3) indicated structural and electrostatic differences between the alleles, which could cause functional differences. To test, whether structural characteristics might influence enzymatic activity of *Pain-1* invertase alleles, biochemical characterization was performed.

From yeast *SUC2* transformants complemented with *Pain-1* cDNA alleles total protein was extracted. To test soluble acid invertase activity, a modified protocol based on ZRENNER ET AL. (1995) was used. Invertase assays were carried out at 30°C. Additionally, *Pain-1* alleles were assayed at 4°C. The analysis at 4°C was performed to study possible differences of enzyme kinetics due to allelic amino acid composition in response to cold storage conditions. It was previously reported that cold storage influences transcriptional changes regarding vacuolar invertases (ZRENNER ET AL., 1996; ZHOU ET AL., 2004; BAGNARESI ET AL., 2008), but no study investigated the effect of low temperatures on the enzyme activity itself. The *Pain-1* alleles displayed in Table 3.1.14 were used for biochemical characterization at 30°C and 4°C.

Table 3.1.14: *Pain-1* alleles used for biochemical characterization.

Tetraploid genotypes	Allele	Diploid genotypes	Allele
‘Satina’	<i>Pain_SA</i>	P18	<i>Pain_P18N1</i>
	<i>Pain_SN</i>		<i>Pain_P18N2</i>
‘Diana’	<i>Pain_DA</i>	P40	<i>Pain_P40N1</i>
	<i>Pain_DN1</i>		<i>Pain_P40N2</i>
	<i>Pain_DN2</i>	P54	<i>Pain_P54N</i>
‘Theresa’	<i>Pain_TN1</i>		
	<i>Pain_TN2</i>		

The biochemical parameters Michaelis constant (K_m) and the maximal velocity (v_{max}) of invertase reaction were determined.

Results of the biochemical analysis at 30°C and 4°C are shown in Table 3.1.15.

Table 3.1.15: K_m (mM) and v_{max} (mmol/h*mg protein⁻¹) of *Pain-I* invertase alleles at 30°C and 4°C.

Genotype	Allele	K_m (30°C)	K_m (4°C)	v_{max} (30°C)	v_{max} (4°C)
‘Satina’	<i>Pain_SA</i>	22.1±1.4	2.3±0.3	4.7±1.0	1.7±0.1
	<i>Pain_SN</i>	23.0±1.4	3.5±0.3	4.1±1.0	1.5±0.1
‘Diana’	<i>Pain_DA</i>	19.9±1.4	4.0±0.3	12.4±0.8	2.8±0.1
	<i>Pain_DN1</i>	19.6±1.4	2.7±0.3	11.2±0.8	2.7±0.1
	<i>Pain_DN2</i>	15.6±1.4	3.7±0.3	6.2±1.0	2.2±0.1
‘Theresa’	<i>Pain_TN1</i>	19.8±1.4	3.0±0.3	3.4±1.0	1.5±0.1
	<i>Pain_TN2</i>	21.6±1.4	na	2.0±1.4	na
P18	<i>Pain_P18N1</i>	20.4±1.4	2.7±0.3	5.5±1.0	2.1±0.1
	<i>Pain_P18N2</i>	16.9±1.4	4.7±0.4	2.7±1.0	1.9±0.1
P40	<i>Pain_P40N1</i>	17.2±1.4	3.4±0.3	5.8±1.0	2.1±0.1
	<i>Pain_P40N2</i>	14.9±1.4	4.4±0.3	6.8±1.0	2.6±0.1
P54	<i>Pain_P54N</i>	19.1±1.4	2.7±0.4	3.7±1.0	1.6±0.1
<i>FY 1679</i>		21.0±1.6	21.1±2.5	23.9±4.5	7.7±0.9

Standard deviations are derived from three biological replicates for the associated alleles *Pain_SA* and *Pain_DA*, and the wild type reference strain *FY 1679*, and from two biological replicates for the other alleles done in technical replicates to obtain six measurements. To make assays of different invertase isoforms comparable, the yeast reference strain *FY 1679* was used as positive control.

Tables 3.1.16, 3.1.17, 3.1.18 and 3.1.19 summarize the significance values for differences between the K_m and v_{max} values measured for the *Pain-I* alleles at 30°C and 4°C. The statistical calculation was performed by Benjamin Stich, MPIZ/Köln.

Table 3.1.16: Overview of statistical significance levels of K_m values from the *Pain-I* invertase alleles at 30°C.

Allele	<i>Pain_SA</i>	<i>Pain_SN</i>	<i>Pain_DA</i>	<i>Pain_DN1</i>	<i>Pain_DN2</i>	<i>Pain_TN1</i>	<i>Pain_TN2</i>	<i>Pain_P18N1</i>	<i>Pain_P18N2</i>	<i>Pain_P40N1</i>	<i>Pain_P40N2</i>	<i>Pain_P54N</i>
<i>Pain_SA</i>	---	0.63	0.28	0.22	0.0058	0.26	0.79	0.39	0.019	0.028	0.0029	0.15
<i>Pain_SN</i>	---	---	0.13	0.10	0.0024	0.12	0.47	0.20	0.008	0.011	0.0012	0.067
<i>Pain_DA</i>	---	---	---	0.89	0.048	0.98	0.40	0.80	0.15	0.20	0.024	0.71
<i>Pain_DN1</i>	---	---	---	---	0.06	0.91	0.33	0.70	0.19	0.25	0.03	0.81
<i>Pain_DN2</i>	---	---	---	---	---	0.051	0.009	0.03	0.53	0.42	0.71	0.095
<i>Pain_TN1</i>	---	---	---	---	---	---	0.38	0.78	0.16	0.21	0.025	0.73
<i>Pain_TN2</i>	---	---	---	---	---	---	---	0.55	0.032	0.045	0.0047	0.23
<i>Pain_P18N1</i>	---	---	---	---	---	---	---	---	0.09	0.13	0.015	0.53
<i>Pain_P18N2</i>	---	---	---	---	---	---	---	---	---	0.86	0.32	0.27
<i>Pain_P40N1</i>	---	---	---	---	---	---	---	---	---	---	0.25	0.35
<i>Pain_P40N2</i>	---	---	---	---	---	---	---	---	---	---	---	0.048
<i>Pain_P54N</i>	---	---	---	---	---	---	---	---	---	---	---	---

Significant differences are defined by P-values <0.05 and are orange coloured, no significant differences are described by P-values >0.05.

Table 3.1.17: Overview of statistical significance levels of K_m values from the *Pain-I* invertase alleles at 4°C.

Allele	<i>Pain SA</i>	<i>Pain SN</i>	<i>Pain DA</i>	<i>Pain DNI</i>	<i>Pain DN2</i>	<i>Pain TNI</i>	<i>Pain TN2</i>	<i>Pain P18N1</i>	<i>Pain P18N2</i>	<i>Pain P40N1</i>	<i>Pain P40N2</i>	<i>Pain P54N</i>
<i>Pain SA</i>	---	0.014	0.0019	0.36	0.0078	0.11	na	0.298	0.0003	0.023	0.0004	0.34
<i>Pain SN</i>	---	---	0.24	0.080	0.65	0.33	na	0.1	0.026	0.798	0.06	0.14
<i>Pain DA</i>	---	---	---	0.0099	0.47	0.053	na	0.013	0.21	0.16	0.44	0.022
<i>Pain DNI</i>	---	---	---	---	0.042	0.43	na	0.89	0.0012	0.13	0.0021	0.89
<i>Pain DN2</i>	---	---	---	---	---	0.18	na	0.052	0.066	0.49	0.15	0.079
<i>Pain TNI</i>	---	---	---	---	---	---	na	0.51	0.0058	0.46	0.012	0.57
<i>Pain TN2</i>	---	---	---	---	---	---	---	na	na	na	na	na
<i>Pain P18N1</i>	---	---	---	---	---	---	---	---	0.0014	0.16	0.0027	0.98
<i>Pain P18N2</i>	---	---	---	---	---	---	---	---	---	0.017	0.59	0.0028
<i>Pain P40N1</i>	---	---	---	---	---	---	---	---	---	---	0.038	0.2085
<i>Pain P40N2</i>	---	---	---	---	---	---	---	---	---	---	---	0.0055
<i>Pain P54N</i>	---	---	---	---	---	---	---	---	---	---	---	---

Significant differences are defined by P-values <0.05 and are orange coloured, no significant differences are described by P-values >0.05.

Table 3.1.18: Overview of statistical significance levels of v_{max} values from the *Pain-I* invertase alleles at 30°C.

Allele	<i>Pain SA</i>	<i>Pain SN</i>	<i>Pain DA</i>	<i>Pain DNI</i>	<i>Pain DN2</i>	<i>Pain TNI</i>	<i>Pain TN2</i>	<i>Pain P18N1</i>	<i>Pain P18N2</i>	<i>Pain P40N1</i>	<i>Pain P40N2</i>	<i>Pain P54N</i>
<i>Pain SA</i>	---	0.696	5.80E-05	0.0003	0.29	0.38	0.15	0.58	0.177	0.43	0.15	0.52
<i>Pain SN</i>	---	---	3.01E-05	0.0001	0.16	0.62	0.25	0.35	0.32	0.25	0.077	0.79
<i>Pain DA</i>	---	---	---	0.32	0.0004	1.35E-05	3.11E-05	0.0002	6.2E-06	0.0002	0.0009	1.98E-05
<i>Pain DNI</i>	---	---	---	---	0.0021	5.26E-05	9.68E-05	0.0007	2.28E-05	0.0012	0.0049	7.98E-05
<i>Pain DN2</i>	---	---	---	---	---	0.0653	0.03	0.599	0.026	0.77	0.68	0.1
<i>Pain TNI</i>	---	---	---	---	---	---	0.44	0.16	0.61	0.11	0.03	0.81
<i>Pain TN2</i>	---	---	---	---	---	---	---	0.067	0.72	0.047	0.016	0.34
<i>Pain P18N1</i>	---	---	---	---	---	---	---	---	0.068	0.813	0.356	0.249
<i>Pain P18N2</i>	---	---	---	---	---	---	---	---	---	0.044	0.012	0.46
<i>Pain P40N1</i>	---	---	---	---	---	---	---	---	---	---	0.49	0.16
<i>Pain P40N2</i>	---	---	---	---	---	---	---	---	---	---	---	0.048
<i>Pain P54N</i>	---	---	---	---	---	---	---	---	---	---	---	---

Significant differences are defined by P-values <0.05 and are orange coloured, no significant differences are described by P-values >0.05.

Table 3.1.19: Overview of statistical significance levels of v_{\max} values from the *Pain-1* invertase alleles at 4°C.

Allele	<i>Pain SA</i>	<i>Pain SN</i>	<i>Pain DA</i>	<i>Pain DNI</i>	<i>Pain DN2</i>	<i>Pain TNI</i>	<i>Pain TN2</i>	<i>Pain P18N1</i>	<i>Pain P18N2</i>	<i>Pain P40N1</i>	<i>Pain P40N2</i>	<i>Pain P54N</i>
<i>Pain SA</i>	---	0.06	4.05 E-06	8.19 E-06	0.0093	0.22	na	0.013	0.25	0.013	3.20 E-05	0.55
<i>Pain SN</i>	---	---	3.74 E-07	6.11 E-07	0.0003	0.5	na	0.0003	0.0097	0.0003	2.18 E-06	0.23
<i>Pain DA</i>	---	---	---	0.41	0.0006	1.22 E-06	na	0.0003	5.86 E-05	0.0003	0.2	4.80 E-06
<i>Pain DNI</i>	---	---	---	---	0.0019	2.20E-06	na	0.0008	0.0002	0.0008	0.58	9.54 E-06
<i>Pain DN2</i>	---	---	---	---	---	0.0012	na	0.78	0.12	0.77	0.0073	0.0052
<i>Pain TNI</i>	---	---	---	---	---	---	na	0.0015	0.037	0.0015	7.64 E-06	0.56
<i>Pain TN2</i>	---	---	---	---	---	---	---	na	na	na	na	na
<i>Pain P18N1</i>	---	---	---	---	---	---	---	---	0.17	0.99	0.0034	0.0069
<i>Pain P18N2</i>	---	---	---	---	---	---	---	---	---	0.18	0.0005	0.12
<i>Pain P40N1</i>	---	---	---	---	---	---	---	---	---	---	0.0034	0.0067
<i>Pain P40N2</i>	---	---	---	---	---	---	---	---	---	---	---	3.14 E-05
<i>Pain P54N</i>	---	---	---	---	---	---	---	---	---	---	---	---

Significant differences are defined by P-values <0.05 and are orange coloured, no significant differences are described by P-values >0.05.

The 30°C assay of *Pain-1* alleles showed that the substrate affinity ranged between 15mM of the allele *Pain_P40N2* and 23mM of the allele *Pain_SN*. Maximal velocities of the alleles varied between 2mmol*h⁻¹*mg protein⁻¹ in the case of the allele *Pain_TN2* and 12mmol*h⁻¹*mg protein⁻¹ for the allele *Pain_DA*. The enzymatic characteristics of the analyzed alleles from the cultivar ‘Satina’ *Pain_SA* and *Pain_SN* showed no significant differences. In the K_m and v_{\max} values of the cultivar ‘Diana’ differences were displayed in respect to the allele *Pain_DN2*. *Pain_DN2* showed the highest substrate affinity with a K_m of approximately 16mM, and the slowest substrate conversion with a v_{\max} of around 6mmol*h⁻¹*mg protein⁻¹. The other two analyzed alleles *Pain_DA* and *Pain_DNI* displayed substrate affinities of approximately 20mM and maximal velocities of around 12mmol*h⁻¹*mg protein⁻¹. Comparison of the associated *Pain-1* alleles *Pain_SA* and *Pain_DA*, which are not identical at amino acid level, revealed no significant differences regarding their affinity to sucrose, but showed strong differences in the rate of sucrose conversion. The allele *Pain_DA* converts sucrose approximately 2.5 times faster than the allele *Pain_SA*.

Looking at the other analyzed *Pain-1* alleles, the two ‘Theresa’ alleles showed similar enzymatic characteristics and did not differ significantly. K_m values varied from 20 to 21mM, and v_{\max} values ranged from 2 to 3mmol*h⁻¹*mg protein⁻¹. Biochemical analysis of the alleles

from the diploid potato genotypes P18 and P40 did not display any significant K_m and v_{max} differences. K_m and v_{max} values of *Pain_P18N1* and *Pain_P18N2* ranged from 17 to 20mM, and 3 to 5.5mmol*h⁻¹*mg protein⁻¹, respectively. The alleles *Pain_P40N1* and *Pain_P40N2* displayed substrate affinities between 15 and 17mM, and v_{max} values between 6 and 7mmol*h⁻¹*mg protein⁻¹.

Biochemical analysis of the enzymatic characteristics of the *Pain-I* alleles at 4°C showed a dramatic increase in the enzyme's affinity to sucrose. The K_m values decreased approximately 5.5 times compared to K_m values measured at 30°C. Also the maximal velocities of the alleles were affected at 4°C. By trend v_{max} values were around 2mmol*h⁻¹*mg protein⁻¹.

Additionally, biochemical characteristics were determined for two nucleotide variants of the 'Satina' allele *Pain_SN* to measure codon influences in the heterologous system yeast. The variants differed in one to three nucleotides to *Pain_SN* (Table 3.1.20), (Appendix A 3.1.25).

Table 3.1.20: SNPs of the nucleotide variants of the allele *Pain_SN*.

SNP position	<i>Pain_SN</i>	<i>Pain_SN</i> *	<i>Pain_SN</i> **
69	T	T	C
75	T	C	T
1050	C	T	C
1350	A	G	A

SNP position numbering refers to cDNA sequence where '1' represents the adenine of the start codon ATG and the polymorphism is described. The nucleotide exchanges are synonymous, not causing an amino acid exchange.

It was shown that all nucleotide variants of *Pain_SN* displayed similar K_m and v_{max} values (Table 3.1.21), showing that a codon usage independent comparison of different alleles was possible.

Table 3.1.21: K_m (mM) and v_{max} (mmol/h*mg protein⁻¹) of *Pain-SN1* nucleotide variants at 30°C and 4°C.

Allele	K_m (30°C)	K_m (4°C)	v_{max} (30°C)	v_{max} (4°C)
<i>Pain SN</i>	23.0±1.4	3.5±0.3	4.1±1.0	1.5±0.1
<i>Pain SN</i> *	23.0±1.7	3.0±0.3	5.7±1.0	1.8±0.1
<i>Pain SN</i> **	24.5±1.5	2.5±0.4	7.8±1.4	1.7±0.1

Standard deviations are derived from two biological replicates done in technical triplicates.

The Tables 3.1.22 and 23 summarize the significance values for differences between the K_m and v_{max} values measured for the nucleotide variants of the allele *Pain_SN* at 30°C and 4°C. The statistical calculation was performed by Benjamin Stich, MPIZ/Köln.

Table 3.1.22: Overview of statistical significance levels of K_m values from the *Pain-SNI* nucleotide variants at 30°C and 4°C.

Allele	30°C			4°C		
	<i>Pain</i> <i>SN</i>	<i>Pain</i> <i>SN*</i>	<i>Pain</i> <i>SN**</i>	<i>Pain</i> <i>SN</i>	<i>Pain</i> <i>SN*</i>	<i>Pain</i> <i>SN**</i>
<i>Pain</i> <i>SN</i>	---	0.1	0.48	---	0.28	0.06
<i>Pain</i> <i>SN*</i>	---	---	0.53	---	---	0.35
<i>Pain</i> <i>SN**</i>	---	---	---	---	---	---

Significant differences are defined by P-values <0.05 and are orange coloured, no significant differences are described by P-values >0.05.

Table 3.1.23: Overview of statistical significance levels of v_{max} values from the *Pain-SNI* nucleotide variants at 30°C and 4°C.

Allele	30°C			4°C		
	<i>Pain</i> <i>SN</i>	<i>Pain</i> <i>SN*</i>	<i>Pain</i> <i>SN**</i>	<i>Pain</i> <i>SN</i>	<i>Pain</i> <i>SN*</i>	<i>Pain</i> <i>SN**</i>
<i>Pain</i> <i>SN</i>	---	0.27	0.048	---	0.04	0.084
<i>Pain</i> <i>SN*</i>	---	---	0.24	---	---	0.83
<i>Pain</i> <i>SN**</i>	---	---	---	---	---	---

Significant differences are defined by P-values <0.05 and are orange coloured, no significant differences are described by P-values >0.05.

3.1.2.4 Western blot analysis

To analyze, whether the observed differences of invertase enzyme activity and yeast growth behaviour are not due to changes in protein quantity, immunoblot quantification was performed.

As a loading control the blotted membrane was stained with Ponceau S (Figure 3.1.24). The concentration of total yeast protein extract was the same within the loaded samples.

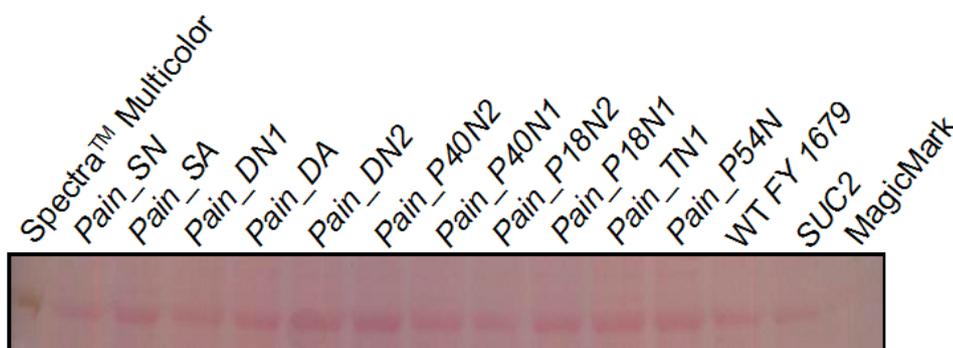


Figure 3.1.24: Ponceau S stained blot membrane. The corresponding alleles are given by their name above every lane. 15µg of total protein were loaded. A representative protein band out of the blotted membrane is shown as loading control.

From all tested invertase antibodies (chapter 2, Table 2.2.15), the antibody against a 58kDa vacuolar invertase of potato (BURCH ET AL., 1992) detected *Pain-1* protein most reliable and, therefore, was used in subsequent Western blot analysis. The invertase protein content of a set of allelic *Pain-1* yeast *SUC2* transformants was analyzed representatively (Figure 3.1.25).

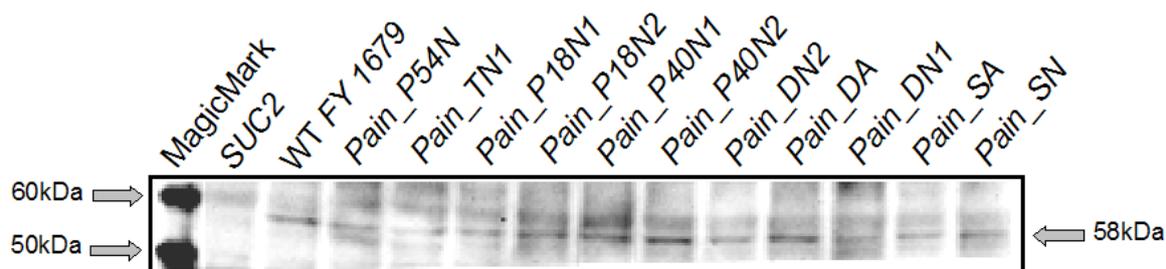


Figure 3.1.25: Western blot analysis of *Pain-1* alleles using an antibody against a 58kDa vacuolar invertase of potato. The corresponding alleles are given by their name above every lane. 15 μ g of total protein were loaded. Additionally, yeast invertase mutant *SUC2* and yeast reference strain WT *FY 1679* were blotted. Left arrows stand for size bands of the MagicMark Marker, the right arrow refers to the 58kDa band where the invertase protein was detected.

Western blot analysis showed that invertase protein content of *SUC2* transformants containing different *Pain-1* alleles did not differ significantly. Additionally, the invertase of the yeast reference strain WT *FY 1679* was detected by the potato antibody compared to the yeast invertase mutant *SUC2* where no protein was detectable.

3.2 The *Invap-b* locus on chromosome IX

The *Invap-b* locus was intensively studied and described by MADDISON ET AL., (1999). The locus consists of two invertase genes *invGE* and *invGF* linked in direct tandem repeat and has a size of approximately 8.6kb. Both genes exhibit a similar exon/intron structure composed of six exons and five introns. This exon/intron structure is identical to the two potato cell wall-bound invertases *pCD111* and *pCD141* on chromosome X (HEDLEY ET AL., 1993, 1994), and slightly different from the *Pain-1* invertase gene on chromosome III (ZHOU ET AL., 1994). Expression analysis showed *invGE* transcription in the leaf, stem, root, tuber, and floral tissue, whilst *invGF* expression is restricted to floral tissues (MADDISON ET AL., 1999).

The genes *invGE* and *invGF* encode cell wall-bound invertase isoforms. These acidic insoluble invertases act in a pH optimum range of 4.5 to 5.0 and are functional in the apoplast (TYMOWSKA-LALANNE & KREIS, 1998).

3.2.1 Structural characterization of the genes *invGE* and *invGF*

3.2.1.1 Identification of associated *invGE* and *invGF* alleles

The *Invap-b* locus maps to potato chromosome IX (CHEN ET AL., 2001) in a region associated with tuber quality trait,s where a QTL for potato tuber sugar content, *Sug9a*, was identified (MENÉNDEZ ET AL., 2002). In the latter study, the genes *invGE* and *invGF* were directly mapped in the mapping population used and showed linkage to the QTL *Sug9a*.

Single-strand conformation polymorphism (SSCP) analysis revealed an association of *invGE* and *invGF* alleles with starch and sugar content of potato tubers. SSCP fragments found to be associated with better potato chips quality were named *invGE-6f* and *invGF-4d*, respectively (LI ET AL., 2005). The occurrence of those fragments in the genotypes was highly correlated indicating that *invGE-6f* and *invGF-4d* are in LD. The analysis of protein sequences of allelic *invGE-6f* fragments revealed a unique histidine instead of proline at amino acid position 368 (LI ET AL., 2005). *invGE-6f* fragments containing histidine at position 368 were associated with better potato chips quality. Histidine 368 is encoded by the cDNA SNP at nucleotide position 1103* (section 3.2.1.2, Table 3.2.3) where cytosine changes to adenine. Therefore, cloned *invGE* cDNA alleles were named based on the presence (A) or absence (N) of SNP 1103*.

Assigning cloned *invGF* alleles to the SSCP fragment *invGF-4d* and detecting the underlying SNPs are subject of ongoing investigations in the research project.

3.2.1.2 Molecular cloning of *invGE* and *invGF* invertase cDNA alleles

Regarding *invGE* and *invGF* expression patterns (MADDISON ET AL., 1999), molecular cloning of *invGE* alleles was performed using leaf and floral tissue, whilst *invGF* allele cloning was initially carried out with floral tissue. During this study *invGF* alleles were obtained also from leaf material demonstrating that the restriction of floral *invGF* expression is genotype dependent.

❖ Genotype dependent *invGF* expression in leaves

Following the approach to detect *invGF* transcripts as described by MADDISON ET AL. (1999), gene specific primers amplifying a small fragment of the gene from the tetraploid genotypes ‘Désirée’, ‘Saturna’, ‘Diana’, ‘Theresa’, and from the diploid genotypes P40 and P54 were designed. MADDISON ET AL. (1999) restricted the expression analysis consisting of a histochemical GUS assay of *invGF* expression in transgenic plants and RT-PCR from mature flowers, flower bud, source, and sink leaf, respectively to the genotypes ‘Désirée’ and ‘Saturna’. The *invGF* primers (chapter 2, Table 2.1.7) were selected spanning from exon I to exon III, which should generate a product of 402bp from cDNA. In contrast, any product generated from contaminating genomic DNA would include sequences from two intermediate introns and the mini-exon II, amounting to 704bp. RT-PCR with the latter primers using total RNA prepared from both mature flowers and leaves as template generated a product of the expected size (Figure 3.2.1) indicating expression of *invGF* in these organs.

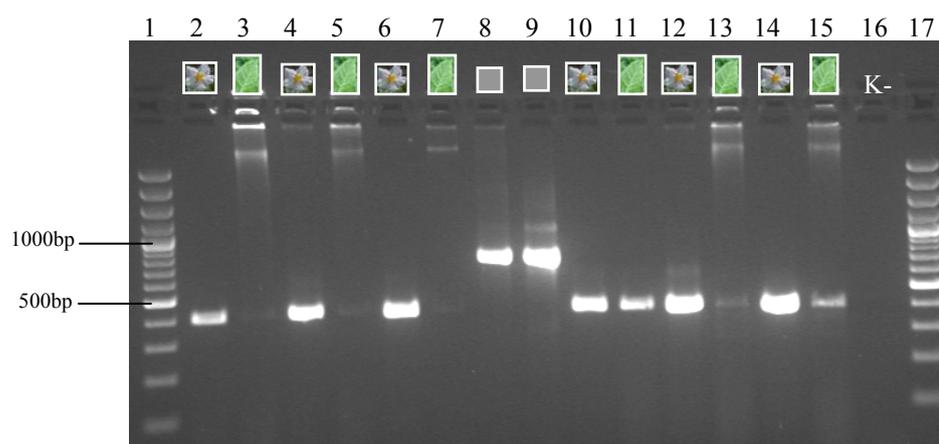


Figure 3.2.1: Amplification of a 402bp *invGF* fragment following the approach described by MADDISON ET AL. (1999). As template cDNA from leaves and flowers, and genomic DNA from leaves were used. 🌸 cDNA template from flowers, 🌿 cDNA template from leaves, 🟤 genomic DNA from leaves. Lane 1: Fermentas ladder 100bp plus, lane 2: floral cDNA ‘Saturna’, lane 3: leaf cDNA ‘Saturna’, lane 4: floral cDNA ‘Désirée’, lane 5, leaf cDNA ‘Désirée’, lane 6: floral cDNA P40, lane 7: leaf cDNA P40, lane 8: genomic DNA P40, lane 9: genomic DNA ‘Diana’, lane 10: floral cDNA ‘Diana’, lane 11: leaf cDNA ‘Diana’, lane 12: floral cDNA ‘Theresa’, lane 13: leaf cDNA ‘Theresa’, lane 14: floral cDNA P54, lane 15: leaf cDNA P54, lane 16: negative control for leaf cDNA, lane 17: Fermentas ladder 100bp plus.

To obtain full-length *invGF* alleles, PCR amplification using full-length gene specific primers was accomplished. cDNA and genomic DNA of flowers and leaves from the genotypes ‘Satina’, ‘Diana’, ‘Theresa’, P18, P40, and P54 served as template. Additionally, full-length PCR was carried out for cDNA and genomic DNA from flowers and leaves of the cultivar ‘Saturna’ (Figure 3.2.2).

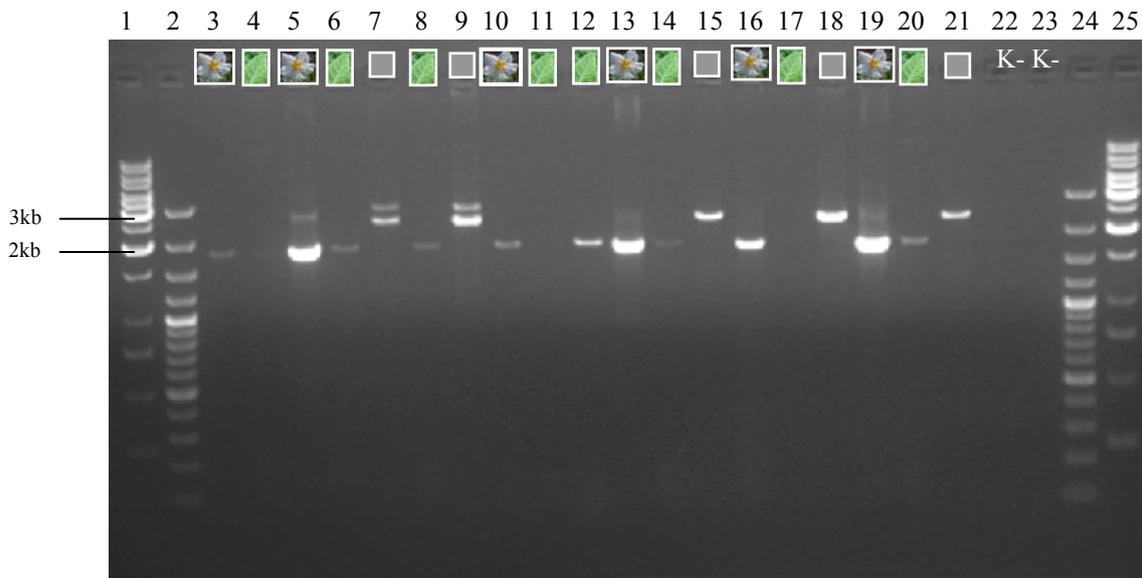


Figure 3.2.2: Full-length amplification of the gene *invGF* in different genotypes. As template cDNA from leaves and flowers, and genomic DNA from leaves were used. Full-length amplification was carried out using specific *invGF* primers (chapter 2, Table 2.1.2, *invGF*-F-fulgth/*invGF*-R-fulgth). A positive control was included using specific primers for the gene *pCD141* (chapter 2, Table 2.1.2, *CD141fl*_F/*CD141fl*_R) and cDNA from leaves to check cDNA integrity (lane 12). 🌸 cDNA template from flowers, 🌿 cDNA template from leaves, 📄 genomic DNA from leaves. Lane1: 1kb Fermentas ladder, lane 2: Fermentas ladder 100bp plus, lane 3: floral cDNA ‘Satina’, lane 4: leaf cDNA ‘Satina’, lane 5: floral cDNA ‘Diana’, lane 6: leaf cDNA ‘Diana’, lane 7: genomic DNA ‘Diana’, lane 8: leaf cDNA ‘Theresa’, lane 9: genomic DNA ‘Theresa’, lane 10: floral cDNA ‘Saturna’, lane 11: leaf cDNA ‘Saturna’, lane 12: leaf cDNA ‘Saturna’ with *pCD141* specific primers as positive control, lane 13: floral cDNA P18, lane 14: leaf cDNA P18, lane 15: genomic DNA P18, lane 16: floral cDNA P40, lane 17: leaf cDNA P40, lane 18: genomic DNA P40, lane 19: floral cDNA P54, lane 20: leaf cDNA P54; lane 21: genomic DNA P54, lane 22: negative control for leaf cDNA, lane 23: negative control for genomic DNA, lane 24: Fermentas ladder 100bp plus, lane 25: 1kb Fermentas ladder.

Both PCRs with short and full-length primers revealed genotype depending *invGF* expression. *invGF* transcripts were detected in leaves of the genotypes ‘Diana’, ‘Theresa’, P18, and P54. The gene specific expression in the genotypes ‘Diana’ and P54 were stronger compared to ‘Theresa’ and P18. The genotype ‘Satina’ showed a very weak *invGF* expression (Figure 3.2.2 lane 4), No *invGF* transcripts were detectable in ‘Désirée’ (Figure 3.2.1 lane 5), ‘Saturna’ (Figure 3.2.1 lane 3, Figure 3.2.2 lane 11), and P40 (Figure 3.2.1 lane 7, Figure 3.2.2 lane 17).

Cloning and sequencing of the entire RT-PCR products confirmed their identity to and, therefore, its origin from *invGF*.

❖ *invGE* and *invGF* cDNA alleles

Using full-length gene specific primers, cDNA invertase alleles of *invGE* and *invGF* were cloned and sequenced from the three tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’ and from three diploid potato genotypes P18, P40, and P54.

All tetraploid genotypes harbour the associated SSCP fragments of the genes *invGE* and *invGF* (LI ET AL., 2005; Table 3.2.1) and, therefore, were chosen in this study. The diploid genotypes were included because they were parents of the QTL analysis for cold sweetening of potato tubers (MENÉNDEZ ET AL., 2002).

Table 3.2.1: Distribution of the associated SSCP fragments *invGE-6f* and *invGF-4d* present in the genotypes ‘Satina’, ‘Diana’, and ‘Theresa’.

Genotype	<i>invGE-6f</i>	<i>invGF-4d</i>
‘Satina’	1	1
‘Diana’	1	1
‘Theresa’	1	1

1=SSCP fragment is present.

Out of 80 cloned cDNA sequences per genotype, a sequence was defined as an allele when it was found twice in two independent PCRs⁴. The consensus sequence of all alleles found in one genotype was then used for allele definition when variable sequence polymorphisms occurred.

invGE and *invGF* invertase alleles obtained from each genotype are listed in Table 3.2.2.

Table 3.2.2: Overview of *invGE* and *invGF* alleles.

Genotype	Full-length <i>invGE</i> clones	<i>invGE</i> alleles	Full-length <i>invGF</i> clones	<i>invGF</i> alleles
‘Satina’	10	<i>E_SA</i> <i>E_SN1</i> <i>E_SN2</i> <i>E_SN3</i>	14	<i>F_SN1</i> <i>F_SN2</i> <i>F_SN3</i> <i>F_SN4</i>
‘Diana’	8	<i>E_DA</i> <i>E_DN1</i> <i>E_DN2</i>	4	<i>F_DN1</i> <i>F_DN2</i>
‘Theresa’	19	<i>E_TA</i> <i>E_TN1</i> <i>E_TN2</i> <i>E_TN3</i>	4	<i>F_TN1</i> <i>F_TN2</i>
P18	9	<i>E_P18N1</i> <i>E_P18N2</i>	2	<i>F_P18N</i>
P40	8	<i>E_P40N1</i> <i>E_P40N2</i>	4	<i>F_P40N1</i> <i>F_P40N2</i>
P54	5	<i>E_P54N1</i> <i>E_P54N2</i>	10	<i>F_P54N1</i> <i>F_P54N2</i>

The ‘A’ in the allele name stands for ‘association with better potato chips quality’ and refers to clones containing SNP 1103* (Table 3.2.3), which leads to a histidine at protein position 368. The ‘N’ in the allele name means ‘not associated’ with tuber starch and sugar content. The full-length clone number refers to the number of fully sequenced clones from each genotype used for allele definition. PCR amplification was carried out using gene specific full-length primers: *invGE*-*invGE*-F-fulgth/*invGE*-R-fulgth (chapter 2, Table 2.1.2); *invGF*-*invGF*-F-fulgth/*invGF*-R-fulgth (chapter 2, Table 2.1.2).

From all six genotypes analyzed in this study, 17 *invGE* and 13 *invGF* alleles were identified.

⁴ Exceptions are listed in Appendix A 3.2.

3.2.1.2.1 *invGE* cDNA alleles of the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’⁵

Cloning and sequencing of ‘Satina’ cDNA resulted in ten full-length clones, from which four *invGE* alleles *E_SA*, *E_SNI*, *E_SN2*, and *E_SN3* were defined. From the genotype ‘Diana’ eight full-length clones were obtained, and of those three different alleles *E_DA*, *E_DNI*, and *E_DN2* were identified. For the cultivar ‘Theresa’ 19 full-length clones were isolated and four alleles *E_TA*, *E_TN1*, *E_TN2*, and *E_TN3* were defined.

The nucleotide sequence comparison (Appendix A 3.2.13) of the alleles described above detected 125 SNPs, which resulted in 43 amino acid exchanges (Table 3.2.3; Figure 3.2.3).

Table 3.2.3: SNPs present in ‘Satina’, ‘Diana’, and ‘Theresa’ alleles

Position cDNA SNP	<i>E</i> <i>SA</i>	<i>E</i> <i>SNI</i>	<i>E</i> <i>SN2</i>	<i>E</i> <i>SN3</i>	<i>E</i> <i>DA</i>	<i>E</i> <i>DNI</i>	<i>E</i> <i>DN2</i>	<i>E</i> <i>TA</i>	<i>E</i> <i>TN1</i>	<i>E</i> <i>TN2</i>	<i>E</i> <i>TN3</i>	aa
58	T	C	C	T	T	C	C	T	T	C	C	T/C F20L
85	A	G	G	A	A	G	G	A	G	G	G	A/G K29G
86	A	G	G	A	A	G	G	A	G	G	G	s.
95	G	C	C	C	G	C	C	C	C	C	C	G/C G32A
106	G	A	A	A	G	A	A	A	A	A	A	s.
108	T	T	A	T	T	T	A	T	T	T	T	A/T V36I
133	G	G	G	G	G	G	G	G	G	G	C	C/G P45A
135	T	T	T	A	T	T	T	A	T	T	T	s.
162	T	T	T	T	T	T	T	T	G	T	T	s.
163	G	G	G	A	G	G	G	A	G	G	G	A/G S55G
187	C	C	C	T	C	C	C	T	T	T	C	T/C Y63H
204	T	T	T	T	T	T	T	T	A	T	T	s.
231	A	A	A	A	A	A	A	A	G	A	A	s.
276	T	C	C	C	T	C	C	C	C	C	T	s.
345	C	T	T	T	C	T	T	T	T	T	T	s.
351	C	T	T	T	C	T	T	T	T	T	T	s.
390	C	A	A	A	C	A	A	A	A	A	A	s.
402	T	A	A	T	T	A	A	T	T	A	A	s.
411	G	A	A	A	G	G	A	A	A	G	A	s.
414	T	C	C	T	T	C	C	T	T	C	T	s.
415	G	G	A	G	G	G	A	G	G	G	G	A/G I139V
420	T	C	C	C	T	C	C	C	C	C	C	s.
429	T	C	C	C	T	C	C	C	C	C	C	s.

⁵ Alleles of each genotype were defined by alignment of all fully sequenced cDNA clones: ‘Satina’ (Appendix A 3.2.1), ‘Diana’ (Appendix A 3.2.2), ‘Theresa’ (Appendix A 3.2.3). The nucleotide sequence for one allele was defined by aligning all obtained nucleotide sequences for this allele and using the consensus sequence when SNPs occurred: *E_SA* (Appendix A 3.2.4), *E_SNI* (Appendix A 3.2.5), *E_SN3* (Appendix A 3.2.6), *E_DA* (Appendix A 3.2.7), *E_DNI* (Appendix A 3.2.8), *E_TA* (Appendix A 3.2.9), *E_TN1* (Appendix A 3.2.10), *E_TN2* (Appendix A 3.2.11), *E_TN3* (Appendix A 3.2.12). For the alleles *E_SN2* and *E_DN2* only one sequence was obtained, respectively. It has been shown that both alleles are real because allele specific SNPs were detected by pyrosequencing assay of cDNA and genomic DNA.

Position cDNA SNP	<i>E</i> <i>SA</i>	<i>E</i> <i>SN1</i>	<i>E</i> <i>SN2</i>	<i>E</i> <i>SN3</i>	<i>E</i> <i>DA</i>	<i>E</i> <i>DNI</i>	<i>E</i> <i>DN2</i>	<i>E</i> <i>TA</i>	<i>E</i> <i>TNI</i>	<i>E</i> <i>TN2</i>	<i>E</i> <i>TN3</i>	aa
444	T	C	C	C	T	C	C	C	C	C	C	s.
451	A	T	T	T	A	T	T	T	T	T	T	T/C T151S
456	A	A	A	G	A	A	A	G	G	A	A	s.
462	A	A	A	G	A	A	A	G	G	A	A	s.
465	C	T	T	T	C	T	T	T	T	T	T	s.
471	C	A	G	A	C	A	A	A	A	A	A	s.
477	A	G	G	G	A	G	G	G	A	G	G	s.
495	G	A	A	A	G	A	A	A	A	A	A	s.
511	G	A	A	A	G	A	A	A	A	A	A	G/A V171I
519	C	T	T	T	C	T	T	T	T	T	T	s.
531	G	G	A	G	G	G	A	G	G	G	G	s.
538	A	G	G	G	A	G	G	G	G	G	G	A/G I180V
540	T	T	A	A	T	A	A	A	T	A	T	s.
576	T	T	T	T	T	T	T	T	T	T	C	s.
588	T	A	A	T	T	A	A	T	T	A	A	s.
597	T	C	C	C	T	C	C	C	C	C	C	s.
598	G	C	C	C	G	C	C	C	C	C	C	s.
599	T	A	A	A	T	A	A	A	A	A	A	T/A V200Q
607	G	C	C	C	G	C	C	C	C	C	C	G/C V203L
615	G	A	A	A	G	A	A	A	A	A	A	s.
639	A	A	C	A	A	A	C	A	A	A	A	C/A N213K
645	A	A	A	A	A	A	A	A	T	A	A	T/A S215R
658	T	T	T	T	T	T	G	T	T	T	T	G/T V220L
688	G	G	A	G	G	G	A	G	G	G	G	A/G T230A/ V
689	T	C	C	C	T	C	C	C	C	C	C	T/C V230A/ T
702	C	T	T	T	C	T	T	T	T	T	T	s.
753	C	C	C	T	C	T	C	T	T	T	T	s.
768	T	A	A	A	T	A	A	A	A	A	A	T/A N256K
774	T	T	T	T	T	A	T	T	T	A	T	s.
780	T	C	C	C	T	C	C	C	C	C	C	s.
807	---	A	A	---	---	---	A	---	---	---	---	K269
808	---	A	A	---	---	---	A	---	---	---	---	K269
809	---	A	A	---	---	---	A	---	---	---	---	K269
810*	T	A	T	T	T	T	T	T	T	T	T	A/T K270N
817*	T	C	C	C	T	T	C	T	C	T	T	C/T H273Y
855*	T	G	T	G	T	G	G	T	T	G	G	T/G D285E
858*	C	T	T	T	C	T	T	C	T	T	T	s.
864*	T	T	T	T	T	T	A	T	T	T	T	s.
870*	C	T	T	T	C	T	T	C	T	T	T	s.
884*	G	A	A	A	G	A	A	G	A	A	A	G/A R295K

Position cDNA SNP	<i>E</i> <i>SA</i>	<i>E</i> <i>SN1</i>	<i>E</i> <i>SN2</i>	<i>E</i> <i>SN3</i>	<i>E</i> <i>DA</i>	<i>E</i> <i>DN1</i>	<i>E</i> <i>DN2</i>	<i>E</i> <i>TA</i>	<i>E</i> <i>TN1</i>	<i>E</i> <i>TN2</i>	<i>E</i> <i>TN3</i>	aa
909*	C	C	C	C	C	C	C	C	C	C	T	s.
924*	T	T	T	T	T	T	C	T	T	T	T	s.
926*	G	C	C	C	G	C	C	G	C	C	C	s.
927*	T	G	G	G	T	G	G	T	G	G	G	T/G C309S
945*	T	C	C	C	T	C	C	T	C	C	C	s.
975*	T	C	C	T	T	C	C	T	T	C	C	s.
981*	T	C	C	C	T	C	C	T	C	C	C	s.
983*	C	C	C	C	C	C	T	C	C	C	C	T/C L328P
986*	C	T	T	T	C	T	T	C	T	T	T	s.
987*	T	G	G	G	T	G	G	T	G	G	G	T/G T329M
1005*	G	A	A	A	G	A	A	G	A	A	A	s.
1010*	G	G	G	G	G	G	G	G	C	G	G	C/G A337G
1016*	C	C	C	C	C	C	C	C	T	C	C	T/C I340T
1017*	C	A	A	A	C	A	A	C	A	A	A	s.
1065*	T	T	T	A	T	T	T	T	A	T	T	s.
1068*	A	T	A	A	A	A	A	A	A	A	A	s.
1083*	G	G	G	A	G	G	G	G	A	G	G	s.
1086*	A	T	T	T	A	T	T	A	T	T	T	s.
1101*	T	C	C	C	T	C	C	T	C	C	C	s.
1103*	A	C	C	C	A	C	C	A	C	C	C	A/C H368P
1110*	T	T	T	C	T	T	T	T	T	T	T	s.
1117*	T	T	T	T	T	C	T	T	T	C	T	s.
1149*	C	C	A	A	C	A	A	C	A	A	A	s.
1152*	A	G	A	A	A	A	A	A	A	A	A	s.
1158*	A	G	G	G	A	G	G	A	G	G	G	s.
1167*	C	T	T	T	C	T	T	C	T	T	T	s.
1168*	---	---	A	---	---	A	A	---	---	A	---	I389
1169*	---	---	T	---	---	T	T	---	---	T	---	I389
1170*	---	---	T	---	---	T	T	---	---	T	---	I389
1179**	T	C	C	C	T	C	C	T	C	C	C	s.
1182**	C	C	C	C	C	C	C	C	C	C	G	G/C K394N
1191**	G	G	G	G	G	G	G	G	A	G	G	s.
1216**	A	A	A	G	A	A	A	A	G	A	A	G/A E406K
1237**	T	G	G	G	T	G	G	T	G	G	G	T/G S413A
1254**	A	G	G	G	A	G	G	A	G	G	G	s.
1257**	T	C	C	C	T	C	C	T	C	C	C	s.
1272**	G	G	A	G	G	G	A	G	G	G	G	s.
1276**	A	G	A	A	A	A	A	A	A	A	A	G/A E426K
1278**	A	G	G	G	A	G	G	A	G	G	G	s.
1281**	T	C	C	C	T	C	C	T	C	C	C	s.
1299**	T	A	A	A	T	A	A	T	A	A	A	T/A N433K
1305**	T	C	C	T	T	C	C	T	C	C	C	s.
1332**	C	C	C	C	C	C	T	C	C	C	C	s.
1335**	A	T	A	T	A	A	A	A	T	A	A	s.
1365**	A	G	A	A	A	A	G	A	A	A	A	s.

Position cDNA SNP	<i>E</i> <i>SA</i>	<i>E</i> <i>SN1</i>	<i>E</i> <i>SN2</i>	<i>E</i> <i>SN3</i>	<i>E</i> <i>DA</i>	<i>E</i> <i>DN1</i>	<i>E</i> <i>DN2</i>	<i>E</i> <i>TA</i>	<i>E</i> <i>TN1</i>	<i>E</i> <i>TN2</i>	<i>E</i> <i>TN3</i>	aa
1379**	C	C	T	C	C	T	C	C	C	T	T	T/C V460A
1380**	A	G	G	G	A	G	G	A	G	G	G	
1384**	T	T	T	G	T	T	T	T	T	T	T	G/T V462L
1395**	A	A	A	G	A	A	A	A	G	A	A	s.
1410**	T	C	C	C	T	C	C	T	C	C	C	s.
1413**	A	A	A	A	A	A	G	A	A	A	A	s.
1427**	G	G	G	G	G	G	G	G	G	G	A	A/G Q476R
1456**	G	A	G	G	G	G	G	G	G	G	G	A/G I486V
1476**	T	T	T	T	T	T	T	T	T	T	G	s.
1556**	T	T	C	T	T	C	C	T	T	C	T	C/T T519M
1617**	A	T	T	T	A	T	T	A	T	T	T	s.
1653**	A	G	G	G	A	G	G	A	G	G	G	s.
1659**	A	G	G	G	A	G	G	A	G	G	G	s.
1665**	T	T	T	T	T	C	C	T	T	T	C	s.
1666**	G	A	A	A	G	A	A	G	A	A	A	G/A D556N
1692**	T	C	T	T	T	T	T	T	T	T	T	s.
1723**	C	C	C	C	C	C	C	C	A	C	C	A/C I575L

SNP position numbering refers to cDNA sequence where ‘1’ represents the adenine of the start codon ATG and the polymorphism is described. * and **: SNP positions changed because of amino acid insertions, numbers refer to alignment nomenclature and not to the SNP positions of the cDNA for standardized comparison. aa=amino acid exchanges ; s.=synonymous nucleotide exchange that does not lead to an amino acid exchange. Non synonymous nucleotide exchanges lead to an amino acid exchange and are coloured in orange.

The four ‘Satina’ alleles displayed a total of 95 SNPs, of which 30 caused an amino acid exchange. In the three allelic sequences of ‘Diana’ 87 SNPs occurred, from which 26 resulted in an amino acid exchange. The four ‘Theresa’ alleles differed in 70 SNPs. 22 of them led to an amino acid exchanges. The alleles *E_SN1*, *E_SN2*, and *E_DN2* contain the additional amino acid lysine (K) at position 259. An amino acid insertion also occurs at position 390* where the alleles *E_SN2*, *E_DN1*, *E_DN2*, and *E_TN2* contain an additional isoleucine (I).

The amino acid alignment shows the polymorphisms between all 11 *invGE* alleles from the three tetraploid potato cultivars. The comparison of these deduced protein sequences revealed 42 variable amino acid positions in the different genotypes, which are highlighted in colour (Figure 3.2.3).

Figure 3.2.3: Amino acid alignment of ‘Satina’, ‘Diana’, and ‘Theresa’ *invGE* alleles. Amino acid exchanges are shown in colour. Additional amino acids are coloured in grey bars. At protein position 230 three different amino acids are displayed.

The amino acid alignment of *invGE* alleles of the tetraploid genotypes showed that ‘Satina’ and ‘Diana’ contain an allele identical at amino acid level ($E_SA=E_DA$).

3.2.1.2.2 *invGE* cDNA alleles of the diploid potato genotypes P18, P40, and P54⁶

From the genotype P18 nine full-length clones were obtained, and of those two alleles E_P18N1 and E_P18N2 were defined. Cloning and sequencing of P40 cDNA resulted in eight full-length clones, from which two different alleles E_P40N1 and E_P40N2 were identified. From the genotype P54 five full-length clones were isolated, and two alleles E_P54N1 and E_P54N2 were determined.

The alleles contain at nucleotide level (Appendix A 3.2.23) 48 SNPs. These sequence polymorphisms caused 23 amino acid exchanges (Table 3.2.4; Figure 3.2.4).

Table 3.2.4: SNPs present in P18, P40, and P54 alleles.

Position cDNA SNP	E_P18N1	E_P18N2	E_P40N1	E_P40N2	E_P54N1	E_P54N2	aa
58	C	C	C	T	C	T	T/C F20L
83	A	A	A	A	A	T	T/A I28N
85	G	G	G	A	G	A	A/G K29G
86	G	G	G	A	G	A	A/G K29G
108	A	T	A	T	T	T	s.
187	C	T	C	C	C	A	T/A/C Y63N/H
255	T	T	T	C	T	T	s.
402	A	A	A	T	A	T	s.
411	A	G	A	A	G	A	s.
414	C	C	C	T	C	T	s.
415	A	G	A	G	G	G	A/G I139V
426	C	C	C	T	C	C	s.
456	A	A	A	G	A	G	s.
462	A	A	A	G	A	G	s.
531	A	G	A	G	G	G	s.
540	A	A	A	T	A	T	s.
565	A	A	A	G	A	G	G/A E189K
619	G	G	G	A	G	G	A/G I207V
639	C	A	C	A	A	A	C/A N213K

⁶ Alleles of each genotype were defined by alignment of all fully sequenced cDNA clones: P18 (Appendix A 3.2.14), P40 (Appendix A 3.2.15), P54 (Appendix A 3.2.16). The nucleotide sequence for one allele was defined by aligning all obtained nucleotide sequences for this allele and using the consensus sequence when SNPs occurred: E_P18N1 (Appendix A 3.2.17), E_P18N2 (Appendix A 3.2.18), E_P40N1 (Appendix A 3.2.19), E_P40N2 (Appendix A 3.2.20), E_P54N1 (Appendix A 3.2.21), E_P54N2 (Appendix A 3.2.22).

Position cDNA SNP	<i>E_P18N1</i>	<i>E_P18N2</i>	<i>E_P40N1</i>	<i>E_P40N2</i>	<i>E_P54N1</i>	<i>E_P54N2</i>	aa
645	A	A	A	A	A	G	s.
688	A	G	A	G	G	G	A/G T230A
753	C	T	C	T	T	C	s.
774	T	A	T	T	A	T	s.
807	A	---	A	---	---	---	K259
808	A	---	A	---	---	---	K259
809	A	---	A	---	---	---	K259
817*	C	T	C	C	T	C	T/C Y273H
855*	G	G	G	T	G	T	T/G D285E
975*	C	C	C	T	C	T	s.
1031*	T	T	T	T	T	G	G/T G344V
1101*	C	C	C	T	C	C	s.
1117*	T	C	T	T	C	T	s.
1168*	A	A	A	---	A	---	I389
1169*	T	T	T	---	T	---	I389
1170*	T	T	T	---	T	---	I389
1216**	A	A	A	G	A	G	G/A E406K
1272**	A	G	A	G	G	G	s.
1335**	A	A	A	T	A	T	s.
1379**	T	T	T	C	T	C	C/T A460V
1395**	A	A	A	G	A	G	s.
1462**	A	A	A	C	A	A	s.
1464**	G	G	G	A	G	G	A/G L488M
1471**	G	G	G	G	G	A	A/G N491D
1556**	C	C	C	T	C	T	T/C M519T
1616**	G	G	G	A	G	G	A/G D539G
1652**	C	C	C	C	C	T	T/C M551T
1665**	T	T	T	T	C	T	s.
1712**	C	C	C	T	C	C	T/C I561T

SNP position numbering refers to cDNA sequence where '1' represents the adenine of the start codon ATG and the polymorphism is described. * and **: SNP positions changed because of amino acid insertions, numbers refer to alignment nomenclature and not to the SNP positions of the cDNA for standardized comparison. aa=amino acid exchanges ; s.=synonymous nucleotide exchange that does not lead to an amino acid exchange. Non synonymous nucleotide exchanges lead to an amino acid exchange and are coloured in orange.

In the two allelic sequences of P18 12 SNPs occurred, from which five resulted in an amino acid exchange. The two P40 alleles differed in 30 SNPs. 14 of them led to changes in the protein sequence. From 26 SNPs of the two P54 alleles, 13 caused an amino acid substitution. The alleles *E_P18N1* and *E_P40N1* contain the additional amino acid lysine (K) at position 259. An amino acid insertion also occurs at position 390*, where the alleles *E_P18N1*, *E_P18N2*, *E_P40N1*, and *E_P54N1* contain an additional isoleucine (I).

The amino acid alignment displays the differences of the six cloned *invGE* alleles from the three diploid potato genotypes (Figure 3.2.4). The comparison of the protein sequences revealed 22 variable amino acid positions.

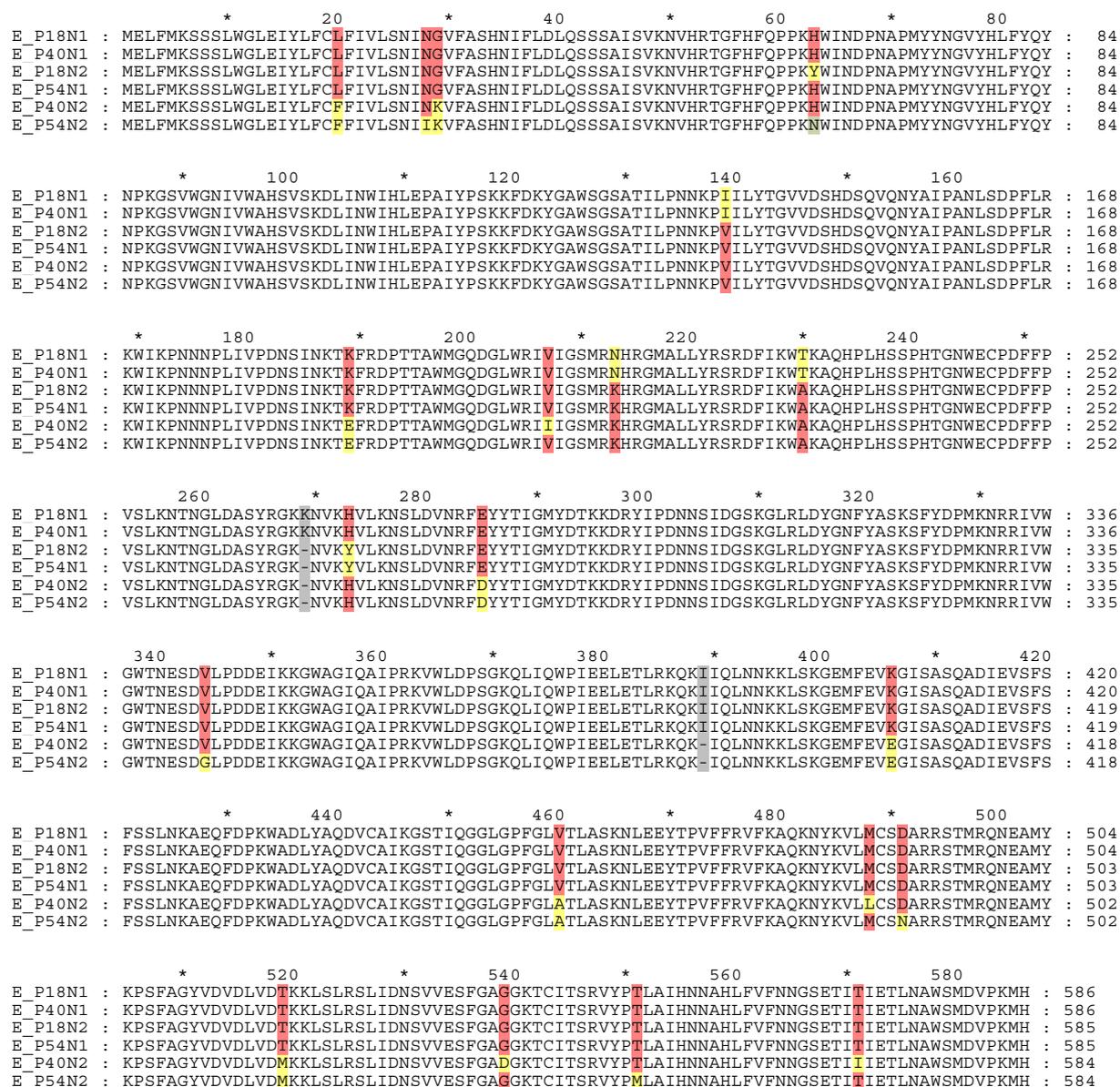


Figure 3.2.4: Amino acid alignment of P18, P40, and P54 *invGE* alleles. Amino acid exchanges are shown in colour. Additional amino acids are coloured in grey bars. At protein position 63 three different amino acids are displayed.

3.2.1.2.3 Amino acid alignment of all *invGE* invertase alleles of the analyzed potato genotypes

Multiple alignment of allelic *invGE* protein sequences revealed 53 variable amino acid polymorphisms between six genotypes (Figure 3.2.5). 21 amino acid exchanges were found to be genotype specific occurring only once.


```

*          440          *          460          *          480          *          500
E_DN1      : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLVTLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 503
E_P54N1    : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLVTLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 503
E_TN2     : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLVTLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 503
E_P18N2   : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLVTLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 503
E_TN3     : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLVTLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 502
E_SN2     : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLVTLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 504
E_P18N1   : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLVTLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 504
E_P40N1   : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLVTLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 504
E_DN2     : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 504
E_SN1     : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 503
E_SN3     : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 502
E_TN1     : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 502
E_P40N2   : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 502
E_P54N2   : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 502
E_SA      : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 502
E_DA      : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 502
E_TA      : FSSLNKAEQFDPKWADLYAQDVCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLMLCSDARRSTMRQNEAMY : 502

*          520          *          540          *          560          *          580
E_DN1      : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 585
E_P54N1    : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 585
E_TN2     : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 585
E_P18N2   : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 585
E_TN3     : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 584
E_SN2     : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 586
E_P18N1   : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 586
E_P40N1   : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 586
E_DN2     : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 586
E_SN1     : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 585
E_SN3     : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 584
E_TN1     : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 584
E_P40N2   : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 584
E_P54N2   : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 584
E_SA      : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 584
E_DA      : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 584
E_TA      : KPSFAGYVDVLDVTKKLSLRSLIDNSVVESFGAGGKTCITSRVYPTLAIHNNNAHLFVFNNGSETITTIETLNAWSMDVPMKH : 584

```

Figure 3.2.5: Amino acid alignment of all cloned *invGE* alleles of all genotypes. Amino acid exchanges are shown in colour. Additional amino acids are coloured in grey bars. At protein positions 63 and 230 three different amino acids are displayed.

Comparison of allelic cDNA sequences of the *invGE* gene from the three tetraploid and the three diploid genotypes revealed that different genotypes harbour amino acid sequence identical alleles. The alleles *E_SN2*, *E_P18N1*, and *E_P40N1*, the alleles *E_DN1* and *E_P54N1*, and the alleles *E_TN2* and *E_P18N2* have the same amino acid sequences.

3.2.1.2.4 Phenetic trees of all *invGE* invertase alleles of the analyzed potato genotypes

In addition to the multiple amino acid alignment (3.2.1.2.3), the phenetic tree analysis was applied to group the invertase alleles according to their similarity at amino acid as well as at nucleotide level. Using the neighbour-joining method, the allelic classification visualized that *invGE* alleles from all six genotypes group in two clades and multiple subclades (Figure 3.2.6, Figure 3.2.7).

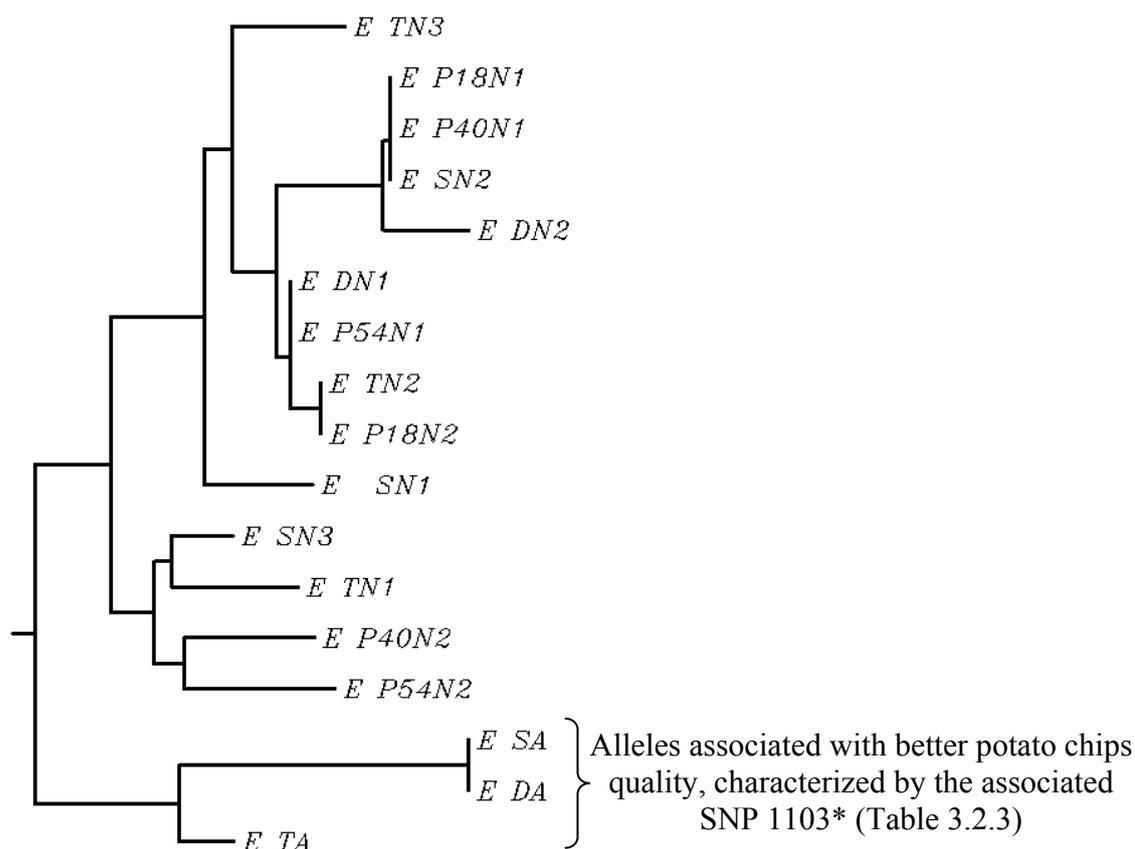


Figure 3.2.6: Amino acid based phenetic tree (Neighbour-joining method) of all cloned *invGE* invertase alleles.

The amino acid based phenetic tree has two clades. One clade contains most of the *invGE* alleles, and the other one the alleles *E_SA*, *E_DA*, and *E_TA*, which were found to be associated with superior potato chips quality.

The first clade then splits into diverse subclades due to the high number of amino acid polymorphisms between the different genotypes, although different genotypes contain amino acid sequence identical alleles. The alleles *E_SN2*, *E_P18N1*, and *E_P40N1*, the alleles *E_DN1* and *E_P54N1*, and the alleles *E_TN2* and *E_P18N2* have the same amino acid sequences.

The second clade consists of the alleles *E_SA*, *E_DA*, and *E_TA*. The two alleles *E_SA* and *E_DA* are identical at amino acid level, whilst *E_TA* differs in its amino acid sequence compared to the other two. However, all three alleles are characterized by the nucleotide adenine at cDNA SNP 1103* (Table 3.2.3), which is associated with better potato chips quality.

Cloning and sequencing of *invGE* alleles showed that different genotypes contain alleles identical at amino acid level but different regarding their nucleotide sequence. The allelic nucleotide composition was defined based on the consensus sequence of multiple alignments

of full-length clones obtained from each genotype (Table 3.2.2). Although SNPs are present at the mentioned cDNA positions, the nucleotide polymorphisms resulted in one and the same amino acid sequence.

Nucleotide comparison (Appendix A 3.2.24) showed that the associated alleles *E_SA* and *E_DA* consist of an identical nucleotide sequence as well as the alleles *E_TN2* and *E_P18N2*. The nucleotide sequences of the alleles and *E_P18N1* and *E_P40N1* are also identical, whilst the sequence of *E_SN2* differs at position 471. At position 471 a synonymous nucleotide exchange from A in the alleles *E_P18N1* and *E_P40N1* to G in allele *E_SN2* arose. Also one synonymous nucleotide variation was detected between the alleles *E_DN1* and *E_P54N1*. Allele *E_DN1* contains nucleotide A and allele *E_P54N1* consists of nucleotide G at position 1408**. Allele specific SNPs were detected in at least two sequences and used for comparing the allelic nucleotide polymorphisms.

The phenetic tree analysis showed the nucleotide polymorphisms between all alleles of the six different genotypes (Figure 3.2.7).

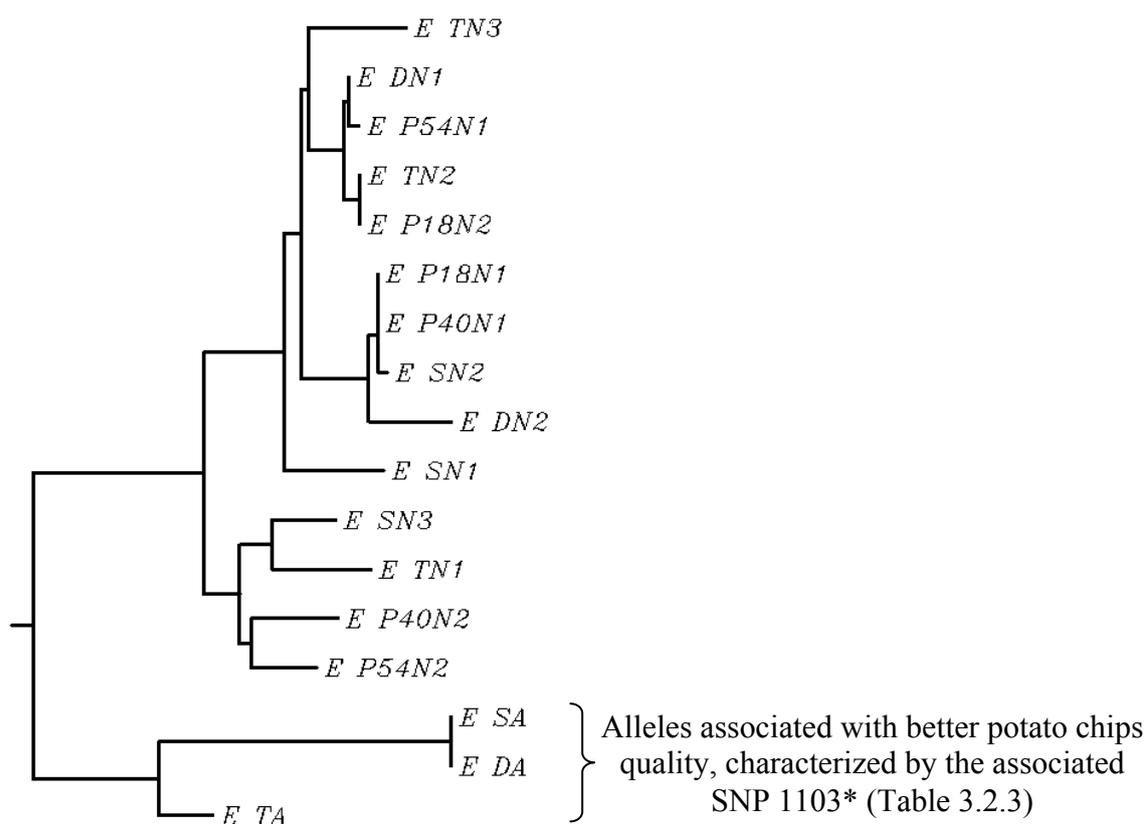


Figure 3.2.7: Nucleotide sequence based phenetic tree (Neighbour-joining tree) of all cloned *invGE* invertase alleles.

Although being very similar, the nucleotide based tree is characterized by an extended number of subclades due to a higher number of nucleotide polymorphisms in contrast to the amino acid based phenetic tree (Figure 3.2.6).

3.2.1.2.5 *invGF* cDNA alleles of the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’⁷

From the genotype ‘Satina’ 14 full-length clones were obtained, and of those four different alleles *F_SN1*, *F_SN2*, *F_SN3*, and *F_SN4* were identified. For ‘Diana’ four full-length clones were isolated, and two alleles *F_DN1* and *F_DN2* were determined. Cloning and sequencing of ‘Theresa’ cDNA resulted in four full-length clones, from which two *invGF* alleles *F_TN1* and *F_TN2* were defined.

The nucleotide comparison (Appendix A 3.2.35) of the alleles described above detected 86 SNPs, of which 19 were non synonymous and caused amino acid exchanges (Table 3.2.5; Figure 3.2.8).

Table 3.2.5: SNPs present in ‘Satina’, ‘Diana’, and ‘Theresa’ alleles.

Position cDNA SNP	<i>F_SN1</i>	<i>F_SN2</i>	<i>F_SN3</i>	<i>F_SN4</i>	<i>F_DN1</i>	<i>F_DN2</i>	<i>F_TN1</i>	<i>F_TN2</i>	aa
28	G	G	G	G	A	G	G	G	A/G T10A
58	A	A	A	G	A	A	G	A	G/A V20I
96	C	C	C	T	T	C	T	C	s.
111	T	C	C	C	C	C	C	C	s.
117	G	A	A	A	A	A	A	A	s.
141	A	C	A	C	C	A	C	A	A/C Q47H
162	C	C	C	T	T	C	T	C	s.
223	G	A	G	G	G	G	G	G	A/G I75V
225	C	T	T	C	C	C	C	C	s.
228	C	C	C	T	T	T	T	T	s.
249	C	C	C	C	C	T	C	T	s.
255	T	C	C	C	C	C	C	C	s.
273	T	C	T	C	C	T	C	T	s.
279	T	T	T	T	T	G	T	G	s.
345	C	T	T	T	T	C	T	C	s.
351	C	A	A	A	A	A	A	A	s.
363	C	C	C	T	T	C	T	C	s.
369	G	G	G	A	A	G	A	G	s.
378	A	A	G	A	A	A	A	A	s.

⁷ Alleles of each genotype were defined by alignment of all fully sequenced cDNA clones: ‘Satina’ (Appendix A 3.2.25), ‘Diana’ (Appendix A 3.2.26), ‘Theresa’ (Appendix A 3.2.27). The nucleotide sequence for one allele was defined by aligning all obtained nucleotide sequences for this allele and using the consensus sequence when SNPs occurred: *F_SN1* (Appendix A 3.2.28), *F_SN2* (Appendix A 3.2.29), *F_SN3* (Appendix A 3.2.30), *F_SN4* (Appendix A 3.2.31), *F_DN2* (Appendix A 3.2.32), *F_TN1* (Appendix A 3.2.33), *F_TN2* (Appendix A 3.2.34). For the allele *F_DN1* two nucleotide sequences were obtained, from which one showed an alternative stop codon leading to no termination of the sequence. Therefore, the nucleotide alignment of both sequences is not shown. *F_DN1* specific SNPs were shown to be real by pyrosequencing analysis of cDNA and genomic DNA.

Position cDNA SNP	F_SN1	F_SN2	F_SN3	F_SN4	F_DN1	F_DN2	F_TN1	F_TN2	aa
381	T	T	T	A	A	T	A	T	s.
405	G	G	G	A	A	G	A	G	s.
429	A	A	A	A	A	T	A	A	s.
432	A	A	A	G	G	A	G	A	s.
438	C	C	C	T	T	C	T	C	s.
459	C	T	C	C	C	C	C	C	s.
466	A	A	A	G	G	A	G	G	A/G I156V
478	T	G	G	A	A	T	A	T	T/G/A L160V/I
489	A	T	T	T	T	A	T	A	s.
491	T	T	T	T	T	T	T	C	C/T H164Y
495	C	C	C	A	A	T	A	T	s.
519	C	C	T	C	C	C	C	T	s.
542	C	A	A	C	C	C	C	C	A/C D181A
546	C	T	T	C	C	T	C	T	s.
555	G	A	G	G	G	G	G	G	s.
579	C	T	T	C	C	C	C	C	s.
582	A	A	A	T	T	A	T	A	s.
630	G	A	A	A	G	A	A	A	s.
651	T	A	A	T	T	T	T	T	s.
663	A	A	A	A	A	A	A	G	s.
672	T	T	T	C	C	T	C	T	s.
675	C	C	T	C	C	C	C	C	s.
717	T	T	T	C	C	T	C	C	s.
729	C	T	T	T	T	T	T	T	s.
750	C	T	T	C	C	C	C	C	s.
753	T	T	T	C	C	T	C	T	s.
778	A	A	A	C	C	A	C	A	s.
779	T	T	T	A	A	T	A	T	T/A I260Q
799	T	T	T	C	C	T	C	T	s.
801	T	T	T	C	C	T	C	T	C/T H267Y
805	T	T	T	T	T	T	T	A	A/T N269Y
820	A	A	A	A	A	A	A	G	G/A G274S
864	C	T	C	C	C	C	C	C	s.
876	G	A	A	A	A	A	A	A	s.
885	T	T	T	C	C	T	C	C	s.
886	G	G	G	A	A	G	A	G	A/G I296V
888	T	T	T	A	A	T	A	A	s.
903	T	T	C	T	T	T	T	T	s.
933	T	T	T	C	C	T	C	T	s.
958	A	A	A	T	T	A	T	A	T/A S320T
970	A	A	A	C	C	A	C	A	C/A P324T
981	C	C	C	T	T	C	T	C	s.
1029	G	A	A	G	G	G	G	G	s.
1038	T	T	T	C	C	T	C	T	s.
1041	G	G	G	T	T	G	T	G	s.
1047	A	A	A	A	A	A	A	G	s.

Position cDNA SNP	<i>F_SN1</i>	<i>F_SN2</i>	<i>F_SN3</i>	<i>F_SN4</i>	<i>F_DN1</i>	<i>F_DN2</i>	<i>F_TN1</i>	<i>F_TN2</i>	aa
1089	C	C	C	T	T	T	T	C	s.
1108	G	G	G	A	A	A	A	A	G/A V370I
1113	G	G	A	A	A	A	A	A	s.
1164	T	T	T	C	C	T	C	T	s.
1170	A	A	A	G	G	A	G	G	s.
1173	A	A	A	G	G	G	G	G	s.
1206	A	A	A	G	G	A	G	G	s.
1263	A	A	A	G	G	A	G	G	s.
1271	C	C	C	C	C	C	C	T	T/C L424S
1279	T	T	T	C	C	C	C	T	T/C S427P
1320	C	C	C	C	C	T	C	C	s.
1323	G	G	G	A	A	G	A	G	s.
1335	A	A	A	T	T	T	T	A	s.
1452	T	T	T	C	C	T	C	C	s.
1458	C	C	C	T	T	C	T	C	s.
1536	A	A	A	A	A	T	A	A	s.
1623	G	G	G	G	G	A	G	G	s.
1636	T	T	T	T	T	C	T	T	s.
1638	G	G	G	G	G	G	G	A	s.
1650	G	G	G	C	C	C	C	G	G/C E550D
1745	G	G	G	G	G	G	G	A	s.

SNP position numbering refers to cDNA sequence where ‘1’ represents the adenine of the start codon ATG and the polymorphism is described. aa=amino acid exchanges ; s.=synonymous nucleotide exchange that does not lead to an amino acid exchange. Non synonymous nucleotide exchanges lead to an amino acid exchange and are coloured in orange.

The four ‘Satina’ alleles differed in 70 SNPs, 14 of those led to amino acid exchanges. In the two allelic sequences of ‘Diana’ 47 SNPs were found, from which nine resulted in an amino acid substitution. The two alleles of ‘Theresa’ had a total of 47 SNPs, and 14 of those resulted in an amino acid difference.

The amino acid alignment shows the polymorphisms of all eight *invGF* alleles from the three tetraploid potato cultivars. The comparison of deduced protein sequences revealed 19 variable amino acid positions in the different genotypes displayed in coloured columns (Figure 3.2.8).

3.2.1.2.6 *invGF* cDNA alleles of the diploid potato genotypes P18, P40, and P54⁸

From P18 two full-length clones were isolated, and one allele *F_P18N* was determined. 20 partial sequenced P18 clones showed identical sequences to *F_P18N* but exhibited frame shifts or other modifications and, therefore, were not completely sequenced. From the genotype P40 four full-length clones were obtained, and of those two different alleles *F_P40N1* and *F_P40N2* were identified. Cloning and sequencing of P54 cDNA resulted in ten full-length clones, from which two *invGF* alleles *F_P54N1* and *F_P54N2* were defined.

31 SNPs for all alleles of the diploid genotypes were detected by nucleotide sequence comparison (Appendix A 3.2.43). Six of the identified polymorphisms caused an amino acid exchange (Table 3.2.6; Figure 3.2.9).

Table 3.2.6: SNPs present in P18, P40, and P54 alleles.

Position cDNA SNP	<i>F_P18N</i>	<i>F_P40N1</i>	<i>F_P40N2</i>	<i>F_P54N1</i>	<i>F_P54N2</i>	aa
111	T	T	C	T	T	s.
223	G	G	A	G	G	s.
225	C	C	T	C	C	T/C I75V
255	T	T	C	T	T	s.
351	C	C	A	C	C	s.
363	C	C	T	C	C	s.
542	C	C	A	C	C	A/C D181A
546	C	C	T	C	C	s.
579	C	C	T	C	C	s.
630	G	G	A	G	G	s.
651	T	T	A	T	T	s.
717	T	T	C	T	T	s.
729	C	C	T	C	C	s.
750	C	C	T	C	C	s.
791	G	G	A	G	G	A/G D264G
942	T	T	C	T	T	s.
1089	T	T	C	T	T	s.
1090	C	C	G	C	C	G/C A364P
1206	A	A	G	A	A	s.
1224	A	G	A	A	G	s.
1238	C	T	C	C	T	T/C I413T
1242	T	T	C	T	T	s.
1263	G	A	A	G	A	s.
1320	C	T	C	C	T	s.
1386	G	A	A	G	A	s.
1446	T	C	C	T	C	s.
1452	C	C	T	C	C	s.
1534	G	G	A	G	G	s.
1536	T	T	A	T	T	A/T T512A
1668	G	A	G	G	A	s.
1677	T	C	C	T	C	s.

⁸ Alleles of each genotype were defined by alignment of all fully sequenced cDNA clones: P18 (Appendix A 3.2.36), P40 (Appendix A 3.2.37), P54 (Appendix A 3.2.38). The nucleotide sequence for one allele was defined by aligning all obtained nucleotide sequences for this allele and using the consensus sequence when SNPs occurred: *F_P18N* (Appendix A 3.2.39), *F_P40N2* (Appendix A 3.2.40), *F_P54N1* (Appendix A 3.2.41), *F_P54N2* (Appendix A 3.2.42).

SNP position numbering refers to cDNA sequence where '1' represents the adenine of the start codon ATG and the polymorphism is described. aa=amino acid exchanges ; s.=synonymous nucleotide exchange that does not lead to an amino acid exchange. Non synonymous nucleotide exchanges lead to an amino acid exchange and are coloured in orange.

In the two allelic sequences of P40 27 SNPs occurred, six of those resulted in an amino acid exchange. The two P54 alleles differed in eight SNPs, and of those one led to an exchange in the amino acid sequence.

The amino acid alignment of the five *invGF* alleles from the three diploid potato genotypes showed the variability of six positions where amino acids differed (Figure 3.2.9).

```

F_P40N1 : MDYSSNSRWALPVILVCFEIVLLSNNVVFASHKVF IHLQSQNAVNVQTVHRTGYHFQPEKHWINDPNAPMYFNGV YHLFYQYNP : 84
F_P54N2 : MDYSSNSRWALPVILVCFEIVLLSNNVVFASHKVF IHLQSQNAVNVQTVHRTGYHFQPEKHWINDPNAPMYFNGV YHLFYQYNP : 84
F_P18N : MDYSSNSRWALPVILVCFEIVLLSNNVVFASHKVF IHLQSQNAVNVQTVHRTGYHFQPEKHWINDPNAPMYFNGV YHLFYQYNP : 84
F_P54N1 : MDYSSNSRWALPVILVCFEIVLLSNNVVFASHKVF IHLQSQNAVNVQTVHRTGYHFQPEKHWINDPNAPMYFNGV YHLFYQYNP : 84
F_P40N2 : MDYSSNSRWALPVILVCFEIVLLSNNVVFASHKVF IHLQSQNAVNVQTVHRTGYHFQPEKHWINDPNAPMYFNGV YHLFYQYNP : 84

F_P40N1 : NGSVWGNIVWAHSVSKDLINWINLEPAIYPSKPFQDQFTWGSATILPGNKPVILYTGIVDANQTQVQNYAIPANLSDPYLREW : 168
F_P54N2 : NGSVWGNIVWAHSVSKDLINWINLEPAIYPSKPFQDQFTWGSATILPGNKPVILYTGIVDANQTQVQNYAIPANLSDPYLREW : 168
F_P18N : NGSVWGNIVWAHSVSKDLINWINLEPAIYPSKPFQDQFTWGSATILPGNKPVILYTGIVDANQTQVQNYAIPANLSDPYLREW : 168
F_P54N1 : NGSVWGNIVWAHSVSKDLINWINLEPAIYPSKPFQDQFTWGSATILPGNKPVILYTGIVDANQTQVQNYAIPANLSDPYLREW : 168
F_P40N2 : NGSVWGNIVWAHSVSKDLINWINLEPAIYPSKPFQDQFTWGSATILPGNKPVILYTGIVDANQTQVQNYAIPANLSDPYLREW : 168

F_P40N1 : IKPDNNPLIVADASINKTKFRDPTTAWMGKDGHWIRIVMGSRLKHSRGLAIMYRSKDFMKVWVAKHPLHSTNGTGNWECDFFPV : 252
F_P54N2 : IKPDNNPLIVADASINKTKFRDPTTAWMGKDGHWIRIVMGSRLKHSRGLAIMYRSKDFMKVWVAKHPLHSTNGTGNWECDFFPV : 252
F_P18N : IKPDNNPLIVADASINKTKFRDPTTAWMGKDGHWIRIVMGSRLKHSRGLAIMYRSKDFMKVWVAKHPLHSTNGTGNWECDFFPV : 252
F_P54N1 : IKPDNNPLIVADASINKTKFRDPTTAWMGKDGHWIRIVMGSRLKHSRGLAIMYRSKDFMKVWVAKHPLHSTNGTGNWECDFFPV : 252
F_P40N2 : IKPDNNPLIVADASINKTKFRDPTTAWMGKDGHWIRIVMGSRLKHSRGLAIMYRSKDFMKVWVAKHPLHSTNGTGNWECDFFPV : 252

F_P40N1 : ALKGTNGIDQYGEYKYVVLKNSMDLTRFEYITLTKYDTKKDRYVDPVGSIDSWKGLRFDYGNFYASKTFYDTSKNRRVIWGSN : 336
F_P54N2 : ALKGTNGIDQYGEYKYVVLKNSMDLTRFEYITLTKYDTKKDRYVDPVGSIDSWKGLRFDYGNFYASKTFYDTSKNRRVIWGSN : 336
F_P18N : ALKGTNGIDQYGEYKYVVLKNSMDLTRFEYITLTKYDTKKDRYVDPVGSIDSWKGLRFDYGNFYASKTFYDTSKNRRVIWGSN : 336
F_P54N1 : ALKGTNGIDQYGEYKYVVLKNSMDLTRFEYITLTKYDTKKDRYVDPVGSIDSWKGLRFDYGNFYASKTFYDTSKNRRVIWGSN : 336
F_P40N2 : ALKGTNGIDQYGEYKYVVLKNSMDLTRFEYITLTKYDTKKDRYVDPVGSIDSWKGLRFDYGNFYASKTFYDTSKNRRVIWGSN : 336

F_P40N1 : ESDIFPEDDPAKQWAGIQLIPRKVWLDPSGKQLVQWPVEELETLRQKQVLSNKKLNNGEKVEVTGITPAQADVEVTFSPASLD : 420
F_P54N2 : ESDIFPEDDPAKQWAGIQLIPRKVWLDPSGKQLVQWPVEELETLRQKQVLSNKKLNNGEKVEVTGITPAQADVEVTFSPASLD : 420
F_P18N : ESDIFPEDDPAKQWAGIQLIPRKVWLDPSGKQLVQWPVEELETLRQKQVLSNKKLNNGEKVEVTGITPAQADVEVTFSPASLD : 420
F_P54N1 : ESDIFPEDDPAKQWAGIQLIPRKVWLDPSGKQLVQWPVEELETLRQKQVLSNKKLNNGEKVEVTGITPAQADVEVTFSPASLD : 420
F_P40N2 : ESDIFPEDDPAKQWAGIQLIPRKVWLDPSGKQLVQWPVEELETLRQKQVLSNKKLNNGEKVEVTGITPAQADVEVTFSPASLD : 420

F_P40N1 : KAESFDSSWTDMYAQDVCGLGADVQGGGLGPFGLATLATENLEENTPVFFRVFKAQQNYKVLCCSDAKRSTLKFNETMYKVSA : 504
F_P54N2 : KAESFDSSWTDMYAQDVCGLGADVQGGGLGPFGLATLATENLEENTPVFFRVFKAQQNYKVLCCSDAKRSTLKFNETMYKVSA : 504
F_P18N : KAESFDSSWTDMYAQDVCGLGADVQGGGLGPFGLATLATENLEENTPVFFRVFKAQQNYKVLCCSDAKRSTLKFNETMYKVSA : 504
F_P54N1 : KAESFDSSWTDMYAQDVCGLGADVQGGGLGPFGLATLATENLEENTPVFFRVFKAQQNYKVLCCSDAKRSTLKFNETMYKVSA : 504
F_P40N2 : KAESFDSSWTDMYAQDVCGLGADVQGGGLGPFGLATLATENLEENTPVFFRVFKAQQNYKVLCCSDAKRSTLKFNETMYKVSA : 504

F_P40N1 : GFVDVLDADKKLSLRSIDNSVIESFGAGGKTCITSRVYPTLAINEKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_P54N2 : GFVDVLDADKKLSLRSIDNSVIESFGAGGKTCITSRVYPTLAINEKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_P18N : GFVDVLDADKKLSLRSIDNSVIESFGAGGKTCITSRVYPTLAINEKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_P54N1 : GFVDVLDADKKLSLRSIDNSVIESFGAGGKTCITSRVYPTLAINEKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_P40N2 : GFVDVLDADKKLSLRSIDNSVIESFGAGGKTCITSRVYPTLAINEKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581

```

Figure 3.2.9: Amino acid alignment of P18, P40, and P54 *invGF* alleles. Amino acid exchanges are highlighted in colour.

Comparison of allelic cDNA sequences of the gene *invGF* from the three diploid genotypes revealed that the alleles *F_P18N* and *F_P54N1*, and the alleles *F_P40N1* and *F_P54N2* are identical at amino acid level.

3.2.1.2.7 Amino acid alignment of all *invGF* invertase alleles of the analyzed potato genotypes

The variability of amino acid positions, which exhibit exchanges was visualized by multiple sequence alignment of *invGF* invertase alleles (Figure 3.2.10). Several amino acid exchanges occur in different genotypes at the same position.

		*	20	*	40	*	60	*	80			
F_SN2	:	MDYSSNSRWALPVLVLCFFI	VLLSNNVVFASHKVF	IHLQSQNAVNV	HTVHRTGYHFQPEKHW	INDPNAPMYFNG	IYHLFPYQYNP	:	84			
F_P40N2	:	MDYSSNSRWALPVLVLCFFI	VLLSNNVVFASHKVF	IHLQSQNAVNV	QTVHRTGYHFQPEKHW	INDPNAPMYFNG	IYHLFPYQYNP	:	84			
F_SN3	:	MDYSSNSRWALPVLVLCFFI	VLLSNNVVFASHKVF	IHLQSQNAVNV	QTVHRTGYHFQPEKHW	INDPNAPMYFNG	IYHLFPYQYNP	:	84			
F_P40N1	:	MDYSSNSRWALPVLVLCFFI	VLLSNNVVFASHKVF	IHLQSQNAVNV	QTVHRTGYHFQPEKHW	INDPNAPMYFNG	IYHLFPYQYNP	:	84			
F_P54N2	:	MDYSSNSRWALPVLVLCFFI	VLLSNNVVFASHKVF	IHLQSQNAVNV	QTVHRTGYHFQPEKHW	INDPNAPMYFNG	IYHLFPYQYNP	:	84			
F_P54N1	:	MDYSSNSRWALPVLVLCFFI	VLLSNNVVFASHKVF	IHLQSQNAVNV	QTVHRTGYHFQPEKHW	INDPNAPMYFNG	IYHLFPYQYNP	:	84			
F_P18N	:	MDYSSNSRWALPVLVLCFFI	VLLSNNVVFASHKVF	IHLQSQNAVNV	QTVHRTGYHFQPEKHW	INDPNAPMYFNG	IYHLFPYQYNP	:	84			
F_SN1	:	MDYSSNSRWALPVLVLCFFI	VLLSNNVVFASHKVF	IHLQSQNAVNV	QTVHRTGYHFQPEKHW	INDPNAPMYFNG	IYHLFPYQYNP	:	84			
F_TN2	:	MDYSSNSRWALPVLVLCFFI	VLLSNNVVFASHKVF	IHLQSQNAVNV	QTVHRTGYHFQPEKHW	INDPNAPMYFNG	IYHLFPYQYNP	:	84			
F_DN2	:	MDYSSNSRWALPVLVLCFFI	VLLSNNVVFASHKVF	IHLQSQNAVNV	QTVHRTGYHFQPEKHW	INDPNAPMYFNG	IYHLFPYQYNP	:	84			
F_SN4	:	MDYSSNSRWALPVLVLCFFI	VLLSNNVVFASHKVF	IHLQSQNAVNV	HTVHRTGYHFQPEKHW	INDPNAPMYFNG	IYHLFPYQYNP	:	84			
F_TN1	:	MDYSSNSRWALPVLVLCFFI	VLLSNNVVFASHKVF	IHLQSQNAVNV	HTVHRTGYHFQPEKHW	INDPNAPMYFNG	IYHLFPYQYNP	:	84			
F_DN1	:	MDYSSNSRWALPVLVLCFFI	VLLSNNVVFASHKVF	IHLQSQNAVNV	HTVHRTGYHFQPEKHW	INDPNAPMYFNG	IYHLFPYQYNP	:	84			
		*	100	*	120	*	140	*	160			
F_SN2	:	NGSVWGNIVVAHVSVDKDLINW	INLEPAIYPSKPPDQFGTWSGSAT	ILPGNKPVILYTGIVDANQTQVQNYA	IPANLSDPVLREW	:	168					
F_P40N2	:	NGSVWGNIVVAHVSVDKDLINW	INLEPAIYPSKPPDQFGTWSGSAT	ILPGNKPVILYTGIVDANQTQVQNYA	IPANLSDPVLREW	:	168					
F_SN3	:	NGSVWGNIVVAHVSVDKDLINW	INLEPAIYPSKPPDQFGTWSGSAT	ILPGNKPVILYTGIVDANQTQVQNYA	IPANLSDPVLREW	:	168					
F_P40N1	:	NGSVWGNIVVAHVSVDKDLINW	INLEPAIYPSKPPDQFGTWSGSAT	ILPGNKPVILYTGIVDANQTQVQNYA	IPANLSDPVLREW	:	168					
F_P54N2	:	NGSVWGNIVVAHVSVDKDLINW	INLEPAIYPSKPPDQFGTWSGSAT	ILPGNKPVILYTGIVDANQTQVQNYA	IPANLSDPVLREW	:	168					
F_P54N1	:	NGSVWGNIVVAHVSVDKDLINW	INLEPAIYPSKPPDQFGTWSGSAT	ILPGNKPVILYTGIVDANQTQVQNYA	IPANLSDPVLREW	:	168					
F_P18N	:	NGSVWGNIVVAHVSVDKDLINW	INLEPAIYPSKPPDQFGTWSGSAT	ILPGNKPVILYTGIVDANQTQVQNYA	IPANLSDPVLREW	:	168					
F_SN1	:	NGSVWGNIVVAHVSVDKDLINW	INLEPAIYPSKPPDQFGTWSGSAT	ILPGNKPVILYTGIVDANQTQVQNYA	IPANLSDPVLREW	:	168					
F_TN2	:	NGSVWGNIVVAHVSVDKDLINW	INLEPAIYPSKPPDQFGTWSGSAT	ILPGNKPVILYTGIVDANQTQVQNYA	IPANLSDPVLREW	:	168					
F_DN2	:	NGSVWGNIVVAHVSVDKDLINW	INLEPAIYPSKPPDQFGTWSGSAT	ILPGNKPVILYTGIVDANQTQVQNYA	IPANLSDPVLREW	:	168					
F_SN4	:	NGSVWGNIVVAHVSVDKDLINW	INLEPAIYPSKPPDQFGTWSGSAT	ILPGNKPVILYTGIVDANQTQVQNYA	IPANLSDPVLREW	:	168					
F_TN1	:	NGSVWGNIVVAHVSVDKDLINW	INLEPAIYPSKPPDQFGTWSGSAT	ILPGNKPVILYTGIVDANQTQVQNYA	IPANLSDPVLREW	:	168					
F_DN1	:	NGSVWGNIVVAHVSVDKDLINW	INLEPAIYPSKPPDQFGTWSGSAT	ILPGNKPVILYTGIVDANQTQVQNYA	IPANLSDPVLREW	:	168					
		*	180	*	200	*	220	*	240	*		
F_SN2	:	IKPDNNPLIVAD	SINKTKFRDPTTAWMGKDGHWIR	IVMGSRLRKHRSGLAIMYRSKDFMKVWVAKKPHLHSTNGTGNWECDFFPV	:	252						
F_P40N2	:	IKPDNNPLIVAD	SINKTKFRDPTTAWMGKDGHWIR	IVMGSRLRKHRSGLAIMYRSKDFMKVWVAKKPHLHSTNGTGNWECDFFPV	:	252						
F_SN3	:	IKPDNNPLIVAD	SINKTKFRDPTTAWMGKDGHWIR	IVMGSRLRKHRSGLAIMYRSKDFMKVWVAKKPHLHSTNGTGNWECDFFPV	:	252						
F_P40N1	:	IKPDNNPLIVAD	SINKTKFRDPTTAWMGKDGHWIR	IVMGSRLRKHRSGLAIMYRSKDFMKVWVAKKPHLHSTNGTGNWECDFFPV	:	252						
F_P54N2	:	IKPDNNPLIVAD	SINKTKFRDPTTAWMGKDGHWIR	IVMGSRLRKHRSGLAIMYRSKDFMKVWVAKKPHLHSTNGTGNWECDFFPV	:	252						
F_P54N1	:	IKPDNNPLIVAD	SINKTKFRDPTTAWMGKDGHWIR	IVMGSRLRKHRSGLAIMYRSKDFMKVWVAKKPHLHSTNGTGNWECDFFPV	:	252						
F_P18N	:	IKPDNNPLIVAD	SINKTKFRDPTTAWMGKDGHWIR	IVMGSRLRKHRSGLAIMYRSKDFMKVWVAKKPHLHSTNGTGNWECDFFPV	:	252						
F_SN1	:	IKPDNNPLIVAD	SINKTKFRDPTTAWMGKDGHWIR	IVMGSRLRKHRSGLAIMYRSKDFMKVWVAKKPHLHSTNGTGNWECDFFPV	:	252						
F_TN2	:	IKPDNNPLIVAD	SINKTKFRDPTTAWMGKDGHWIR	IVMGSRLRKHRSGLAIMYRSKDFMKVWVAKKPHLHSTNGTGNWECDFFPV	:	252						
F_DN2	:	IKPDNNPLIVAD	SINKTKFRDPTTAWMGKDGHWIR	IVMGSRLRKHRSGLAIMYRSKDFMKVWVAKKPHLHSTNGTGNWECDFFPV	:	252						
F_SN4	:	IKPDNNPLIVAD	SINKTKFRDPTTAWMGKDGHWIR	IVMGSRLRKHRSGLAIMYRSKDFMKVWVAKKPHLHSTNGTGNWECDFFPV	:	252						
F_TN1	:	IKPDNNPLIVAD	SINKTKFRDPTTAWMGKDGHWIR	IVMGSRLRKHRSGLAIMYRSKDFMKVWVAKKPHLHSTNGTGNWECDFFPV	:	252						
F_DN1	:	IKPDNNPLIVAD	SINKTKFRDPTTAWMGKDGHWIR	IVMGSRLRKHRSGLAIMYRSKDFMKVWVAKKPHLHSTNGTGNWECDFFPV	:	252						
		*	260	*	280	*	300	*	320	*		
F_SN2	:	ALKGTNGIDQY	GEEYKVLKNSMDL	TRFEYTLGKYDTKKDRY	VPDVGSDSWKGLRFDYGNFYASKTFYD	TSKNRRVIWGSN	:	336				
F_P40N2	:	ALKGTNGIDQY	GEEYKVLKNSMDL	TRFEYTLGKYDTKKDRY	VPDVGSDSWKGLRFDYGNFYASKTFYD	TSKNRRVIWGSN	:	336				
F_SN3	:	ALKGTNGIDQY	GEEYKVLKNSMDL	TRFEYTLGKYDTKKDRY	VPDVGSDSWKGLRFDYGNFYASKTFYD	TSKNRRVIWGSN	:	336				
F_P40N1	:	ALKGTNGIDQY	GEEYKVLKNSMDL	TRFEYTLGKYDTKKDRY	VPDVGSDSWKGLRFDYGNFYASKTFYD	TSKNRRVIWGSN	:	336				
F_P54N2	:	ALKGTNGIDQY	GEEYKVLKNSMDL	TRFEYTLGKYDTKKDRY	VPDVGSDSWKGLRFDYGNFYASKTFYD	TSKNRRVIWGSN	:	336				
F_P54N1	:	ALKGTNGIDQY	GEEYKVLKNSMDL	TRFEYTLGKYDTKKDRY	VPDVGSDSWKGLRFDYGNFYASKTFYD	TSKNRRVIWGSN	:	336				
F_P18N	:	ALKGTNGIDQY	GEEYKVLKNSMDL	TRFEYTLGKYDTKKDRY	VPDVGSDSWKGLRFDYGNFYASKTFYD	TSKNRRVIWGSN	:	336				
F_SN1	:	ALKGTNGIDQY	GEEYKVLKNSMDL	TRFEYTLGKYDTKKDRY	VPDVGSDSWKGLRFDYGNFYASKTFYD	TSKNRRVIWGSN	:	336				
F_TN2	:	ALKGTNGIDQY	GEEYKVLKNSMDL	TRFEYTLGKYDTKKDRY	VPDVGSDSWKGLRFDYGNFYASKTFYD	TSKNRRVIWGSN	:	336				
F_DN2	:	ALKGTNGIDQY	GEEYKVLKNSMDL	TRFEYTLGKYDTKKDRY	VPDVGSDSWKGLRFDYGNFYASKTFYD	TSKNRRVIWGSN	:	336				
F_SN4	:	ALKGTNGIDQY	GEEYKVLKNSMDL	TRFEYTLGKYDTKKDRY	VPDVGSDSWKGLRFDYGNFYASKTFYD	TSKNRRVIWGSN	:	336				
F_TN1	:	ALKGTNGIDQY	GEEYKVLKNSMDL	TRFEYTLGKYDTKKDRY	VPDVGSDSWKGLRFDYGNFYASKTFYD	TSKNRRVIWGSN	:	336				
F_DN1	:	ALKGTNGIDQY	GEEYKVLKNSMDL	TRFEYTLGKYDTKKDRY	VPDVGSDSWKGLRFDYGNFYASKTFYD	TSKNRRVIWGSN	:	336				
		*	340	*	360	*	380	*	400	*		
F_SN2	:	ESDIFPEDD	NAKGWAGIQLIPRKVWLD	PSGKQLQWPV	VEELET	LR	TQKVQLSNKKLNNGEKVEVTG	ITPAQADVEV	TF	FSASLD	:	420
F_P40N2	:	ESDIFPEDD	NAKGWAGIQLIPRKVWLD	PSGKQLQWPV	VEELET	LR	TQKVQLSNKKLNNGEKVEVTG	ITPAQADVEV	TF	FSASLD	:	420
F_SN3	:	ESDIFPEDD	NAKGWAGIQLIPRKVWLD	PSGKQLQWPV	VEELET	LR	TQKVQLSNKKLNNGEKVEVTG	ITPAQADVEV	TF	FSASLD	:	420
F_P40N1	:	ESDIFPEDD	NAKGWAGIQLIPRKVWLD	PSGKQLQWPV	VEELET	LR	TQKVQLSNKKLNNGEKVEVTG	ITPAQADVEV	TF	FSASLD	:	420
F_P54N2	:	ESDIFPEDD	NAKGWAGIQLIPRKVWLD	PSGKQLQWPV	VEELET	LR	TQKVQLSNKKLNNGEKVEVTG	ITPAQADVEV	TF	FSASLD	:	420
F_P54N1	:	ESDIFPEDD	NAKGWAGIQLIPRKVWLD	PSGKQLQWPV	VEELET	LR	TQKVQLSNKKLNNGEKVEVTG	ITPAQADVEV	TF	FSASLD	:	420
F_P18N	:	ESDIFPEDD	NAKGWAGIQLIPRKVWLD	PSGKQLQWPV	VEELET	LR	TQKVQLSNKKLNNGEKVEVTG	ITPAQADVEV	TF	FSASLD	:	420
F_SN1	:	ESDIFPEDD	NAKGWAGIQLIPRKVWLD	PSGKQLQWPV	VEELET	LR	TQKVQLSNKKLNNGEKVEVTG	ITPAQADVEV	TF	FSASLD	:	420
F_TN2	:	ESDIFPEDD	NAKGWAGIQLIPRKVWLD	PSGKQLQWPV	VEELET	LR	TQKVQLSNKKLNNGEKVEVTG	ITPAQADVEV	TF	FSASLD	:	420
F_DN2	:	ESDIFPEDD	NAKGWAGIQLIPRKVWLD	PSGKQLQWPV	VEELET	LR	TQKVQLSNKKLNNGEKVEVTG	ITPAQADVEV	TF	FSASLD	:	420
F_SN4	:	ESDIFPEDD	NAKGWAGIQLIPRKVWLD	PSGKQLQWPV	VEELET	LR	TQKVQLSNKKLNNGEKVEVTG	ITPAQADVEV	TF	FSASLD	:	420
F_TN1	:	ESDIFPEDD	NAKGWAGIQLIPRKVWLD	PSGKQLQWPV	VEELET	LR	TQKVQLSNKKLNNGEKVEVTG	ITPAQADVEV	TF	FSASLD	:	420
F_DN1	:	ESDIFPEDD	NAKGWAGIQLIPRKVWLD	PSGKQLQWPV	VEELET	LR	TQKVQLSNKKLNNGEKVEVTG	ITPAQADVEV	TF	FSASLD	:	420

```

*           440           *           460           *           480           *           500
F_SN2      : KAESFDSSWTDMYAQDVCGLKGADVQGGGLGPPFGLATLATENLEENTPVFFRVFKAQQNYKVLLCSDAKRSTLKFNETMYKVSFA : 504
F_P40N2    : KAESFDSSWTDMYAQDVCGLKGADVQGGGLGPPFGLATLATENLEENTPVFFRVFKAQQNYKVLLCSDAKRSTLKFNETMYKVSFA : 504
F_SN3      : KAESFDSSWTDMYAQDVCGLKGADVQGGGLGPPFGLATLATENLEENTPVFFRVFKAQQNYKVLLCSDAKRSTLKFNETMYKVSFA : 504
F_P40N1    : KAESFDSSWTDMYAQDVCGLKGADVQGGGLGPPFGLATLATENLEENTPVFFRVFKAQQNYKVLLCSDAKRSTLKFNETMYKVSFA : 504
F_P54N2    : KAESFDSSWTDMYAQDVCGLKGADVQGGGLGPPFGLATLATENLEENTPVFFRVFKAQQNYKVLLCSDAKRSTLKFNETMYKVSFA : 504
F_P54N1    : KAESFDSSWTDMYAQDVCGLKGADVQGGGLGPPFGLATLATENLEENTPVFFRVFKAQQNYKVLLCSDAKRSTLKFNETMYKVSFA : 504
F_P18N     : KAESFDSSWTDMYAQDVCGLKGADVQGGGLGPPFGLATLATENLEENTPVFFRVFKAQQNYKVLLCSDAKRSTLKFNETMYKVSFA : 504
F_SN1      : KAESFDSSWTDMYAQDVCGLKGADVQGGGLGPPFGLATLATENLEENTPVFFRVFKAQQNYKVLLCSDAKRSTLKFNETMYKVSFA : 504
F_TN2      : KAELFDSSWTDMYAQDVCGLKGADVQGGGLGPPFGLATLATENLEENTPVFFRVFKAQQNYKVLLCSDAKRSTLKFNETMYKVSFA : 504
F_DN2      : KAESFDPSWTDMYAQDVCGLKGADVQGGGLGPPFGLATLATENLEENTPVFFRVFKAQQNYKVLLCSDAKRSTLKFNETMYKVSFA : 504
F_SN4      : KAESFDPSWTDMYAQDVCGLKGADVQGGGLGPPFGLATLATENLEENTPVFFRVFKAQQNYKVLLCSDAKRSTLKFNETMYKVSFA : 504
F_TN1      : KAESFDPSWTDMYAQDVCGLKGADVQGGGLGPPFGLATLATENLEENTPVFFRVFKAQQNYKVLLCSDAKRSTLKFNETMYKVSFA : 504
F_DN1      : KAESFDPSWTDMYAQDVCGLKGADVQGGGLGPPFGLATLATENLEENTPVFFRVFKAQQNYKVLLCSDAKRSTLKFNETMYKVSFA : 504

*           520           *           540           *           560           *           580
F_SN2      : GFVDVDLADKKLSLRSRLIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_P40N2    : GFVDVDLADKKLSLRSRLIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_SN3      : GFVDVDLADKKLSLRSRLIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_P40N1    : GFVDVDLADKKLSLRSRLIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_P54N2    : GFVDVDLADKKLSLRSRLIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_P54N1    : GFVDVDLADKKLSLRSRLIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_P18N     : GFVDVDLADKKLSLRSRLIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_SN1      : GFVDVDLADKKLSLRSRLIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_TN2      : GFVDVDLADKKLSLRSRLIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_DN2      : GFVDVDLADKKLSLRSRLIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_SN4      : GFVDVDLADKKLSLRSRLIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_TN1      : GFVDVDLADKKLSLRSRLIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581
F_DN1      : GFVDVDLADKKLSLRSRLIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNNGTEPITITETLDAWSMGKAKIQY : 581

```

Figure 3.2.10: Amino acid alignment of all cloned *invGF* invertase alleles. Amino acid exchanges are highlighted in colour. At protein position 160 three different amino acids are displayed.

Protein sequence comparison of the deduced *invGF* alleles from all analyzed genotypes showed that amino acids differed at 23 positions, of which eight were genotype specific and occurred only once.

3.2.1.2.8 Phenetic trees of all *invGF* invertase alleles of the analyzed potato genotypes

The further characterization of *invGF* alleles included, besides multiple amino acid alignment (section 3.2.1.2.7), a phenetic tree analysis in order to group the invertase alleles according to their similarity at amino acid as well as at nucleotide level. The neighbour-joining method was used for allelic classification and showed that *invGF* alleles from all analyzed potato genotypes group in different clades and subclades (Figure 3.2.11, Figure 3.2.12).

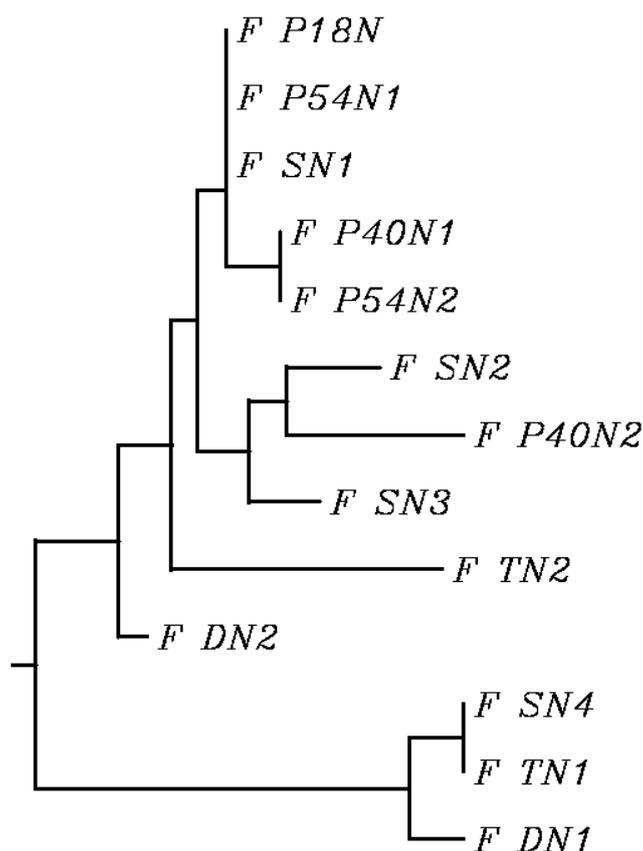


Figure 3.2.11: Amino acid based phenetic tree (Neighbour-joining method) of all cloned *invGF* invertase alleles.

The amino acid based phenetic tree splits into two main clades, from which the first one contains most of the *invGF* alleles. It is build of quite diverse subclades due to the high number of amino acid polymorphisms between the different genotypes. Amino acid comparison revealed sequence identical alleles in different genotypes. The alleles *F_SN1*, *F_P18N*, and *F_P54N1*, and the alleles *F_P40N1* and *F_P54N2* show the same amino acid sequence.

The second clade consists of the alleles *F_SN4*, *F_DN1*, and *F_TN1*. The two alleles *F_SN4* and *F_TN1* are identical at amino acid level, whilst *F_DN1* differs in its amino acid sequence. Cloning and sequencing of *invGF* alleles showed that different genotypes contain alleles identical at amino acid level but different at nucleic acid level (Appendix A 3.2.44). Table 3.2.7 shows a nucleotide comparison of amino acid identical alleles of different genotypes. The allelic nucleotide sequence was defined based on the consensus sequence of multiple alignments of full-length clones obtained from each genotype (Table 3.2.2). Although SNPs are present at the mentioned cDNA positions the nucleotide polymorphisms resulted in one and the same amino acid sequence.

Table 3.2.7: Genotype specific nucleotide differences of alleles *F_SNI*, *F_P18N*, and *F_P54N1*.

Position of cDNA SNP	<i>F_SNI</i>	<i>F_P18N</i>	<i>F_P54N1</i>
117	G	A	A
876	G	A	A
1089	C	T	T
1113	G	A	A
1173	A	G	G
1242	C	T	T
1263	A	G	G
1386	A	G	G
1446	C	T	T
1452	T	C	C
1536	A	T	T
1677	C	T	T

Nucleotide sequence comparison showed that the alleles *F_P40N1* and *F_P54N2*, and the alleles *F_SN4* and *F_TN1* were identical. The nucleotide sequences of the alleles *F_P18N* and *F_P54N1* were also identical, whilst the sequence of *F_SNI* differed in 12 positions. Allele specific SNPs were detected in at least two sequences and used for comparing the allelic nucleotide polymorphisms.

The nucleotide polymorphisms between all *invGF* alleles of the six analyzed genotypes were visualized using the phenetic tree analysis (Figure 3.2.12).

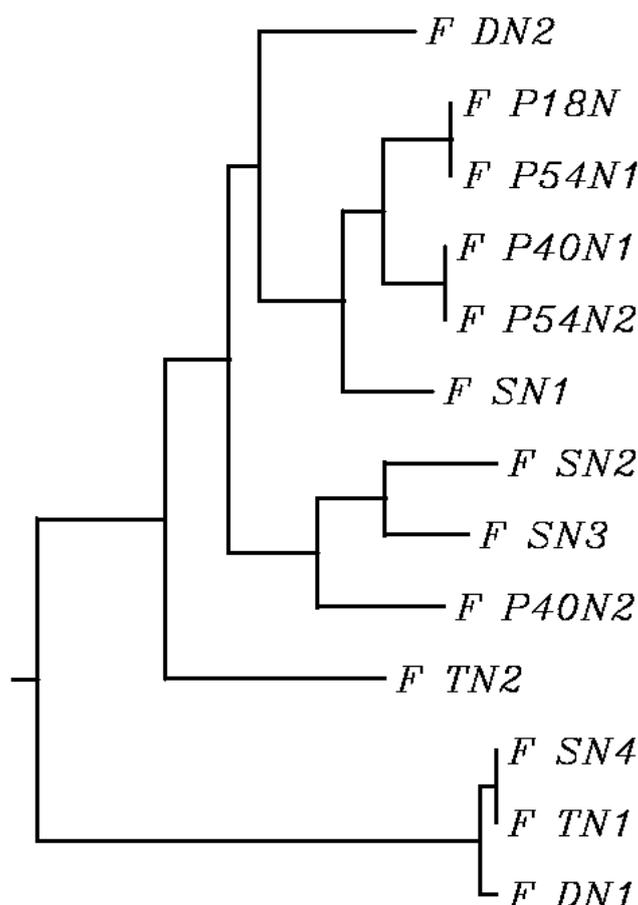


Figure 3.2.12: Nucleotide sequence based phenetic tree (Neighbour-joining tree) of all cloned *invGF* invertase alleles.

The alleles group regarding their similarity in the same clades and subclades as observed in the amino acid sequences based phenetic tree (Figure 3.2.11). Subclades are more subdivided because of nucleotide polymorphisms.

3.2.1.3 Three-dimensional modelling of *invGE* and *invGF* alleles

The 3D-modelling was performed by Pawel Durek, MPIMP/Golm. The allelic invertase molecular structures were modelled after the invertase 3D and crystal structure of cyanobacteria (ALBERTO ET AL., 2004). The models are comparative, superimposing two allelic sequences. Differences of the alleles are highlighted. The models include the putative sucrose binding site with the substrate sucrose. In addition to the structural visualization of amino acid exchanges, also the electrostatic potential (EP) of the molecules was mapped at pH 4.7 mimicking the apoplasmic conditions.

❖ Structural modelling of *invGE* alleles of the cultivars ‘Satina’ and ‘Theresa’

Analysis of allelic molecular structures was performed with the associated ‘Satina’ allele *E_SA* compared to the allele *E_SN3*, and with the associated ‘Theresa’ allele *E_TA* compared to the allele *E_TN1*. The models contrast the allelic sequences with each other, meaning that

one sequence superimposes the other and vice versa (Figure 3.2.14). Based on protein sequence alignments (Figure 3.2.3), regions, which are affected directly or indirectly by amino acid exchanges, were identified (Figure 3.2.13).

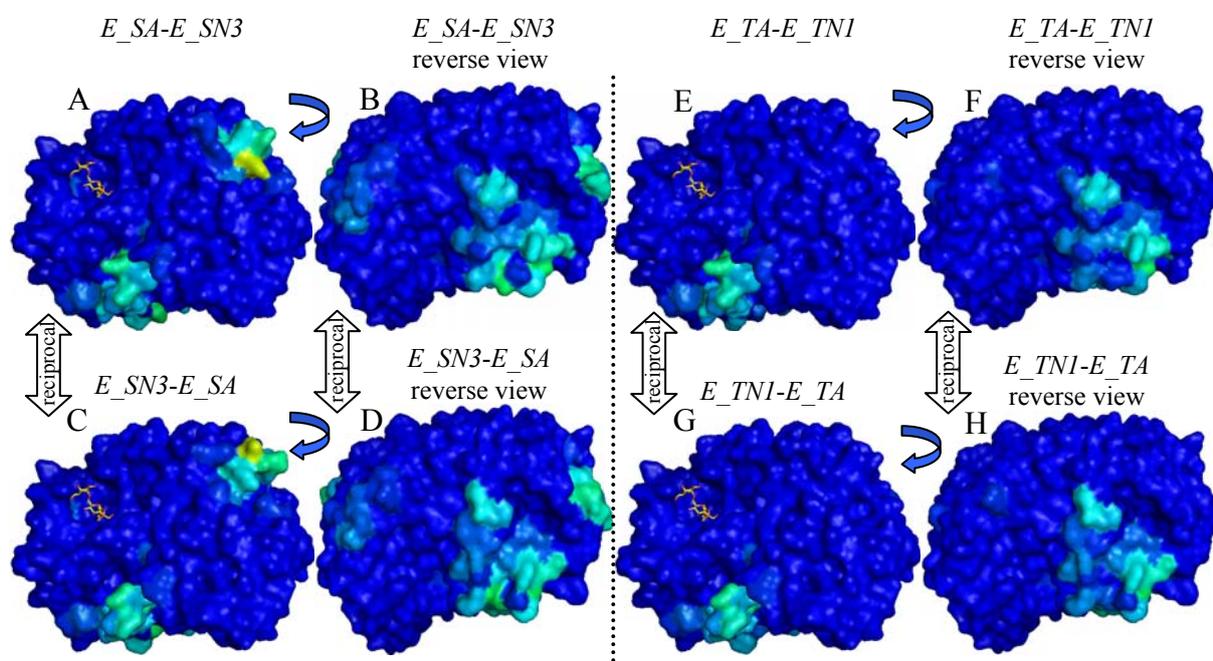
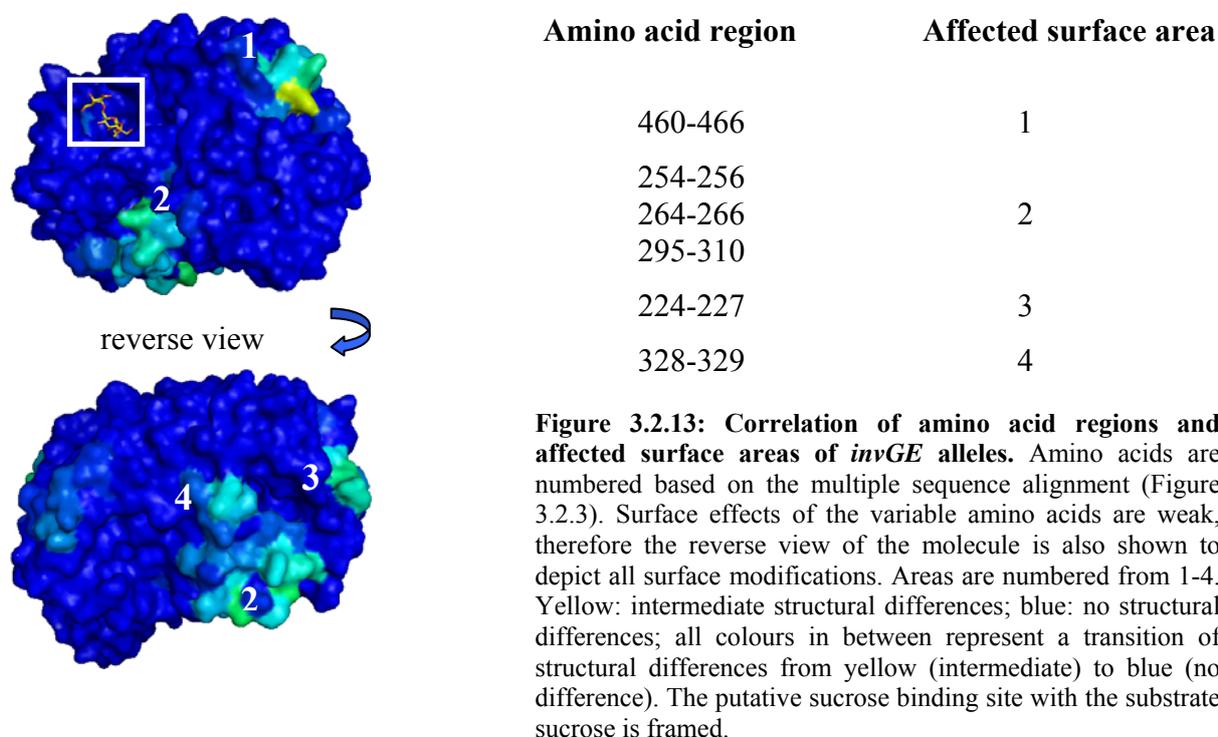


Figure 3.2.14: Structural comparison of ‘Satina’ alleles *E_SA*, *E_SN3* and ‘Theresa’ alleles *E_TA*, *E_TN1*. A: *E_SA* superimposes *E_SN3*; B: *E_SA* superimposes *E_SN3*, reverse view; C: *E_SN3* superimposes *E_SA*; D: *E_SN3* superimposes *E_SA*, reverse view; E: *E_TA* superimposes *E_TN1*; F: *E_TA* superimposes *E_TN1*, reverse view; G: *E_TN1* superimposes *E_TA*; H: *E_TN1* superimposes *E_TA*, reverse view. Yellow: intermediate structural differences; blue: no structural differences; all colours in between represent a transition of structural differences from yellow (intermediate) to blue (no difference).

The models of the invertase alleles *E_SA*, *E_SN3* and *E_TA*, *E_TNI* showed structural differences on the enzyme's surface. The analysis of the allelic molecules *E_SA* and *E_SN3* revealed differences in regions 1, 2, 3, and 4 (Figure 3.2.13). Area 1 is defined by amino acids 460-466 showing alanine (A) in allele *E_SA* and valine (V) in allele *E_SN3* at position 462. Three amino acid exchanges between the two alleles occur in region 2 where at positions 256, 295 and 309 the protein sequence differs. The allele *E_SA* contains at position 256 asparagine (N) versus lysine (K) in allele *E_SN3*, at position 295 arginine (R) versus lysine (K), and at position 309 cysteine (C) versus serine (S). Region 3 is defined by amino acids 224-227. Here, the protein sequences of both 'Satina' alleles are identical. In the vicinity of this region, at position 230, an exchange from valine (V) to alanine (A) in the alleles *E_SA* and *E_SN3* respectively, occurs. This difference might influence surrounding areas and lead to variable protein folding. Amino acids 328 and 329 determine region 4, where at position 329 an amino acid exchange from threonine (T) in allele *E_SA* to methionine (M) in allele *E_SN3* appears. The models of the two 'Theresa' alleles *E_TA* and *E_TNI* show surface differences in the regions 2 and 4. Differences are absent in area 1 and 3 because of identical amino acid composition of both alleles. In region 2, which is defined by amino acids 254-256, 264-266, and 295-310 two amino acid exchanges occur. The allele *E_TA* contains at position 295 arginine (R) versus lysine (K) in allele *E_TNI*, and at position 309 cysteine (C) versus serine (S). These two substitutions are also present in allele *E_SA* from 'Satina'.

❖ **Comparison of 3D-structures of the 'Satina' and 'Theresa' *invGE* alleles, which are associated with better chips quality**

The tetraploid potato cultivars 'Satina' and 'Theresa' harbour alleles, which are not identical at amino acid level but exhibit the nucleotide adenine at cDNA SNP 1103* (Table 3.2.3), which is associated with better potato chips quality. Comparison of the 3D-structures of *Pain_SA* and *Pain_TA* molecules revealed minor surface differences between the two protein models (Figure 3.2.15).

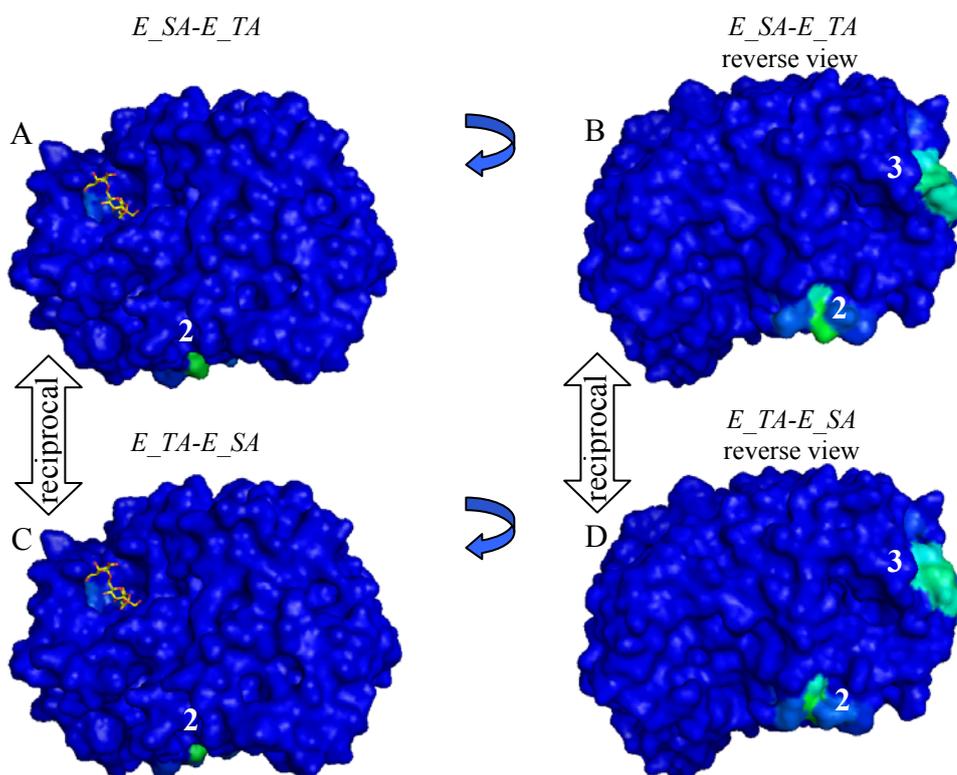


Figure 3.2.15: Comparative 3-D structures of the associated alleles E_{SA} and E_{TA} . A: E_{SA} superimposes E_{TA} ; B: E_{SA} superimposes E_{TA} , reverse view; C: E_{TA} superimposes E_{SA} ; D: E_{TA} superimposes E_{SA} , reverse view. Green: weak structural differences; blue: no structural differences; all colours in between represent a transition of structural differences from green (weak) to blue (no difference).

Alleles E_{SA} and E_{TA} differ at 11 amino acid positions. The models of both allelic molecules revealed weak structural differences on the enzyme's surface in regions 2 and 3. Region 2 is defined by three amino acid stretches 254-256, where at position 256 an amino acid exchange was detected. The allele E_{SA} exhibits asparagine (N), whilst E_{TA} has lysine (K). Except this exchange, both alleles are identical in region 2 explaining the limited extension of this area compared to the other analyzed molecules (Figure 3.2.14). Region 3, defined by the amino acids 224-227, is identical in both alleles. The visible structural effect might be caused by the altered sequence at position 230 in the vicinity of region 3, which substitutes valine (V) to alanine (A) in the alleles E_{SA} and E_{TA} .

❖ Modelling the electrostatic potential (EP) of *invGE* alleles from the cultivars 'Satina' and 'Theresa'

The mapping of the EP of the *invGE* alleles E_{SA} , E_{SN3} , E_{TA} , and E_{TN1} revealed minor charge differences of the molecules at pH 4.7 (Figure 3.2.16).

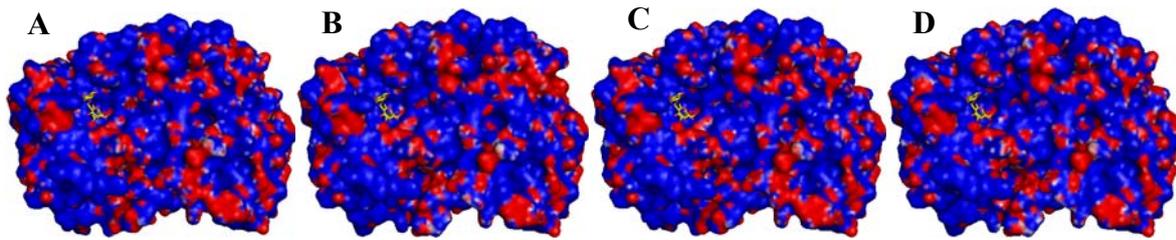


Figure 3.2.16: EP of the ‘Satina’ and ‘Theresa’ alleles. A: E_{SA} ; B: E_{SN3} ; C: E_{TA} ; D: E_{TNI} . Red: negatively charged; blue: positively charged; white: neutrally charged.

Focusing on the EP of the putative sucrose binding site

The putative sucrose binding site (Figure 3.2.13) is positively charged matching the partial negative charge of the substrate sucrose due to its hydroxyl groups.

Zooming into the putative sucrose binding domain revealed weak charge differences between the molecules E_{SA} (A) and E_{SN3} (B). The alleles E_{TA} (C) and E_{TNI} (D) showed no visible differences (Figure 3.2.17).

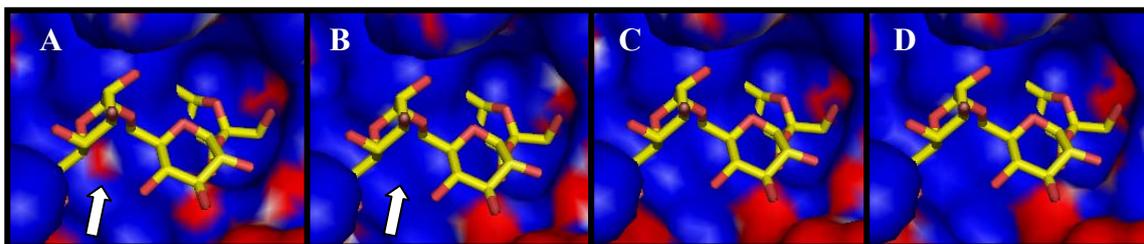


Figure 3.2.17: Focusing on the EP of the putative sucrose binding site of the ‘Satina’ and ‘Theresa’ alleles. A: E_{SA} ; B: E_{SN3} ; C: E_{TA} ; D: E_{TNI} . Red: negatively charged; blue: positively charged; white: neutrally charged.

The EP changes of the putative sucrose binding site of the two alleles from ‘Satina’ were weak. The EP of the associated allele E_{SA} (A) exhibits a small negatively charged area compared to the allele E_{SN3} (B). The charge of the ‘Theresa’ alleles E_{TA} (C) and E_{TNI} (D) were similar.

❖ **Structural modelling of *invGF* alleles of the cultivar ‘Satina’**

Molecular analysis of allelic structures was conducted using the two ‘Satina’ alleles F_{SN3} and F_{SN4} . The models compare the allelic sequences with each other by means of superimposing sequences of the different alleles (Figure 3.2.19). Doing so, differences between the alleles are visualized. Multiple alignment (Figure 3.2.8) of allelic protein sequences allowed to identify the regions, which are directly or indirectly affected by amino acid exchanges (Figure 3.2.18).

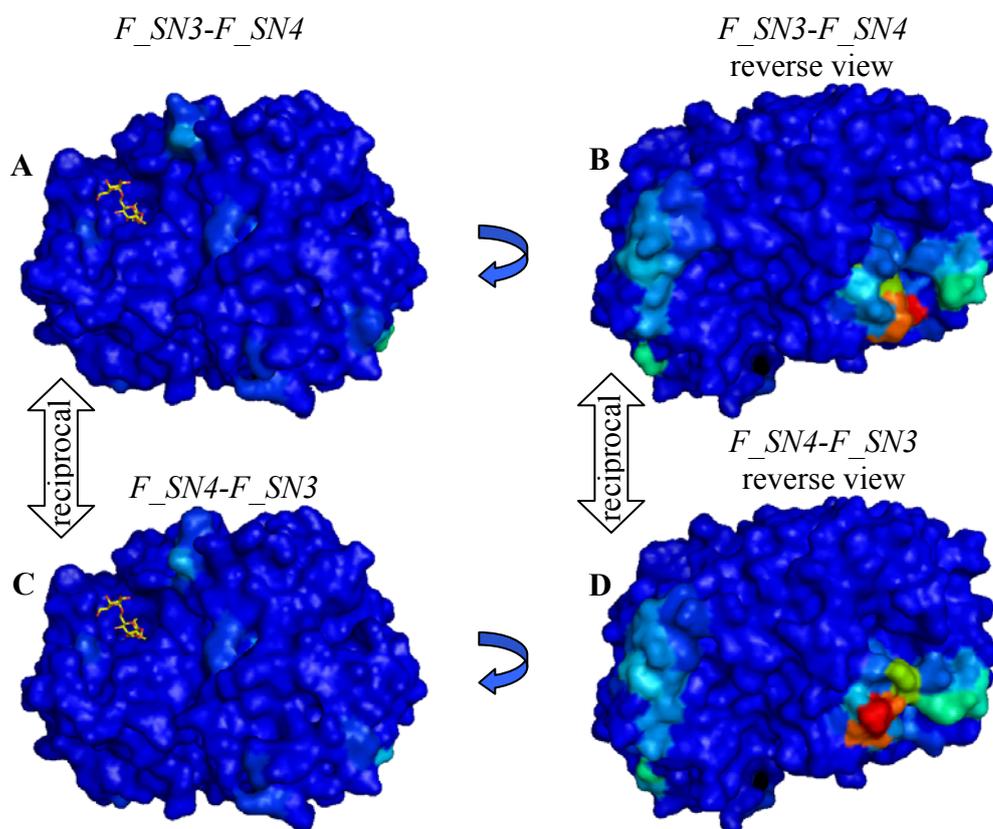
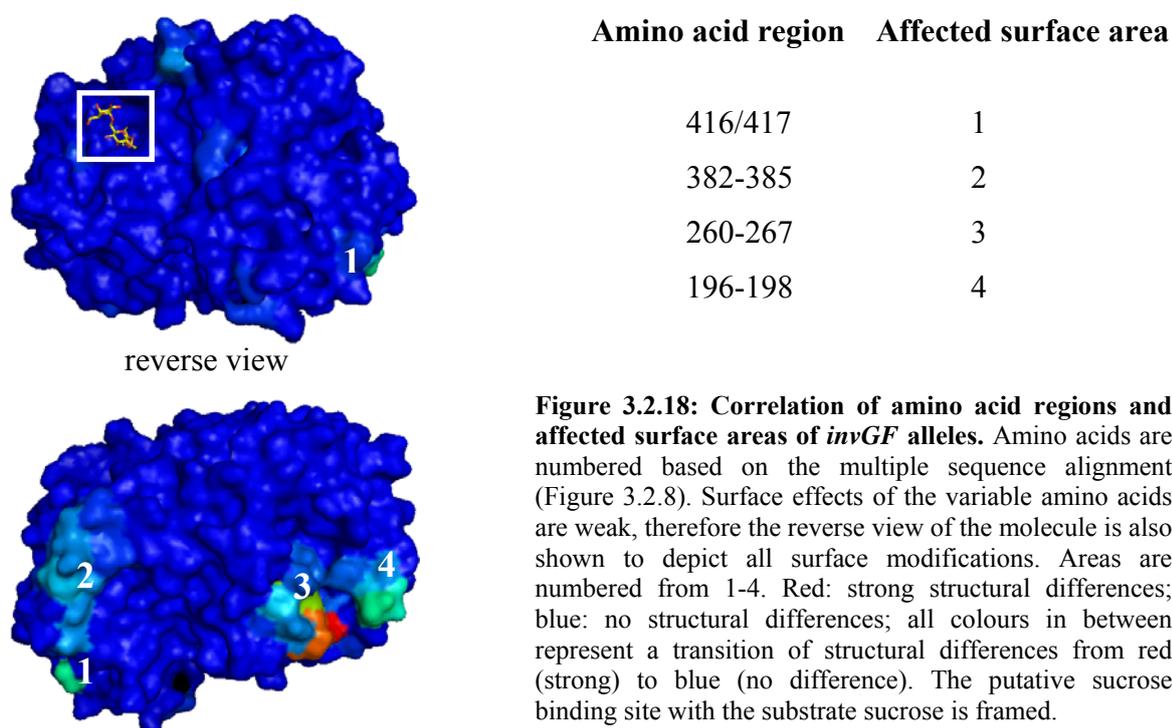


Figure 3.2.19: Structural comparison of ‘Satina’ alleles *F_SN3* and *F_SN4*. **A:** *F_SN3* superimposes *F_SN4*; **B:** *F_SN3* superimposes *F_SN4*, reverse view; **C:** *F_SN4* superimposes *F_SN3*; **D:** *F_SN4* superimposes *F_SN3*, reverse view. Red: strong structural differences; blue: no structural differences; all colours in between represent a transition of structural differences from red (strong) to blue (no difference).

The molecular models of the ‘Satina’ alleles *F_SN3* and *F_SN4* showed structural differences on the enzyme’s surface. Those differences were assigned to regions 1, 2, 3, and 4 (Figure 3.2.18). Area 1 is characterized by amino acids 416 and 417 exhibiting no differences between the two alleles. Region 2 is determined by the amino acids 382-385. Both alleles are identical in this region. Also area 4 consists of identical amino acids at positions 196-198. Although amino acids in the corresponding regions 1, 2, and 4 do not differ between the alleles, sterical differences occur. The reasons for the mild structural effects may be surrounding allelic sequences causing a variable lobing of the corresponding regions of the putative 3D invertase structure.

Region 3, which is defined by the amino acids 260-267 shows exchanges between the alleles. The allele *F_SN3* contains at position 260 isoleucine (I) versus glutamine (Q) in allele *F_SN4* and at position 267 tyrosine (Y) versus histidine (H).

❖ **Modelling the electrostatic potential (EP) of *invGF* alleles from the cultivar ‘Satina’**

The mapping of the EP of the *invGF* alleles *F_SN3* and *F_SN4* revealed minor charge differences of the molecules at pH 4.7 (Figure 3.2.20).

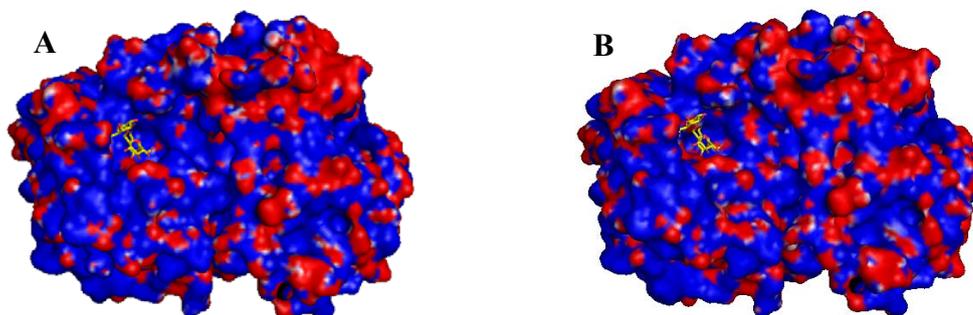


Figure 3.2.20: EP of the alleles *F_SN3* and *F_SN4*. **A:** *F_SN3*; **B:** *F_SN4*. Red: negatively charged; blue: positively charged; white: neutrally charged.

Focusing on the EP of the putative sucrose binding site

The putative sucrose binding site (Figure 3.2.18) has a positive charge matching the partial negative charge of the substrate sucrose due to its hydroxyl groups.

Zooming into the putative sucrose binding domain revealed charge differences between the molecules *F_SN3* (Figure 3.2.21, A) and *F_SN4* (Figure 3.2.21, B).

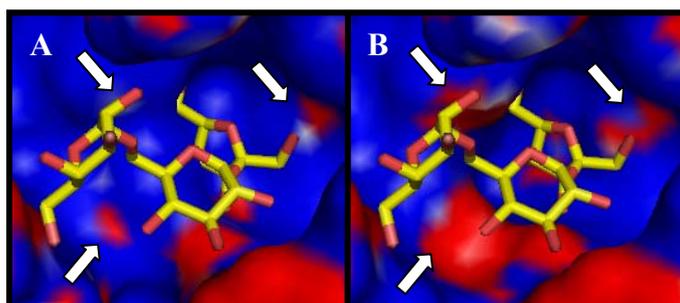


Figure 3.2.21: Focusing of the EP of the putative sucrose binding site of the alleles *F_SN3* and *F_SN4*. A: *F_SN3*; B: *F_SN4*. Red: negatively charged; blue: positively charged; white: neutrally charged.

The EP of the allele *F_SN4* (B) switched from a positive to a more negative charge caused by its allelic composition.

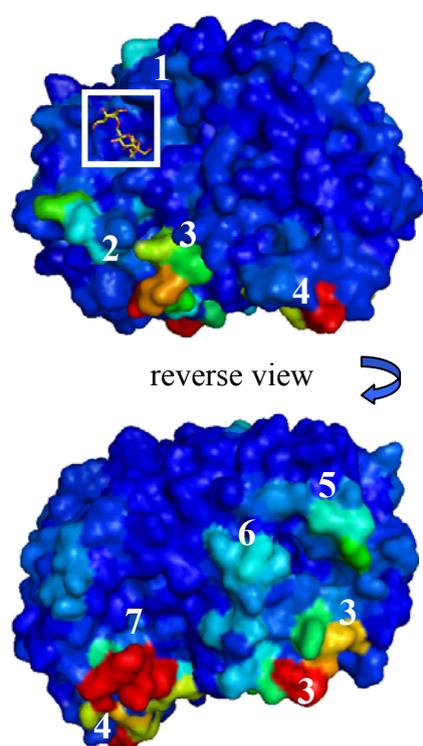
❖ Comparison of 3D-structures of *invGE* and *invGF* alleles

Association analysis showed that two SSCP fragments of the genes *invGE* (*invGE-6f*) and *invGF* (*invGF-4d*) are associated with better potato chips quality. These fragments are in LD showing nearly identical distributions within the population used (LI ET AL., 2005). Amino acid alignments (Figure 3.2.3) and phenetic tree analysis (Figure 3.2.6) showed the similarity of the three associated *invGE* alleles. Also three alleles of the gene *invGF* show high similarity to each other (Figure 3.2.8) and group in a separate clade compared to the other *invGF* alleles (Figure 3.2.11). Out of these three *invGF* alleles, which showed similar grouping as the associated *invGE* alleles and, therefore, might represent the associated *invGF* alleles, the allele *F_SN4* was selected for comparative 3D-modelling.

The associated *invGE* alleles *E_SA* and *E_TA* were compared against *F_SN4*, which was found to be amino acid and nucleotide identical to *F_TN1*. The *invGF* alleles *F_SN4*, *F_DNI*, and *F_TN1* are distinctive from the other *invGF* alleles.

Comparison of the 3D molecular structures of *E_SA* and *F_SN4*, and of *E_TA* and *F_SN4* revealed similar surface differences of both analyzed groups due to minor structural differences between the two associated *invGE* alleles *E_SA* and *E_TA*. The structural models are shown representatively from the comparison of *E_SA* and *F_SN4*.

A multiple alignment (Appendix A 3.2.45) of the allelic protein sequences allowed the identification of regions, which are directly or indirectly affected by amino acid exchanges (Figure 3.2.22).



Amino acid region	Affected surface area
86-89	1
133-135	2
210-213	2
262-268	3
300-309	3
419/420	4
476-480	4
133-136	5
326-329	6
510-519	7

Figure 3.2.22: Correlation of amino acid regions and affected surface areas of comparative *invGE* and *invGF* alleles. Amino acids are numbered based on multiple sequence alignment (Appendix A 3.2.19). The reverse view of the molecule is shown because of the manifestation of allelic amino acid differences at the whole surface. Areas are numbered from 1-7. Red: strong structural differences; blue: no structural differences; all colours in between represent a transition of structural differences from red (strong) to blue (no difference). The putative sucrose-binding site with the substrate sucrose is framed.

Comparison of the 3D molecular structures of *E_SA* and *F_SN4*, and of *E_TA* and *F_SN4* revealed surface differences between both analyzed enzymes (Figure 3.2.23).

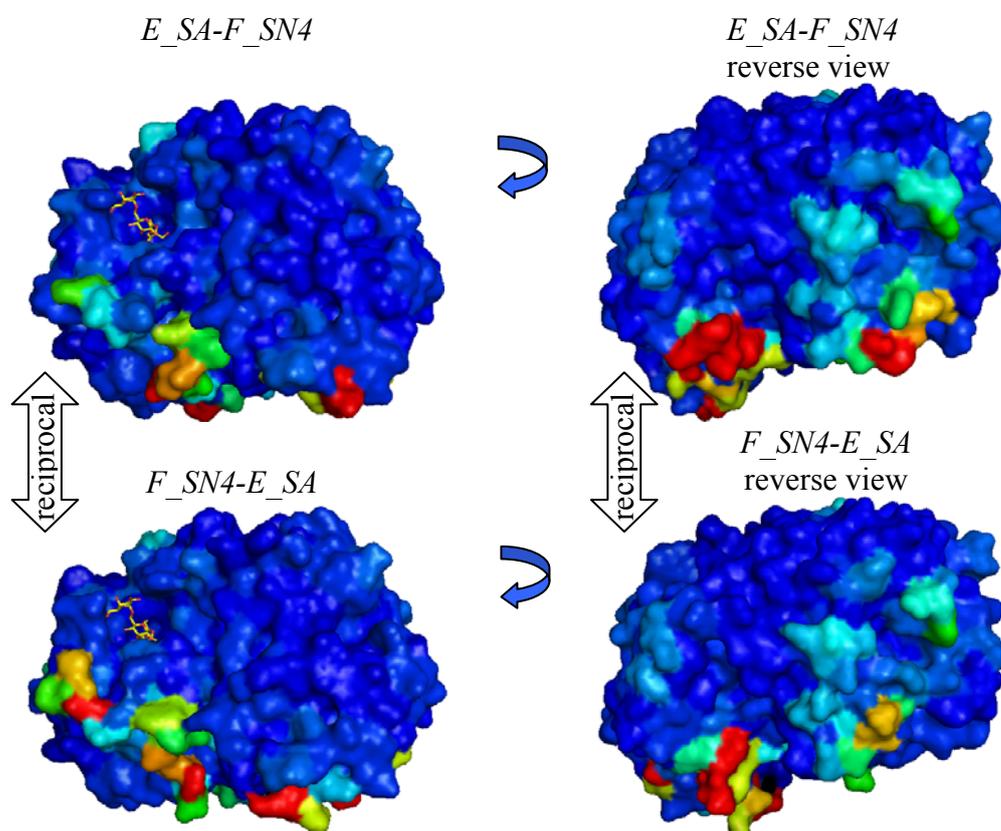


Figure 3.2.23: Structural comparison of *invGE* allele *E_SA* and *invGF* allele *F_SN4*. A: *E_SA* superimposes *F_SN4*; B: *E_SA* superimposes *F_SN4*, reverse view; C: *F_SN4* superimposes *E_SA*; D: *F_SN4* superimposes *E_SA*, reverse view. Red: strong structural differences; blue: no structural differences; all colours in between represent a transition of structural differences from red (strong) to blue (no difference).

The analysis of the corresponding allelic molecules revealed differences in the regions 1, 2, 3, 4, 5, 6, and 7 (area definition: Figure 3.2.22). Region 1 is defined by the amino acids 86-89. At position 87 an amino acid exchange occurs between both alleles. The allele *E_SA* contains lysine (K), whilst *F_SN4* consists of asparagine (N). The amino acids 133-135 and 210-213 represent the surface area 2. Amino acid exchanges at positions 135 and 211 cause structural differences. The allelic sequences change from asparagine (N135) in allele *E_SA* to glycine (G135) in allele *F_SN4*. At position 211 *E_SA* exhibits methionine (M) compared to leucine (L) in *F_SN4*. Region 3 is defined by the amino acids 262-268 and 300-309. Five regional amino acid exchanges are found between the two alleles. Protein sequence comparison revealed the following *E_SA* to *F_SN4* exchanges: A267Q, N302V, N303G, G307S and C308W. In the surface area 4 two amino acid substitutions occur. The first exchange is at position 420 where *E_SA* possesses serine (S), whilst *F_SN4* has alanine (A). The second exchange at position 480 shows a difference from lysine (K) in *E_SA* to glutamine (Q) in *F_SN4*. Region 5 is defined by the amino acids 133-136. At position 135 *E_SA* contains asparagine (N), *F_SN4* consists of lysine (G). The amino acids 326-329 are located in region 6 where an exchange at position 328 from threonine (T) in *E_SA* to serine (S) in *F_SN4* occurs. Region 7 is defined by the amino acids 510-519. In this area two exchanges at positions 515 and 517 were detected. The allele *E_SA* contains at position 515 valine (V) compared to alanine (A) in *F_SN4* and at position 517 a methionine (M), whilst *F_SN4* does not exhibit a comparable residue at this position.

The amino acid exchanges in the described regions manifest sterically and influence the lobing of the putative invertase molecules.

3.2.2 Functional characterization of the genes *invGE* and *invGF*

3.2.2.1 Differential expression analysis of *invGE* and *invGF* alleles

Referring to the expression pattern of the genes *invGE* and *invGF* observed by MADDISON ET AL. (1999), allele specific expression analysis was carried out in pyrosequencing assays using leaf tissues for *invGE* analysis and floral tissues for *invGF* characterization.

The pyrosequencing analysis was performed using cDNA from leaves and flowers and leaf and floral genomic DNA to determine the relative frequency of *invGE* and *invGF* alleles. Comparison of both samples revealed specific expression patterns not just for each genotype but also between different alleles of the same genotype. In the following Figures the relationship between the presence of alleles in the genome and their transcription levels are

illustrated. Accordingly, both values are shown together in terms of the relative expression level.

Additionally, plasmids harbouring one allele of complementary SNPs of corresponding genotypes were mixed in different ratios to monitor the accuracy of pyrosequencing analysis. Plasmid based measurements, working as positive controls, showed that SNP dependent variations of $\pm 5\%$ occurred. Values of cDNA and genomic dosages of the analysed alleles as determined by pyrosequencing were corrected for the observed SNP specific variations.

3.2.2.1.1 Expression patterns of *invGE* alleles in leaves of the tetraploid genotypes

By means of pyrosequencing the distribution of allele specific SNPs was analyzed (Table 3.2.3). The following Table 3.2.8 displays an overview of the SNPs selected from each tetraploid cultivar.

Table 3.2.8: Allele specific SNPs analyzed by pyrosequencing.

Genotype	Allele	SNP position	Allele specific SNP
'Satina'	<i>E_SA</i>	SNP 1237	<i>E_SA/E_SNI/E_SN2/E_SN3</i> T/G/G/G
	<i>E_SNI</i>	SNP 1366	A/G/A/A
	<i>E_SN2</i>	SNP 1379	C/C/T/C
	<i>E_SN3</i>	SNP 1216	A/A/A/G
'Diana'	<i>E_DA</i>	SNP 1086	<i>E_DA/E_DNI/E_DN2</i> A/T/T
	<i>E_DNI</i>	SNP 1117	T/C/T
	<i>E_DN2</i>	SNP 924	T/T/C
'Theresa'	<i>E_TA</i>	SNP 1615	<i>E_TA/E_TNI/E_TN2/E_TN3</i> A/T/T/T
	<i>E_TNI</i>	SNP 1720	C/A/C/C
	<i>E_TN2</i>	SNP 1553	T/T/C/T
	<i>E_TN3</i>	SNP 1473	T/T/T/G

SNP positions refer to cDNA sequence where '1' represents the adenine of the start codon ATG. Primers used for pyrosequencing are listed in chapter 2 (Table 2.1.5 and Table 2.2.9). Allele specific SNPs are highlighted in bold capitals.

The pyrosequencing analysis was performed using cDNA from leaves and leaf genomic DNA to measure the dosages of the alleles (Figure 3.2.24).

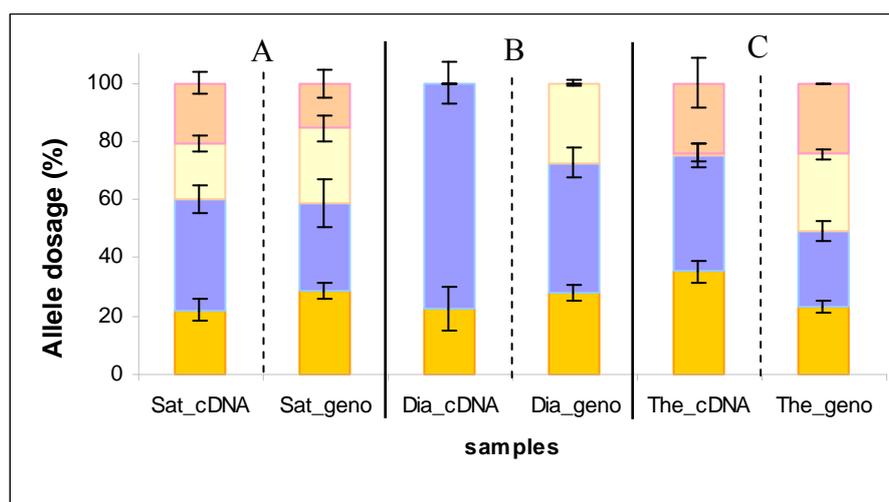


Figure 3.2.24: Pyrosequencing analysis of *invGE* alleles from the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’. **A:** Pyrosequencing of ‘Satina’ alleles. E_{SA} : yellow; E_{SNI} : pink; E_{SN2} : light yellow; E_{SN4} : blue. **B:** Pyrosequencing of ‘Diana’ alleles. E_{DA} : yellow; E_{DNI} : blue; E_{DN2} : light yellow. **C:** Pyrosequencing of ‘Theresa’ alleles. E_{TA} : yellow; E_{TNI} : blue; E_{TN2} : pink; E_{TN3} : light yellow. 100% is defined as the sum of alternative peaks measuring allele specific SNPs. In addition to pyrosequencing analysis of cDNA samples, the genomic dosage of the alleles was determined using genomic DNA of leaves. Percentages of the expression level of the cDNA samples are related to the genomic dosage of the alleles. Standard deviations are derived from two biological replicates done in technical triplicates.

Pyrosequencing analysis of genomic DNA showed that the three associated alleles E_{SA} , E_{DA} , and E_{TA} from the different cultivars are present in simplex (25%) in the corresponding genotype. For the cultivar ‘Satina’ four *invGE* alleles were found. If the alleles were transcribed according to the allele dosage, each allele is expected to contribute 25% of the total transcripts. Comparison of genomic allele dosage and allele transcription revealed that the alleles E_{SNI} and E_{SN4} were slightly over-represented, whilst the alleles E_{SA} and E_{SN2} were less abundant in cDNA samples.

In the cultivar ‘Diana’ three alleles were identified. Pyrosequencing analysis of genomic ‘Diana’ DNA showed allele dosages of 25% E_{DA} , 25% E_{DN2} and 50% of E_{DNI} .

However, the fraction represented by E_{DNI} could consist of two alleles since ‘Diana’ is tetraploid. In the pyrosequencing assay of ‘Diana’ leaf cDNA no transcripts for the allele E_{DN2} were detected due to a transcription rate below detection level, whilst the cDNA fraction represented by E_{DNI} showed a strong over-representation up to 80%. The abundance of the associated allele E_{DA} decreased from 25% of its genomic dosage to approximately 20% in cDNA.

The cultivar ‘Theresa’ has four *invGE* alleles E_{TA} , E_{TNI} , E_{TN2} , and E_{TN3} . Each allele is present in simplex (25%). Pyrosequencing analysis of leaf cDNA showed that the transcripts of the allele E_{TN3} were below the detection level. The abundance of the associated allele E_{TA} increased slightly from 25% genomic dosage to about 38% in leaf

cDNA. The strongest uprating was observed for the allele *E_TN1* that increased up to 40% when compared to the genomic dosage. Expression of the allele *E_TN2* did not differ compared to its genomic dosage and remained at 25%.

3.2.2.1.2 Expression pattern of *invGE* alleles in leaves of the diploid genotypes

For allele specific separation within the diploid potato genotypes P18, P40, and P54 allele specific SNPs were identified based on multiple nucleotide alignments (Table 3.2.4). SNP at position 108 was used to separate the two P18 alleles *E_P18N1* and *E_P18N2*. Using the SNP at position 58 the P40 alleles *E_P40N1*, *E_P40N2* and the P54 alleles *E_P54N1*, *E_P54N2* were distinguished (Table 3.2.9).

Table 3.2.9: Allele specific SNPs analyzed by pyrosequencing.

Genotype	Allele	SNP position	Allele specific SNP
P18	<i>E_P18N1</i>	SNP 108	A
	<i>E_P18N2</i>		T
P40	<i>E_P40N1</i>	SNP 58	C
	<i>E_P40N2</i>		T
P54	<i>E_P54N1</i>	SNP 58	C
	<i>E_P54N2</i>		T

SNP positions refer to cDNA sequence where '1' represents the adenine of the start codon ATG. Primers used for pyrosequencing are listed in chapter 2 (Table 2.1.5 and Table 2.2.10).

Pyrosequencing analysis was performed using cDNA from leaves (Figure 3.2.25).

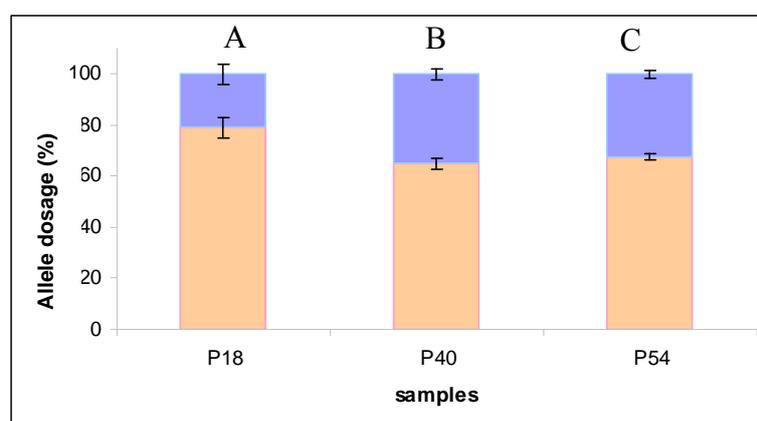


Figure 3.2.25: Pyrosequencing analysis of *invGE* alleles from the diploid genotypes P18, P40, and P54. A: Pyrosequencing of P18 alleles. ■: *E_P18N1*; ■: *E_P18N2*. **B:** Pyrosequencing of P40 alleles. ■: *E_P40N1*; ■: *E_P40N2*. **C:** Pyrosequencing of P54 alleles. ■: *E_P54N1*; ■: *E_P54N2*. 100% is defined as the sum of alternative peaks measuring allele specific SNPs. Percentages of the expression level of the cDNA samples are related to the genomic allelic distribution being 50% because of the heterozygous diploid genotype. Genomic DNA of the corresponding genotypes was not analyzed in pyrosequencing assay. Standard deviations are derived from two biological replicates done in technical triplicates.

The three potato genotypes are diploid, meaning that they can be homozygous harbouring one *invGE* allele or heterozygous consisting of two *invGE* alleles. For P18, P40, and P54 two different *invGE* alleles were identified, respectively. Consequently, each allele per genotype is

present at 50%. Pyrosequencing analysis of leaf cDNA showed transcriptional changes of the genotype specific *invGE* alleles compared to their allele dosage. The allele *E_P18N1* revealed an increased abundance from 50% of its genomic distribution to 80% in cDNA.

In P40 the allele *E_P40N1* was under-represented in cDNA samples compared to its genomic dosage, whilst the allele *E_P40N2* showed an abundance increase up to 62% compared to its genomic dosage.

The two P54 alleles exhibit a similar expression pattern to P40. The allele order was inverted meaning that *E_P54N1* was over-represented, whilst *E_P54N2* was under-represented compared to the genomic allele dosage.

3.2.2.1.3 Expression pattern of *invGF* alleles in flowers of the tetraploid genotypes

Allele specific SNPs were selected based on multiple nucleotide alignment to separate different *invGF* alleles in the tetraploid genotypes (Table 3.2.5). The following Table 3.2.10 summarizes the selected SNPs from each cultivar.

Table 3.2.10: Allele specific SNPs analyzed by pyrosequencing.

Genotype	Allele	SNP position	Allele specific SNP
			<i>F_SN1/F_SN2/F_SN3/F_SN4</i>
‘Satina’	<i>F_SN1</i>	SNP 111	T /C/C/C
	<i>F_SN2</i>	SNP 459	C/ T /C/C
	<i>F_SN3</i>	SNP 378	A/A/ G /A
	<i>F_SN4</i>	SNP 96	C/C/C/ T
‘Diana’	<i>F_DN1</i>	SNP 96	T
	<i>F_DN2</i>		C
‘Theresa’	<i>F_TN1</i>	SNP 96	T
	<i>F_TN2</i>		C

SNP positions refer to cDNA sequence where ‘1’ represents the adenine of the start codon ATG. Primers used for pyrosequencing are listed in chapter 2 (Table 2.1.6 and Table 2.2.11). Allele specific SNPs are highlighted in bold capitals.

The pyrosequencing analysis was performed using cDNA and genomic DNA from flowers to measure the dosages of the alleles (Figure 3.2.26).

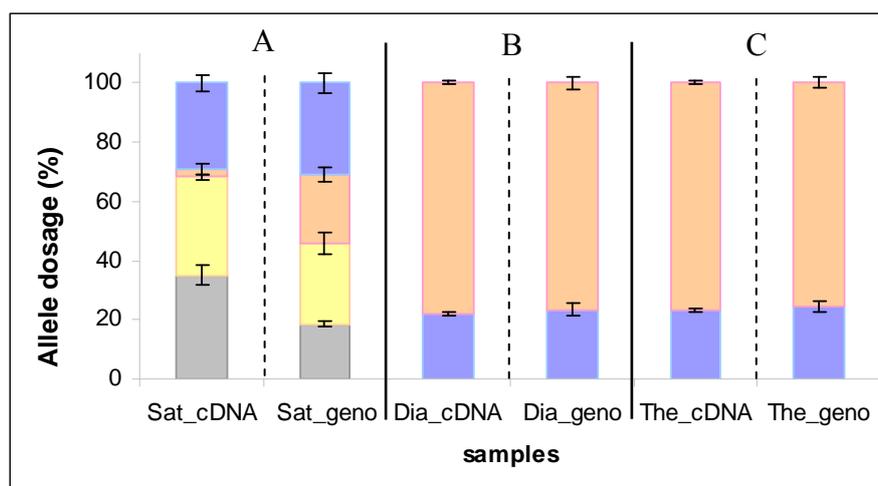


Figure 3.2.26: Pyrosequencing analysis of *invGF* alleles from the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’. **A:** Pyrosequencing of ‘Satina’ alleles. \square : *F_SNI*; \square : *F_SN2*; \square : *F_SN3*; \square : *F_SN4*. **B:** Pyrosequencing of ‘Diana’ alleles. \square : *F_DNI*; \square : *F_DN2*. **C:** Pyrosequencing of ‘Theresa’ alleles. \square : *F_TNI*; \square : *F_TN2*. 100% is defined as the sum of alternative peaks measuring allele specific SNPs. In addition to pyrosequencing analysis of cDNA samples, the genomic dosage of the alleles was determined using genomic DNA of flowers. Percentages of the expression level of the cDNA samples are related to the genomic allelic distribution. Standard deviations are derived from two biological replicates done in technical triplicates.

For the cultivar ‘Satina’ four *invGF* alleles were found. If the alleles were transcribed according to the allele dosage, each allele is expected to contribute 25% of the total transcripts. In the cDNA pyrosequencing assay marginal transcript was detectable for the allele *F_SN3*. The alleles *F_SNI* and *F_SN2* were more abundant in cDNA compared to their genomic dosage, whilst the presence of allele *F_SN4* remained stable at 25% according to its genomic distribution.

In the cultivar ‘Diana’ two alleles were identified. The allele *F_DNI* was present in simplex (25%), whilst the allele *F_DN2* was triplex (75%). However, *F_DN2* could possibly consist of up to three diverse alleles due to the tetraploidy of ‘Diana’. Pyrosequencing of flower cDNA revealed no differences in the expression pattern of both alleles compared to their genomic dosage.

The alleles *F_TNI* and *F_TN2* were defined for the cultivar ‘Theresa’. The allele *F_TNI* existed in simplex (25%), whilst the allele *F_TN2* was present in triplex (75%). Considering the tetraploidy, the fraction represented by the allele *F_TN2* might consist of more than one allele. Similar to the expression pattern of the two ‘Diana’ alleles, also the ‘Theresa’ alleles did not show any changes in their expression compared to their genomic dosage.

3.2.2.1.4 Expression pattern of *invGF* alleles in flowers of the diploid genotypes

From the potato genotype P18 one *invGF* allele was identified, whilst for P40 and P54 two alleles were found. In the pyrosequencing assay the distribution of allele specific SNPs were

analyzed (Table 3.2.6). In the following Table 3.2.11 discriminative SNPs of P40 and P54 alleles are listed.

Table 3.2.11: Allele specific SNPs analyzed by pyrosequencing.

Genotype	Allele	SNP position	Allele specific SNP
P40	<i>F_P40N1</i>	SNP 1534	G
	<i>F_P40N2</i>		A
P54	<i>F_P54N1</i>	SNP 1446	T
	<i>F_P54N2</i>		C

SNP positions refer to cDNA sequence where 1 represents the adenine of the start codon ATG. Primers used for pyrosequencing are listed in chapter 2 (Table 2.1.6 and Table 2.2.12).

The pyrosequencing analysis was performed using cDNA and genomic DNA from flowers to measure the dosages of the alleles (Figure 3.2.27)

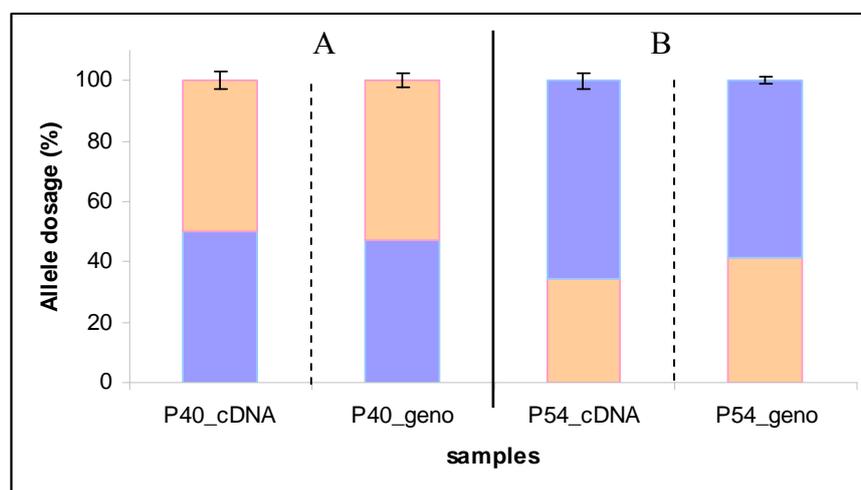


Figure 3.2.27: Pyrosequencing analysis of *invGF* alleles from the diploid genotypes P40 and P54. A: Pyrosequencing of P40 alleles. ■: *F_P40N1*; ■: *F_P40N2*. **B:** Pyrosequencing of P54 alleles. ■: *F_P54N1*; ■: *F_P54N2*. 100% is defined as the sum of alternative peaks measuring allele specific SNPs. In addition to pyrosequencing analysis of cDNA samples, the genomic dosage of the alleles was determined using genomic DNA of flowers. Percentages of the expression level of the cDNA samples are related to the genomic allelic distribution. Standard deviations are derived from two biological replicates done in technical triplicates.

Regarding the diploidy of the potato genotypes, the two alleles per genotype are expected to be equally frequent at genomic level. The expression pattern of the two P40 alleles *F_P40N1* and *F_P40N2* remained unchanged compared to their genomic dosage. Transcript levels of both alleles were consistent at approximately 50%.

In P54 the allele *F_P54N1* showed a decreased abundance of 15% compared to its genomic dosage. The allele *F_P54N2* was over-represented up to approximately 65% in cDNA compared to its genomic dosage.

3.2.2.2 Functional complementation of the yeast invertase mutant *SUC2*

Similar to the previous functional complementation analysis of the yeast invertase mutant *SUC2* with *Pain-1* cDNA alleles (section 3.1.2.2), cDNA alleles of the genes *invGE* and *invGF* were used for yeast transformation. Transforming *SUC2* with *invGE* and *invGF* cDNA alleles resulted in yeast transformants that were able to grow on sucrose as carbohydrate source, indicating functional complementation of the *SUC2* mutation.

The *invGE* and *invGF* cDNA alleles listed below (Figure 3.2.28) were used for complementation of *SUC2* and subsequent analysis of invertase activity (section 3.2.2.3).

Genotype	<i>invGE</i> alleles	
'Satina'	<i>E_SA</i>	
	<i>E_SN1</i>	
	<i>E_SN3</i>	
'Theresa'	<i>E_TA</i>	
	<i>E_TN1</i>	
P18	<i>E_P18N1</i>	
P40	<i>E_P40N1</i>	
	<i>E_P40N2</i>	
P54	<i>E_P54N1</i>	
	<i>E_P54N2</i>	
	<i>invGF</i> alleles	
'Satina'	<i>F_SN1</i>	
	<i>F_SN2</i>	
	<i>F_SN3</i>	
	<i>F_SN4</i>	
'Diana'	<i>F_DN1</i>	
	<i>F_DN2</i>	
P18	<i>F_P18N</i>	
P40	<i>F_P40N1</i>	
	<i>F_P40N2</i>	
P54	<i>F_P54N1</i>	
	<i>F_P54N2</i>	

Representative yeast transformants complemented with *invGE* and *invGF* invertase alleles were spotted on solid yeast minimal broth with 2% sucrose as carbon source. The wild type *FY 1479* was plated as positive control, whilst the invertase mutant *SUC2* was the negative control.

Figure 3.2.28: cDNA alleles used for *SUC2* complementation and *SUC2* transformants on solid yeast minimal media.

All *SUC2* invertase transformants exhibited substantial growth on sucrose and were used for the biochemical characterization of the *invGE* and *invGF* alleles. The potato alleles complemented the invertase deficiency of the yeast mutant not this good as the wild type *FY 1479* and displayed less colonies.

3.2.2.3 Biochemical characterization of *invGE* and *invGF* alleles

Putative 3D-models of allelic *invGE* and *invGF* molecules (section 3.2.1.3) indicated structural and electrostatic differences between the alleles, which could cause functional

differences. To test, whether structural characteristics might influence enzymatic activity of *invGE* and *invGF* invertase alleles, biochemical characterization was performed.

From yeast *SUC2* transformants complemented with *invGE* and *invGF* cDNA alleles, total protein was extracted. To test invertase activity, a modified protocol based on ZRENNER ET AL. (1995) was used. Invertase assays were carried out at 30°C. The *invGE* and *invGF* alleles displayed in Table 3.2.12 were used for biochemical characterization.

Table 3.2.12: *invGE* and *invGF* alleles used for biochemical characterization.

Genotype	<i>invGE</i> alleles	Genotype	<i>invGF</i> alleles
‘Satina’	<i>E_SA</i>	‘Satina’	<i>F_SN1</i>
	<i>E_SN1</i>		<i>F_SN2</i>
	<i>E_SN3</i>		<i>F_SN3</i>
	<i>F_SN4</i>		
‘Theresa’	<i>E_TA</i>	‘Diana’	<i>F_DN1</i>
	<i>E_TN1</i>		<i>F_DN2</i>
P18	<i>E_P18N1</i>	P18	<i>F_P18N</i>
P40	<i>E_P40N1</i>	P40	<i>F_P40N1</i>
	<i>E_P40N2</i>		<i>F_P40N2</i>
P54	<i>E_P54N1</i>	P54	<i>F_P54N1</i>
	<i>E_P54N2</i>		<i>F_P54N2</i>

The biochemical parameters Michaelis constant (K_m) and the maximal velocity (v_{max}) of invertase reaction were determined.

K_m values are independent from the enzyme concentration, whereas v_{max} values depend on the enzyme concentration. Due to the lack of an appropriate antibody against *invGE* and *invGF* cell wall-bound invertase isoforms, immunoblot analysis could not be performed (section 3.2.2.4). Therefore, v_{max} values of *invGE* and *invGF* alleles cannot be taken into account until protein quantification can be carried out.

❖ Biochemical characterization of *invGE* alleles

Results of the biochemical analysis for *invGE* alleles are shown in Table 3.2.13.

Table 3.2.13: K_m (mM) of *invGE* invertase alleles.

Genotype	<i>invGE</i> allele	K_m
‘Satina’	<i>E_SA</i>	19.66±0.93
	<i>E_SN1</i>	16.99±0.85
	<i>E_SN3</i>	23.97±0.93
‘Theresa’	<i>E_TA</i>	21.32±0.93
	<i>E_TN1</i>	18.64±0.85
P18	<i>E_P18N1</i>	24.4±0.93
P40	<i>E_P40N1</i>	17.13±0.85
	<i>E_P40N2</i>	17.1±0.85
P54	<i>E_P54N1</i>	22.73±0.85
	<i>E_P54N2</i>	19.51±0.85
<i>FY 1679</i>		24.25±2.5

Standard deviations are derived from three biological replicates for the associated alleles *E_SA* and *E_TA*, and the wild type reference strain *FY 1679* and from two biological replicates for the other alleles done in technical replicates to obtain six measurements. To make assays of different invertase isoforms comparable, the yeast reference strain *FY 1679* was used as positive control.

Table 3.2.14 summarizes the significance values for differences between the K_m values measured for the *invGE* alleles. The statistical calculation was performed by Benjamin Stich, MPIZ/Köln.

Table 3.2.14: Statistical significance levels of K_m values from the *invGE* invertase alleles.

Allele	<i>E_SA</i>	<i>E_SNI</i>	<i>E_SN3</i>	<i>E_TA</i>	<i>E_TNI</i>	<i>E_P18NI</i>	<i>E_P40NI</i>	<i>E_P40N2</i>	<i>E_P54NI</i>	<i>E_P54N2</i>
<i>E_SA</i>	---	0.056	0.006	0.23	0.43	0.0036	0.067	0.065	0.032	0.91
<i>E_SNI</i>	---	---	0.0001	0.0050	0.19	7.66E-05	0.92	0.93	0.0004	0.06
<i>E_SN3</i>	---	---	---	0.067	0.001	0.75	0.0002	0.0001	0.34	0.004
<i>E_TA</i>	---	---	---	---	0.055	0.038	0.006	0.006	0.29	0.18
<i>E_TNI</i>	---	---	---	---	---	0.0006	0.23	0.22	0.005	0.48
<i>E_P18NI</i>	---	---	---	---	---	---	8.97E-05	8.70E-05	0.21	0.002
<i>E_P40NI</i>	---	---	---	---	---	---	---	0.98	0.0005	0.07
<i>E_P40N2</i>	---	---	---	---	---	---	---	---	0.0005	0.067
<i>E_P54NI</i>	---	---	---	---	---	---	---	---	---	0.02
<i>E_P54N2</i>	---	---	---	---	---	---	---	---	---	---

Significant differences are defined by P-values <0.05 and are coloured in orange, no significant differences are described by P-values >0.05.

The K_m values of the ‘Satina’ alleles *E_SA* and *E_SNI* differed not significantly but showed a significant difference to *E_SN3*. The allele *E_SN3* revealed the highest K_m of approximately 24mM meaning that this allele shows a lower affinity to its substrate sucrose compared to the other two ‘Satina’ alleles.

K_m values of the two ‘Theresa’ alleles did not differ significantly. The allele *E_TA* had a K_m value of approximately 21mM, whilst the K_m of allele *E_TNI* was around 19mM.

Comparison of K_m values of the two P40 alleles *E_P40NI* and *E_P40N2* revealed no significant change in enzyme affinity to its substrate. K_m values of both enzymes were approximately 17mM. The K_m values of P54 alleles *E_P54NI* and *E_P54N2* differed significantly. *E_P54NI* displayed a K_m value around 23mM and *E_P54N2* of approximately 20mM. The K_m value of the yeast wild type reference strain *FY 1679* was approximately 24mM.

❖ Biochemical characterization of *invGF* alleles

Results of the biochemical analysis for *invGF* alleles are shown in Table 3.2.15

Table 3.2.15: K_m (mM) of *invGF* invertase alleles.

Genotype	<i>invGF</i> allele	K_m
‘Satina’	<i>F_SNI</i>	12.72±1.41
	<i>F_SN2</i>	12.30±1.41
	<i>F_SN3</i>	14.73±1.41
	<i>F_SN4</i>	17.71±1.23
‘Diana’	<i>F_DNI</i>	13.10±1.41
	<i>F_DN2</i>	14.45±1.45
P18	<i>F_P18N</i>	13.90±1.41
P40	<i>F_P40NI</i>	21.41±1.41
	<i>F_P40N2</i>	17.03±1.41
P54	<i>F_P54NI</i>	18.02±1.41
	<i>F_P54N2</i>	16.65±1.41
<i>FY 1679</i>		20.1±3.1

Standard deviations are derived from two biological replicates done in technical triplicates. To make assays of different invertase isoforms comparable, the yeast reference strain *FY 1679* was used as positive control.

Table 3.2.16 summarizes the significance values for differences between the K_m values measured for the *invGF* alleles. The statistical calculation was performed by Benjamin Stich, MPIZ/Köln.

Table 3.2.16: Statistical significance levels of K_m values from the *invGF* invertase alleles.

Allele	<i>F</i> <i>SN1</i>	<i>F</i> <i>SN2</i>	<i>F</i> <i>SN3</i>	<i>F</i> <i>SN4</i>	<i>F</i> <i>DN1</i>	<i>F</i> <i>DN2</i>	<i>F</i> <i>P18N</i>	<i>F</i> <i>P40N1</i>	<i>F</i> <i>P40N2</i>	<i>F</i> <i>P54N1</i>	<i>F</i> <i>P54N2</i>
<i>F</i> _{<i>SN1</i>}	---	0.83	0.33	0.02	0.87	0.41	0.56	0.0009	0.05	0.02	0.07
<i>F</i> _{<i>SN2</i>}	---	---	0.24	0.01	0.71	0.31	0.44	0.0006	0.035	0.014	0.05
<i>F</i> _{<i>SN3</i>}	---	---	---	0.14	0.03	0.1	0.06	0.07	0.72	0.12	0.35
<i>F</i> _{<i>SN4</i>}	---	---	---	---	0.03	0.11	0.06	0.07	0.72	0.87	0.58
<i>F</i> _{<i>DN1</i>}	---	---	---	---	---	0.51	0.68	0.001	0.07	0.03	0.1
<i>F</i> _{<i>DN2</i>}	---	---	---	---	---	---	0.8	0.005	0.23	0.1	0.3
<i>F</i> _{<i>P18N</i>}	---	---	---	---	---	---	---	0.003	0.14	0.06	0.2
<i>F</i> _{<i>P40N1</i>}	---	---	---	---	---	---	---	---	0.048	0.11	0.034
<i>F</i> _{<i>P40N2</i>}	---	---	---	---	---	---	---	---	---	0.63	0.85
<i>F</i> _{<i>P54N1</i>}	---	---	---	---	---	---	---	---	---	---	0.51
<i>F</i> _{<i>P54N2</i>}	---	---	---	---	---	---	---	---	---	---	---

Significant differences are defined by P-values <0.05 and are coloured in orange, no significant differences are described by P-values >0.05.

The K_m values of the ‘Satina’ alleles *F*_{*SN1*}, *F*_{*SN2*}, and *F*_{*SN3*} were similar, whilst the K_m value of allele *F*_{*SN4*} differed significantly. The allele *F*_{*SN4*} showed the lowest substrate affinity compared to the other ‘Satina’ alleles characterized by a K_m value of approximately 18mM. The enzymatic characteristics of the two ‘Diana’ alleles *F*_{*DN1*} (13mM) and *F*_{*DN2*} (≈14mM) were similar to each other, no significant differences were detected.

The K_m values of the P40 alleles *F*_{*P40N1*} and *F*_{*P40N2*} differed significantly. The allele *F*_{*P40N1*} displayed a lower affinity (≈21mM) to sucrose than allele *F*_{*P40N2*} (≈17mM). The K_m values of the two P54 alleles *F*_{*P54N1*} and *F*_{*P54N2*} showed similarity to each other, no enzymatic differences were observed. The K_m value of *F*_{*P54N1*} was 18mM and of *F*_{*P54N2*} approximately 17mM. The K_m value of the yeast wild type reference strain *FY 1679* was approximately 20mM.

❖ Comparison of biochemical *invGE* and *invGF* characteristics

Both *invGE* and *invGF* genes encode cell wall-bound invertase isoforms functional in the apoplast. Biochemical analysis of *invGE* and *invGF* alleles showed differences in enzyme affinity to the substrate sucrose. The significance values for differences between the K_m values measured for *invGE* alleles compared to *invGF* alleles are listed in Table 3.2.17.

Table 3.2.17: Statistical significance levels of K_m values from the *invGE* and *invGF* invertase alleles.

Allele	<i>E</i> <i>SA</i>	<i>E</i> <i>SN1</i>	<i>E</i> <i>SN3</i>	<i>E</i> <i>TA</i>	<i>E</i> <i>TNI</i>	<i>E</i> <i>P18N1</i>	<i>E</i> <i>P40N1</i>	<i>E</i> <i>P40N2</i>	<i>E</i> <i>P54N1</i>	<i>E</i> <i>P54N2</i>
<i>F_SN1</i>	0.002	0.029	4.30 E-05	0.0005	0.005	3.08 E-05	0.026	0.026	0.0003	0.004
<i>F_SN2</i>	0.001	0.019	3.03 E-05	0.0003	0.003	2.19 E-05	0.016	0.017	0.0002	0.003
<i>F_SN3</i>	0.016	0.215	0.0002	0.003	0.043	0.0002	0.192	0.196	0.003	0.049
<i>F_SN4</i>	0.28	0.685	0.0042	0.0669	0.595	0.0028	0.739	0.728	0.1027	0.8082
<i>F_DN1</i>	0.001	0.042	5.71 E-05	0.001	0.0072	4.07 E-05	0.037	0.038	0.001	0.007
<i>F_DN2</i>	0.017	0.205	0.0003	0.004	0.0438	0.0002	0.183	0.187	0.004	0.051
<i>F_P18N</i>	0.007	0.099	0.0001	0.002	0.0179	8.31 E-05	0.087	0.089	0.001	0.018
<i>F_P40N1</i>	0.358	0.025	0.18	0.953	0.1351	0.125	0.029	0.028	0.512	0.051
<i>F_P40N2</i>	0.16	0.985	0.002	0.036	0.3699	0.0015	0.957	0.968	0.049	0.506

Significant differences are defined by P-values <0.05 and are coloured in orange, no significant differences are described by P-values >0.05.

Significant differences in K_m values were detected for the *invGE* and *invGF* alleles of the cultivar ‘Satina’. The alleles *E_SA* and *E_SN1* were significantly different to *F_SN1*, *F_SN2*, and *F_SN3*, whilst *F_SN4* showed no significant differences. The allele *E_SN3* differed significantly from all four *invGF* alleles.

Biochemical analysis of the P18 *invGE* and *invGF* alleles showed significant differences for both alleles. The allele *E_P40N1* converted sucrose with a lower affinity than the allele *F_P40N2*. The *invGF* alleles of P54 possessed a slightly lower affinity to sucrose than the P54 *invGE* alleles.

3.2.2.4 Western blot analysis of *invGE* and *invGF* invertase proteins

To analyze whether the observed differences of invertase activity of *invGE* and *invGF* alleles are due to changes in protein quantity, it is necessary to perform immunoblot quantification. From all invertase antibodies tested (chapter 2, Table 2.2.15), no satisfying *invGE* and *invGF* protein detection pattern were obtained. Therefore, a specific antibody against potato invertases was custom produced by BioGenes, Gesellschaft für Biopolymere mbH (Berlin) and is subject of ongoing investigations of this project.

3.3 The *Invap-a* locus on chromosome X

The *Inv_{ap}-a* locus consists of the two invertase genes *pCD111* and *pCD141*, and encodes cell wall-bound invertase isoforms. These acidic insoluble invertases act in a pH optimum range of 3.5 to 5.1, and are functional in the apoplast (HEDLEY ET AL., 1993, 1994). cDNA of *pCD111* (incomplete sequence; accession: Z21486) and *pCD141* (accession: Z22645) have been cloned and structurally characterized (HEDLEY ET AL. 1993, 1994).

3.3.1 Structural characterization of the genes *pCD111* and *pCD141*

3.3.1.1 Molecular cloning of *pCD111* and *pCD141* invertase cDNA alleles from leaf tissue

Using full-length gene specific primers, cDNA invertase alleles of *pCD111* and *pCD141* were cloned and sequenced from the three tetraploid potato cultivars ‘Satina’, ‘Diana’ and ‘Theresa’, and from the three diploid potato genotypes P18, P40, and P54. The tetraploid genotypes were selected based on the presence of the associated SSCP fragment from the *pCD141* gene (LI ET AL., 2008; Table 3.3.1) and, therefore, were chosen in this study. The diploid potato genotypes were included because they were used as parents for mapping QTLs and candidate genes for cold sweetening of potato tubers (MENÉNDEZ ET AL., 2002).

Table 3.3.1: Distribution of the associated SSCP fragment *pCD141_3c* present in the tetraploid genotypes ‘Satina’, ‘Diana’, and ‘Theresa’.

Genotype	<i>pCD141-3c</i>
‘Satina’	1
‘Diana’	0
‘Theresa’	0

0=SSCP fragment is absent, 1=SSCP fragment is present. Primers used for the amplification of the fragment *pCD141-3* are listed in chapter 2 (Table 2.1.8).

Out of 40 cloned cDNA sequences per genotype, a sequence was defined as an allele when it was detected twice in two independent PCRs⁹. Additionally, the consensus sequence of all alleles found in one genotype was used for allele definition when variable sequence polymorphisms occurred. For the genes *pCD111* and *pCD141* not every allele was detected twice in two independent PCRs due to the focus on the genes *Pain-1* (section 3.1), *invGE*, and *invGF* (section 3.2) because at the beginning of this work less information about association and putative allelic effects on potato chips quality of *pCD111* and *pCD141* was available. *pCD111* and *pCD141* invertase alleles obtained from each genotype are listed in Table 3.3.2.

⁹ Exceptions are listed in Appendix A 3.3.

Table 3.3.2: Overview of *pCD111* and *pCD141* alleles.

Genotype	Full-length clones	<i>pCD111</i> alleles	Full-length clones	<i>pCD141</i> alleles
‘Satina’	5	<i>pCD111_S1</i> <i>pCD111_S2</i> <i>pCD111_S3</i>	6	<i>pCD141_S1</i> <i>pCD141_S2</i> <i>pCD141_S3</i>
‘Diana’	1	<i>pCD111_D1</i>	5	<i>pCD141_D1</i> <i>pCD141_D2</i>
‘Theresa’	4	<i>pCD111_T1</i> <i>pCD111_T2</i>	6	<i>pCD141_T1</i> <i>pCD141_T2</i> <i>pCD141_T3</i>
P18	0	0	5	<i>pCD141_P18_1</i> <i>pCD141_P18_2</i>
P40	1	<i>pCD111_P40_1</i>	2	<i>pCD141_P40_1</i>
P54	3	<i>pCD111_P54_1</i> <i>pCD111_P54_2</i>	4	<i>pCD141_P54_1</i> <i>pCD141_P54_2</i>

The full-length clone number refers to the number of fully sequenced clones from each genotype used for allele definition. PCR amplification was carried out using gene specific full-length primers: *pCD111*-CD111fl_F/CD111fl_R (chapter 2, Table 2.1.2), *pCD141*-CD141fl_F/CD141fl_R (chapter 2, Table 2.1.2).

In ‘Theresa’, P18, and P40 cDNA sequences were found, which contained internal frame shifts and missing or modified start or stop codons in gene *pCD111*. These sequences were excluded from the analysis. From all six genotypes selected in this study, nine *pCD111* and 13 *pCD141* alleles were identified

3.3.1.1.1 *pCD111* cDNA alleles of the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’,¹⁰

From five full-length cDNA clones of the genotype ‘Satina’ three different alleles *pCD111_S1*, *pCD111_S2*, and *pCD111_S3* were identified. For the cultivar ‘Diana’ one full-length clone was obtained and named *pCD111_D1*. Cloning and sequencing of ‘Theresa’ cDNA resulted in four full-length clones, from which two *pCD111* alleles *pCD111_T1* and *pCD111_T2* were defined.

Comparing all six alleles at nucleotide level (Appendix A 3.3.6), 47 single nucleotide polymorphisms (SNPs) were detected, 30 of them resulted in an amino acid exchange (Table 3.3.3; Figure 3.3.1).

¹⁰ Alleles of each genotype were defined by alignment of all fully sequenced cDNA clones: ‘Satina’ (Appendix A 3.3.1), ‘Theresa’ (Appendix A 3.3.2). The nucleotide sequence for one allele was defined by aligning all obtained nucleotide sequences for this allele and using the consensus sequence when SNPs occurred: *pCD111_S1* (Appendix A 3.3.3), *pCD111_S2* (Appendix A 3.3.4), *pCD111_T2* (Appendix A 3.3.5). For the alleles *pCD111_S3*, *pCD111_D1*, and *pCD111_T1* only one nucleotide sequence was obtained, respectively.

Table 3.3.3: SNPs present in ‘Satina’, ‘Diana’, and ‘Theresa’ *pCD111* alleles.

Position of cDNA SNP	<i>pCD111</i> S1	<i>pCD111</i> S2	<i>pCD111</i> S3	<i>pCD111</i> D1	<i>pCD111</i> T1	<i>pCD111</i> T2	aa
181	A	A	A	A	C	A	C/A Q61N
183	A	T	A	A	A	T	A/T K61N
207	C	C	T	T	C	C	s.
267	T	T	A	A	A	T	s.
303	C	C	G	G	C	C	s.
357	T	C	T	T	T	C	s.
478	A	G	G	G	G	G	A/G N160D
635	G	A	G	G	G	A	G/A G212E
640	C	A	C	C	C	A	C/A Q214K
668	A	G	A	A	A	G	A/G K223R
670	A	G	A	A	A	G	A/G N224D
675	C	C	C	C	A	C	C/A L225F
691	A	G	A	A	A	G	A/G I231V
693	T	C	T	T	T	C	T/C I231V
697	C	G	C	C	C	G	C/G H233D
750	T	C	T	T	T	C	s.
780	G	A	G	G	G	A	s.
815	C	C	T	T	T	C	T/C V272A
858	A	C	A	A	C	C	s.
865	A	A	A	A	C	A	C/A Q289K
866	T	A	T	T	A	A	T/A I289K
897	A	C	A	A	A	C	s.
920	G	T	G	G	G	G	G/T W307
924	G	C	G	G	G	C	G/C K308N
939	C	C	T	T	C	C	s.
972	T	T	C	C	T	T	s.
977	C	T	C	C	C	T	C/T P326L
981	C	G	C	C	C	G	C/G S327R
1039	G	G	A	A	G	G	G/A V347I
1056	A	G	G	G	A	G	s.
1081	T	C	T	T	T	C	s.
1104	G	G	A	A	G	G	s.
1110	G	G	A	A	G	G	s.
1141	C	C	T	T	C	C	C/T L381F
1143	A	A	T	T	A	A	A/T L381F

Position of cDNA SNP	<i>pCD111</i> S1	<i>pCD111</i> S2	<i>pCD111</i> S3	<i>pCD111</i> D1	<i>pCD111</i> T1	<i>pCD111</i> T2	aa
1182	G	C	C	C	C	C	G/C K394N
1245	T	C	C	C	T	C	s.
1252	T	T	T	T	A	T	A/T T418S
1273	C	C	C	C	A	C	A/C T425P
1286	A	A	G	A	A	A	A/G N429S
1292	A	C	C	C	A	C	A/C D431A
1334	C	C	C	C	T	C	T/C M445T
1401	G	A	G	G	G	A	s.
1426	G	G	A	A	G	G	G/A A476T
1432	G	A	G	G	G	A	G/A D478N
1464	A	A	C	C	A	A	s.
1538	T	C	C	C	C	C	T/C I513T

SNP position numbering refers to cDNA sequence where '1' represents the adenine of the start codon ATG and the polymorphism is described. aa=amino acid exchanges ; s.=synonymous nucleotide exchange that does not lead to an amino acid exchange. Non synonymous nucleotide exchanges lead to an amino acid exchange and are orange coloured.

The six *pCD111* alleles from the three tetraploid potato cultivars displayed 47 SNPs resulting in 26 variable amino acid positions because at protein sequence positions 61 and 289 three different amino acids are displayed (Figure 3.3.1). The three alleles of 'Satina' had a total of 41 SNPs, of which 24 caused an amino acid exchange. In the two allelic sequences of 'Theresa' 29 SNPs occurred, from which 19 resulted in amino acid substitutions.

The amino acid alignment shows the polymorphisms of all six *pCD111* alleles from the three tetraploid potato cultivars. The comparison of these deduced protein sequences revealed variable amino acid positions in the different genotypes seen in red, yellow, and grey (Figure 3.3.1).

```

CD111_S3 : MDCLKSSLSFSLPIFLLYFSIILSFNNGVNASHKVFPGQLQSTSTVDVKNVHRTGYHFQPPKNWINDPNAPMYNGVYHLYFYQY : 83
CD111_D1 : MDCLKSSLSFSLPIFLLYFSIILSFNNGVNASHKVFPGQLQSTSTVDVKNVHRTGYHFQPPKNWINDPNAPMYNGVYHLYFYQY : 83
CD111_S1 : MDCLKSSLSFSLPIFLLYFSIILSFNNGVNASHKVFPGQLQSTSTVDVKNVHRTGYHFQPPKNWINDPNAPMYNGVYHLYFYQY : 83
CD111_T1 : MDCLKSSLSFSLPIFLLYFSIILSFNNGVNASHKVFPGQLQSTSTVDVKNVHRTGYHFQPPKNWINDPNAPMYNGVYHLYFYQY : 83
CD111_S2 : MDCLKSSLSFSLPIFLLYFSIILSFNNGVNASHKVFPGQLQSTSTVDVKNVHRTGYHFQPPKNWINDPNAPMYNGVYHLYFYQY : 83
CD111_T2 : MDCLKSSLSFSLPIFLLYFSIILSFNNGVNASHKVFPGQLQSTSTVDVKNVHRTGYHFQPPKNWINDPNAPMYNGVYHLYFYQY : 83

*          20          40          60          80
CD111_S3 : NPYGSVWGNIVWAHSVSTDLINWIPLPFGIYPSEVFDKYGTWGSATILPNNKPIILYTGIVDAKNTQVQNYAIPADISDPFL : 166
CD111_D1 : NPYGSVWGNIVWAHSVSTDLINWIPLPFGIYPSEVFDKYGTWGSATILPNNKPIILYTGIVDAKNTQVQNYAIPADISDPFL : 166
CD111_S1 : NPYGSVWGNIVWAHSVSTDLINWIPLPFGIYPSEVFDKYGTWGSATILPNNKPIILYTGIVDAKNTQVQNYAIPADISDPFL : 166
CD111_T1 : NPYGSVWGNIVWAHSVSTDLINWIPLPFGIYPSEVFDKYGTWGSATILPNNKPIILYTGIVDAKNTQVQNYAIPADISDPFL : 166
CD111_S2 : NPYGSVWGNIVWAHSVSTDLINWIPLPFGIYPSEVFDKYGTWGSATILPNNKPIILYTGIVDAKNTQVQNYAIPADISDPFL : 166
CD111_T2 : NPYGSVWGNIVWAHSVSTDLINWIPLPFGIYPSEVFDKYGTWGSATILPNNKPIILYTGIVDAKNTQVQNYAIPADISDPFL : 166

*          100          120          140          160
CD111_S3 : RKWIKPDNNPLIVADV SINKTQFRDPTTCWLGDGYWRTLIGSVWVKQGLAILYKSKNFMKWTKIQHPLHSVDTGTGNWECPDF : 249
CD111_D1 : RKWIKPDNNPLIVADV SINKTQFRDPTTCWLGDGYWRTLIGSVWVKQGLAILYKSKNFMKWTKIQHPLHSVDTGTGNWECPDF : 249
CD111_S1 : RKWIKPDNNPLIVADV SINKTQFRDPTTCWLGDGYWRTLIGSVWVKQGLAILYKSKNFMKWTKIQHPLHSVDTGTGNWECPDF : 249
CD111_T1 : RKWIKPDNNPLIVADV SINKTQFRDPTTCWLGDGYWRTLIGSVWVKQGLAILYKSKNFMKWTKIQHPLHSVDTGTGNWECPDF : 249
CD111_S2 : RKWIKPDNNPLIVADV SINKTQFRDPTTCWLGDGYWRTLIGSVWVKQGLAILYKSKNFMKWTKIQHPLHSVDTGTGNWECPDF : 249
CD111_T2 : RKWIKPDNNPLIVADV SINKTQFRDPTTCWLGDGYWRTLIGSVWVKQGLAILYKSKNFMKWTKIQHPLHSVDTGTGNWECPDF : 249

*          180          200          220          240
CD111_S3 : FVLLHGTNGLDASYNKKNIKHVLKVSVDVTRFEYTVGKYDTKKDRYIPDKTSDGWNGLRLDYGNYASKSFYDPSKNRRI : 332
CD111_D1 : FVLLHGTNGLDASYNKKNIKHVLKVSVDVTRFEYTVGKYDTKKDRYIPDKTSDGWNGLRLDYGNYASKSFYDPSKNRRI : 332
CD111_S1 : FVLLHGTNGLDASYNKKNIKHVLKVSVDVTRFEYTVGKYDTKKDRYIPDKTSDGWNGLRLDYGNYASKSFYDPSKNRRI : 332
CD111_T1 : FVLLHGTNGLDASYNKKNIKHVLKVSVDVTRFEYTVGKYDTKKDRYIPDKTSDGWNGLRLDYGNYASKSFYDPSKNRRI : 332
CD111_S2 : FVLLHGTNGLDASYNKKNIKHVLKVSVDVTRFEYTVGKYDTKKDRYIPDKTSDGWNGLRLDYGNYASKSFYDPSKNRRI : 332
CD111_T2 : FVLLHGTNGLDASYNKKNIKHVLKVSVDVTRFEYTVGKYDTKKDRYIPDKTSDGWNGLRLDYGNYASKSFYDPSKNRRI : 332

*          260          280          300          320
CD111_S3 : MWGWANESDVTVNDVKKGWAGIQTIIPRKLWLDPSGKQLVQWPVEELETREQKVQLSNRKLKGGDKIEVKGITPAQADVEVTF : 415
CD111_D1 : MWGWANESDVTVNDVKKGWAGIQTIIPRKLWLDPSGKQLVQWPVEELETREQKVQLSNRKLKGGDKIEVKGITPAQADVEVTF : 415
CD111_S1 : MWGWANESDVTVNDVKKGWAGIQTIIPRKLWLDPSGKQLVQWPVEELETREQKVQLSNRKLKGGDKIEVKGITPAQADVEVTF : 415
CD111_T1 : MWGWANESDVTVNDVKKGWAGIQTIIPRKLWLDPSGKQLVQWPVEELETREQKVQLSNRKLKGGDKIEVKGITPAQADVEVTF : 415
CD111_S2 : MWGWANESDVTVNDVKKGWAGIQTIIPRKLWLDPSGKQLVQWPVEELETREQKVQLSNRKLKGGDKIEVKGITPAQADVEVTF : 415
CD111_T2 : MWGWANESDVTVNDVKKGWAGIQTIIPRKLWLDPSGKQLVQWPVEELETREQKVQLSNRKLKGGDKIEVKGITPAQADVEVTF : 415

*          340          360          380          400
CD111_S3 : SFLDKAEPFDPNWNANLYAQDVCAIKGSTVQGGGLPFGLLTLASQNLEEYTPVFFRVFKAQDKYKVLKMSDASRSTLKNDKT : 498
CD111_D1 : SFLDKAEPFDPNWNANLYAQDVCAIKGSTVQGGGLPFGLLTLASQNLEEYTPVFFRVFKAQDKYKVLKMSDASRSTLKNDKT : 498
CD111_S1 : SFLDKAEPFDPNWNANLYAQDVCAIKGSTVQGGGLPFGLLTLASQNLEEYTPVFFRVFKAQDKYKVLKMSDASRSTLKNDKT : 498
CD111_T1 : SFLDKAEPFDPNWNANLYAQDVCAIKGSTVQGGGLPFGLLTLASQNLEEYTPVFFRVFKAQDKYKVLKMSDASRSTLKNDKT : 498
CD111_S2 : SFLDKAEPFDPNWNANLYAQDVCAIKGSTVQGGGLPFGLLTLASQNLEEYTPVFFRVFKAQDKYKVLKMSDASRSTLKNDKT : 498
CD111_T2 : SFLDKAEPFDPNWNANLYAQDVCAIKGSTVQGGGLPFGLLTLASQNLEEYTPVFFRVFKAQDKYKVLKMSDASRSTLKNDKT : 498

*          420          440          460          480          500
CD111_S3 : MYKPSFAGYVDVLDLTKNTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKIKSLNAWTMGKPKMN : 581
CD111_D1 : MYKPSFAGYVDVLDLTKNTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKIKSLNAWTMGKPKMN : 581
CD111_S1 : MYKPSFAGYVDVLDLTKNTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKIKSLNAWTMGKPKMN : 581
CD111_T1 : MYKPSFAGYVDVLDLTKNTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKIKSLNAWTMGKPKMN : 581
CD111_S2 : MYKPSFAGYVDVLDLTKNTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKIKSLNAWTMGKPKMN : 581
CD111_T2 : MYKPSFAGYVDVLDLTKNTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKIKSLNAWTMGKPKMN : 581

*          520          540          560          580
CD111_S3 : WSGFHSSY : 589
CD111_D1 : WSGFHSSY : 589
CD111_S1 : WSGFHSSY : 589
CD111_T1 : WSGFHSSY : 589
CD111_S2 : WSGFHSSY : 589
CD111_T2 : WSGFHSSY : 589

```

Figure 3.3.1: Amino acid alignment of *pCD111* alleles from the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’. Amino acid exchanges are highlighted in colour. At the amino acid positions 61 and 289 three different amino acids are displayed.

Comparison of allelic cDNA sequences of the gene *pCD111* from the three tetraploid cultivars revealed that none of the six alleles is present in different genotypes.

3.3.1.1.2 *pCD111* cDNA alleles of the diploid potato genotypes P18, P40, and P54¹¹

From the genotype P18 no full-length cDNA alleles were obtained. The cloned sequences either did not feature the start codon of the gene, or the reading frame was shifted resulted in a sequence, which could not be used for further analysis. Cloning and sequencing of P40 cDNA

¹¹ Alleles of each genotype were defined by alignment of all fully sequenced cDNA clones: P54 (Appendix A 3.3.7). The nucleotide sequence for one allele was defined by aligning all obtained nucleotide sequences for this allele and using the consensus sequence when SNPs occurred: *pCD111_P54_1* (Appendix A 3.3.8). For the alleles *pCD111_P40_1* and *pCD111_P54_2* only one full-length nucleotide sequence was obtained, respectively.

produced one full-length clone. The allele was named *pCD111_P40_1*. In P54 three full-length clones were found, and two different alleles, *pCD111_P54_1* and *pCD111_P54_2*, were identified.

The nucleotide sequence comparison (Appendix A 3.3.9) of the alleles described above detected 42 SNPs. 22 of them led to amino acid exchanges (Table 3.3.4; Figure 3.3.2).

Table 3.3.4: SNPs present in P40 and P54 alleles.

Position of cDNA SNP	<i>pCD111_P40_1</i>	<i>pCD111_P54_1</i>	<i>pCD111_P54_2</i>	aa
183	A	T	T	A/T K61N
207	T	C	C	s.
267	A	T	T	s.
303	G	C	G	s.
357	T	C	C	s.
360	C	C	T	s.
389	C	C	T	C/T P133L
407	A	A	C	A/C K136T
421	T	T	C	T/C Y141H
633	G	G	C	G/C W211C
635	G	A	A	G/A G212E
640	C	A	A	C/A Q214K
668	A	G	G	A/G K223R
670	A	G	G	A/G N224D
691	A	G	G	s.
693	T	C	C	T/C I231V
697	C	G	G	C/G H233D
750	T	C	C	s.
780	G	A	G	s.
815	T	C	T	C/T A272V
858	A	C	C	s.
866	T	A	A	T/A I289K
897	A	C	C	s.
924	G	C	G	C/G N308K
939	T	C	C	s.
972	C	T	T	s.
977	C	T	T	C/T P326L
981	C	G	G	C/G S327R
1039	A	G	G	A/G I347V
1081	T	C	C	s.
1104	A	G	G	s.
1110	A	G	G	s.

Position of cDNA SNP	<i>pCD111_P40_1</i>	<i>pCD111_P54_1</i>	<i>pCD111_P54_2</i>	aa
1141	T	C	C	s.
1143	T	A	A	T/A F381L
1302	T	T	C	s.
1382	A	A	G	A/G Q461R
1401	G	A	A	s.
1404	G	G	A	s.
1426	A	G	G	A/G T476A
1432	G	A	G	A/G N478D
1464	C	A	A	s.
1757	G	A	A	G/A R586H

SNP position numbering refers to cDNA sequence where '1' represents the adenine of the start codon ATG and the polymorphism is described. aa=amino acid exchanges ; s.=synonymous nucleotide exchange that does not lead to an amino acid exchange. Non synonymous nucleotide exchanges lead to an amino acid exchange and are orange coloured.

In the two P54 allelic sequences *pCD111_P54_1* and *pCD111_P54_2* 10 SNPs occurred. Eight of them caused an amino acid substitution.

The amino acid alignment of the P40 allele *pCD111_P40_1*, and the P54 alleles *pCD111_P54_1* and *pCD111_P54_2* showed 22 polymorphisms. (Figure 3.3.2).

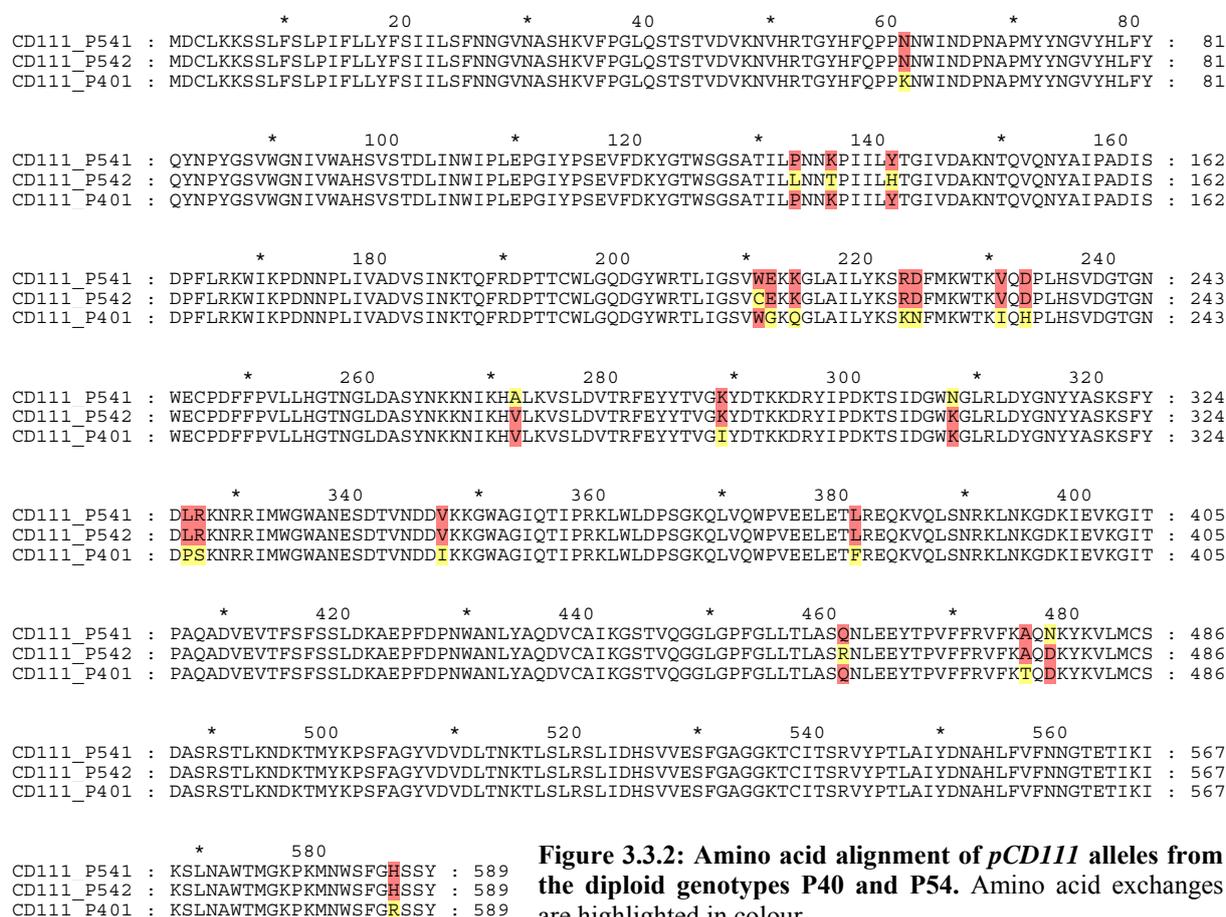


Figure 3.3.2: Amino acid alignment of *pCD111* alleles from the diploid genotypes P40 and P54. Amino acid exchanges are highlighted in colour.

3.3.1.1.3 Amino acid alignment of all *pCD111* invertase alleles of the analyzed potato genotypes

Multiple alignment of the nine *pCD111* deduced protein sequences revealed partially overlapping amino acid polymorphisms between the tetraploid and diploid genotypes (Figure 3.3.3). Several amino acid exchanges occur in different genotypes at the same position. The comparison of the protein sequences showed that amino acids differed at 32 positions, of which 17 were genotype specific and occurred only once.

		*	20	*	40	*	60	*	80	
CD111_S3	:	MDCLKSSLSLPIFLLYFSI	ILSFNNGVNASHKVFPGLQSTSTVDVKNVHRTGYHFQPP	KNWINDPNAPMYNGVYHLFY	:	81				
CD111_P401	:	MDCLKSSLSLPIFLLYFSI	ILSFNNGVNASHKVFPGLQSTSTVDVKNVHRTGYHFQPP	KNWINDPNAPMYNGVYHLFY	:	81				
CD111_D1	:	MDCLKSSLSLPIFLLYFSI	ILSFNNGVNASHKVFPGLQSTSTVDVKNVHRTGYHFQPP	KNWINDPNAPMYNGVYHLFY	:	81				
CD111_S1	:	MDCLKSSLSLPIFLLYFSI	ILSFNNGVNASHKVFPGLQSTSTVDVKNVHRTGYHFQPP	KNWINDPNAPMYNGVYHLFY	:	81				
CD111_T1	:	MDCLKSSLSLPIFLLYFSI	ILSFNNGVNASHKVFPGLQSTSTVDVKNVHRTGYHFQPP	KNWINDPNAPMYNGVYHLFY	:	81				
CD111_S2	:	MDCLKSSLSLPIFLLYFSI	ILSFNNGVNASHKVFPGLQSTSTVDVKNVHRTGYHFQPP	KNWINDPNAPMYNGVYHLFY	:	81				
CD111_T2	:	MDCLKSSLSLPIFLLYFSI	ILSFNNGVNASHKVFPGLQSTSTVDVKNVHRTGYHFQPP	KNWINDPNAPMYNGVYHLFY	:	81				
CD111_P541	:	MDCLKSSLSLPIFLLYFSI	ILSFNNGVNASHKVFPGLQSTSTVDVKNVHRTGYHFQPP	KNWINDPNAPMYNGVYHLFY	:	81				
CD111_P542	:	MDCLKSSLSLPIFLLYFSI	ILSFNNGVNASHKVFPGLQSTSTVDVKNVHRTGYHFQPP	KNWINDPNAPMYNGVYHLFY	:	81				
		*	100	*	120	*	140	*	160	
CD111_S3	:	QYNPYGSVWGNIVWAHSVSTDLINWI	PLEPGIYPSEVFDKYGTWGSATILPNNKPIILY	TGIVDAKNTQVQNYAIPAD	IS	:	162			
CD111_P401	:	QYNPYGSVWGNIVWAHSVSTDLINWI	PLEPGIYPSEVFDKYGTWGSATILPNNKPIILY	TGIVDAKNTQVQNYAIPAD	IS	:	162			
CD111_D1	:	QYNPYGSVWGNIVWAHSVSTDLINWI	PLEPGIYPSEVFDKYGTWGSATILPNNKPIILY	TGIVDAKNTQVQNYAIPAD	IS	:	162			
CD111_S1	:	QYNPYGSVWGNIVWAHSVSTDLINWI	PLEPGIYPSEVFDKYGTWGSATILPNNKPIILY	TGIVDAKNTQVQNYAIPAD	IS	:	162			
CD111_T1	:	QYNPYGSVWGNIVWAHSVSTDLINWI	PLEPGIYPSEVFDKYGTWGSATILPNNKPIILY	TGIVDAKNTQVQNYAIPAD	IS	:	162			
CD111_S2	:	QYNPYGSVWGNIVWAHSVSTDLINWI	PLEPGIYPSEVFDKYGTWGSATILPNNKPIILY	TGIVDAKNTQVQNYAIPAD	IS	:	162			
CD111_T2	:	QYNPYGSVWGNIVWAHSVSTDLINWI	PLEPGIYPSEVFDKYGTWGSATILPNNKPIILY	TGIVDAKNTQVQNYAIPAD	IS	:	162			
CD111_P541	:	QYNPYGSVWGNIVWAHSVSTDLINWI	PLEPGIYPSEVFDKYGTWGSATILPNNKPIILY	TGIVDAKNTQVQNYAIPAD	IS	:	162			
CD111_P542	:	QYNPYGSVWGNIVWAHSVSTDLINWI	PLEPGIYPSEVFDKYGTWGSATILPNNKPIILY	TGIVDAKNTQVQNYAIPAD	IS	:	162			
		*	180	*	200	*	220	*	240	
CD111_S3	:	DPFLRKWIKPDNNPLIVADVS	INKTQFRDPTTCWLGQDGYWRTLIGSVW	EKKGLAILEYKSKNFMKWT	KIQHPLHSDVDTGN	:	243			
CD111_P401	:	DPFLRKWIKPDNNPLIVADVS	INKTQFRDPTTCWLGQDGYWRTLIGSVW	EKKGLAILEYKSKNFMKWT	KIQHPLHSDVDTGN	:	243			
CD111_D1	:	DPFLRKWIKPDNNPLIVADVS	INKTQFRDPTTCWLGQDGYWRTLIGSVW	EKKGLAILEYKSKNFMKWT	KIQHPLHSDVDTGN	:	243			
CD111_S1	:	DPFLRKWIKPDNNPLIVADVS	INKTQFRDPTTCWLGQDGYWRTLIGSVW	EKKGLAILEYKSKNFMKWT	KIQHPLHSDVDTGN	:	243			
CD111_T1	:	DPFLRKWIKPDNNPLIVADVS	INKTQFRDPTTCWLGQDGYWRTLIGSVW	EKKGLAILEYKSKNFMKWT	KIQHPLHSDVDTGN	:	243			
CD111_S2	:	DPFLRKWIKPDNNPLIVADVS	INKTQFRDPTTCWLGQDGYWRTLIGSVW	EKKGLAILEYKSKNFMKWT	KIQHPLHSDVDTGN	:	243			
CD111_T2	:	DPFLRKWIKPDNNPLIVADVS	INKTQFRDPTTCWLGQDGYWRTLIGSVW	EKKGLAILEYKSKNFMKWT	KIQHPLHSDVDTGN	:	243			
CD111_P541	:	DPFLRKWIKPDNNPLIVADVS	INKTQFRDPTTCWLGQDGYWRTLIGSVW	EKKGLAILEYKSKNFMKWT	KIQHPLHSDVDTGN	:	243			
CD111_P542	:	DPFLRKWIKPDNNPLIVADVS	INKTQFRDPTTCWLGQDGYWRTLIGSVW	EKKGLAILEYKSKNFMKWT	KIQHPLHSDVDTGN	:	243			
		*	260	*	280	*	300	*	320	
CD111_S3	:	WECPDFFPVLLHGTNGLDAS	YNKKNIKHVLKVS	LDVTRFEYTVG	IYDTKKDRY	IPDKT	SIDG	WGLRLDYGNY	YASKSFY	:
CD111_P401	:	WECPDFFPVLLHGTNGLDAS	YNKKNIKHVLKVS	LDVTRFEYTVG	IYDTKKDRY	IPDKT	SIDG	WGLRLDYGNY	YASKSFY	:
CD111_D1	:	WECPDFFPVLLHGTNGLDAS	YNKKNIKHVLKVS	LDVTRFEYTVG	IYDTKKDRY	IPDKT	SIDG	WGLRLDYGNY	YASKSFY	:
CD111_S1	:	WECPDFFPVLLHGTNGLDAS	YNKKNIKHVLKVS	LDVTRFEYTVG	IYDTKKDRY	IPDKT	SIDG	WGLRLDYGNY	YASKSFY	:
CD111_T1	:	WECPDFFPVLLHGTNGLDAS	YNKKNIKHVLKVS	LDVTRFEYTVG	IYDTKKDRY	IPDKT	SIDG	WGLRLDYGNY	YASKSFY	:
CD111_S2	:	WECPDFFPVLLHGTNGLDAS	YNKKNIKHVLKVS	LDVTRFEYTVG	IYDTKKDRY	IPDKT	SIDG	WGLRLDYGNY	YASKSFY	:
CD111_T2	:	WECPDFFPVLLHGTNGLDAS	YNKKNIKHVLKVS	LDVTRFEYTVG	IYDTKKDRY	IPDKT	SIDG	WGLRLDYGNY	YASKSFY	:
CD111_P541	:	WECPDFFPVLLHGTNGLDAS	YNKKNIKHVLKVS	LDVTRFEYTVG	IYDTKKDRY	IPDKT	SIDG	WGLRLDYGNY	YASKSFY	:
CD111_P542	:	WECPDFFPVLLHGTNGLDAS	YNKKNIKHVLKVS	LDVTRFEYTVG	IYDTKKDRY	IPDKT	SIDG	WGLRLDYGNY	YASKSFY	:
		*	340	*	360	*	380	*	400	
CD111_S3	:	DPSKNRRIMWGWANES	DTVND	IKKGWAGI	QTI	PRKLWLD	PSGKQLVQWPVEELET	FREQKVLSNRKLN	KGDKIEVKGIT	:
CD111_P401	:	DPSKNRRIMWGWANES	DTVND	IKKGWAGI	QTI	PRKLWLD	PSGKQLVQWPVEELET	FREQKVLSNRKLN	KGDKIEVKGIT	:
CD111_D1	:	DPSKNRRIMWGWANES	DTVND	IKKGWAGI	QTI	PRKLWLD	PSGKQLVQWPVEELET	FREQKVLSNRKLN	KGDKIEVKGIT	:
CD111_S1	:	DPSKNRRIMWGWANES	DTVND	IKKGWAGI	QTI	PRKLWLD	PSGKQLVQWPVEELET	FREQKVLSNRKLN	KGDKIEVKGIT	:
CD111_T1	:	DPSKNRRIMWGWANES	DTVND	IKKGWAGI	QTI	PRKLWLD	PSGKQLVQWPVEELET	FREQKVLSNRKLN	KGDKIEVKGIT	:
CD111_S2	:	DPSKNRRIMWGWANES	DTVND	IKKGWAGI	QTI	PRKLWLD	PSGKQLVQWPVEELET	FREQKVLSNRKLN	KGDKIEVKGIT	:
CD111_T2	:	DPSKNRRIMWGWANES	DTVND	IKKGWAGI	QTI	PRKLWLD	PSGKQLVQWPVEELET	FREQKVLSNRKLN	KGDKIEVKGIT	:
CD111_P541	:	DPSKNRRIMWGWANES	DTVND	IKKGWAGI	QTI	PRKLWLD	PSGKQLVQWPVEELET	FREQKVLSNRKLN	KGDKIEVKGIT	:
CD111_P542	:	DPSKNRRIMWGWANES	DTVND	IKKGWAGI	QTI	PRKLWLD	PSGKQLVQWPVEELET	FREQKVLSNRKLN	KGDKIEVKGIT	:
		*	420	*	440	*	460	*	480	
CD111_S3	:	PAQADVEVTF	SFLDKAE	FD	PNWAN	LYAQDVCAIKG	STVQGG	LPFGLLTLAS	ONLEEYTPV	FRVFK
CD111_P401	:	PAQADVEVTF	SFLDKAE	FD	PNWAN	LYAQDVCAIKG	STVQGG	LPFGLLTLAS	ONLEEYTPV	FRVFK
CD111_D1	:	PAQADVEVTF	SFLDKAE	FD	PNWAN	LYAQDVCAIKG	STVQGG	LPFGLLTLAS	ONLEEYTPV	FRVFK
CD111_S1	:	PAQADVEVTF	SFLDKAE	FD	PNWAN	LYAQDVCAIKG	STVQGG	LPFGLLTLAS	ONLEEYTPV	FRVFK
CD111_T1	:	PAQADVEVTF	SFLDKAE	FD	PNWAN	LYAQDVCAIKG	STVQGG	LPFGLLTLAS	ONLEEYTPV	FRVFK
CD111_S2	:	PAQADVEVTF	SFLDKAE	FD	PNWAN	LYAQDVCAIKG	STVQGG	LPFGLLTLAS	ONLEEYTPV	FRVFK
CD111_T2	:	PAQADVEVTF	SFLDKAE	FD	PNWAN	LYAQDVCAIKG	STVQGG	LPFGLLTLAS	ONLEEYTPV	FRVFK
CD111_P541	:	PAQADVEVTF	SFLDKAE	FD	PNWAN	LYAQDVCAIKG	STVQGG	LPFGLLTLAS	ONLEEYTPV	FRVFK
CD111_P542	:	PAQADVEVTF	SFLDKAE	FD	PNWAN	LYAQDVCAIKG	STVQGG	LPFGLLTLAS	ONLEEYTPV	FRVFK

```

*           500           *           520           *           540           *           560
CD111_S3   : DASRSTLKNDKTMYKPSFAGYVDVDLTKNKTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKI : 567
CD111_P401 : DASRSTLKNDKTMYKPSFAGYVDVDLTKNKTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKI : 567
CD111_D1   : DASRSTLKNDKTMYKPSFAGYVDVDLTKNKTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKI : 567
CD111_S1   : DASRSTLKNDKTMYKPSFAGYVDVDLTKNKTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKI : 567
CD111_T1   : DASRSTLKNDKTMYKPSFAGYVDVDLTKNKTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKI : 567
CD111_S2   : DASRSTLKNDKTMYKPSFAGYVDVDLTKNKTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKI : 567
CD111_T2   : DASRSTLKNDKTMYKPSFAGYVDVDLTKNKTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKI : 567
CD111_P541 : DASRSTLKNDKTMYKPSFAGYVDVDLTKNKTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKI : 567
CD111_P542 : DASRSTLKNDKTMYKPSFAGYVDVDLTKNKTLSLRSLIDHSVVESFGAGGKTCITSRVYPTLAIYDNAHLFVFNNGTETIKI : 567

*           580
CD111_S3   : KSLNAWTMGKPKMNWSFGHSSY : 589
CD111_P401 : KSLNAWTMGKPKMNWSFGHSSY : 589
CD111_D1   : KSLNAWTMGKPKMNWSFGHSSY : 589
CD111_S1   : KSLNAWTMGKPKMNWSFGHSSY : 589
CD111_T1   : KSLNAWTMGKPKMNWSFGHSSY : 589
CD111_S2   : KSLNAWTMGKPKMNWSFGHSSY : 589
CD111_T2   : KSLNAWTMGKPKMNWSFGHSSY : 589
CD111_P541 : KSLNAWTMGKPKMNWSFGHSSY : 589
CD111_P542 : KSLNAWTMGKPKMNWSFGHSSY : 589

```

Figure 3.3.3: Amino acid alignment of all cloned *pCD111* invertase alleles. Amino acid exchanges are highlighted in colour. At the amino acid positions 61 and 289 three different amino acids are displayed.

Comparison of cDNA sequences revealed that the alleles *pCD111_T2* and *pCD111_P54_1* are identical at amino acid level.

3.3.1.1.4 Phenetic trees of all *pCD111* invertase alleles of the analyzed potato genotypes

In addition to the multiple amino acid alignment (3.3.1.1.3), the phenetic tree analysis was used to group the invertase alleles according to similarity. Using the neighbour-joining method showed that *pCD111* alleles from all analyzed potato genotypes grouped in two clades and five subclades (Figure 3.3.4).

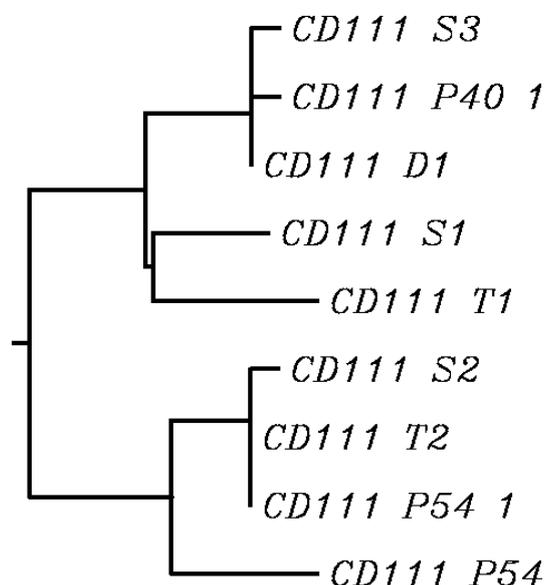


Figure 3.3.4: Amino acid based phenetic tree (Neighbour-joining tree) of all cloned *pCD111* invertase alleles.

The first clade includes alleles *pCD111_S1*, *pCD111_S3*, *pCD111_D1*, *pCD111_T1*, and *pCD111_P40_1*. The second clade consists of *pCD111_S2*, *pCD111_T2*, and the two alleles from the diploid genotype P54 *pCD111_P54_1* and *pCD111_P54_2*. Most alleles of both

clades differ in one or more amino acid positions. The alleles *pCD111_T2* and *pCD111_P54_1* are identical at amino acid level.

Cloning and sequencing of *pCD111* alleles showed that the tetraploid genotype ‘Theresa’ and the diploid genotype P54 contained an allele identical in amino acid sequence but different at nucleotide level. The following Table 3.3.5 summarizes the nucleotide comparison of the two amino acid identical alleles *pCD111_T2* and *pCD111_P54_1*. The allelic nucleotide sequence was defined based on the consensus sequence of the alignment of full-length clones obtained from each genotype (Table 3.3.2). Although SNPs are present at four positions in the cDNAs, the nucleotide polymorphisms resulted in one and the same amino acid sequence.

Table 3.3.5: Genotype specific nucleotide differences of alleles identical at amino acid level.

Position of cDNA SNP	<i>pCD111_T2</i>	<i>pCD111_P54_1</i>
21	C	T
111	A	T
681	A	G
801	G	A

The nucleotide polymorphisms between all *pCD111* (Appendix A 3.3.10) were visualized using the phenetic tree analysis (Figure 3.3.5).

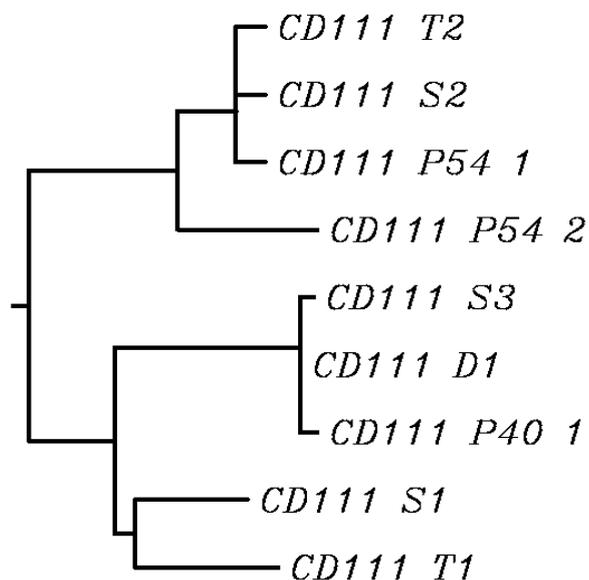


Figure 3.3.5: Nucleotide sequence based phenetic tree (Neighbour-joining tree) of all cloned *pCD111* invertase alleles.

The two phenetic trees are very similar, just displaying more subclades due to a higher number of nucleotide polymorphisms as compared to the amino acid exchanges (Figure 3.3.4).

3.3.1.1.5 *pCD141* cDNA alleles of the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’¹²

Cloning and sequencing of cDNA of the cultivar ‘Satina’ resulted in six full-length clones, from which three *pCD141* alleles *pCD141_S1*, *pCD141_S2*, and *pCD141_S3* were identified. For the cultivar ‘Diana’ five full-length clones were obtained, and of those two different alleles *pCD141_D1* and *pCD141_D2* were defined. From the cultivar ‘Theresa’ six full-length clones were isolated and led to the definition of three different alleles *pCD141_T1*, *pCD141_T2*, and *pCD141_T3*.

At nucleotide level (Appendix A 3.3.19) the alleles contain 62 SNPs but not all of them are causative for amino acid exchanges (Table 3.3.6; Figure 3.3.6).

Table 3.3.6: SNPs present in ‘Satina’, ‘Diana’, and ‘Theresa’ *pCD141* alleles.

Position cDNA SNP	<i>pCD141</i> <i>S1</i>	<i>pCD141</i> <i>S2</i>	<i>pCD141</i> <i>S3</i>	<i>pCD141</i> <i>D1</i>	<i>pCD141</i> <i>D2</i>	<i>pCD141</i> <i>T1</i>	<i>pCD141</i> <i>T2</i>	<i>pCD141</i> <i>T3</i>	aa
57	C	T	C	C	C	T	C	C	s.
140	C	T	C	C	C	T	C	C	T/C V47A
178	C	C	T	C	C	C	C	C	C/T P60S
186	C	T	C	C	C	T	C	C	s.
207	T	T	T	C	C	T	C	C	s.
276	C	T	T	T	T	T	T	T	s.
280	A	G	G	G	G	G	G	G	A/G I94V
378	C	C	C	T	T	C	T	T	s.
426	C	T	T	T	T	C	T	T	s.
440	C	C	C	G	G	C	G	G	C/G A147G
444	T	C	C	T	T	T	T	T	s.
462	T	C	T	C	C	C	C	C	s.
474	G	A	G	G	G	G	G	G	s.
483	G	A	G	G	G	G	G	G	G/A M161I
508	A	A	A	A	A	A	A	G	A/G I170V
582	A	A	A	C	C	A	C	C	s.
601	G	G	A	G	G	G	G	G	G/A G201R
621	T	T	T	A	A	A	A	A	s.
624	G	G	G	C	C	C	C	C	s.
667	A	G	A	A	A	A	A	A	A/G N223D

¹² Alleles of each genotype were defined by alignment of all fully sequenced cDNA clones: ‘Satina’ (Appendix A 3.3.11), ‘Diana’ (Appendix A 3.3.12), ‘Theresa’ (Appendix A 3.3.13). The nucleotide sequence for one allele was defined by aligning all obtained nucleotide sequences for this allele and using the consensus sequence when SNPs occurred: *pCD141_S2* (Appendix A 3.3.14), *pCD141_S3* (Appendix A 3.3.15), *pCD141_D1* (Appendix A 3.3.16), *pCD141_T1* (Appendix A 3.3.17), *pCD141_T2* (Appendix A 3.3.18). For the alleles *pCD141_S1*, *pCD141_D2*, and *pCD141_T3* only one full-length nucleotide sequence was obtained, respectively.

Position cDNA SNP	<i>pCD141</i> <i>S1</i>	<i>pCD141</i> <i>S2</i>	<i>pCD141</i> <i>S3</i>	<i>pCD141</i> <i>D1</i>	<i>pCD141</i> <i>D2</i>	<i>pCD141</i> <i>T1</i>	<i>pCD141</i> <i>IT2</i>	<i>pCD141</i> <i>T3</i>	aa
673	A	G	A	A	A	A	A	A	A/G N225D
714	A	C	A	A	A	A	A	A	s.
717	T	C	C	C	C	C	C	C	s.
720	C	G	C	C	C	C	C	C	s.
765	A	G	A	A	A	A	A	A	s.
775	G	A	G	G	G	G	G	G	G/A D259N
798	T	T	T	C	C	C	C	C	s.
843	T	T	T	A	A	A	A	A	s.
862	A	G	A	A	A	A	A	A	A/G I288V
889	A	A	A	C	C	C	C	C	s.
891	G	A	A	G	G	G	G	G	s.
892	C	T	T	T	T	T	T	T	C/T H298Y
905	A	A	A	G	G	G	G	G	A/G N302S
939	T	T	T	C	C	C	C	C	s.
980	G	A	G	G	G	G	G	G	G/A S327N
1029	T	T	G	T	T	T	T	T	s.
1030	C	G	G	G	G	G	G	G	C/G R344V
1031	G	T	T	T	T	T	T	T	G/T R344V
1059	C	T	C	C	C	C	C	C	s.
1096	T	C	C	C	C	C	C	C	T/C S366P
1135	T	T	C	T	T	T	T	T	s.
1188	G	A	G	G	G	G	G	G	s.
1224	A	T	A	A	A	A	A	A	s.
1255	G	A	A	A	A	A	A	A	G/A A419T
1266	A	T	T	C	C	C	C	C	A/T E422D
1277	T	C	C	C	C	C	C	C	T/C L426P
1368	C	C	C	A	A	A	A	A	s.
1416	T	T	T	C	C	C	C	C	s.
1434	A	A	A	T	T	T	T	T	A/T Q478H
1446	G	A	A	A	A	A	A	A	s.
1467	C	T	T	C	C	C	C	C	s.
1503	T	C	C	T	T	T	T	T	s.
1541	C	C	C	C	T	C	C	C	C/T A514V
1542	A	G	G	G	G	G	G	G	s.
1560	T	T	T	C	C	C	C	C	s.
1582	G	G	G	A	A	A	A	A	G/A V528I
1614	G	A	A	A	A	A	A	A	s.
1629	G	G	G	A	A	A	A	A	s.
1641	A	G	G	G	G	G	G	G	s.
1674	C	A	A	A	A	A	A	A	s.
1680	C	C	T	C	C	C	C	C	s.
1683	T	C	C	C	C	C	C	C	s.

SNP position numbering refers to cDNA sequence where ‘1’ represents the adenine of the start codon ATG and the polymorphism is described. aa=amino acid exchanges ; s.=synonymous nucleotide exchange that does not lead to an amino acid exchange. Non synonymous nucleotide exchanges lead to an amino acid exchange and are orange coloured.

The eight *pCD141* alleles of the tetraploid cultivars differed in 62 SNPs, from which 23 resulted in an amino acid substitution. From 41 SNPs of the three ‘Satina’ alleles, 17 caused an amino acid exchange. The two ‘Diana’ alleles possessed two SNPs, from which one resulted in an amino acid substitution. The three ‘Theresa’ alleles differed in nine SNPs. Three of them led to an amino acid exchange.

The amino acid alignment highlights the differences of all cloned *pCD141* alleles from the three tetraploid potato cultivars (Figure 3.3.6). Positions of genotype dependent amino acid exchanges were revealed.

```

CD141_T2 : MEILRRSSSLWVLPILLLLCFINNGVFDASHKVMHLQSTTSHVDASKVHRTGYHFQPKNWINDPNGMPYNGVYHLFYQY : 83
CD141_T3 : MEILRRSSSLWVLPILLLLCFINNGVFDASHKVMHLQSTTSHVDASKVHRTGYHFQPKNWINDPNGMPYNGVYHLFYQY : 83
CD141_D1 : MEILRRSSSLWVLPILLLLCFINNGVFDASHKVMHLQSTTSHVDASKVHRTGYHFQPKNWINDPNGMPYNGVYHLFYQY : 83
CD141_D2 : MEILRRSSSLWVLPILLLLCFINNGVFDASHKVMHLQSTTSHVDASKVHRTGYHFQPKNWINDPNGMPYNGVYHLFYQY : 83
CD141_T1 : MEILRRSSSLWVLPILLLLCFINNGVFDASHKVMHLQSTTSHVDASKVHRTGYHFQPKNWINDPNGMPYNGVYHLFYQY : 83
CD141_S2 : MEILRRSSSLWVLPILLLLCFINNGVFDASHKVMHLQSTTSHVDASKVHRTGYHFQPKNWINDPNGMPYNGVYHLFYQY : 83
CD141_S3 : MEILRRSSSLWVLPILLLLCFINNGVFDASHKVMHLQSTTSHVDASKVHRTGYHFQPKNWINDPNGMPYNGVYHLFYQY : 83
CD141_S1 : MEILRRSSSLWVLPILLLLCFINNGVFDASHKVMHLQSTTSHVDASKVHRTGYHFQPKNWINDPNGMPYNGVYHLFYQY : 83

          *          20          *          40          *          60          *          80
CD141_T2 : NPKGAIWGNIVWAHSVSKDLINWIPLPAIYPSKVPDKYGTWGSATILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYL : 166
CD141_T3 : NPKGAIWGNIVWAHSVSKDLINWIPLPAIYPSKVPDKYGTWGSATILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYL : 166
CD141_D1 : NPKGAIWGNIVWAHSVSKDLINWIPLPAIYPSKVPDKYGTWGSATILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYL : 166
CD141_D2 : NPKGAIWGNIVWAHSVSKDLINWIPLPAIYPSKVPDKYGTWGSATILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYL : 166
CD141_T1 : NPKGAIWGNIVWAHSVSKDLINWIPLPAIYPSKVPDKYGTWGSATILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYL : 166
CD141_S2 : NPKGAIWGNIVWAHSVSKDLINWIPLPAIYPSKVPDKYGTWGSATILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYL : 166
CD141_S3 : NPKGAIWGNIVWAHSVSKDLINWIPLPAIYPSKVPDKYGTWGSATILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYL : 166
CD141_S1 : NPKGAIWGNIVWAHSVSKDLINWIPLPAIYPSKVPDKYGTWGSATILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYL : 166

          *          100          *          120          *          140          *          160
CD141_T2 : RKWKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVIYKSNKFMKWTAKAKHPLHSAPGTGNWECPD : 249
CD141_T3 : RKWKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVIYKSNKFMKWTAKAKHPLHSAPGTGNWECPD : 249
CD141_D1 : RKWKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVIYKSNKFMKWTAKAKHPLHSAPGTGNWECPD : 249
CD141_D2 : RKWKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVIYKSNKFMKWTAKAKHPLHSAPGTGNWECPD : 249
CD141_T1 : RKWKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVIYKSNKFMKWTAKAKHPLHSAPGTGNWECPD : 249
CD141_S2 : RKWKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVIYKSNKFMKWTAKAKHPLHSAPGTGNWECPD : 249
CD141_S3 : RKWKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVIYKSNKFMKWTAKAKHPLHSAPGTGNWECPD : 249
CD141_S1 : RKWKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVIYKSNKFMKWTAKAKHPLHSAPGTGNWECPD : 249

          *          180          *          200          *          220          *          240
CD141_T2 : FFPVSLKKNKGLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYASKTFPDSGKNRR : 332
CD141_T3 : FFPVSLKKNKGLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYASKTFPDSGKNRR : 332
CD141_D1 : FFPVSLKKNKGLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYASKTFPDSGKNRR : 332
CD141_D2 : FFPVSLKKNKGLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYASKTFPDSGKNRR : 332
CD141_T1 : FFPVSLKKNKGLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYASKTFPDSGKNRR : 332
CD141_S2 : FFPVSLKKNKGLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYASKTFPDSGKNRR : 332
CD141_S3 : FFPVSLKKNKGLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYASKTFPDSGKNRR : 332
CD141_S1 : FFPVSLKKNKGLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYASKTFPDSGKNRR : 332

          *          260          *          280          *          300          *          320          *
CD141_T2 : ILLGWANESDVTDNDVRKGWAGVHPIPRKIWLDPESGKQLVQWPVQELETLRKKKVQLNNKLNKGEKVEIKGITVAQADVEVI : 415
CD141_T3 : ILLGWANESDVTDNDVRKGWAGVHPIPRKIWLDPESGKQLVQWPVQELETLRKKKVQLNNKLNKGEKVEIKGITVAQADVEVI : 415
CD141_D1 : ILLGWANESDVTDNDVRKGWAGVHPIPRKIWLDPESGKQLVQWPVQELETLRKKKVQLNNKLNKGEKVEIKGITVAQADVEVI : 415
CD141_D2 : ILLGWANESDVTDNDVRKGWAGVHPIPRKIWLDPESGKQLVQWPVQELETLRKKKVQLNNKLNKGEKVEIKGITVAQADVEVI : 415
CD141_T1 : ILLGWANESDVTDNDVRKGWAGVHPIPRKIWLDPESGKQLVQWPVQELETLRKKKVQLNNKLNKGEKVEIKGITVAQADVEVI : 415
CD141_S2 : ILLGWANESDVTDNDVRKGWAGVHPIPRKIWLDPESGKQLVQWPVQELETLRKKKVQLNNKLNKGEKVEIKGITVAQADVEVI : 415
CD141_S3 : ILLGWANESDVTDNDVRKGWAGVHPIPRKIWLDPESGKQLVQWPVQELETLRKKKVQLNNKLNKGEKVEIKGITVAQADVEVI : 415
CD141_S1 : ILLGWANESDVTDNDVRKGWAGVHPIPRKIWLDPESGKQLVQWPVQELETLRKKKVQLNNKLNKGEKVEIKGITVAQADVEVI : 415

          *          340          *          360          *          380          *          400          *
CD141_T2 : FSPFSLDKAEFPDPSWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKVLKMSDASRSSLKNET : 498
CD141_T3 : FSPFSLDKAEFPDPSWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKVLKMSDASRSSLKNET : 498
CD141_D1 : FSPFSLDKAEFPDPSWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKVLKMSDASRSSLKNET : 498
CD141_D2 : FSPFSLDKAEFPDPSWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKVLKMSDASRSSLKNET : 498
CD141_T1 : FSPFSLDKAEFPDPSWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKVLKMSDASRSSLKNET : 498
CD141_S2 : FSPFSLDKAEFPDPSWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKVLKMSDASRSSLKNET : 498
CD141_S3 : FSPFSLDKAEFPDPSWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKVLKMSDASRSSLKNET : 498
CD141_S1 : FSPFSLDKAEFPDPSWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKVLKMSDASRSSLKNET : 498

```

```

00          *          520          *          540          *          560          *          580
CD141_T2 : TMYKPSFAGYVDVLDLADKKLSLRSLIDHSTIVESFGAGGKTCITSRVYPTLAI FDKAHLFAFNNGAERIT IETLNAWSMANAKL : 581
CD141_T3 : TMYKPSFAGYVDVLDLADKKLSLRSLIDHSTIVESFGAGGKTCITSRVYPTLAI FDKAHLFAFNNGAERIT IETLNAWSMANAKL : 581
CD141_D1 : TMYKPSFAGYVDVLDLADKKLSLRSLIDHSTIVESFGAGGKTCITSRVYPTLAI FDKAHLFAFNNGAERIT IETLNAWSMANAKL : 581
CD141_D2 : TMYKPSFAGYVDVLDLADKKLSLRSLIDHSTIVESFGAGGKTCITSRVYPTLAI FDKAHLFAFNNGAERIT IETLNAWSMANAKL : 581
CD141_T1 : TMYKPSFAGYVDVLDLADKKLSLRSLIDHSTIVESFGAGGKTCITSRVYPTLAI FDKAHLFAFNNGAERIT IETLNAWSMANAKL : 581
CD141_S2 : TMYKPSFAGYVDVLDLADKKLSLRSLIDHSTIVESFGAGGKTCITSRVYPTLAI FDKAHLFAFNNGAERIT IETLNAWSMANAKL : 581
CD141_S3 : TMYKPSFAGYVDVLDLADKKLSLRSLIDHSTIVESFGAGGKTCITSRVYPTLAI FDKAHLFAFNNGAERIT IETLNAWSMANAKL : 581
CD141_S1 : TMYKPSFAGYVDVLDLADKKLSLRSLIDHSTIVESFGAGGKTCITSRVYPTLAI FDKAHLFAFNNGAERIT IETLNAWSMANAKL : 581

```

```

CD141_T2 : H : 582
CD141_T3 : H : 582
CD141_D1 : H : 582
CD141_D2 : H : 582
CD141_T1 : H : 582
CD141_S2 : H : 582
CD141_S3 : H : 582
CD141_S1 : H : 582

```

Figure 3.3.6: Amino acid alignment of *pCD141* alleles from the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’. Amino acid exchanges are highlighted in colour.

Comparison of allelic amino acid sequences from the tetraploid cultivars revealed that the genotypes ‘Diana’ and ‘Theresa’ harbour an allele identical at amino acid level (*CD141_D1*=*CD141_T2*).

3.3.1.1.6 *pCD141* cDNA alleles of the diploid potato genotypes P18, P40, and P54¹³

From the genotype P18 five full-length clones were obtained, from which two different cDNA alleles *pCD141_P18_1* and *pCD141_P18_2* were identified. Cloning and sequencing of P40 cDNA resulted in two full-length clones identical at amino acid level. The allele was named *pCD141_P40_1*. Sequence comparison suggested a second P40 allele but the clones harbouring an allelic sequence either did not feature the start codon of the gene or the reading frame was shifted, and, therefore, were not used in further analysis. From the genotype P54 four full-length clones were isolated, from which two different alleles *pCD141_P54_1* and *pCD141_P54_2* were defined.

The alleles contain at nucleotide level 64 SNPs (Appendix A 3.3.28). These sequence polymorphisms can cause amino acid exchanges (Table 3.3.7; Figure 3.3.7).

Table 3.3.7: SNPs present in P18, P40, and P54 *pCD141* alleles.

Position of cDNA SNP	<i>pCD141_P18_1</i>	<i>pCD141_P18_2</i>	<i>pCD141_P40_1</i>	<i>pCD141_P54_1</i>	<i>pCD141_P54_2</i>	aa
57	T	C	C	C	C	s.
101	T	T	T	T	C	T/C A34V
140	T	C	C	C	C	T/C V47A
141	T	T	T	T	A	s.
153	T	T	T	C	T	s.

¹³ Alleles of each genotype were defined by alignment of all fully sequenced cDNA clones: P18 (Appendix A 3.3.20), P40 (Appendix A 3.3.21), P54 (Appendix A 3.3.22). The nucleotide sequence for one allele was defined by aligning all obtained nucleotide sequences for this allele and using the consensus sequence when SNPs occurred: *pCD141_P18_1* (Appendix A 3.3.23), *pCD141_P18_2* (Appendix A 3.3.24), *pCD141_P40_1* (Appendix A 3.3.25), *pCD141_P54_1* (Appendix A 3.3.26), *pCD141_P54_2* (Appendix A 3.3.27). For the allele *pCD141_P40_2* only one full-length nucleotide sequence was obtained.

Position of cDNA SNP	pCD141 P18_1	pCD141 P18_2	pCD141 P40_1	pCD141 P54_1	pCD141 P54_2	aa
186	T	C	C	C	C	s.
195	C	C	C	T	C	s.
207	C	T	C	C	T	s.
231	T	C	T	T	C	s.
276	T	C	T	T	C	s.
378	C	C	T	C	C	s.
440	C	C	G	C	C	G/C G147A
444	C	C	T	T	T	s.
462	T	T	C	C	C	s.
582	A	C	C	C	C	s.
585	A	T	A	T	T	s.
621	T	A	A	T	T	s.
624	G	G	C	G	G	s.
701	A	T	A	A	A	A/T H234I
714	G	G	A	A	A	s.
717	C	C	C	C	T	s.
720	G	C	C	G	G	s.
761	C	C	C	C	T	C/T S254L
765	G	A	A	G	G	s.
775	A	G	G	A	A	A/G N259D
798	T	T	C	T	T	s.
843	T	A	A	A	A	s.
862	G	A	A	A	A	G/A V288I
889	A	A	C	A	A	C/A K297Q
891	A	G	G	A	A	s.
895	T	A	T	T	T	T/A F299I
905	A	A	G	A	A	A/G N302S
913	A	A	A	A	G	A/G I305V
939	T	T	C	T	T	s.
980	A	G	G	G	G	A/G N327S
1043	T	T	T	T	C	T/C V348A
1143	A	T	T	T	T	s.
1146	G	A	A	A	A	s.
1158	A	G	G	G	G	s.
1188	A	A	G	G	G	s.
1192	G	G	G	A	A	G/A E398K
1224	A	T	A	A	A	s.
1266	T	T	C	T	T	s.
1368	A	C	A	C	C	s.
1390	T	G	T	T	T	T/G L464V
1412	T	T	T	T	C	T/C F417S
1416	C	T	C	T	C	s.

Position of cDNA SNP	<i>pCD141</i> <i>P18_1</i>	<i>pCD141</i> <i>P18_2</i>	<i>pCD141</i> <i>P40_1</i>	<i>pCD141</i> <i>P54_1</i>	<i>pCD141</i> <i>P54_2</i>	aa
1417	A	A	A	A	C	s.
1429	A	G	G	G	G	A/G T477A
1434	T	A	T	T	T	T/A H478Q
1446	G	A	A	G	G	s.
1452	T	T	T	T	C	s.
1461	C	C	C	T	C	s.
1467	C	T	C	C	C	s.
1503	T	C	T	T	T	s.
1542	A	G	G	G	G	s.
1560	T	T	C	T	T	s.
1582	G	G	A	G	G	G/A V528I
1587	G	G	G	C	G	s.
1602	T	T	C	C	T	s.
1613	C	C	C	C	T	C/T T538I
1629	G	G	A	G	G	s.
1641	G	G	G	A	G	s.
1673	C	C	C	T	C	T/C V558A
1674	A	A	A	G	A	s.
1683	C	C	C	C	T	s.
1689	G	G	G	A	G	s.

SNP position numbering refers to cDNA sequence where '1' represents the adenine of the start codon ATG and the polymorphism is described. aa=amino acid exchanges ; s.=synonymous nucleotide exchange that does not lead to an amino acid exchange. Non synonymous nucleotide exchanges lead to an amino acid exchange and are orange coloured.

The five alleles obtained from the three diploid genotypes differed in 64 SNPs, from which 21 resulted in an amino acid exchange. The two P18 alleles differed in 30 SNPs. Nine of them led to an amino acid substitution. In the two P54 allelic sequences 18 SNPs occurred, from which four resulted in an amino acid exchange.

Multiple alignment of the protein sequences of all alleles from the three potato genotypes P18, P40, and P54 showed the variability of positions where amino acids differed (Figure 3.3.7).

```

*           20           *           40           *           60           *           80
CD141_P182 : MEILRRSSSLWVLPILLLCFFINNGVFDASHKVYMHLQSTTSHVDASKVHRTGYHFQPPKNWINDPNGPMYYNGVYHLFY : 81
CD141_P401 : MEILRRSSSLWVLPILLLCFFINNGVFDASHKVYMHLQSTTSHVDASKVHRTGYHFQPPKNWINDPNGPMYYNGVYHLFY : 81
CD141_P181 : MEILRRSSSLWVLPILLLCFFINNGVFDASHKVYMHLQSTTSHVDVSKVHRTGYHFQPPKNWINDPNGPMYYNGVYHLFY : 81
CD141_P541 : MEILRRSSSLWVLPILLLCFFINNGVFDASHKVYMHLQSTTSHVDASKVHRTGYHFQPPKNWINDPNGPMYYNGVYHLFY : 81
CD141_P542 : MEILRRSSSLWVLPILLLCFFINNGVFDASHKVYMHLQSTTSHVDASKVHRTGYHFQPPKNWINDPNGPMYYNGVYHLFY : 81

*           100          *           120          *           140          *           160
CD141_P182 : QYNPKGAIWGNIVWAHVSVDKDLINWIPLEPAIYPSKVFDKYGTWSGSATILPGNKPVILYTGIVDANKTQVQNYAIPANMS : 162
CD141_P401 : QYNPKGAIWGNIVWAHVSVDKDLINWIPLEPAIYPSKVFDKYGTWSGSATILPGNKPVILYTGIVDGNKTQVQNYAIPANMS : 162
CD141_P181 : QYNPKGAIWGNIVWAHVSVDKDLINWIPLEPAIYPSKVFDKYGTWSGSATILPGNKPVILYTGIVDANKTQVQNYAIPANMS : 162
CD141_P541 : QYNPKGAIWGNIVWAHVSVDKDLINWIPLEPAIYPSKVFDKYGTWSGSATILPGNKPVILYTGIVDANKTQVQNYAIPANMS : 162
CD141_P542 : QYNPKGAIWGNIVWAHVSVDKDLINWIPLEPAIYPSKVFDKYGTWSGSATILPGNKPVILYTGIVDANKTQVQNYAIPANMS : 162

*           180          *           200          *           220          *           240
CD141_P182 : DPYLRKWKIPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVNRHRGKVIMYKSNKNFMKWTKAKHPLHSAPGTG : 243
CD141_P401 : DPYLRKWKIPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVNRHRGKVIMYKSNKNFMKWTKAKHPLHSAPGTG : 243
CD141_P181 : DPYLRKWKIPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVNRHRGKVIMYKSNKNFMKWTKAKHPLHSAPGTG : 243
CD141_P541 : DPYLRKWKIPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVNRHRGKVIMYKSNKNFMKWTKAKHPLHSAPGTG : 243
CD141_P542 : DPYLRKWKIPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVNRHRGKVIMYKSNKNFMKWTKAKHPLHSAPGTG : 243

*           260          *           280          *           300          *           320
CD141_P182 : NWECPDFFPVSLKNKDGLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTTKKDKYFPDNTSIDGWKGLRLDYGNYYASKTF : 324
CD141_P401 : NWECPDFFPVSLKNKDGLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTTKKDQFPDSTSIDGWKGLRLDYGNYYASKTF : 324
CD141_P181 : NWECPDFFPVSLKNKNGLDTSYNGKDIKHVLKVSFDVTRFDHYTVGTYDTTKKDKYPDNTSIDGWKGLRLDYGNYYASKTF : 324
CD141_P541 : NWECPDFFPVSLKNKNGLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTTKKDKYPDNTSIDGWKGLRLDYGNYYASKTF : 324
CD141_P542 : NWECPDFFPVLLKNKNGLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTTKKDKYPDNTSVDGWKGLRLDYGNYYASKTF : 324

*           340          *           360          *           380          *           400
CD141_P182 : FDSGKNRRILLGWANESDTVDNDVRKGWAGVHPIPRKIWLDPSGKQLVQWPVQELETLRKKKVQLNNKLNKGEKVEIKGI : 405
CD141_P401 : FDSGKNRRILLGWANESDTVDNDVRKGWAGVHPIPRKIWLDPSGKQLVQWPVQELETLRKKKVQLNNKLNKGEKVEIKGI : 405
CD141_P181 : FDNGKNRRILLGWANESDTVDNDVRKGWAGVHPIPRKIWLDPSGKQLVQWPVQELETLRKKKVQLNNKLNKGEKVEIKGI : 405
CD141_P541 : FDSGKNRRILLGWANESDTVDNDVRKGWAGVHPIPRKIWLDPSGKQLVQWPVQELETLRKKKVQLNNKLNKGKKVEIKGI : 405
CD141_P542 : FDSGKNRRILLGWANESDTVDNDVRKGWAGVHPIPRKIWLDPSGKQLVQWPVQELETLRKKKVQLNNKLNKGKKVEIKGI : 405

*           420          *           440          *           460          *           480
CD141_P182 : TVAQADVEVIFSFTSLDKAEPFDPSWADLYAQDVCAIKGSTVQGGLGPFGLLTLASKNVEEYTPVFFRIFKAHDKYKVLMC : 486
CD141_P401 : TVAQADVEVIFSFTSLDKAEPFDPSWADLYAQDVCAIKGSTVQGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKVLMC : 486
CD141_P181 : TVAQADVEVIFSFTSLDKAEPFDPSWADLYAQDVCAIKGSTVQGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKVLMC : 486
CD141_P541 : TVAQADVEVIFSFTSLDKAEPFDPSWADLYAQDVCAIKGSTVQGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKVLMC : 486
CD141_P542 : TVAQADVEVIFSFTSLDKAEPFDPSWADLYAQDVCAIKGSTVQGGLGPFGLLTLASKNLEEYTPVSFRIFKAHDKYKVLMC : 486

*           500          *           520          *           540          *           560
CD141_P182 : SDASRSSLKNETTMYKPSFAGYVDVLADKKLSRSLIDHSVVESFGAGGTCITSRVYPTLAIFDKAHLFAFNNGAERIT : 567
CD141_P401 : SDASRSSLKNETTMYKPSFAGYVDVLADKKLSRSLIDHSVVESFGAGGTCITSRVYPTLAIFDKAHLFAFNNGAERIT : 567
CD141_P181 : SDASRSSLKNETTMYKPSFAGYVDVLADKKLSRSLIDHSVVESFGAGGTCITSRVYPTLAIFDKAHLFAFNNGAERIT : 567
CD141_P541 : SDASRSSLKNETTMYKPSFAGYVDVLADKKLSRSLIDHSVVESFGAGGTCITSRVYPTLAIFDKAHLFVFNNGAERIT : 567
CD141_P542 : SDASRSSLKNETTMYKPSFAGYVDVLADKKLSRSLIDHSVVESFGAGGTCITSRVYPTLAIFDKAHLFAFNNGAERIT : 567

*           580
CD141_P182 : IETLNAWSMANAKLH : 582
CD141_P401 : IETLNAWSMANAKLH : 582
CD141_P181 : IETLNAWSMANAKLH : 582
CD141_P541 : IETLNAWSMANAKLH : 582
CD141_P542 : IETLNAWSMANAKLH : 582

```

Figure 3.3.7: Amino acid alignment of *pCD141* alleles from the diploid genotypes P18, P40, and P54. Amino acid exchanges are highlighted in colour.

Comparison of allelic cDNA sequences of the gene *pCD141* from the three diploid genotypes revealed that none of the five alleles is present in different genotypes.

3.3.1.1.7 Amino acid alignment of all *pCD141* invertase alleles of the analyzed potato genotypes

The variability of amino acid positions, which exhibit exchanges, was visualized by multiple sequence alignment of *pCD141* invertase alleles (Figure 3.3.8). Several amino acid exchanges occur in different genotypes at the same position. The comparison of the protein sequences showed that amino acids differed at 35 positions, of which 25 were genotype specific and occurred only once.

Comparison of allelic amino acid sequences from the three tetraploid and the three diploid genotypes revealed that the genotypes ‘Diana’, ‘Theresa’, and P40 harbour an allele identical at amino acid level ($CD141_D1=CD141_T2=CD141_P40_1$).

```

*          20          *          40          *          60          *          80
CD141_S1 : MEILRRSSSLWVLPILLCCFFINNGVFDASHKVMYHLQSTTSHVDASKVHRTGYHFQPKKNWINDPNGPMYYNGVYHLF : 80
CD141_S3 : MEILRRSSSLWVLPILLCCFFINNGVFDASHKVMYHLQSTTSHVDASKVHRTGYHFQPKKNWINDPNGPMYYNGVYHLF : 80
CD141_P182 : MEILRRSSSLWVLPILLCCFFINNGVFDASHKVMYHLQSTTSHVDASKVHRTGYHFQPKKNWINDPNGPMYYNGVYHLF : 80
CD141_T2 : MEILRRSSSLWVLPILLCCFFINNGVFDASHKVMYHLQSTTSHVDASKVHRTGYHFQPKKNWINDPNGPMYYNGVYHLF : 80
CD141_T3 : MEILRRSSSLWVLPILLCCFFINNGVFDASHKVMYHLQSTTSHVDASKVHRTGYHFQPKKNWINDPNGPMYYNGVYHLF : 80
CD141_D1 : MEILRRSSSLWVLPILLCCFFINNGVFDASHKVMYHLQSTTSHVDASKVHRTGYHFQPKKNWINDPNGPMYYNGVYHLF : 80
CD141_D2 : MEILRRSSSLWVLPILLCCFFINNGVFDASHKVMYHLQSTTSHVDASKVHRTGYHFQPKKNWINDPNGPMYYNGVYHLF : 80
CD141_P401 : MEILRRSSSLWVLPILLCCFFINNGVFDASHKVMYHLQSTTSHVDASKVHRTGYHFQPKKNWINDPNGPMYYNGVYHLF : 80
CD141_T1 : MEILRRSSSLWVLPILLCCFFINNGVFDASHKVMYHLQSTTSHVDVSKVHRTGYHFQPKKNWINDPNGPMYYNGVYHLF : 80
CD141_S2 : MEILRRSSSLWVLPILLCCFFINNGVFDASHKVMYHLQSTTSHVDVSKVHRTGYHFQPKKNWINDPNGPMYYNGVYHLF : 80
CD141_P181 : MEILRRSSSLWVLPILLCCFFINNGVFDASHKVMYHLQSTTSHVDVSKVHRTGYHFQPKKNWINDPNGPMYYNGVYHLF : 80
CD141_P541 : MEILRRSSSLWVLPILLCCFFINNGVFDASHKVMYHLQSTTSHVDASKVHRTGYHFQPKKNWINDPNGPMYYNGVYHLF : 80
CD141_P542 : MEILRRSSSLWVLPILLCCFFINNGVFDASHKVMYHLQSTTSHVDASKVHRTGYHFQPKKNWINDPNGPMYYNGVYHLF : 80

*          100         *          120         *          140         *          160
CD141_S1 : YQYNPKGAIWGNIVWAHSVSKDLINWIPEPAIYPSKVFDKYGTWSSGATILPGNKPVILYTGIVDANKTQVQNYAIPAN : 160
CD141_S3 : YQYNPKGAIWGNIVWAHSVSKDLINWIPEPAIYPSKVFDKYGTWSSGATILPGNKPVILYTGIVDANKTQVQNYAIPAN : 160
CD141_P182 : YQYNPKGAIWGNIVWAHSVSKDLINWIPEPAIYPSKVFDKYGTWSSGATILPGNKPVILYTGIVDANKTQVQNYAIPAN : 160
CD141_T2 : YQYNPKGAIWGNIVWAHSVSKDLINWIPEPAIYPSKVFDKYGTWSSGATILPGNKPVILYTGIVDANKTQVQNYAIPAN : 160
CD141_T3 : YQYNPKGAIWGNIVWAHSVSKDLINWIPEPAIYPSKVFDKYGTWSSGATILPGNKPVILYTGIVDANKTQVQNYAIPAN : 160
CD141_D1 : YQYNPKGAIWGNIVWAHSVSKDLINWIPEPAIYPSKVFDKYGTWSSGATILPGNKPVILYTGIVDANKTQVQNYAIPAN : 160
CD141_D2 : YQYNPKGAIWGNIVWAHSVSKDLINWIPEPAIYPSKVFDKYGTWSSGATILPGNKPVILYTGIVDANKTQVQNYAIPAN : 160
CD141_P401 : YQYNPKGAIWGNIVWAHSVSKDLINWIPEPAIYPSKVFDKYGTWSSGATILPGNKPVILYTGIVDANKTQVQNYAIPAN : 160
CD141_T1 : YQYNPKGAIWGNIVWAHSVSKDLINWIPEPAIYPSKVFDKYGTWSSGATILPGNKPVILYTGIVDANKTQVQNYAIPAN : 160
CD141_S2 : YQYNPKGAIWGNIVWAHSVSKDLINWIPEPAIYPSKVFDKYGTWSSGATILPGNKPVILYTGIVDANKTQVQNYAIPAN : 160
CD141_P181 : YQYNPKGAIWGNIVWAHSVSKDLINWIPEPAIYPSKVFDKYGTWSSGATILPGNKPVILYTGIVDANKTQVQNYAIPAN : 160
CD141_P541 : YQYNPKGAIWGNIVWAHSVSKDLINWIPEPAIYPSKVFDKYGTWSSGATILPGNKPVILYTGIVDANKTQVQNYAIPAN : 160
CD141_P542 : YQYNPKGAIWGNIVWAHSVSKDLINWIPEPAIYPSKVFDKYGTWSSGATILPGNKPVILYTGIVDANKTQVQNYAIPAN : 160

*          180         *          200         *          220         *          240
CD141_S1 : MSDPYLRKWIKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVMYKSNKNFMKWTKAKHPLHSAP : 240
CD141_S3 : MSDPYLRKWIKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVMYKSNKNFMKWTKAKHPLHSAP : 240
CD141_P182 : MSDPYLRKWIKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVMYKSNKNFMKWTKAKHPLHSAP : 240
CD141_T2 : MSDPYLRKWIKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVMYKSNKNFMKWTKAKHPLHSAP : 240
CD141_T3 : MSDPYLRKWIKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVMYKSNKNFMKWTKAKHPLHSAP : 240
CD141_D1 : MSDPYLRKWIKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVMYKSNKNFMKWTKAKHPLHSAP : 240
CD141_D2 : MSDPYLRKWIKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVMYKSNKNFMKWTKAKHPLHSAP : 240
CD141_P401 : MSDPYLRKWIKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVMYKSNKNFMKWTKAKHPLHSAP : 240
CD141_T1 : MSDPYLRKWIKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVMYKSNKNFMKWTKAKHPLHSAP : 240
CD141_S2 : MSDPYLRKWIKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVMYKSNKNFMKWTKAKHPLHSAP : 240
CD141_P181 : MSDPYLRKWIKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVMYKSNKNFMKWTKAKHPLHSAP : 240
CD141_P541 : MSDPYLRKWIKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVMYKSNKNFMKWTKAKHPLHSAP : 240
CD141_P542 : MSDPYLRKWIKPDNNPLIVADKTINKSQFRDPTTAWMGRDGNWRILVGSVRNHRGKVMYKSNKNFMKWTKAKHPLHSAP : 240

```

```

CD141_S1 : GTGNWECDFDFPVSLK260GLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDK300HF*PDNTSIDGWKGLRLDYGNYA : 320
CD141_S3 : GTGNWECDFDFPVSLK260GLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDK300YF*PDNTSIDGWKGLRLDYGNYA : 320
CD141_P182 : GTGNWECDFDFPVSLK260GLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDK300YI*PDNTSIDGWKGLRLDYGNYA : 320
CD141_T2 : GTGNWECDFDFPVSLK260GLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDK300YF*PDNTSIDGWKGLRLDYGNYA : 320
CD141_T3 : GTGNWECDFDFPVSLK260GLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDK300YF*PDNTSIDGWKGLRLDYGNYA : 320
CD141_D1 : GTGNWECDFDFPVSLK260GLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDK300YF*PDNTSIDGWKGLRLDYGNYA : 320
CD141_D2 : GTGNWECDFDFPVSLK260GLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDK300YF*PDNTSIDGWKGLRLDYGNYA : 320
CD141_P401 : GTGNWECDFDFPVSLK260GLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDK300YF*PDNTSIDGWKGLRLDYGNYA : 320
CD141_T1 : GTGNWECDFDFPVSLK260GLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDK300YF*PDNTSIDGWKGLRLDYGNYA : 320
CD141_S2 : GTGNWECDFDFPVSLK260GLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDK300YF*PDNTSIDGWKGLRLDYGNYA : 320
CD141_P181 : GTGNWECDFDFPVSLK260GLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDK300YF*PDNTSIDGWKGLRLDYGNYA : 320
CD141_P541 : GTGNWECDFDFPVSLK260GLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDK300YF*PDNTSIDGWKGLRLDYGNYA : 320
CD141_P542 : GTGNWECDFDFPVSLK260GLDTSYNGKDIKHVLKVSFDVTRFDHYTIGTYDTKKDK300YF*PDNTSIDGWKGLRLDYGNYA : 320

CD141_S1 : SKTFFD340SGKNRRILLGWANESDTR360DNDVRKKGWAGVHPIPRKIWLDS380SGKQLVQWPVQELETLRKKKQV400LNNKLNKGEKV : 400
CD141_S3 : SKTFFD340SGKNRRILLGWANESDTR360DNDVRKKGWAGVHPIPRKIWLDS380SGKQLVQWPVQELETLRKKKQV400LNNKLNKGEKV : 400
CD141_P182 : SKTFFD340SGKNRRILLGWANESDTR360DNDVRKKGWAGVHPIPRKIWLDS380SGKQLVQWPVQELETLRKKKQV400LNNKLNKGEKV : 400
CD141_T2 : SKTFFD340SGKNRRILLGWANESDTR360DNDVRKKGWAGVHPIPRKIWLDS380SGKQLVQWPVQELETLRKKKQV400LNNKLNKGEKV : 400
CD141_T3 : SKTFFD340SGKNRRILLGWANESDTR360DNDVRKKGWAGVHPIPRKIWLDS380SGKQLVQWPVQELETLRKKKQV400LNNKLNKGEKV : 400
CD141_D1 : SKTFFD340SGKNRRILLGWANESDTR360DNDVRKKGWAGVHPIPRKIWLDS380SGKQLVQWPVQELETLRKKKQV400LNNKLNKGEKV : 400
CD141_D2 : SKTFFD340SGKNRRILLGWANESDTR360DNDVRKKGWAGVHPIPRKIWLDS380SGKQLVQWPVQELETLRKKKQV400LNNKLNKGEKV : 400
CD141_P401 : SKTFFD340SGKNRRILLGWANESDTR360DNDVRKKGWAGVHPIPRKIWLDS380SGKQLVQWPVQELETLRKKKQV400LNNKLNKGEKV : 400
CD141_T1 : SKTFFD340SGKNRRILLGWANESDTR360DNDVRKKGWAGVHPIPRKIWLDS380SGKQLVQWPVQELETLRKKKQV400LNNKLNKGEKV : 400
CD141_S2 : SKTFFD340SGKNRRILLGWANESDTR360DNDVRKKGWAGVHPIPRKIWLDS380SGKQLVQWPVQELETLRKKKQV400LNNKLNKGEKV : 400
CD141_P181 : SKTFFD340SGKNRRILLGWANESDTR360DNDVRKKGWAGVHPIPRKIWLDS380SGKQLVQWPVQELETLRKKKQV400LNNKLNKGEKV : 400
CD141_P541 : SKTFFD340SGKNRRILLGWANESDTR360DNDVRKKGWAGVHPIPRKIWLDS380SGKQLVQWPVQELETLRKKKQV400LNNKLNKGEKV : 400
CD141_P542 : SKTFFD340SGKNRRILLGWANESDTR360DNDVRKKGWAGVHPIPRKIWLDS380SGKQLVQWPVQELETLRKKKQV400LNNKLNKGEKV : 400

CD141_S1 : EIKGITVAQADVEVIFSF420ASLEKAE440LPDPSWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKN460LEEYTPV480FRIFKAQDK : 480
CD141_S3 : EIKGITVAQADVEVIFSF420SLDKAE440PFDP*SWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKN460LEEYTPV480FRIFKAQDK : 480
CD141_P182 : EIKGITVAQADVEVIFSF420SLDKAE440PFDP*SWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKN460VEEYTPV480FRIFKAQDK : 480
CD141_T2 : EIKGITVAQADVEVIFSF420SLDKAE440PFDP*SWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKN460LEEYTPV480FRIFKAHDK : 480
CD141_T3 : EIKGITVAQADVEVIFSF420SLDKAE440PFDP*SWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKN460LEEYTPV480FRIFKAHDK : 480
CD141_D1 : EIKGITVAQADVEVIFSF420SLDKAE440PFDP*SWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKN460LEEYTPV480FRIFKAHDK : 480
CD141_D2 : EIKGITVAQADVEVIFSF420SLDKAE440PFDP*SWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKN460LEEYTPV480FRIFKAHDK : 480
CD141_P401 : EIKGITVAQADVEVIFSF420SLDKAE440PFDP*SWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKN460LEEYTPV480FRIFKAHDK : 480
CD141_T1 : EIKGITVAQADVEVIFSF420SLDKAE440PFDP*SWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKN460LEEYTPV480FRIFKAHDK : 480
CD141_S2 : EIKGITVAQADVEVIFSF420SLDKAE440PFDP*SWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKN460LEEYTPV480FRIFKAQDK : 480
CD141_P181 : EIKGITVAQADVEVIFSF420SLDKAE440PFDP*SWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKN460LEEYTPV480FRIFKAHDK : 480
CD141_P541 : EIKGITVAQADVEVIFSF420SLDKAE440PFDP*SWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKN460LEEYTPV480FRIFKAHDK : 480
CD141_P542 : EIKGITVAQADVEVIFSF420SLDKAE440PFDP*SWADLYAQDVCAIKGSTVQGGGLGPFGLLTLASKN460LEEYTPV480FRIFKAHDK : 480

CD141_S1 : YKVL500MCDASRSSLKNETTMYKPSFAGYVDVLD520ADKKLSLRS540SLIDHS560VVESFGAGGKT*CITSRVYPTLAIFDKAHLFAFN : 560
CD141_S3 : YKVL500MCDASRSSLKNETTMYKPSFAGYVDVLD520ADKKLSLRS540SLIDHS560VVESFGAGGKT*CITSRVYPTLAIFDKAHLFAFN : 560
CD141_P182 : YKVL500MCDASRSSLKNETTMYKPSFAGYVDVLD520ADKKLSLRS540SLIDHS560VVESFGAGGKT*CITSRVYPTLAIFDKAHLFAFN : 560
CD141_T2 : YKVL500MCDASRSSLKNETTMYKPSFAGYVDVLD520ADKKLSLRS540SLIDHS560VVESFGAGGKT*CITSRVYPTLAIFDKAHLFAFN : 560
CD141_T3 : YKVL500MCDASRSSLKNETTMYKPSFAGYVDVLD520ADKKLSLRS540SLIDHS560VVESFGAGGKT*CITSRVYPTLAIFDKAHLFAFN : 560
CD141_D1 : YKVL500MCDASRSSLKNETTMYKPSFAGYVDVLD520ADKKLSLRS540SLIDHS560VVESFGAGGKT*CITSRVYPTLAIFDKAHLFAFN : 560
CD141_D2 : YKVL500MCDASRSSLKNETTMYKPSFAGYVDVLD520ADKKLSLRS540SLIDHS560VVESFGAGGKT*CITSRVYPTLAIFDKAHLFAFN : 560
CD141_P401 : YKVL500MCDASRSSLKNETTMYKPSFAGYVDVLD520ADKKLSLRS540SLIDHS560VVESFGAGGKT*CITSRVYPTLAIFDKAHLFAFN : 560
CD141_T1 : YKVL500MCDASRSSLKNETTMYKPSFAGYVDVLD520ADKKLSLRS540SLIDHS560VVESFGAGGKT*CITSRVYPTLAIFDKAHLFAFN : 560
CD141_S2 : YKVL500MCDASRSSLKNETTMYKPSFAGYVDVLD520ADKKLSLRS540SLIDHS560VVESFGAGGKT*CITSRVYPTLAIFDKAHLFAFN : 560
CD141_P181 : YKVL500MCDASRSSLKNETTMYKPSFAGYVDVLD520ADKKLSLRS540SLIDHS560VVESFGAGGKT*CITSRVYPTLAIFDKAHLFAFN : 560
CD141_P541 : YKVL500MCDASRSSLKNETTMYKPSFAGYVDVLD520ADKKLSLRS540SLIDHS560VVESFGAGGKT*CITSRVYPTLAIFDKAHLFAFN : 560
CD141_P542 : YKVL500MCDASRSSLKNETTMYKPSFAGYVDVLD520ADKKLSLRS540SLIDHS560VVESFGAGGKT*CITSRVYPTLAIFDKAHLFAFN : 560

CD141_S1 : NGAERIT580IETLNAWSMANAKLH : 582
CD141_S3 : NGAERIT580IETLNAWSMANAKLH : 582
CD141_P182 : NGAERIT580IETLNAWSMANAKLH : 582
CD141_T2 : NGAERIT580IETLNAWSMANAKLH : 582
CD141_T3 : NGAERIT580IETLNAWSMANAKLH : 582
CD141_D1 : NGAERIT580IETLNAWSMANAKLH : 582
CD141_D2 : NGAERIT580IETLNAWSMANAKLH : 582
CD141_P401 : NGAERIT580IETLNAWSMANAKLH : 582
CD141_T1 : NGAERIT580IETLNAWSMANAKLH : 582
CD141_S2 : NGAERIT580IETLNAWSMANAKLH : 582
CD141_P181 : NGAERIT580IETLNAWSMANAKLH : 582
CD141_P541 : NGAERIT580IETLNAWSMANAKLH : 582
CD141_P542 : NGAERIT580IETLNAWSMANAKLH : 582

```

Figure 3.3.8: Amino acid alignment of all cloned *pCD141* invertase alleles. Amino acid exchanges are highlighted in colour.

3.3.1.1.8 Phenetic trees of all *pCD141* invertase alleles of the analyzed potato genotypes

To further characterize the invertase alleles in addition to the multiple amino acid alignment (3.3.1.1.7), a phenetic tree analysis was used. Similarity based grouping of *pCD141* alleles using the neighbour-joining method visualized a complex distribution, resulting in two clades and multiple subclades (Figure 3.3.9).

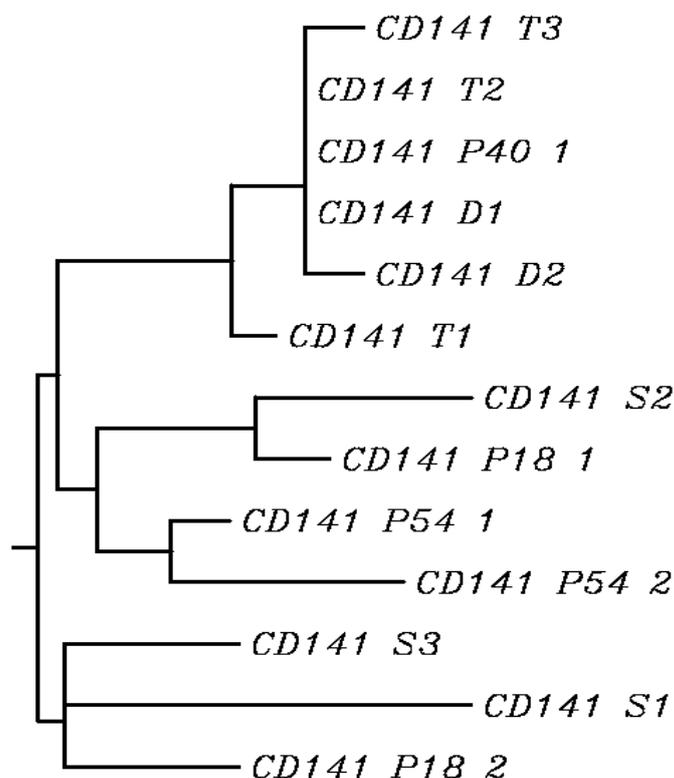


Figure 3.3.9: Amino acid based phenetic tree (Neighbour-joining tree) of all cloned *pCD141* invertase alleles.

The phenetic tree as well as the multiple amino acid alignment revealed the high diversity within the cloned *pCD141* alleles from six different genotypes. The alleles group in two different clades from which the first one consists of two subclades dividing further on.

The first subclade of the first main clade comprises the two ‘Diana’ alleles, the three ‘Theresa’ alleles as well as the allele of the diploid potato genotype P40. The alleles *pCD141_D1*, *pCD141_T2*, and *pCD141_P40_1* are identical at amino acid level and, therefore, group together. The second subclade of the first main clade consists of the alleles *pCD141_S2*, *pCD141_P18_1*, and of the two P54 alleles *pCD141_P54_1* and *pCD141_P54_2*. The second main clade contains the alleles *pCD141_S1*, *pCD141_S3*, and *pCD141_P18_2*.

Cloning and sequencing of *pCD141* alleles showed that the alleles *pCD141_D1*, *pCD141_T2*, and *pCD141_P40_1* are identical at amino acid level. Comparison of the nucleotide

sequences revealed that *pCD141_P40_1* differs in one SNP at cDNA position 1602. The P40 allele contains at the given position nucleotide C, whilst *pCD141_D1* and *pCD141_T2* exhibit the nucleotide T.

The nucleotide polymorphisms between all *pCD141* (Appendix A 3.3.29) were visualized using the phenetic tree analysis (Figure 3.3.10).

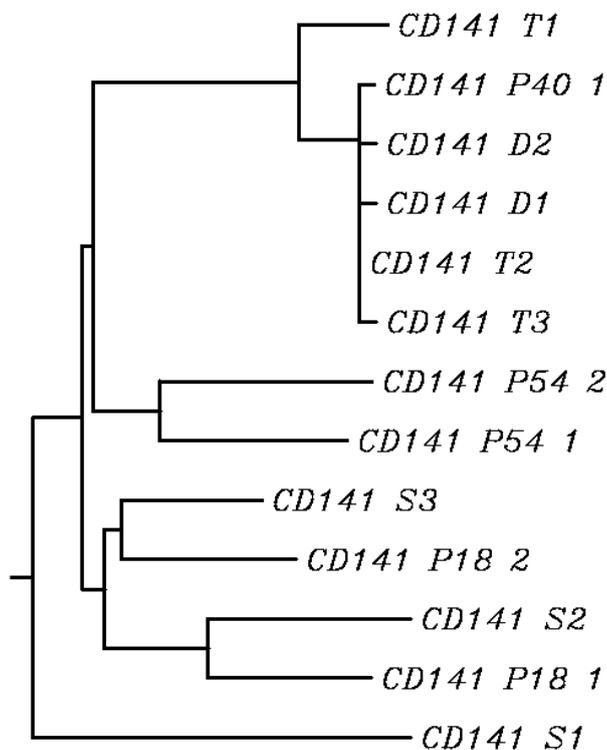


Figure 3.3.10: Nucleotide sequence based phenetic tree (Neighbour-joining tree) of all cloned *pCD141* invertase alleles.

The alleles group regarding their similarity in the same clades and subclades as observed in the amino acid sequences based phenetic tree (Figure 3.3.9). Subclades are more subdivided because of nucleotide polymorphisms.

3.3.1.2 Classification of alleles of the genes *pCD111* and *pCD141*

The genes *pCD111* and *pCD141* map to potato chromosome X (CHEN ET AL., 2001) in a region associated with tuber quality traits where a QTL for potato tuber sugar content, *Sug10a*, was identified (MENÉNDEZ ET AL., 2002). In the latter study, the genes *pCD111* and *pCD141* (referred to *Inv_{ap-a}*) were directly mapped, and showed linkage to the QTL *Sug10a*.

Single-strand conformation polymorphism (SSCP) analysis was carried out for the gene *pCD141* and revealed an association of *pCD141* SSCP fragments with starch and sugar content of potato tubers (LI ET AL., 2008). The SSCP fragment *pCD141_3c* was found to have a negative effect on potato chips quality and tuber starch content. To assign one of the cloned

pCD141 allele (section 3.3.1.1.5/6) to the corresponding associated SSCP fragment, 12 potato cultivars from BNA, SARA, and NOR, respectively were analyzed. To date in none of these 36 genotypes, defined as standards and characterized regarding *pCD141_3c* distribution, associated SNPs could be identified. This is a subject of ongoing investigations in the research project.

3.3.1.3 Genomic organization of the *Invap-a* locus

❖ BAC library screens

The genomic sequence and gene organization of the gene pair *pCD111/pCD141* were identified using high density BAC library screens with two different PCR generated probes. Using the primers CD111S2_For/CD111S1_Rev and pCD141-3F/pCD141-3R (chapter 2, Table 2.1.10A), Probe 1 for the gene *pCD111* and Probe 2 for the gene *pCD141* were generated. Both probes consisted of exon based sequences (HEDLEY ET AL. 1993, 1994) of the genes *pCD111* and *pCD141*, respectively.

The screening of two different BAC libraries (BA and BC, BALLVORA ET AL. 2002, 2007) constructed with genomic DNA of the same diploid genotype resulted in six positive BAC clones for the gene *pCD141* (Table 3.3.8). From the *pCD141* positive BACs one clone (BAC BC3) showed full-length PCR amplification of the gene *pCD111*. BAC screens with the specific *pCD111* Probe 1 were negative.

Table 3.3.8: Positive BAC clones for the genes *pCD111* and *pCD141*.

Library	BAC clones <i>pCD141</i>	PCR <i>pCD141</i>	PCR <i>pCD111</i>
BA BAC library	BA1: Plate 28K15	yes	not analyzed
	BA2: Plate 40M19	yes	not analyzed
	BA3: Plate 59E10	yes	not analyzed
BC BAC library	BC1: Plate 37C23	yes	no
	BC2: Plate 146K10	yes	no
	BC3: Plate 163L15	yes	no

PCR for the genes *pCD111* and *pCD141* were performed with gene specific full-length gene primers: *pCD111*-CD111fl_F/CD111fl_R (chapter 2, Table 2.1.2), *pCD141*-CD141fl_F/CD141fl_R (chapter 2, Table 2.1.2). Positive BACs are numbered and their position in the *E. coli* microtiter plate is listed.

BAC library screening and full-length PCR amplification of *pCD111* and *pCD141* within BAC BC3 suggested that this clone contains both invertase genes of interest. BAC insert (size: 130kb) sequencing was carried out by MWG Biotech AG, Ebersberg using the 454 sequencing technique on the GS FLX system.

❖ BAC Annotation

Full-length sequencing of the BAC insert revealed sequence and structural information of the genes *pCD111* and *pCD141* and of flanking genes (Table 3.3.9).

Table 3.3.9: BC3 sequence annotation.

Strand	Apollo name	Position on the BAC insert (bp)	Description
+	gene 10	2,068-2454	response to auxin stimulus
+	gene 20	4,655-13,035	kinesin
+	gene 30	31,961-36,971	cell wall invertase <i>pCD111</i>, beta-fructofuranosidase (glycoside hydrolase family 32)
+	gene 40	44,311-48,793	cell wall invertase <i>pCD141</i>, beta-fructofuranosidase (glycoside hydrolase family 32)
+	gene 50	62,479-64,535	GTP-binding protein
+	gene 60	69,953-71,012	unknown
+	gene 70	73,520-74,558	putative integral membrane family protein
+	gene 80	75,343-78,806	putative RNA-binding protein
+	gene 90	85,626-88,464	putative embryo defective protein
+	gene 100	122,735-125,703	unknown
-	gene 110	54,022-51,302	putative ribosomal protein
-	gene 120	58,824-55,722	putative esterase lipase
-	gene 130	68,349-66,377	putative dynamin
-	gene 140	81,608-80,442	unknown
-	gene 150	94,506-89,268	DNA-binding protein
-	gene 160	109,512-106,330	Pre-mRNA splicing factor

The invertase genes were named ‘gene 30’ (*pCD111*) and ‘gene 40’ (*pCD141*) in the Apollo BAC sequence characterization. BAC annotation was carried out using the software Apollo Genome Annotation and Curation Tool, version 1.9.8. Both invertase genes are written in bold.

The screened BAC libraries BA and BC harbour genomic DNA of the same diploid genotype P6/210, which is a hybrid derived from the cross of the parental genotypes P40 x P41 (LEISTER ET AL., 1996). The genotype P40 was also selected in this study for invertase allele characterization. Sequence alignments of P40 cDNA alleles from *pCD111* and *pCD141* with the genomic sequences of the genes 30 (*pCD111*, Appendix A 3.3.30) and 40 (*pCD141*, Appendix A 3.3.31) from the BAC BC3 showed no sequence identity. Since only one P40 cDNA allele from *pCD111* and *pCD141* respectively, was cloned it is possible that the second not detected P40 allele corresponds to the BAC sequences, or that the detected BAC allele for the genes *pCD111* and *pCD141* originate from the other parental genotype P41.

❖ Structural characterization of the genes *pCD111* and *pCD141*

The exon and intron organization of the genes *pCD111* and *pCD141* was determined by aligning the *pCD111* (Appendix A 3.3.32¹⁴) and *pCD141* (Appendix A 3.3.33¹⁵) cDNA alleles and the genomic sequences of the corresponding genes from BAC BC3. Both genes consist of six exons and five introns (Figure 3.3.11). The gene *pCD111* has a length of 5012bp, whilst the gene size of *pCD141* is 4478bp.

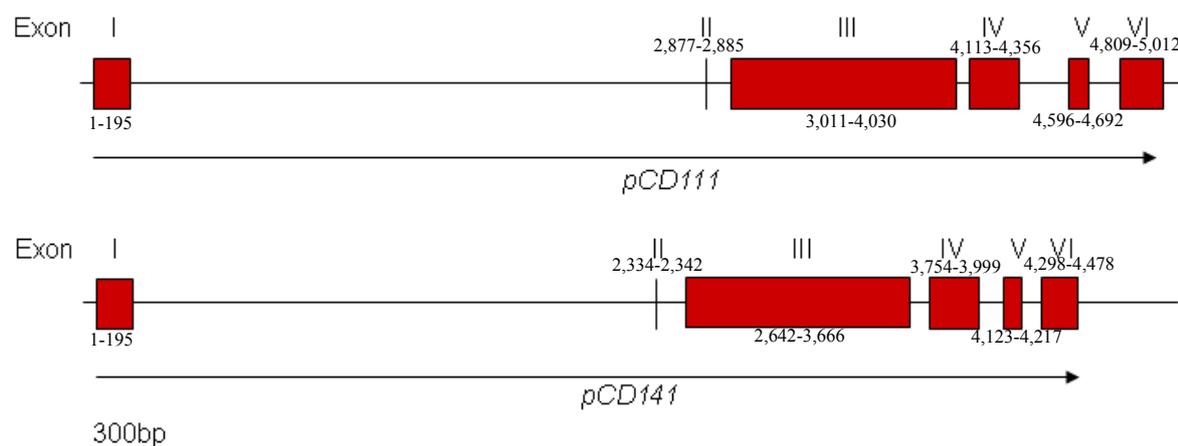


Figure 3.3.11: Genomic organization of the genes *pCD111* and *pCD141*. Exons are drawn in red and numbered from I to VI. The arrows symbolize the whole length of the particular gene without promoter and terminator sequences.

The range of individual *pCD111* and *pCD141* exons and introns are summarized in Table 3.3.10.

Table 3.3.10: Ranges of exons and introns of the genes *pCD111* and *pCD141*.

Exon number	Range (bp)	Intron number	Range (bp)
<i>pCD111</i>			
I	1-195	I	196-2,876
II	2,877-2,885	II	2,886-3,010
III	3,011-4,030	III	4,031-4,112
IV	4,113-4,356	IV	4,357-4,595
V	4,596-4,692	V	4,693-4,808
VI	4,809-5,012		
<i>pCD141</i>			
I	1-195	I	196-2,333
II	2,334-2,342	II	2,343-2,641
III	2,642-3,666	III	3,667-3,753
IV	3,754-3,999	IV	4,000-4,122
V	4,123-4,217	V	4,218-4,297
VI	4,298-4,478		

¹⁴ The alignment program Multalin version 5.4.1 (<http://bioinfo.genetoul.fr>) used revealed problems in the comparison of the genomic *pCD141* sequence with the *pCD141* cDNA sequence due to SNPs in both sequences. The mini-exon II was not aligned properly, two additional nucleotides occurred, whilst another one was connected with the first exon.

¹⁵ As described above in footnote 14.

It is known from the literature that the genomic structure of higher plant invertases is fairly conserved and consists of six to eight exons. As it is the case for the invertases *Pain-1* (chapter 3.1), *invGE* and *invGF* (chapter 3.2), also the genes *pCD111* and *pCD141* show this exon-intron structure and exhibit the extremely small exon II, which only codes for the core tripeptide DPN of the conserved β -fructosidase motif NDPNG (TYMOWSKA-LALANNE & KREIS, 1998).

It has been shown that potato invertase loci encoding cell wall-bound isoforms are characterized by a direct tandem repeat organization where two linked genes are separated by about 2,3kb (MADDISON ET AL., 1999). The *Inv_{ap}-a* locus consisting of the gene pair *pCD111/pCD141* showed this kind of genomic structure with approximately 8kb separating the corresponding invertase genes (Figure 3.3.12).

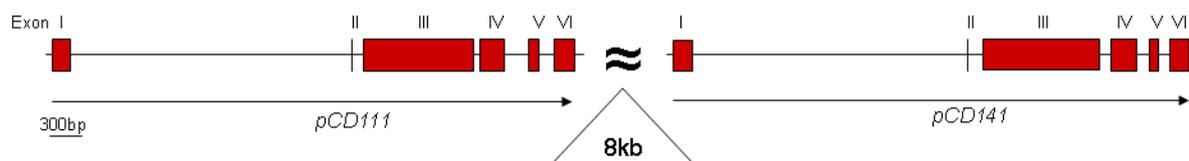


Figure 3.3.12: Tandem repeat linkage of the genes *pCD111* and *pCD141*. Exons are drawn in red and numbered from I to VI. The arrows symbolize the whole length of the genes without promoter and terminator sequences.

4 Discussion¹⁶

4.1 The physiological impact of potato invertases on tuber chips quality

The interest of this study was to characterize the underlying mechanisms of the trait ‘potato chips quality’, which is influenced by the starch and sugar content of potato tubers. A high content of the reducing sugars glucose and fructose, which are accumulating during tuber cold storage (cold-sweetening), results in inferior chips quality e.g. a dark colour, a bitter taste and a high acrylamide concentration. Starch and sugar content of potato tubers are quantitative traits, which can be considered as model traits using the candidate gene approach to unravel the molecular basis of quantitative trait loci (QTL). QTL for tuber starch and sugar content or potato chips colour have been mapped in potato (DOUCHES & FREYRE, 1994; MENÉNDEZ ET AL., 2002). A number of candidate genes have been identified regarding the fact of their co-localisation with QTLs on molecular maps, as well as being functional in the biosynthesis, degradation, or transport of starch and sugars in potato and other plants (CHEN ET AL., 2001; MENÉNDEZ ET AL., 2002). Among others, invertase genes were identified as positional candidates for cold-sweetening QTLs. Furthermore, association analysis showed a significant correlation between alleles of vacuolar and cell wall-bound invertase isoforms and better potato chips quality as well as other tuber traits (LI ET AL., 2005, 2008). In potato three invertase loci *Pain-1*, *Inv_{ap}-a* and *Inv_{ap}-b* are known. The gene *Pain-1* on chromosome III encodes a vacuolar invertase. The two gene pairs *invGE/invGF* of the locus *Inv_{ap}-b* and *pCD111/pCD141* of the locus *Inv_{ap}-a* on potato chromosomes IX and X respectively, code for cell wall-bound invertase isoforms. The genes *invGE* and *invGF* are linked in a direct tandem repeat and separated by approximately 2.3kb from each other. The size of the *Inv_{ap}-b* locus is approximately 8.6kb (MADDISON ET AL., 1999). Comparative analysis of the potato genetic map revealed that the gene pair *pCD111/pCD141* on chromosome X arose by partial chromosome duplication of the gene pair *invGE/invGF* of chromosome IX and, therefore, might also be organized in a direct tandem repeat (GEBHARDT ET AL., 2003; FRIDMAN ET AL., 2003). The present study showed that the genes *pCD111/pCD141* are arranged in a direct tandem repeat and separated by approximately 8kb. The size of the *Inv_{ap}-a* locus is approximately 17.5kb (chapter 3.3, section 3.3.1.3).

¹⁶ Due to the complexity of the present work, a summary of the project and the main results were included to facilitate reading and understanding.

Besides being positional candidates, invertases are also functional candidates. Invertases are enzymes, which catalyze the last step in the carbohydrate breakdown chain. The products of the invertase reaction are the reducing sugars glucose and fructose, which directly interfere with potato chips quality (SHALLENBERGER ET AL., 1959). In consequence invertases fulfil both criteria, being candidate genes in the genetic and biochemical sense.

Part of the phenotypic differences of potato chips quality can be explained by allelic variation of invertases as identified by association analysis (LI ET AL., 2005, 2008). FRIDMAN ET AL. (2004) identified the tomato invertase gene *LIN5* as causal for the QTL *Brix9-2-5* for sugar yield of tomato fruits, comparing differences between *LIN5* alleles of the cultivated tomato (*Solanum lycopersicum*) and of wild species (*Solanum pennellii*). The tomato fruits of the wild species showed a dramatic reduction of the sugar content compared to fruits of the cultivated tomato. The present study aimed to elucidate whether allelic variation of functional potato invertase alleles in different genotypes can be identified and to what extent this natural variation accounts for the observed phenotypic diversity regarding chips quality. The question was whether allelic variation manifested itself at functional level as functional differences that could be characterized, like observed in tomato for the fruit sugar content (FRIDMAN ET AL., 2004).

To analyze allelic composition and functional relevance of invertase alleles, six different genotypes were selected. Based on association analysis the tetraploid cultivars ‘Satina’, ‘Diana’, and ‘Theresa’ were chosen due to the presence or absence of associated invertase SSCP fragments (LI ET AL., 2005, 2008). Additionally, three diploid potato genotypes were analyzed previously being used as parents for mapping QTLs and candidate genes for cold sweetening of potato tubers (MENÉNDEZ ET AL., 2002).

4.2 Structural characterization of potato invertase alleles

Invertase alleles of the five genes *Pain-1*, *invGE/invGF*, and *pCD111/pCD141* were structurally analyzed focusing on their cDNA and genomic sequences, their exon/intron structures and their gene organization. Additionally, *Pain-1* and *invGE/invGF* alleles were characterized using 3D-modelling to visualize putative effects of allelic amino acid differences. The genes *pCD111* and *pCD141* were not modelled because less information about association and putative allelic effects on potato chips quality was available at the beginning of this work.

❖ Molecular cloning of invertase cDNA alleles

cDNA alleles of *Pain-1* were isolated from tuber tissue, *invGE*, *pCD111*, and *pCD141* cDNA alleles were obtained from leaf tissue. In the case of *invGF*, alleles were isolated using floral as well as leaf tissue. In the following Table 4.1 all invertase cDNA alleles cloned from the six genotypes are listed.

Table 4.1: Overview of isolated invertase cDNA alleles.

Genotype	<i>Pain-1</i> alleles	<i>invGE</i> alleles	<i>invGF</i> alleles	<i>pCD111</i> alleles	<i>pCD141</i> alleles
‘Satina’	2	4	4	3	3
‘Diana’	3	3	2	1	2
‘Theresa’	2	4	2	2	3
P18	2	2	1	0	2
P40	2	2	2	1	1
P54	1	2	2	2	2

PCR based cDNA cloning of the five known potato invertase genes *Pain-1* on chromosome III, *invGE/invGF* on chromosome IX and *pCD111/pCD141* on chromosome X resulted in 64 distinct alleles.

Specific PCR amplification of the five invertase genes *Pain-1*, *invGE*, *invGF*, *pCD111*, and *pCD141* was indicated by the presence of corresponding alleles amplified in one course of PCR using a given gene specific primer pair. In Table 4.2 the sequences of full-length primers for all five genes are listed showing gene specific sequence polymorphisms that resulted in gene specific amplification.

Table 4.2: Full-length primers used for gene specific PCR amplification.

Invertase gene	Chr. number	Forward primer (in 5'-3'orientation)	Reverse primer (in 5'-3'orientation)
<i>Pain-1</i>	III	ATG GCC ACG CAG TAC C	GAT GAA TTA CAA GTC TTG CAA GGG
<i>invGE</i>	IX	ATG GAA TTA TTT ATG AAA AGC TCT TCT CTT TGG GGG T	TTA GTG CAT CTT AGG TAC ATC CAT GCT CCA AGC
<i>invGF</i>	IX	ATG GAT TAT TCA TCT AAT TCT CGT TGG GCT TTG CCA G	TCA ATA TTG TAT CTT AGC TTT GCC CAT ACT CCA TGC
<i>pCD111</i>	X	ATG GAT TGT TTA AAA AAG TCT TCT C	TCA ATA AGA AGA GTG ACC AAA TGA CCA ATT CA
<i>pCD141</i>	X	ATG GAG ATT TTA AGA AGA TCT TCT TCT CTT TGG GTT	CTA GTG CAA CTT TGC ATT AGC CAT GCT CCA AGC

In the course of cloning and sequencing of cDNA alleles, quite a number of singular nucleotide variants (singletons) or non functional clones showing internal frame shifts and missing start and stop codons, were isolated. As the cloning of cDNA alleles was PCR based, a proofreading *Taq*-Polymerase, which is characterized by a high fidelity (approximately 5.5×10^{-5} mismatches per base pair per PCR cycle) was used to minimize PCR derived errors. Hereby four times better amplification results compared to common *Taq* DNA-Polymerase were achieved. Sequence reliability was similar to other commercially available proof-reading polymerases (i.e. *Pfu* DNA-Polymerase: 1.5×10^{-5} errors per base pair per PCR cycle, Invitrogen, Karlsruhe). Another source of *in-vitro* invertase sequence variability might have been the conversion of RNA to cDNA by Superscript II (Invitrogen, Karlsruhe), which possesses an error rate of 1×10^{-4} per nucleotide.

Randomly occurring sequencing errors were eliminated by checking forward and reverse sequence of the affected area. Furthermore, in the present study PCR did not show equal amplification of invertase alleles, and allele detection needed several PCRs. This phenomenon is known as allelic dropout and was observed in diverse studies in human genetics as well as in plant microsatellite genotyping (FINDLAY ET AL., 1995; TABERLET ET AL., 1996, BROQUET & PETIT, 2004; ZHANG ET AL., 2006; SOULSBURRY ET AL., 2007). Allelic dropout is the failure of PCR amplification of one allele in a heterozygous organism. Therefore, allele identification might be incomplete, and there is the possibility that not all existing invertase alleles of one genotype have been detected. Another aspect of allele mining by PCR was the definition of cDNA alleles, which were not detected as actively transcribed by pyrosequencing analysis of cDNA (section 3.2.2.1.1, Figure 3.2.24). A low number of cDNA clones represented these alleles and some occurred only once in PCR amplification. However, pyrosequencing analysis of genomic DNA showed that the SNPs specific for those alleles are present at genomic level, representing an existing allele of the genotype but is transcribed at such a low level, therefore not being detectable by cDNA pyrosequencing analysis. This is consistent with the rare amplification of these alleles by PCR.

Allele mining for each invertase gene was achieved by using tissues where invertase expression has been demonstrated (ZRENNER ET AL., 1996; MADDISON ET AL., 1999; HEDLEY ET AL., 1993, 1994). For the gene *invGF*, MADDISON ET AL. (1999) detected expression exclusively in floral tissues. Expression profiles of the tomato and the *Arabidopsis invGF* orthologs, *LIN7* and *ATβFRUCT2* respectively, showed also restriction of transcripts to floral tissues (FRIDMAN ET AL., 2003). In contrast to these findings, this study showed that *invGF* expression occurred also cultivar dependent in leaves (section 3.2.1.2, Figures 3.2.1 and 3.2.2). *invGF* alleles isolated from leaves and flowers were identical at amino acid level as well as in their nucleotide sequence.

It has to be pointed out that in the course of this study invertase allele mining using three tetraploid cultivars resulted in a tremendous allelic variation not expected by SSCP analysis that was earlier applied on 240 tetraploid potato cultivars (LI ET AL., 2005, 2008). Besides non synonymous allele specific SNPs, which caused amino acid exchanges, quite a number of synonymous SNPs occurred, leading to alleles identical at amino acid level. As synonymous SNPs do not cause amino acid substitutions, the function of the corresponding protein should not be affected. Nevertheless, several studies in humans showed that synonymous SNPs produced altered mRNA secondary structures affecting mRNA degradation and modification (e.g. splicing) as well as resulting in a reduced amount of translated protein (NACKLEY ET AL., 2006; reviewed in CHAMARY ET AL., 2006). Additionally, KIMCHI-SARFATY ET AL. (2007) showed that synonymous SNPs also affect enzymatic substrate specificities. In this respect, haplotypes showing synonymous SNPs accompanied by similar mRNA and protein levels, led to altered enzyme conformation because of rare codons, resulting from synonymous polymorphisms that affected the timing of co-translational folding and, therefore, the structure of the enzyme. Whether synonymous SNPs detected in potato invertase alleles also alter mRNA secondary structure, mRNA degradation, translation and enzyme conformation needs further investigation.

❖ Phenetic tree analysis of invertase cDNA alleles from all five potato invertase genes

The phenetic tree analysis was applied to group all alleles obtained from the five potato invertase genes according to their similarity at amino acid level (Figure 4.1). This allowed getting an impression of the grouping between the invertase isoforms as well as the clustering of associated and not associated alleles of the latter isoforms to each other.

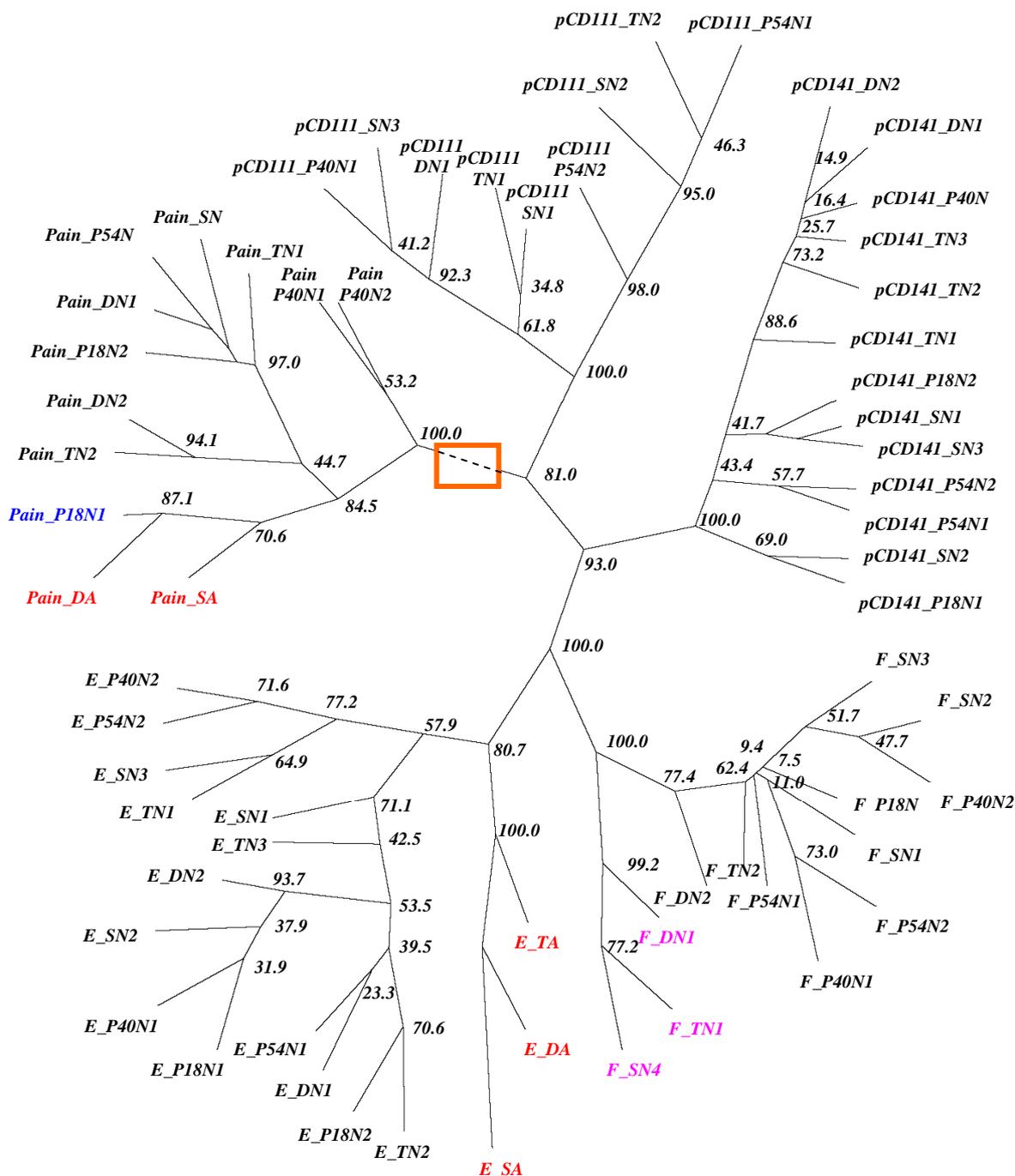


Figure 4.1: Amino acid based phenetic tree of all cloned invertase cDNA alleles. The tree was generated using the maximum parsimony method. A total of 100 bootstrapping runs were performed, and the percent reliability is indicated next to each branch. Allele names comprise the invertase gene, genotype and allele type. Alleles found to be associated with better potato chips quality are coloured in red. The allele *Pain_P18N1*, which is amino acid sequence identical to *Pain_DA* was not tested by association analysis and, therefore, is coloured in blue. The three *invGF* alleles *F_SN4*, *F_DNI*, and *F_TNI* are thought to be representatives of the associated SSCP fragment *invGF-4d* because of similar grouping as observed for the associated *invGE* alleles *E_SA*, *E_DA*, and *E_TA*. The three *invGF* alleles are coloured in pink. The boxed area reflects a truncated branch – this is due to the relatively distant clustering of vacuolar *Pain-1* alleles compared to cell wall-bound invertase isoforms (*invGE*, *invGF*, *pCD111*, and *pCD141*) because of weak sequence homology.

The phenetic tree of invertase alleles generated in this study revealed that the alleles from the different loci group separately. The analysis showed that the gene *Pain-1* is more similar to

the genes *pCD111* and *pCD141* because of closer branching distance in contrast to the genes *invGE* and *invGF*. Peptide based comparison of the different potato invertase isoforms revealed that the cell wall-bound invertases *invGE*, *invGF*, *pCD11,1* and *pCD141* share an amino acid sequence homology of approximately 73%, whilst the vacuolar invertase *Pain-1* displayed a homology of around 38% to the cell wall-bound isoforms explaining the distant clustering. This is in agreement with previous studies where comparison of amino acid sequences of plant invertases demonstrated that cell wall and vacuolar invertases belong to two different classes (TYMOWSKA-LALANNE & KREIS, 1998). Therefore, invertase amino acid sequences of the same isoform originating from different species are more similar to each other than sequences of different isoforms from one and the same species.

❖ Genomic characterization of the genes *Pain-1*, *pCD111*, and *pCD141*

The next step was to elucidate the genomic structures of the genes *Pain-1*, *pCD111*, and *pCD141*. Therefore, high density BAC library screens were performed using two different BAC libraries constructed with genomic DNA of the same diploid genotype (BALLVORA ET AL., 2002, 2007).

Information about genomic potato invertase organization was only available for the genes *invGE* and *invGF* on chromosome IX. MADDISON ET AL. (1999) showed that the *Inv_{ap-b}* locus consists of the genes *invGE* and *invGF* linked in a direct tandem repeat and separated by approximately 2.3kb from each other. Both genes exhibit a similar exon/intron structure composed of six exons and five introns. The size of the *Inv_{ap-b}* locus is approximately 8.6kb (MADDISON ET AL., 1999). Comparative analysis of the potato genetic map revealed that the genome segment harbouring the genes *pCD111* and *pCD141* on chromosome X is related to a genome segment containing the genes *invGE* and *invGF* of chromosome IX, and, therefore, might also be organized in a direct tandem repeat (GEBHARDT ET AL., 2003; FRIDMAN ET AL., 2003). Corresponding studies of orthologous invertase genes of tomato revealed that the syntenic tomato locus to *invGE/invGF*, *LIN5/LIN7* on chromosome IX, also displays the linkage of both genes in a direct tandem repeat (FRIDMAN ET AL., 2003). The tomato invertase genes *LIN8* and *LIN6* on tomato chromosome X, which are orthologs of the potato genes *pCD111* and *pCD141* on potato chromosome X, are also arranged in this manner. Known genomic organizations of above described potato and tomato invertase genes strongly encouraged the expectation that the potato genes *pCD111* and *pCD141* are also linked in a direct tandem repeat.

Sequencing of a BAC insert harbouring the genes *pCD111* and *pCD141* confirmed the latter spatial organization of both genes (section 3.3.1.3, Figures 3.3.11 and 3.3.12) as observed for

their tomato orthologs *LIN8* and *LIN6*. Furthermore, it could be shown that the genes *pCD111* and *pCD141* are separated from each other by approximately 8kb, whilst the tomato genes *LIN8* and *LIN6* are divided by 7,2kb. Additionally, the tomato locus *LIN8/LIN6* is flanked upstream by a kinesin, and downstream by a 40S ribosomal gene. These two genes were also detected flanking the potato gene pair *pCD111/pCD141* of the *Inv_{ap}-a* locus in the same orientation. The size of the *Inv_{ap}-a* locus is approximately 17.5kb.

The potato invertase genes *invGE* and *invGF* as well as the tomato invertase genes *LIN5*, *LIN7*, and *LIN6* consist of six exons and five introns. The tomato invertase gene *LIN8* displays five exons and four introns. However, genomic characterization of the potato genes *pCD111* and *pCD141* showed that both contain six exons and five introns.

The size of the exons and introns is not conserved between different potato invertase genes, except the nine base pair mini-exon II, and varies from 9 to 1,026 and from 83 to 2,682 nucleotides, respectively.

The gene *Pain-1* on chromosome III codes for a vacuolar invertase in contrast to *invGE/invGF* and *pCD111/pCD141*, which all encode cell wall-bound invertase isoforms. One interest of this study was whether *Pain-1* is differently organized than the other two known invertase loci. Searching for *Pain-1* tomato orthologs resulted in one EST on tomato chromosome III (www.sgn.cornell.edu). By sequencing BAC inserts harbouring the *Pain-1* gene no second invertase was detected. Within the BAC inserts *Pain-1* gene surrounding sequences should allow to detect a possible tandem repeat organization (section 3.1.1.4, Figure 3.1.11 A). This possibility was excluded for the *Pain-1* locus. Furthermore, in the corresponding flanking regions no kinesin or ribosomal genes as observed for the genes *pCD111/pCD141* were detected. The *Pain-1* gene consists of seven exons and six introns, and, therefore, has an additional exon and intron when compared to the other four known potato invertase genes. Analysis of invertase isoforms from different species like *Arabidopsis thaliana*, carrot, tomato, maize, tobacco, mung bean, and pea demonstrated that invertases vary in their exon/intron composition (TYMOWSKA-LALANNE & KREIS, 1998). All potato invertases contain the conserved exon II, which only consists of nine nucleotides. This mini-exon, one of the smallest exons known in plants, encodes the residues DPN of the highly conserved β -fructosidase motif, NDPNG (TYMOWSKA-LALANNE & KREIS, 1998). The β -fructosidase motif of the genes *invGE*, *invGF*, and *pCD111* is represented by NDPNA rather than NDPNG. The other potato invertases *Pain-1* and *pCD141* display the residues NDPNG. BOURNAY ET AL. (1996) observed that under cold stress the mini-exon of the *pCD111* cell

wall invertase was skipped in an alternative splicing event. The functional relevance of the splicing effect was not investigated.

Comparison of the amino acid sequences of plant invertases demonstrated that cell wall and vacuolar invertases belong to two different classes (TYMOWSKA-LALANNE & KREIS, 1998). Corresponding invertase amino acid sequences of the same isoform originating from different species are more similar to each other than sequences of different isoforms from one and the same species are. Additionally, typical vacuolar invertase peptide regions or residues were identified that are absent in cell wall-bound isoforms. The peptide domain WECxDF, which is conserved among plant invertases, where x=valine in vacuolar and x=proline in cell wall-bound invertases (TYMOWSKA-LALANNE & KREIS, 1998; STURM, 1999) was also present in the different invertase isoforms of potato.

❖ Structural 3D-analysis of invertase cDNA alleles

In collaboration with Pawel Durek (MPIMP/Golm) the structural consequences of allelic invertase sequence variation was explored by applying a 3D-modelling analysis. The modelling of the allelic invertase structure was based on the 3D crystal structure of cyanobacteria invertase (ALBERTO ET AL., 2004). The models presented included the putative sucrose binding domain. In addition to the structural visualization of amino acid exchanges, also the electric potential (EP) of *Pain-1* and *invGE/invGF* alleles was mapped at pH 4.7 mimicking the vacuolar and apoplastic environment.

The analysis of the molecular structure of the associated ‘Satina’ *Pain-1* allele *Pain_SA* compared to *Pain_SN3* and of the associated ‘Diana’ allele *Pain_DA* compared to *Pain_DN1* and *Pain_DN2* showed that amino acid exchanges were manifested on the surface of the enzyme. None of the modelled molecules showed structural differences within the putative sucrose binding domain (section 3.1.1.3.1, Figure 3.1.7).

Comparative models of ‘Diana’ alleles revealed that models of the two not associated alleles *Pain_DN1* and *Pain_DN2* differed less from each other compared to the associated allele (*Pain_DA* vs. *Pain_DN1*; *Pain_DA* vs. *Pain_DN2*). The SNP 1544 present in the alleles *Pain_SA* and *Pain_DA*, which was found to be associated with better potato chips quality, had a direct effect on the molecule’s surface.

The characterization of the EP showed charge differences among the protein models. The putative sucrose binding site was positively charged matching the partial negative charge of the substrate sucrose due to the hydroxyl groups. A dramatic charge difference of the putative sucrose binding site was observed in the associated *Pain-1* alleles *Pain_SA* and *Pain_DA*, which are not amino acid identical but share the associated SNP at cDNA position 1544

(section 3.1.1.3.2, Figure 3.1.9). The EP switched from positive to neutral compared to the not associated *Pain-1* alleles. The EP changes could not be correlated to amino acid exchanges near the sucrose binding site. The causative amino acids were not yet analyzed, but are subject of ongoing investigations in the research project. Whether these EP differences of the associated alleles compared to the EP of the not associated alleles might influence enzymatic activity remains unclear. Possible effects of the neutral charge might be a weaker binding of sucrose and, therefore, a reduced conversion of sucrose into the reducing sugars glucose and fructose, which influence negatively potato chips quality. This would support the hypothesis that sequence variation of potato invertases leads to functional variation and that in consequence associated alleles produce less reducing sugars in tubers and thus a good potato chips quality.

Modelling *invGE* alleles from the cultivars ‘Satina’ and ‘Theresa’ revealed similar molecular structures of the investigated alleles (section 3.2.1.3, Figure 3.2.14). Only slight differences were visible. Comparative modelling of the associated *invGE* alleles *E_SA* and *E_TA* did not reveal strong differences (Figure 3.2.15). Superimposing the associated *invGE* allele *E_SA* and the *invGF* allele *F_SN4* showed strong structural differences (Figure 3.2.23). The strong structural effects are likely caused by sequence divergence of the two genes. Even though the latter belong to the same locus and arose from gene duplication (GEBHARDT ET AL., 2003; FRIDMAN ET AL., 2003), *invGE* and *invGF* proteins are only 74% similar to each other (MADDISON ET AL., 1999).

EP analysis of *invGE* alleles (Figure 3.2.16 and 3.2.17) revealed only weak charge differences of the associated ‘Satina’ allele *E_SA*, which showed a small negatively charged area compared to the allele *E_SN3*. The associated ‘Theresa’ allele *E_TA* which differs in its amino acid sequences to *E_SA* but contains the associated SNP 1103*, exhibited no charge differences of the putative sucrose binding site compared to *E_TN1*.

For the gene *invGF* two alleles of the cultivar ‘Satina’ *F_SN3* and *F_SN4* were modelled (section 3.2.1.3, Figure 3.2.19). Comparison of both allelic structures showed differences among the molecules. EP analysis showed that especially the putative sucrose binding domain displayed visible changes of its charge between the two alleles. In the allele *F_SN4* the EP of the putative domain switched from positive to negative, influencing possible interactions of substrate and enzyme (Figure 3.2.21). As reported for the *Pain-1* and *invGE* alleles, EP changes are not directly mediated by amino acid exchanges nearby the binding site. It is most likely that surrounding amino acid exchanges cause the charge shift.

In conclusion, 3D-modelling is a tool to gain first insights in the possible structural consequences of different invertase alleles. The models nicely showed that amino acid exchanges can modify the surface of the enzyme. However, no effect on the structure of the putative sucrose binding domain was detected in any of the modelled molecules, but an effect on the charge was observed. The putative sucrose binding site exposed areas of neutral and positive charges in the associated *Pain-1* and *invGE* alleles, *Pain-SA*, *Pain_DA*, and *E_SA*, and in the putative associated *invGF* allele *F_SN4*. Whether these EP differences of the alleles influence enzymatic activity remains unclear. To address this question a biochemical characterization was carried out (*Pain-1*: section 3.1.2.3; *invGE/invGF*: section 3.2.2.3) to determine whether differences in enzymatic activity are present between invertase alleles. The corresponding results will be discussed in one of the following section (4.3: Biochemical analysis of *Pain-1*, *invGE*, and *invGF* alleles).

Extracellular invertase from yeast and alkaline invertase from *Vicia faba* are known to act as oligomers (KERN ET AL., 1992; ROSS ET AL., 1996). Due to 3D-analysis, it might be possible that the vacuolar and cell wall-bound potato invertases might also function as a higher complex of several monomers. ROSS ET AL. (1996) did not investigate the role of allelic subunits in the alkaline invertase complex. Supported by putative 3D structural analysis, there is the possibility that invertase enzyme complexes are formed by allelic proteins (Pawel Durek, personal communication). Considering this information, it is comprehensive that conformational and electrostatic changes on the enzyme's surface affect invertase enzyme complex formation and might lead to altered enzyme activity as reported for other studies about complex formation of enzymes (TETLOW ET AL., 2004a; TETLOW ET AL., 2004b; TETLOW ET AL., 2008).

4.3 Functional characterization of potato invertase alleles

The expression of single genotype specific *Pain-1*, *invGE*, and *invGF* alleles was quantified with pyrosequencing using cDNA. Allele specific transcript abundance was compared to the genomic allele dosages as determined by pyrosequencing using genomic DNA. Additionally, total amounts of *Pain-1* transcripts during tuber cold storage were determined using qRT-PCR.

Pyrosequencing is a robust and quantitative sequencing method, based on real-time detection of pyrophosphate, which is released as a result of nucleotide incorporation in a sequencing-by-synthesis reaction (RONAGHI ET AL., 1996). Assessing allele frequencies in large genomic DNA pools by pyrosequencing has demonstrated the high level of accuracy of this method. WASSON ET AL. (2002) reported the reliable detection of allele frequency differences of 4% between DNA pools from human populations. This result was confirmed by NEVE ET AL. (2002) who estimated that, for large DNA pools, allele frequencies that differ by $\pm 5.2\%$ would be significant. Because of its high accuracy, pyrosequencing has been considered as the method of choice for genotyping SNPs in polyploid species. For example, RICKERT ET AL. (2002) and OEFNER (2002) have shown that the different heterozygous states of a binary SNP in tetraploid potato could reliably be distinguished. Furthermore, RICKERT ET AL. (2002) showed that 82% of the polymorphic sites tested were amenable to allelic discrimination by pyrosequencing, which is by far better than any other SNP genotyping method. Another advantage of pyrosequencing is the possibility to determine multiple SNP frequencies in a single measurement, allowing analysis of more than two alleles simultaneously.

The biochemical analysis of invertase cDNA alleles was performed using yeast as heterologous expression system. The complementation of a yeast invertase mutant with potato cDNA alleles allowed expression of single alleles and their characterization. Invertase activities were assayed to determine substrate affinities (K_m) and reaction rates (v_{max}).

The genes *pCD111* and *pCD141* were not functionally analyzed because of limited information concerning association and putative allelic effects on potato chips quality at the beginning of this work.

In a simple scenario, one can imagine several functional characteristics of an invertase allele influencing positively potato chips quality. Even though potato chips quality is a multigenic trait, invertase was the first gene studied at functional level in terms of allelic differences contributing to this trait. Possible features of a superior allele are summarized in Figure 4.2.

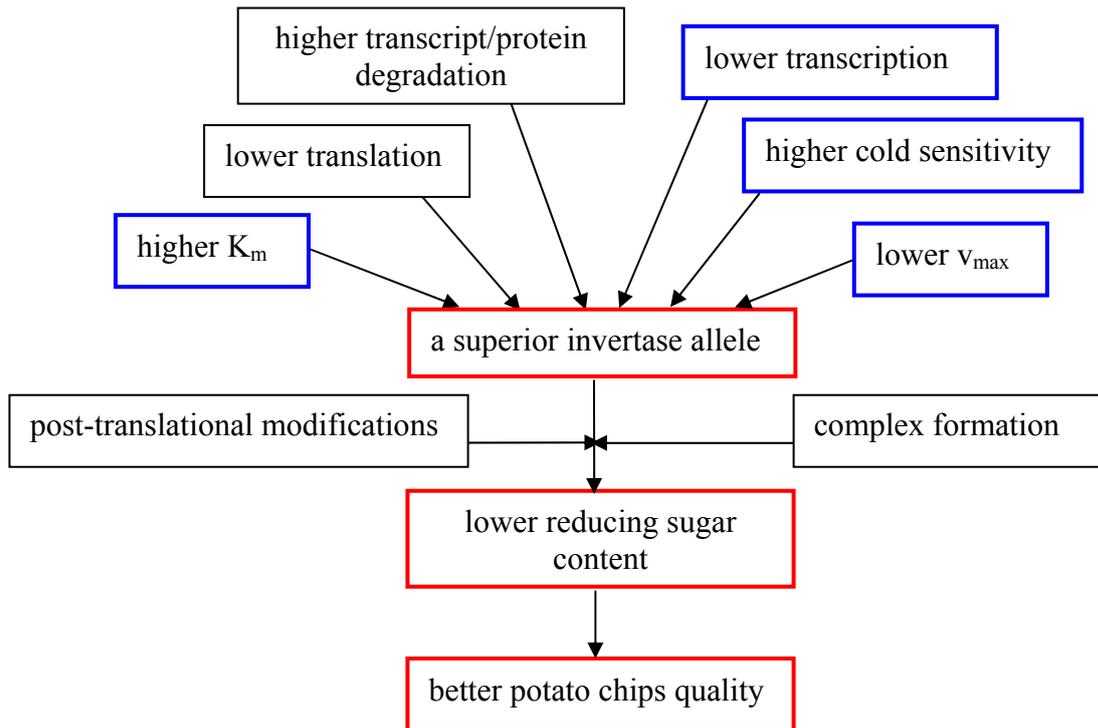


Figure 4.2: Simplified scheme displaying features of an ‘ideal’ invertase allele acting positively on potato chips quality. Red boxes indicate desired phenotypic traits influenced by a superior invertase allele. Blue boxes represent functional characteristics that were studied during this work.

A superior invertase allele should lead to a low reducing sugar content, which in consequence gives rise to a good potato chips quality. Allelic effects can occur at transcriptional, translational, post-translational, and biochemical level. In this agreement, a superior invertase allele would be sparsely transcribed and translated, displaying low protein abundance, and, therefore, having a poor contribution to overall invertase activity consequently leading to a reduced sucrose conversion. Additionally, high levels of transcript and protein degradation would also contribute to low protein content. In addition, a superior invertase allele could be characterized by a high K_m value meaning a low affinity to sucrose combined with a low v_{max} value indicating a slow conversion of sucrose. These biochemical characteristics can be even more prevalent in a cold environment applied to potato tubers to prevent them from sprouting. However, other post-translational modifications like N-glycosylation, protein folding, or allelic complex formation are further possibilities in influencing a superior invertase allele resulting in lower reducing sugar content and, therefore, better potato chips quality.

In the course of this work, out of the mentioned features characterizing a superior invertase allele, allele specific expression and allelic biochemical characteristics were determined to prove whether such alleles are present.

❖ Expression analysis of *Pain-1*, *invGE*, and *invGF* alleles

Besides the possibility of an altered enzymatic activity, sequence variation can also manifest at expression level. A simple assumption consist of a low transcription level of a superior invertase allele resulting in low translation rate contributing to a lower extent to overall invertase activity due to limited enzyme abundance. In the latter case lower invertase protein levels due to a reduced allelic expression would lead to superior chips quality (Figure 4.2).

Differential expression analysis was performed monitoring the genes *Pain-1*, *invGE*, and *invGF*. The study of the expression followed different purposes. The gene *Pain-1* is known to be differentially expressed during tuber cold storage (ZRENNER ET AL., 1996; ZHOU ET AL., 2004; BAGNARESI ET AL., 2008). Using pyrosequencing, genotype specific allelic transcription patterns during a cold storage time course of four weeks were determined compared to additional measured genomic dosages of the alleles. Total amounts of *Pain-1* transcripts during tuber cold storage were measured using qRT-PCR.

The expression of *invGE* and *invGF* alleles was detected by pyrosequencing analysis in comparison to the allelic genomic dosage determined by pyrosequencing using genomic DNA as template.

Expression analysis of *Pain-1*, *invGE*, and *invGF* alleles revealed differential transcriptional regulations of particular alleles. Allele specific expression is a common fact and several studies showed such a regulation on transcriptional level (reviewed in KNIGHT, 2004). Studies in octoploid strawberry showed allele specific expression of a pathogenesis-related gene induced by fungal infection causing fruit rot (SCHAART ET AL., 2005). SPRINGER & STUPAR (2007) used allele specific expression assays to profile the relative allelic expression in seedling tissue derived from maize hybrids. They found evidence for regulatory variation that contributes to biased allelic expression between genotypes and between tissues. Studies in mammals during early stages of development demonstrated the importance of allele specific expression implicating imprinting mechanisms (SZABO & MANN, 1995).

The alleles *Pain_SA* and *Pain_DA* were derived from the genotypes ‘Satina’ and ‘Diana’. It could be shown that the two alleles, having different amino acid sequences but being associated with better potato chips quality, were differentially expressed during tuber cold storage when compared to their genomic dosage (section 3.1.2.1, Figures 3.1.12 and 3.1.14). Whilst *Pain_SA* showed an up-regulation in its expression of approximately 18%, the allele *Pain-DA* revealed a permanent reduction of expression during the cold storage course of four weeks resulting in an overall decrease up to 20% compared to samples that were not stored in the cold. The diploid potato genotype P18 harbours an allele with identical amino acid

sequence to the associated allele *Pain_DA*. The allele *Pain_P18N1* showed a strong transcript increase after one week of tuber cold storage (section 3.1.2.1, Figure 3.1.18). Expression level was 50% higher than in tubers that were not stored in the cold. The differential expression of the associated allele *Pain_SA* and the allele *Pain_P18N1*, which is identical at amino acid level to the associated allele *Pain_DA*, showed that these allelic transcripts are influenced by low temperature leading to higher abundance during tuber cold storage than expected from their genomic dosage. This observation is in contradiction with the hypothesis that a superior invertase allele is characterized by transcriptional regulation implicating a lower expression (Figure 4.2). The associated allele *Pain_DA* exhibited a lower transcription level during tuber cold storage showing the lowest abundance after four weeks in the cold. This is in agreement with the assumption mentioned above that superior alleles display low amounts of transcripts, protein and, therefore, a lower enzymatic activity. However, the impact of the ‘Diana’ allele on overall invertase activity remains unclear since no methods are available to measure the activity of single allelic invertases *in vivo*. Additionally, tissue or clonal specific enzyme activity due to transcriptional changes in specific tissues or single cells might contribute to altered expression patterns and, therefore, lead to a quite variable enzyme activity.

The other alleles not associated with superior chips quality of the genotypes ‘Diana’, ‘Theresa’, and P40 revealed no relevant changes in their expression pattern during tuber cold storage. The allelic expression remained similar compared to the genomic dosage of the alleles.

Allelic differences and the contrasting observations regarding expression of the associated alleles at transcript level might be due to the individual genetic background. Genotype specific transcription factors and other regulatory elements as well as allele specific promoter sequences can result in differences of allele expression. Additionally, observations of allele specific expression patterns are limited due to the limited number of analyzable genotypes in the course of this work.

Using qRT-PCR, total *Pain-1* transcripts were quantified during tuber cold storage. ZRENNER ET AL. (1996) detected soluble acid invertase transcripts after 44h of tuber cold treatment at 4°C using Northern blots. Transcript levels reached a maximum after seven days of cold storage, followed by a decrease throughout the next two to six weeks. In contrast, BAGNARESI ET AL. (2008) detected an increase of soluble acid invertase transcripts at week two to four of cold storage.

In this study expression analysis showed an intense up-regulation of *Pain-1* transcripts after one to two weeks of tuber cold storage at 4°C. After the first week of cold treatment P18

transcripts showed the highest level of relative expression compared to all other analyzed genotypes (section 3.1.2.1, Figure 3.1.19). The cultivar ‘Theresa’ was the only genotype where total invertase transcripts increased again after four weeks of tuber cold storage (section 3.1.2.1, Figure 3.1.17).

The results of the expression analysis of the soluble acid invertase *Pain-1* are in agreement with earlier observations by ZRENNER ET AL. (1996) and BAGNARESI ET AL. (2008). Additionally, it was demonstrated that in different genotypes *Pain-1* alleles were strongly cold influenced regarding their expression, whilst others showed minor changes or stayed unaffected compared to their genomic distribution (section 3.1.2.1, Figures 3.1.14 and 3.1.16).

Pyrosequencing of *invGE* and *invGF* alleles of leaf and floral tissue respectively, showed that genotype specific alleles are differentially expressed compared to their genomic dosage. The question might arise how alleles analyzed in leaves and flowers might contribute to chips quality when not acting directly in the potato tuber. Association analysis of *invGE* and *invGF* genes revealed positive correlations between corresponding allelic fragments and potato chips quality (LI ET AL., 2005). Expression profiles investigated by MADDISON ET AL. (1999) using GUS histochemical expression analysis showed that *invGE* is expressed in several tissues and also under the ‘eyes’ of the tuber. In this study, none of the six selected genotypes allowed *invGE* transcript amplification using cDNA synthesized from mature tuber ‘eye’ RNA. Therefore, leaf tissue has been chosen to identify *invGE* alleles as well as to study their expression. It might be possible that mature tubers were not the appropriate source for *invGE* allele mining even though MADDISON ET AL. (1999) also used mature tubers but from a different cultivar. In this study mature tubers were used to extract RNA. Following the study’s approach analyzing potato chips quality before and after tuber cold storage, tubers were directly processed after harvest or stored in the cold for one to four weeks. MADDISON ET AL. (1999) also showed that *invGF* expression is restricted to floral tissues in the cultivars ‘Désirée’ and ‘Saturna’, which was shown to be not consistent with findings concerning some of the selected genotypes in this study where *invGF* expression was also detected in leaves (section 3.2.1.2, Figures 3.2.1, 3.2.2).

Reasons for the lack of *invGE* transcripts in potato tubers might also be that under the latter conditions *invGE* expression is below the detection limit due to time-dependent expression pattern. Cell wall-bound invertases are known to play an important role in tuber initiation. MINHAS & SAINI (2004/5) showed that under tuber-inducing conditions cell wall invertase activity increases up to 74% during the stolon to tuber transition period. Cell wall-bound invertase has been recognized as a key enzyme in apoplastic phloem unloading, which

switches in the developing tuber to the symplastic mode resulting in the primary function of sucrose synthase (SuSy) in sucrose cleavage. Cell wall invertases regulate the import of the reducing sugars glucose and fructose, which are used for starch synthesis in stolon tips. Assuming a superior invertase allele, less transcribed and translated resulting in low protein abundance accompanied by low activity, might lead to retarded sucrose cleavage and, therefore, less import of sugars in the initiated tuber. Additionally, a less active invertase allele in the apoplast might influence sink strength in a way that phloem unloading is decelerated and further tuber storage compounds are less abundant. Besides playing an important role in tuber initiation, cell wall invertases are generally believed as main determinants of sink strength especially during the initial stages of sink development (ROITSCH, 1999; ROITSCH ET AL., 2000). Various experimental approaches have been used to demonstrate the importance of cell wall-bound invertase isoforms for assimilate partitioning and determining sink strength. These include inhibition of storage tissue development in carrot roots by antisense repression of cell wall invertase (STURM & TANG, 1999; TANG ET AL., 1999), increase in potato tuber size by over-expression of cell wall invertase (TAUBENBERGER ET AL., 1999), arrested seed development in maize mutant lacking cell wall invertase (MILLER & CHOUREY, 1992), induction of sink metabolism in source leaves of transgenic plants by over-expression of a yeast invertase (STITT & SONNEWALD, 1995), and specific expression of a cell wall invertase during pre-storage phase in the thin walled parenchyma of faba bean seed coat (WEBER ET AL., 1995).

A possible explanation for the association of *invGF* alleles transcribed in flowers and leaves might be the fact of LD with associated *invGE* alleles detected in SCCP based analysis (LI ET AL., 2005). It might be comprehensive to think of a haplotype block associated with better chips quality including both genes *invGE* and *invGF*.

The associated *invGE* alleles correlated with better potato chips quality (LI ET AL., 2005) revealed minor changes in their expression in leaves compared to their genomic dosage (section 3.2.2.2.1, Figure 3.2.24). The ‘Satina’ allele *E_SA* and the ‘Diana’ allele *E_DA*, which are identical in their nucleotide and amino acid sequences, are present in simplex (25%) in the corresponding genotypes. Allele expression was reduced by 5% to a level of 20% in leaves. The associated ‘Theresa’ allele *E_TA*, which is present in simplex (25%) and is different in its amino acid sequence to *E_SA* and *E_DA*, showed a slight expression induction up to 35% in leaves. Transcripts for the ‘Diana’ allele *E_DN2* and the ‘Theresa’ allele *E_TN3* were not detected in pyrosequencing analysis possibly due to transcripts below the detection level. These findings indicated an allele specific expression of invertase genes in

potato. Whether this differential expression of *invGE* alleles influences potato chips quality remains unclear since no transcripts in tubers were detectable.

The *invGE* alleles of the diploid potato genotypes P18, P40, and P54 were differentially expressed in leaves as expected from their genomic dosage (section 3.2.2.1.2, Figure 3.2.25). Transcripts of the alleles *E_P18N1*, *E_P40N2*, and *E_P54N1* were more abundant, whilst the other alleles were less present compared to the genomic distribution.

Pyrosequencing based expression analysis of *invGF* alleles showed that the alleles *F_SN4*, *F_DN1*, and *F_TN1*, which group separately from the other *invGF* alleles were not expressed differentially in flowers compared to their genomic dosage (section 3.2.2.1.3, Figure 3.2.26). All three alleles are present in simplex (25%) in the corresponding genotypes. The ‘Satina’ allele *F_SN4* and the ‘Theresa’ allele *F_TN1* are identical at nucleotide and amino acid level. It is speculated that *F_SN4*, *F_DN1*, and *F_TN1* might refer to the *invGF-4d* SSCP fragment, which was found to be associated with better potato chips quality. The speculation is based on the fact that the three alleles show the same separation like the associated *invGE* alleles *E_SA*, *E_DA*, and *E_TA* in phenetic tree analysis (*invGE*: section 3.2.1.2.4, Figure 3.2.6; *invGF*: section 3.2.1.2.8, Figure 3.2.11). Additionally, the ‘Satina’ allele *F_SN3* showed a strong decrease in transcripts, whilst *F_SN1* was the prevalent allele in flowers compared to the allelic genomic distribution. The allocation of the alleles from the genotypes ‘Diana’, ‘Theresa’, and P40 did not differ. The two alleles of P54 revealed minor changes, the allele *F_P54N1* was decreased, whilst the allele *F_P54N2* was increased compared to their genomic distribution.

In conclusion, expression pattern of *Pain-1*, *invGE*, and *invGF* alleles of six genotypes did not correlate with association analysis, which was based on 240 tetraploid individuals (LI ET AL., 2005, 2008). The observation of genotype and invertase gene dependent transcriptional changes might be due to the genetic background. Since no allelic promoters or transcription factors and other modifiers were analyzed in this study the impact of these on allelic invertase expression cannot be clearly assessed. Nevertheless, the results showed that invertase genes are expressed in a genotype specific manner and that associated alleles followed divergent expression patterns. As expression QTLs (eQTLs) are an emerging field of interest in plants such analysis will be an interesting extension of the results gained in the present study, although expression data could not directly be linked to protein abundance and/or enzymatic activity.

❖ The heterologous system yeast

Aiming at the biochemical characterization of single invertase alleles, the use of a heterologous system was necessary to separate alleles from each other. With the knowledge that plant invertases are functional in yeast (FRIDMAN ET AL., 2004), the yeast invertase mutant *SUC2* (GOZALBO & HOHMANN, 1989), which lacks invertase activity was chosen for potato invertase allele expression. FRIDMAN ET AL. (2004) demonstrated the functional complementation of a yeast mutant with tomato cell wall-bound invertases. In this study it was shown that also a vacuolar invertase isoform complements the yeast *SUC2* mutant phenotype. All *SUC2* transformants harbouring *Pain-1*, *invGE*, and *invGF* cDNA alleles were able to grow on sucrose as sole carbohydrate source indicating functional complementation. The cDNA alleles of all three different genes were expressed under the control of the constitutive promoter *Adh1* to obtain high amounts of potato invertase protein.

With respect to the yeast and potato codon usages, it was found that the translation efficiency between both systems is different leading to less potato invertase protein depending on the allelic nucleotide sequence. It was tried to balance this translation deficiencies by incubation of complemented yeast strains for three days achieving equal amounts of potato invertase protein. Immunoblot analysis of *Pain-1* yeast transformants reflected the equal distribution of allelic invertase proteins (section 3.1.2.4, Figures 3.1.24 and 3.1.25).

In yeast, two invertase isoforms occur, which are encoded by the same gene, but originate from differential splicing events. One isoform is active, N-glycosylated, and extracellular, the other isoform is inactive, nonglycosylated, and located in the cytoplasm. The extracellular yeast invertase is targeted for secretion by a signal peptide, which is then removed by a peptidase. Plant vacuolar and cell wall-bound invertases are synthesized as prepropeptides (TYMOWSKA-LALANNE & KREIS, 1998). These peptides consist of N-terminal extensions up to 100 amino acid residues in length, which harbour a signal peptide and an N-terminal propeptide (STURM, 1999). The signal peptide is required for the entry in the endoplasmic reticulum (ER), which leads to the secretory pathway. The N-terminal propeptide as well as a C-terminal extension, the latter being characteristic for vacuolar invertases, are thought to act as vacuolar sorting signals (MATSUOKA & NAKAMURA, 1991). Figure 4.3 illustrates vacuolar and cell wall-bound preproteins and included domains (adopted from TYMOWSKA-LALANNE & KREIS, 1998; STURM, 1999).

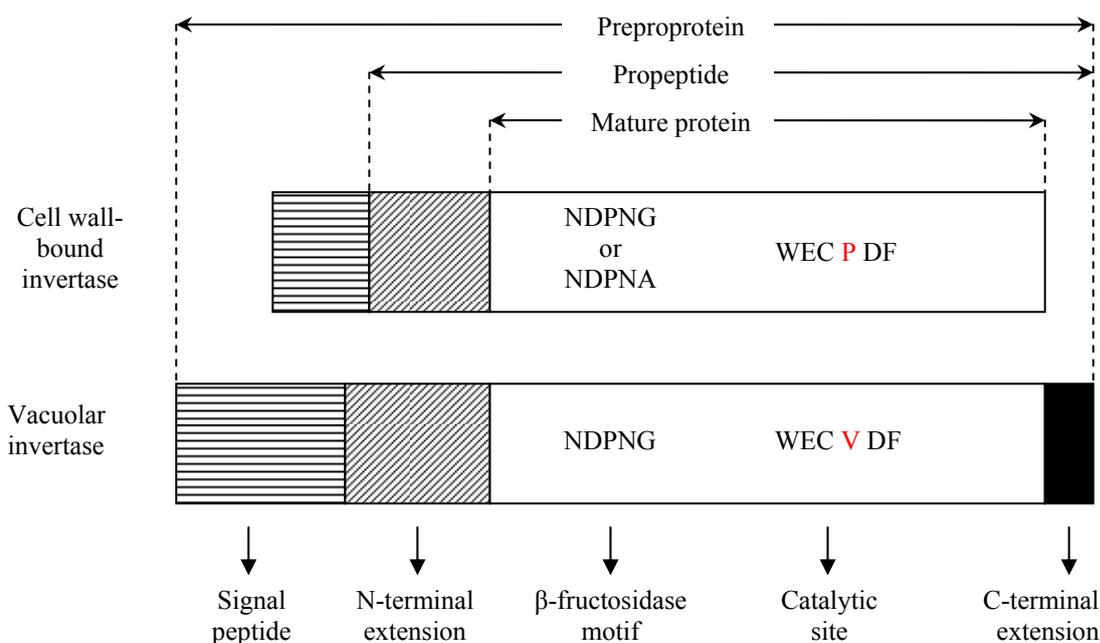


Figure 4.3: Schematic comparison of vacuolar and cell wall-bound preproprotein invertases. The preproprotein sequence includes the signal peptide, N- and C-terminal extensions and the mature protein. The peptide sequences NDPNG/NDPNA and WEC^{P/V}DF represent the β -fructosidase motif and the catalytic site, respectively.

Since yeast has no vacuole or a similar compartment, the question arises how vacuolar allelic potato invertases are processed and targeted in yeast to remain functional. This question can not be answered although functional complementation of the yeast invertase mutant was achieved.

Yeast invertase is known to be an oligomeric glycoprotein with 14 potential N-glycosylation sites located in the sequence (REDDY ET AL., 1999). The carbohydrate chains play a role in structure, function, stability, and folding of glycoproteins (KERN ET AL., 1992). In the yeast Golgi apparatus, N-glycosylation is achieved by a varying number of extended outer polymannose chains, yielding a high-mannose-type glycosylated protein with an average of nine to ten oligosaccharides per peptide chain. The glycosylated proteins are strongly heterogeneous in their carbohydrate content. Studies with yeast invertase demonstrated that glycosylation is a prerequisite for the oligomerization of the enzyme beyond the state of the active dimer. It was shown that core-glycosylation of yeast invertase is necessary for tetramer and octamer formation (KERN ET AL., 1992).

In plants, vacuolar and cell wall-bound invertases are glycosylated, whilst cytoplasmatic invertase isoforms are not glycosylated (STOMMEL & SIMON, 1990). Glycosylation of vacuolar and extracellular glycoproteins are different from each other. Most of the vacuolar glycoproteins described so far were found to be N-glycosylated with modified N-glycans containing fucose and/or xylose residues, but devoid of terminal glucosamine residues. In

contrast, extracellular glycoproteins were found to be N-glycosylated mostly by complex-type N-glycans including large structures with terminal fucose and galactose residues (RAYON ET AL., 1998). Both types of glycoproteins are post-Golgi modified leading to protein maturation in the vacuole or in the extracellular compartment. It was shown that N-glycosylation in plants plays an important role in prevention of proteolytic degradation, induction of correct folding, and biological activity of the protein. Furthermore, N-linked oligosaccharides may contain targeting information, or may be involved in protein recognition (RAYON ET AL., 1998). FAYE & CHRISPEELS (1989) found that unglycosylated cell wall-bound invertase of carrot was degraded in the secretory pathway or immediately after attaining the cell wall. As indicated above, N-glycosylation of proteins in plants is more complex than in yeast. It might be possible that the analyzed potato invertase alleles were not modified according their original status in the heterologous system yeast. Following the argumentation that N-glycosylation plays an important role in correct protein folding, yeast modified potato alleles might not be able to fold correctly. Due to possible folding deficiencies and less complex N-glycosylation, which both is crucial for enzyme activity, it might be also reasonable that measured potato invertase activity does not reflect the native situation in potato. Additionally, the invertase oligomerization must be considered. Yeast invertase was found to be a mixture of dimers, tetramers, and octamers (KERN ET AL., 1992). Studies in *Vicia faba* on alkaline invertase assumed that this enzyme acts as a homotetramer (ROSS ET AL., 1996). Considering this information, it might be possible that soluble acid invertases as well as cell wall-bound invertase isoforms might also function in a complex of subunits. In none of the mentioned oligomeric analysis, the role of allelic monomers was investigated. Assuming that a functional potato invertase complex is build of different allelic subunits in different ratios, yeast transformants containing only one allele do not allow the assembly of ‘multi-allelic’ complexes. VEITIA ET AL. (2008) reviewed cellular reactions to gene dosage imbalances and suppose that stoichiometric imbalances in macromolecular complexes are source of dosage-dependent phenotypes. In this context, it might be reasonable that putative invertase complexes being ‘mono-allelic’ in yeast do not reflect the *in vivo* situation in potato.

❖ Biochemical analysis of *Pain-1*, *invGE*, and *invGF* alleles

Biochemical analysis of plant invertases in heterologous systems was successfully demonstrated for a tomato cell wall-bound invertase isoform by FRIDMAN ET AL. (2004). In that study an invertase allele originating from a wild species (*S. pennellii*), which is barely functional indicated by low fruit sugar content was compared to the homologous invertase allele of the cultivated tomato (*S. lycopersicum*). In contrast, the present study aimed towards

the characterization of functional potato invertase alleles from different genotypes to detect allelic enzyme activities. The possible functional variability of invertases operates under field conditions and, therefore, should not have severe effects on fitness and might be difficult to detect.

Using yeast, the challenge was to determine possible minor enzymatic allelic differences.

The heterologous system yeast made it possible to measure enzyme activity of single potato invertase alleles. The enzymatic parameters Michaelis constant (K_m) and maximal velocity (v_{max}) of the invertase reaction were determined.

The biochemical analysis was carried out for 14 *Pain-1*, 10 *invGE*, and 11 *invGF* invertase alleles. For alleles of the genes *invGE* and *invGF* of the *Invap-b* locus only K_m values are presented because of missing a suitable antibody for determination of the invertase protein levels to evaluate v_{max} values, which are enzyme dependent.

Considering ideal biochemical characteristics of a superior invertase allele, a high K_m value demonstrating the low substrate affinity of the enzyme accompanied by a low v_{max} value representing a slow conversion of sucrose would be a basic working model (Figure 4.2). Additionally, a higher cold sensitivity of the enzyme can intensify the described biochemical parameters leading to even less sucrose conversion into the reducing sugars glucose and fructose and a better potato chips quality.

Biochemical analysis of the *Pain-1* alleles was performed at 30°C and at 4°C. The analysis at 4°C was carried out to study possible differences of enzyme kinetics due to allelic amino acid composition in response to cold storage conditions. It was previously reported that cold storage influences transcriptional changes regarding vacuolar invertases (ZRENNER ET AL., 1996; ZHOU ET AL., 2004; BAGNARESI ET AL., 2008), but no study investigated the effect of low temperatures on the enzyme activity itself. The 30°C assay of *Pain-1* alleles showed that substrate affinity ranged between 15mM of the allele *Pain_P40N2* and 23mM of the allele *Pain_SN* (section 3.1.2.3, Table 3.1.15). Maximal velocities of the alleles varied between 2mmol*h⁻¹*mg protein⁻¹ in the case of the allele *Pain_TN2* and 12mmol*h⁻¹*mg protein⁻¹ for the allele *Pain_DA*. The enzymatic characteristics of the analyzed alleles from the cultivar ‘Satina’ showed no significant differences. In the K_m and v_{max} values of the cultivar ‘Diana’ differences were displayed in respect to the allele *Pain_DN2*. *Pain_DN2* showed the highest substrate affinity with a K_m of approximately 16mM, and the slowest substrate conversion with a v_{max} of around 6mmol*h⁻¹*mg protein⁻¹. The other two analyzed alleles *Pain_DA* and *Pain_DN1* displayed substrate affinities of approximately 20mM and maximal velocities of around 12mmol*h⁻¹*mg protein⁻¹. Comparison of the associated *Pain-1* alleles *Pain_SA* and

Pain_DA, which are not identical at amino acid level, revealed no significant differences regarding the enzyme's affinity to sucrose, but showed strong differences in the rate of sucrose conversion. The allele *Pain_DA* converts sucrose approximately 2.5 times faster than the allele *Pain_SA*.

Looking at the other analyzed *Pain-I* alleles, the two 'Theresa' alleles showed similar enzymatic characteristics and did not differ significantly. K_m values varied from 20 to 21mM and v_{max} values ranged from 2 to 3mmol*h⁻¹*mg protein⁻¹. Biochemical analysis of the alleles from the diploid potato genotypes P18 and P40 did not display any significant K_m and v_{max} differences. K_m - and v_{max} values of *Pain_P18N1* and *Pain_P18N2* ranged from 17 to 20mM and 3 to 5.5mmol*h⁻¹*mg protein⁻¹, respectively. The alleles *Pain_P40N1* and *Pain_P40N2* displayed substrate affinities between 15 and 17mM and v_{max} values between 6 and 7mmol*h⁻¹*mg protein⁻¹.

The biochemical analysis of the enzymatic characteristics of the *Pain-I* alleles at 4°C showed a dramatic increase in the enzyme's affinity to sucrose (section 3.1.2.3, Table 3.1.15). It is accepted that the rate of chemical reaction decreases approximately twofold for each 10°C decrease in temperature (AVERY, 1974). The K_m values decreased approximately 5.5 times compared to K_m values measured at 30°C. Also the maximal velocities of the alleles were affected at 4°C. By trend v_{max} values were around 2mmol*h⁻¹*mg protein⁻¹. The 4°C invertase assay showed the effect of low temperatures towards the vacuolar invertase alleles of potato. In the cold, the alleles showed a higher affinity to sucrose and in agreement a slower maximal velocity due to a stronger binding of the substrate and the general effect of low temperature to slow down enzymatic reaction rates. In contrast, the sucrose affinity of the yeast wild type strain *FY 1679*, used as reference, was not that much affected in the cold. The K_m values remained stable at approximately 22mM. Differences were detected in the rate of sucrose conversion. Yeast invertase slowed down from approximately 24mmol*h⁻¹*mg protein⁻¹ to 7.5mmol*h⁻¹*mg protein⁻¹ indicating a decrease of around threefold.

In conclusion, the associated *Pain-I* alleles *Pain_SA* and *Pain_DA*, which showed in 3D-analysis a charge effect of the sucrose binding site, which might lead to reduced conversion of sucrose, did not display strong physiological differences compared to the other alleles of the corresponding genotypes. The ideal biochemical characteristics outlined in Figure 4.2 for superior allelic invertases associated with superior chips quality were not clearly recognized for vacuolar invertase alleles.

The 30°C assay of *invGE* alleles (section 3.2.2.3, Table 3.2.13) showed that the two associated *invGE* alleles *E_SA* and *E_TA*, which are not amino acid identical but are

characterized by the associated SNP 1103*, displayed similar K_m values of approximately 20mM. From the tetraploid cultivar ‘Satina’ three alleles E_SA , E_SNI , and E_SN3 were analyzed. Regarding the enzyme affinity, E_SN3 displayed the lowest sucrose affinity with a K_m value of approximately 24mM, whilst the allele E_SNI showed the highest substrate affinity with a K_m value of around 17mM. The K_m value of the associated allele E_SA of 20mM was in between and was significantly different from E_SN3 .

From the tetraploid cultivar ‘Theresa’ two alleles E_TA and E_TNI were analyzed. Neither the K_m values nor the v_{max} values of these alleles differed significantly from each other.

Compared to the other *invGE* alleles, the associated alleles E_SA and E_TA did not show strong K_m and v_{max} differences. The lowest K_m value was approximately 17mM (E_SNI), the highest K_m value was 24mM in the case of the allele E_PI8NI .

It could be shown by 3D-analysis that the charge of the putative sucrose binding site of the associated *invGE* alleles did not display any differences like observed for associated *Pain-1* alleles. This is in agreement with determined K_m values, which did not show strong physiological differences. Biochemical parameters of *invGE* alleles did not indicate the presence of obvious superior invertase alleles according to the assumption concerning K_m and v_{max} characteristics mentioned above (Figure 4.2).

Biochemical analysis of *invGF* alleles showed that substrate affinities ranged from approximately 12mM in case of F_SN2 and 21mM for the allele F_P40NI (section 3.2.2.3, Table 3.2.15). The alleles F_SN4 and F_DNI , which clustered phenetically together (section 3.2.1.2.8, Figure 3.2.11) as observed for the associated *invGE* alleles E_SA , E_DA , and E_TA , displayed K_m values of 18mM and 13mM, respectively. Within the tested ‘Satina’ *invGF* alleles F_SN4 revealed the lowest sucrose affinity, which differs significantly from F_SNI and F_SN2 .

The ‘Diana’ allele F_DNI , which is not identical at amino acid level to F_SN4 but belongs to the same outlying group, displayed a K_m value of 13mM. These differences between F_SN4 and F_DNI were significant, whilst the differences within the tested ‘Diana’ alleles were similar and not significant.

The allele F_SN4 , which is considered as a representative of the associated SSCP fragment *invGf-4d*, showed among the tested ‘Satina’ alleles the lowest sucrose affinity. 3D structural analysis of this allele showed that a charge change of the putative sucrose binding site occurred due to the allelic amino acid composition. The electrostatic potential of the site switched from positive to negative, possibly leading to less sucrose conversion reflected by a high K_m value as detected for the allele F_SN4 . To what extent this allele might contribute to

potato chips quality remains unclear since *invGF* is expressed in flowers and leaves and to date no transcripts in tubers were detected.

Comparing the measured K_m values of vacuolar and cell wall-bound invertase isoforms revealed overall similar enzymatic characteristics. Nevertheless, there are K_m and v_{max} values of single *Pain-1*, *invGE*, or *invGF* alleles, which are distinct from the average alleles. Even though the biochemical analysis of *Pain-1*, *invGE*, or *invGF* alleles in the heterologous system yeast revealed only slight allelic differences regarding substrate affinity and substrate conversion, no statement can be made concerning the *in vivo* situation in potato.

The measured invertase affinities towards the substrate sucrose are in general agreement with previously detected K_m values. FRIDMAN ET AL. (2004) measured substrate affinities between approximately 5 and 12mM for a functional cell wall-bound tomato invertase isoform expressed in yeast. The *Arabidopsis* soluble invertase *INV2* displayed a K_m of 12mM (TANG ET AL., 1996). The substrate affinity for a soluble acid invertase from *Triticum aestivum* was set at 19.6mM (KRISHNAN ET AL., 1985).

Convincing evidence that the invertase assay is suitable for the detection of the corresponding enzyme activities is the fact that, concerning the yeast wild type invertase, a sucrose affinity of approximately 22mM was measured. GASCON ET AL. (1968) determined the K_m value of yeast invertase at 25mM, which is nearly identical to the K_m value detected in this study.

Documented K_m values for plant invertases as described above from the BRENDA database (<http://www.brenda-enzymes.org>) were similar to the measured values in this study.

4.4 Potato invertase isoforms and corresponding alleles display a large structural and functional variation and are interesting candidate genes in the trait potato chips quality

The identification of alleles of five different potato invertase genes revealed high allelic diversity within the selected six genotypes. However, there are certain alleles identified shared by more than one genotype, representing basic invertase alleles. It is remarkable that invertase alleles identical at amino acid level displayed synonymous polymorphisms in their nucleotide sequences. These ‘silent’ SNPs not leading to any amino acid exchanges might influence mRNA secondary structure, stability, translational efficiency, and protein folding leading to altered enzyme activity. Possible effects of synonymous SNPs were not subject of this study but the further investigation of these SNPs will clarify if there is a connection between the latter and the trait potato chips quality.

3D-modelling of the alleles gave first insights in possible structural and charge effects regarding amino acid differences between the alleles. It was found that alleles associated with superior potato chips quality showed charge changes in the putative sucrose binding site leading to a putative lower conversion of sucrose into the reducing sugars glucose and fructose. As these reducing sugars are known to interfere with potato chips quality, lower conversion would imply a better chips phenotype. To test, whether allele specific amino acid differences lead to altered enzyme properties, invertase assays were performed in the heterologous system yeast. Determining the enzyme’s affinity to sucrose (K_m) and the maximal rate of substrate conversion (v_{max}) showed that vacuolar and cell wall-bound invertase alleles displayed no differences that could explain the effect on chips quality. The observed differences might reflect the slight differences present *in vivo* since natural occurring alleles were analyzed and no mutant, wild accession, or other impaired alleles were tested like done for tomato (FRIDMAN ET AL., 2004). Genotype dependent alleles were detected, which displayed extreme values regarding their K_m and v_{max} values. The associated alleles were similar to not associated ones, indicating that K_m and v_{max} values do not seem to be relevant for possible reduced sucrose conversion into the sugars glucose and fructose and, therefore, better chips quality. Convincing evidence that the heterologous system yeast was suitable for assaying potato invertase activity was the analysis of two nucleotide variants of the *Pain-1* allele *Pain_SN* (section 3.1.2.3, Tables 3.1.20 and 3.1.21). The variability ranged from one to three nucleotide differences. It was shown that all nucleotide versions displayed similar K_m and v_{max} values, showing that a codon usage independent comparison of different alleles was possible.

Additionally, expression analysis revealed genotype dependent transcription patterns of invertase alleles. Associated alleles showed transcriptional changes for *Pain_SA* and *Pain_P18NI* compared to their genomic distribution. The latter is amino acid identical to *Pain_DA*, which showed no specific expression pattern. The transcript levels of associated *invGE* and the putative associated *invGF* alleles in leaves and flowers, respectively, were similar to their genomic dosages.

Invertase is functional as a complex of allelic subunits, thus it might be possible that allelic structural differences on the enzyme's surface, charge differences of the molecule as well as changed ratios of subunits due to transcriptional and translational regulation, lead to altered enzyme complexes less functional than in other genotypes with an inferior potato chips quality phenotype.

Another regulation of invertase activity is inhibition mediated by the endogenous invertase inhibitor (SCHWIMMER ET AL., 1961; PRESSEY, 1967; EWING AND MCADOO, 1971; WEIL ET AL., 1994; HEIBGES ET AL., 2003) or inhibition from a potato lectin (ISLA ET AL., 1991). The invertase inhibitor forms a non-dissociable complex with the enzyme, whilst the lectin is a dissociable inhibitor. Assuming allelic variants of invertase inhibitors acting differentially on allelic subunits of a putative invertase enzyme complex might lead to altered invertase activity. Also the potato lectin might act dependent on the allelic composition of an invertase complex in different ways to modulate invertase activity.

Association analysis of *Pain-I* allelic fragments revealed LD with allelic fragments of two other genes on chromosome III (LI ET AL., 2008). The first gene of this associated haplotype block located 6cM in the distal of *Pain-I* is the plastidic L-type α -Glucan phosphorylase (*Stp23*). The metabolic role of *Stp23* is the phosphoric degradation of starch. The second gene within the associated block is soluble starch synthase I (*SssI*) 14cM in the proximal of *Pain-I*. *SssI* plays a role in starch synthesis by connecting ADP-glucose to form linear amylose chains. It is possible that alleles of the other two associated genes might be responsible for or contribute to the studied trait potato chips quality. Also additive effects of alleles of all three loci *Pain-I*, *Stp23*, and *SssI* might be reasonable by their role in the carbohydrate metabolism leading to superior chips quality (OMHOLT ET AL., 2000; BIRCHLER ET AL., 2001).

In conclusion, the data presented in this study do not allow to a definite statement about the functional role of invertases on potato chips quality. However, it was shown that tremendous allelic variation of potato invertases exists, which leads to variation of transcript levels and biochemical parameters.

4.5 Future perspectives about the investigation of potato invertases and their natural variation

The results obtained in this thesis revealed important aspects about the diversity of invertase alleles and their regulation at transcriptional and biochemical level.

Considering the information available through this study, the question of ‘associated’ and ‘not associated’ alleles has to be addressed in a new manner. In the course of the work, no consistent functional characteristics of alleles statistically associated with better potato chips quality were determined. The nomenclature of association was based on the SSCP analysis by LI ET AL. (2005, 2008). Having now allele specific SNPs in hands, new alleles not detected so far by SSCP analysis but associated with superior chips quality can be analyzed in a larger population that allows screening for their impact on this trait. Being aware of allelic variability found in this study, new and up to now unidentified invertase alleles not detectable by SSCP analysis can be assessed by PCR based cloning. In consequence deeper biochemical and molecular characterization could enrich the understanding of allelic impact on potato chips quality.

Furthermore, allelic sequence variation strongly implies possible variations in regulatory domains like promoter sequences, enhancer-, and signalling domains. Additional studies of allelic promoter sequences and their influence on transcript levels might be useful to elucidate regulatory mechanisms of specific invertase alleles and identifying eQTLs as well as determining their impact on potato and the quantitative trait chips quality.

Another interesting aspect to follow is how invertase complexes might be build in potato, to what extend allelic monomers are involved in proper complex formation, how allelic monomer abundance due to transcriptional and translational regulations might influence enzyme complexes and, therefore, enzymatic activity.

The information gained by these additional approaches could help to draw a more detailed picture how invertases are regulated and to what extend allelic differences might contribute to a superior potato chips quality.

5 References

ALBERTO, F.; BIGNON, C.; SULZENBACHER, G.; HENRISSAT, B.; CZJZEK, M. (2004): The three-dimensional structure of invertase (beta-fructosidase) from *Thermotoga maritima* reveals a bimodular arrangement and an evolutionary relationship between retaining and inverting glycosidases. *J. Biol. Chem.* (279): 18903-18910.

AP REES, T. (1984): Sucrose metabolism. In: D.H. Lewis (ed) *Storage carbohydrates in vascular plants*. Cambridge University Press, London: 53-73.

AP REES, T.; DIXON, W.L.; POLLOCK, C.J.; FRANKS, F. (1981): Low temperature sweetening of higher plants. *Recent Advances in the Biochemistry of Fruits and Vegetables*. Edited by J. Friend, *Rhodes MJC*. Academic Press, New York, London: 41-61.

APPELDOORN, N.J.G.; DE BRUIJN, S.M.; KOOT-GRONSVELD, E.A.M.; VISSER, R.G.F.; VREUGDENHIL, D.; VAN DER PLAS, L.H.W. (1997): Developmental changes in enzymes involved in conversion of hexose-phosphate and its subsequent metabolites during early tuberization of potato. *Plant Cell Environ.* (22): 1085-1096.

ARAI, M.; MORI, H.; IMASEKI, H. (1991): Roles of sucrose-metabolizing enzymes in growth of seedlings: purification of acid invertase from growing hypocotyls of mung bean seedlings. *Plant Cell Physiol.* (32): 1291-1298.

EVERY, H.E. (1974). *Basic Reaction Kinetics and Mechanisms*. The Macmillan Press Ltd., Hong Kong: p. 174.

BAGNARESI, B.; MOSCHELLA, A.; BERETTA, O.; VITULLI, F.; RANALLI, P. AND PERATA, P. (2008): Heterologous microarray experiments allow the identification of early event associated with potato tuber cold sweetening. *BMC Genomics*.

BAKER, N.A.; SEPT, D.; JOSEPH, S.; HOLST, M.J.; MCCAMMON, J.A. (2001): Electrostatics of nanosystems: application to microtubules and the ribosome. *Proc. Natl. Acad. Sci. USA.* (98): 10037-10041.

- BALLVORA, A.; JÖCKER, A.; VIEHÖVER, P.; ISHIHARA, H.; PAAL, J.; MEKSEM, K.; BRUGGMANN R.; SCHOOF, H.; WEISSHAAR, B.; GEBHARDT, C. (2007): Comparative sequence analysis of Solanum and Arabidopsis in a hot spot for pathogen resistance on potato chromosome V reveals a patchwork of conserved and rapidly evolving genome segments. *BMC Genomics* (8)
- BALLVORA, A.; ERCOLANO, M.R.; WEISS, J.; MEKSEM, K.; BORMANN, C.A.; OBERHAGEMANN, P.; SALAMINI, F.; GEBHARDT, C. (2002): The *R1* gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. *Plant J.* (30):361-371.
- BATES, P.A.; KELLEY, L.A.; MACCALLUM, R.M.; STERNBERG, M.J. (2001): Enhancement of protein modeling by human intervention in applying the automatic programs 3D-JIGSAW and 3D-PSSM. *Proteins* (5): 39-46.
- BEDNAREK, S.Y. AND RAIKHEL, N.V. (1992): Intracellular trafficking of secretory proteins. *Plant Mol. Biol.* (20): 133-150.
- BIRCHLER, J.A. ET AL. (2001): Dosage-dependent gene regulation in multicellular eukaryotes: implications for dosage compensation, aneuploid syndromes, and quantitative traits. *Dev. Biol.* (234), 275-288.
- BOLLER, T. AND KENDE, H. (1979): Hydrolytic Enzymes in the Central Vacuole of Plant Cells. *Plant Physiol.* (63): 1123-1132.
- BONIERBALE, M.W.; PLAISTED, R.L.; TANKSLEY, S.D. (1988): RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. *Genetics* (120): 1095-1104.
- BORMANN, C.A.; RICKERT, A.M.; RUIZ, R.A.; PAAL, J.; LÜBECK, J.; STRAHWALD, J.; BUHR, K.; GEBHARDT, C. (2004): Tagging quantitative trait loci for maturity-corrected late blight resistance in tetraploid potato with PCR-based candidate gene markers. *Mol. Plant Microbe Interact.* (17): 1126-1138.

- BOURNAY, A.-S.; HEDLEY, P.E.; MADDISON, A.; WAUGH, R.; MACHRAY, G.C., (1996): Exon skipping induced by cold stress in a potato invertase gene transcript. *Nucleic Acids Res.* (24):2347-2351.
- BRADFORD, M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities utilizing the principle of protein-dye binding. *Anal. Biochem.* (72): 248-254.
- BROQUET, T. AND PETIT, E. (2004): Quantifying genotyping errors in noninvasive population genetics. *Mol. Ecol.* (13): 3601-3608.
- BRÜCHER, H. (1975): Domestikation und Migration von *Solanum tuberosum* L. *Die Kulturpflanze* (23): 11-47.
- BURCH, L.R.; DAVIES, H.V.; CUTHBERT, E.M.; MACHRAY, G.C.; HEDLEY P.E.; WAUGH, R. (1992): Purification of soluble invertase from potato. *Phytochem.*(31): 190-1904.
- CHAMARY, J.V.; PARMLEY, J.; HURST, L.D. (2006): Hearing silence: non-neutral evolution at synonymous sites in mammals. *Nature* (7): 98-108.
- CHEN, X.; SALAMINI, F.; GEBHARDT, C. (2001): A potato molecular function map for carbohydrate metabolism and transport: *Theor. Appl. Genet.* (102): 284-295.
- CHUDA Y.; ONO H.; YADA H.; OHARA-TAKADA A.; MATSUURA-ENDO C.; MORI M. (2003): Effects of physiological changes in potato tubers (*Solanum tuberosum* L.) after low temperature storage on the level of acrylamide formed in potato chips. *Biosci. Biotechnol. Biochem.* (67): 1188-1190.
- CLAASSEN, P.A.M.; BUDDE, M.A.W.; VANCALKER, M.H. (1993): Increase in phosphorylase activity during cold-induced sugar accumulation in potato tubers. *Potato Res.* (36): 205-217.
- COCHRANE, M.P.; DUFFUS, C.M.; ALLISON, M.J.; MACKAY, G.R. (1991): Amylolytic activity in stored potato tubers. The effect of low temperature storage on the activities of α -amylase and β -amylase and α -glucosidase in potato tubers. *Potato Res.* (34): 333-341.

- COFFIN, R.H.; YADA, A.; PARKIN, K.L.; GRODZINSKI, B.; STANLEY, D.W. (1987): Effect of low temperature storage on sugar concentrations and chip color of certain potato processing potato cultivars and selections. *J. Food Sci.* (52): 639-645.
- DEITING, U.; ZRENNER, R.; STITT, M. (1998): Similar temperature requirement for sugar accumulation and for the induction of new forms of sucrose phosphate synthase and amylase in cold-stored potato tubers. *Plant Cell Environ.* (21): 127-138.
- EWING, E.E. AND MCADOO, M.H., (1971): An examination of methods used to assay potato tuber invertase and its naturally occurring inhibitor. *Plant Physiol.* (48): 366- 370.
- DELANO, W.L. (2002): The PyMOL Molecular Graphics System. *DeLano Scientific*. San Carlos, CA, USA.
- DOLINSKY, T.J.; NIELSEN, J.E.; MCCAMMON, J.A.; BAKER, N.A. (2004): PDB2PQR: an automated pipeline for the setup of Poisson-Boltzmann electrostatics calculations. *Nucleic Acids Res.* (32) (Web Server issue): W665-667.
- DUPLESSIS, P.M.; MARANGONI, A.G.; YADA, R.Y. (1996): A mechanism for low temperature induced sugar accumulation in stored potato tubers: the potential role of the alternative pathway and invertase. *Am. Pot. J.* (73): 483-494.
- FAYE, L. AND CHRISPEELS, M.J. (1989): Apparent inhibition of β -fructosidase secretion by tunicamycin may be explained by breakdown of the unglycosylated protein during secretion. *Plant Physiol.* (89): 845-851.
- FINDLAY, L.; RAY, P.; QUIRKE, P.; RUTHERFORD, A.; LILFORD R. (1995): Allelic drop-out and preferential amplification in single cells and human blastomeres: implications for preimplantation diagnosis of sex and cystic fibrosis. *Mol. Hum. Reprod.* (1): 209-218.
- FREYRE, R. AND DOUCHES, D.S. (1994): Development of a model for markerassisted selection of specific gravity in diploid potato across environments. *Crop Sci.* (34):1361-1368.

- FRIDMAN, E.; CARRARI, F.; LIU, Y-S.; FERNIE, A.R.; ZAMIR, D. (2004): Zooming in on a quantitative trait for tomato yield using interspecific introgressions. *Science* (305): 1786-1789.
- FRIDMAN, E. AND ZAMIR, D. (2003): Functional divergence of a syntenic invertase gene family in tomato, potato, and *Arabidopsis*. *Plant Physiol.* (131): 603-609.
- GASCON, S.; NEUMANN, N.P.; LAMPEN, J.O. (1968): Comparative study of the properties of the purified internal and external invertases from yeast. *J. Biol. Chem.* (243), 1573-1577.
- GEBHARDT, C. (2004): II.8 Potato genetics: molecular maps and more. *Biotechnol. Agricult. Forest.* (55).
- GEBHARDT, C.; RITTER, E.; DEBENER, T.; SCHACHTSCHABEL, U.; WALKEMEIER B. ET AL., (1989): RFLP analysis and linkage mapping in *Solanum tuberosum*. *Theor. Appl. Genet.* (78): 65 - 75.
- GEBHARDT, C.; RITTER, E.; BARONE, A.; DEBENER, T.; WALKEMEIER, B. ET AL. (1991): RFLP maps of potato and their alignment with the homeologous tomato genome. *Theor. Appl. Genet.* (83): 49-57.
- GEBHARDT, C. AND VALKONEN, J.P.T. (2001): Organization of genes controlling disease resistance in the potato genome. *Annu. Rev. Phytopathol* 39: 79-102.
- GEBHARDT, C.; WALKEMEIER, B.; HENSELEWSKI, H.; BARAKAT, A.; DELSENY, M. (2003): Comparative mapping between potato (*Solanum tuberosum*) and *Arabidopsis thaliana* reveals structurally conserved domains and ancient duplications in the potato genome. *Plant J.* (34): 529-541.
- GELDERMANN, H. (1975): Investigations on inheritance of quantitative characters in animals by gene markers. *I. Methods. Theor Appl Genetics* (46): 319-330.
- Gietz, R.D. and Schiestl, R.H. (1995): Transforming Yeast with DNA. (Invited chapter) *Meth. Mol. Cell. Biol.* (5); 255-269.

- GOZALBO, D.; HOHMANN, S. (1989): The naturally occurring silent invertase structural gene *suc2* contains an amber stop codon that is occasionally read through. *Mol. Gen. Genet.* (216): 511-516.
- GREINER, S.; KRAUSGRILL S.; RAUSCH, T. (1998): Cloning of a tobacco apoplastic invertase inhibitor. *Plant Physiol.* (116): 733-742.
- GREINER, M.; RAUSCH, T.; SONNEWALD, U.; HERBERS, K: (1999): Ectopic expression of a tobacco invertase inhibitor homolog prevents cold-induced sweetening of potato tubers. *Nature Biotechnol.* (17): 708-711.
- GUEX, N.; PEITSCH, M.C. (1997): SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis* (18): 2714-2723.
- GOETZ, M. AND ROITSCH, T. (1999): The different pH optima and substrate specificities of extracellular and vacuolar invertases from plants are determined by a single amino-acid substitution. *Plant J.* (20):707-711.
- HANAHAN, D. (1983): Studies on Transformation of Escherichia coli with plasmids. *J. Mol. Biol.* (166): 557-580.
- HAWKES, J. G. (1990): The Potato: Evolution, Biodiversity, and Genetic Resources. *Belhaven Press*, London.
- HEDLEY, P.E.; MACHRAY, G.C.; DAVIES, H.V.; BURCH, L.; WAUGH, R. (1994): Potato (*Solanum tuberosum*) invertase-encoding cDNAs and their differential expression. *Gene* (145): 211-214.
- HEDLEY, P.E.; MACHRAY, G.C.; DAVIES, H.V.; BURCH, L.; WAUGH, R. (1993): cDNA cloning and expression of a potato (*Solanum tuberosum*) invertase. *Plant Mol. Biol.* (22): 917-922.

- HEIBGES, A. HEIBGES; GLACZINSKI, H.; BALLVORA A.; SALAMINI F.; GEBHARDT, C. (2003): Structural diversity and organization of three gene families for Kunitz-type enzyme inhibitors from potato tubers (*Solanum tuberosum* L.). *Mol. Gen. Genomics* (269): 526-534.
- HEIM, U.; WEBER, H.; BAUMLEIN, H.; WOBUS, U. (1993): A sucrosesynthase gene of *Vicia faba* L.: expression pattern in developing seeds in relation to starch synthesis and metabolic regulation. *Planta* (191): 394-401.
- INGRAM, D.S. AND WILLIAMS, P.H. (1991): *Phytophthora infestans*, the cause of late blight of potato. *Adv. in Plant Pathology* (7).
- ISHERWOOD, F.A. (1976): Mechanism of starch-sugar interconversion in *Solanum tuberosum*. *Phytochem.* (15): 33-41.
- ISLA, M.I.; VATTUONE, M.A.; SAMPIETRO A.R. (1991): Modulation of potato invertase activity by fructose. *Phytochem.* (30): 425-426.
- KERN, G.; SCHÜLKE, N.; SCHMID, F.X.; JAENICKE, R. (1992) Stability, quaternary structure, and folding of internal, external, and core-glycosylated invertase from yeast. *Prot. Sci.* (1): 120-131.
- KIMCHI-SARFATY, C.; OH, J.M.; KIM, I.-W.; SAUNA, Z.E.; CALCAGNO, A.M.; AMBUDKAR, S.V.; GOTTESMANN, M.M. (2007): A “silent” polymorphism in the *MDR1* Gene changes substrate specificity. *Science* (315): 525-528.
- KNIGHT, J.C. (2004): Allele-specific gene expression uncovered. *Trends Genet.* (20): 113-116.
- KOCAL, N.; SONNEWALD, U.; SONNEWALD, S. (2008): Cell wall-bound invertase limits sucrose export and is involved in symptom development and inhibition of photosynthesis during compatible interaction between tomato and *Xanthomonas campestris* pv *vesicatoria*. *Plant Physiol.* (148): 1523-1536.

- KRAUSE, K.P.; HILL L.; REIMHOLZ, R.; NIELSEN, T.H.; SONNEWALD, U.; STITT, M. (1998): Sucrose metabolism in cold-stored potato tubers with decreased expression of sucrose phosphate synthase. *Plant Cell Environ.* (21): 285-299.
- KRISHNAN, H.B., BLANCHETTE, J.T.; OKITA, T.W. (1985): Wheat invertases. Characterization of cell wall-bound and soluble forms. *Plant Physiol.* (88): 241-245.
- LEE, H.-S. AND STURM A. (1996): Purification and characterization of neutral and alkaline invertase from carrot. *Plant Physiol.* (112): 1513-1522.
- LEISTER, D.; BALLVORA, A.; SALAMINI, F.; GEBHARDT, C. (1996): A PCR based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. *Nature Genet* 14:421-429.
- LI, H.; ROBERTSON, A.D.; JENSEN, J.H. (2005): Very fast empirical prediction and rationalization of protein pKa values. *Proteins* (61): 704-721.
- LI, L.; PAULO, M.-J.; STRAHWALD, J.; LÜBECK, J.; HOFFERBERT, H.R.; TACKE, E.; JUNGHANS, H.; WUNDER, J.; DRAFFEHN, A.; VAN EEUWIJK, F.; GEBHARDT, C. (2008): Natural DNA variation at candidate loci is associated with potato chip color, tuber starch content, yield and starch yield. *Theor Appl Genet.*
- LI, L.; STRAHWALD, J.; HOFFERBERT, H.R.; LÜBECK, J.; TACKE, E.; JUNGHANS, H.; WUNDER, J.; GEBHARDT, C. (2005): DNA variation at the invertase locus *invGE/GF* is associated with tuber quality traits in populations of potato breeding clones, *Genetics* (170): 813-821.
- LORENTZ, K.; LIENHARD, S.; STURM, A. (1995): Structural organization and differential expression of carrot β -fructofuranosidase genes: identification of a gene coding for a flower bud-specific isoenzyme. *Plant Mol. Biol.* (28): 189-194.
- MADDISON, A.L.; HEDLEY, P.E.; MEYER, C.R.; AZIZ, N.; DAVIDSON, D. (1999): Expression of tandem invertase genes associated with sexual and vegetative growth cycles in potato. *Plant Mol. Biol.* (41): 741-751.

- MALONE, J.G.; MITTOVA, V.; RATCLIFFE, R.G.; KRUGER, N.J. (2006): The response of carbohydrate metabolism in potato tubers to low temperatures. *Plant Cell Physiol.* 47 (9): 1309-1322.
- MATSUOKA K. AND NAKAMURA K. (1991): Propeptide of a precursor to a plant vacuolar protein required for vacuolar targeting. *Proc. Natl. Acad. Sci. USA* (88): 834-838.
- MATSUURA-ENDO C.; KOBAYASHI, A.; WATANABE S.; MIURA H.; KASANO, M.; ISHIBASHI K.; TAKIGAWA S.; NODA T.; YAMAUCHI H.; MORI M. (2006): Effect of the temperature shift on sugar content and activity of vacuolar acid invertase during storage of different potato cultivars. unpublished; accession DQ478950 (<http://www.ncbi.nlm.nih.gov>).
- MATSUURA-ENDO C.; OHARA-TAKADA A.; CHUDA Y.; ONO H.; YADA H.; YOSHIDA M.; KOBAYASHI A.; TSUDA S.; TAKIGAWA S.; NODA T.; YAMAUCHI H.; MORI M. (2006): Effects of storage temperature on the contents of sugars and free amino acids in tubers from different potato cultivars and acrylamide in chips. *Biosci. Biotechnol. Biochem.* (70): 1173-1180.
- MEHLI, L.; SCHAART, J.G.; KJELLEN, T.D.; TRAN, D.H.; SALENTIJN, E.M.J., SCHOUTEN, H.J., IVERSEN, T.-H. (2004): A gene encoding a polygalacturonase-inhibiting protein (PGIP) shows developmental regulation and pathogen-induced expression in strawberry. *New Phytol.* (163): 99-110.
- MELCHER, U. (2003) SSCPS. (<http://opbs.okstate.edu/~melcher/MG/MGW1/MG11129.html>). Accessed 2003 February 17.
- MENÉNDEZ, C.; RITTER, E.; SCHÄGER-PREGL, R.; WALKEMEIER, B.; KALDE, A.; SALAMINI, F. AND GEBHARDT, C. (2002): Cold sweetening in diploid potato: mapping quantitative trait loci and candidate genes. *Genetics* (162): 1423-1434.
- MILLER, M.E.; CHOUREY, P.S. (1992): The maize invertase-deficient miniature-1 seed mutation is associated with aberrant pedicel and endosperm development. *Plant Cell* (4): 297-305.

- MINHAS, J.S.; RAI, V.K.; SAINI, H.S. (2004/5): Carbohydrate metabolism during tuber initiation in potato: a transient surge in invertase activity marks the stolon to tuber transition. *Pot. Res.* (47): 113-126.
- MOTTRAM, D.S.; WEDZICHA, B.L.; DODSON, A.T. (2002): Acrylamide is formed in the Maillard reaction. *Nature* (419): 448-449
- MÜLLER-THURGAU, H. (1882): Über Zuckerrücklage in Pflanzenteilen in Folge niedriger Temperatur. *Landwirtsch. Jahrb. Schweiz* (11): 1229-1238.
- NACKLEY, A.G.; SHABALINA, S.A.; TCHIVILEVA, I.E.; SATTERFIELD, K.; KORCHYNSKYI, O.; MAKAROV, S.S.; MAIXNER, W.; DIATCHENKO, L. (2006): Human catechol-O-Methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science* (314): 1930-1933.
- NEVE, B.; FROGUEL, P.; CORSET, L.; VAILLANT, E.; VATIN, V.; BOUTIN, P. (2002): Rapid SNP allele frequency determination in genomic DNA pools by PyrosequencingTM. *BioTechniques* (32): 1138-1142.
- NICOT, N.; HAUSMAN, J.-F.; HOFFMANN, L.; EVERS D. (2005): Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. *J. Exp. Bot.* (56): 2907-2914.
- OEFFNER, P.J. (2002): Sequence variation and the biological function of genes: methodological and biological considerations. *J. Chromatogr.* (782): 3-25.
- OHARA-TAKADA A.; MATSUURA-ENDO C.; CHUDA Y.; ONO H.; YADA H.; YOSHIDA M.; KOBAYASHI A.; TSUDA S.; TAKIGAWA S.; NODA T.; YAMAUCHI H.; MORI M. (2005): Change in content of sugars and free amino acids in potato tubers under short-term storage at low temperature and the effect on acrylamide level after frying. *Biosci. Biotechnol. Biochem.* (69): 1232-1238.
- OHYAMA, A.; ITO, H.; SATO, T.; NISHIMURA, S.; IMAI, T.; HIRAI, M. (1995): Suppression of acid invertase activity by antisense RNA modifies the sugar composition of tomato fruits. *Plant Cell Physiol.* (36): 369-376.

- OMHOLT, S.W., PLATHE, E., OYEHAUG, L., XIANG, K. (2000): Gene regulatory networks generating the phenomena of additivity, dominance and epistasis. *Genetics* (155): 969-980.
- ORITA, M.; SUZUKI, Y.; SEKIYA, T.; HAYASHI, K. (1989): Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* (5): 874-879.
- PLUTHERO, F. G. (1993): Rapid purification of high-activity *Taq* DNA polymerase. *Nucl. Acids Res.* (21): 4850-4851.
- PFLIEGER, S.; LEFEBVRE V. AND CAUSSE M. (2001): The candidate gene approach in plant genetics: a review. *Mol. Breed.* (7): 275-291.
- PRESSEY, R. (1966): Separation and properties of potato invertase and invertase inhibitor. *Arch. Biochem. Biophys.* (113): 667-674.
- PRESSEY, R. (1967): Invertase inhibitor from potatoes: purification, characterization, and reactivity with plant invertases. *Plant Physiol.* (42): 1780-1786.
- PRESSEY, R. (1969): Role of invertase in accumulation of sugars in cold stored potatoes. *Am. Pot. J.* (46): 291-297
- PRESSEY, R.; SHAW, R. (1966): Effect of temperature on invertase, invertase inhibitor, and sugars in potato tubers: *Plant Physiol.* (41): 1657-1661.
- RICHARDSON, D. L.; DAVIES, H.V.; ROSS H.A.; MACKAY, G.R. (1990): Invertase activity and its relation to hexose accumulation in potato tubers. *J. Exp. Bot.* (41): 95-99.
- PUTZ, B. (1989): Technologie der Chipsherstellung. In: *Kartoffeln, Züchtung, Anbau, Verwertung*. Behr's, Hamburg: 219.
- RAMLOCH-LORENZ K.; KNUDSEN, S. AND STURM, A. (1993): Molecular characterization of the gene for carrot cell wall b-fructosidase. *Plant J.* (4): 545-554.

- RAYON, C.; LEROUGE, P.; FAYE, L. (1998): The protein N-Glycosylation in plants. *J. Exp. Bot.* (49): 1463-1472.
- REDDY, A.; GIBBS, B.S.; LIU, Y.-L.; COWARD, J.K. ; CHANGCHIEN, L.-M. AND MALEY F. (1999): Glycosylation of the overlapping sequons in yeast external invertase: effect of amino acid variation on site selectivity *in vivo* and *in vitro*. *Glycobiology* (9): 547-555.
- RICKERT, A.M.; PREMSTALLER, A.; GEBHARDT, C.; OEFNER, P.J. (2002): Genotyping of SNPs in a polyploid genome by pyrosequencing. *BioTechniques* (32): 592-593, 596-598, 600.
- RITTER, E.; DEBENER, T.; BARONE, A.; SALAMINI, F.; GEBHARDT, C. (1991): RFLP mapping on potato chromosomes of two genes controlling extreme resistance to potato virus X (PVX). *Mol. Gen. Genet.* (227): 81-85.
- ROE, M.A.; FAULKS, R.M.; BELSTEN, J.L. (1990): Role of reducing sugars and amino acids in fry colour of chips from potatoes grown under different nitrogen regimes. *J. Sci. Food. Agric.* (52): 207-214.
- ROITSCH T. (1999): Source–sink regulation by sugar and stress. *Curr. Opin. Plant Biol.* (2): 198-206.
- ROITSCH, T.; EHNEB, R.; GOETZ, M.; HAUSE, B.; HOFMANN, M.; SINHA, A.K. (2000): Regulation and function of extracellular invertase from higher plants in relation to assimilate partitioning, stress responses and sugar signalling. *Austral. J. Plant Physiol.* (27): 815-825.
- ROITSCH, T.; AND GONZÁLES, M.-C. (2004): Function and regulation of plant invertases: sweet sensations. *Trends Plant Sci.* (9): 606-613.
- RONAGHI, M.; KARAMOHAMED, S.; PETTERSON, B.; UHLÉN, M.; NYRÉN, P. (1996): Real-time DNA sequencing using detection of pyro-phosphate release. *Anal. Biochem.* (242): 84-89.
- ROREM, E.S. AND SCHWIMMER, S. (1963): Double pH optima of potato invertase. *Experimenta* (19): 150-151.

- ROSS, H.A.; MCRAE, D.; DAVIS, H.V. (1996): Sucrolytic enzyme activities in cotyledons of the faba bean. *Plant Physiol.* (111): 329-338.
- SAMBROOK, J.; FRITSCH, E.F. AND MANIATIS, T. (1989): Molecular cloning. *A laboratory manual*. Cold Spring Harbor Lab. Press. New York 2nd Edition.
- SAMBROOK J. AND RUSSELL, D.W. (2001): Molecular cloning. *A laboratory manual*. Cold Spring Harbor Lab. Press. New York 3rd Edition.
- SCHAART, J.G.; MEHLI, L.; SCHOUTEN, H.J. (2005): Quantification of allele-specific expression of a gene encoding strawberry polygalacturonase-inhibiting protein (PGIP) using pyrosequencingTM. *Plant J.* (41): 493-500.
- SCHÄFER-PREGL. R.; RITTER. E.; CONCILIO. L.; HESSELBACH. J.; LOVATTI. L. (1998): Analysis of quantitative trait loci (QTL) and quantitative trait alleles (QTA) for potato tuber yield and starch content: *Theor. Appl. Genet.* (97): 834-846.
- SCHOLES J.D.; BUNDOCK, N.; WILDE, R.; ROLFE, S.A. (1996): The impact of reduced vacuolar invertase activity on the photosynthetic and carbohydrate metabolism of tomato. *Planta* (200): 265-272.
- SCHWIMMER, S.; MAKOWER, R.U. AND ROREM, E.S. (1961): Invertase and invertase inhibitor in potato. *Plant Physiol.* (36):313-316.
- SEBKOVA, V., UNGER, C, HARDEGGER, M., STURM, A. (1995): Biochemical, physiological and molecular characterization of sucrose synthase from *Daucus carota*. *Plant Physiol.* (108): 75-83
- SHALLENBERGER, R.S.; SMITH, O.; TREADWAY, R.H. (1959): Role of sugars in the browning reaction in potato chips. *J Agric. Food Chem.* (7): 274-277.

- SIMPSON, C.G.; HEDLEY, P.E.; WATTERS, J.A. ET AL. (2000): Requirements for mini-exon inclusion in potato invertase mRNAs provides evidence for exon-scanning interactions in plants. *RNA* (6): 422-433.
- SMITH, A.M.; ZEEMAN, S.C.; SMITH, S.M. (2005): Starch degradation. *Annu. Rev. Plant Biol.* (56): 73-98
- SOULSBURY, C.D.; IOSSA, G.; EDWARDS, K.J.; BAKER, P.J.; HARRIS, S. (2007): Allelic dropout from a high-quality DNA source. *Conserv. Genet.* (8): 733-738.
- SPRINGER, N.M. AND STUPAR, R.M. (2007): Allele-specific expression patterns reveal biases and embryo-specific parent-of-origin effects in hybrid maize. *Plant Cell* (19): 2391-2402
- STITT, M. AND SONNEWALD, U. (1995): Regulation of metabolism in transgenic plants. *Annu. Rev. Plant Physiol. Mol. Biol.* (46): 341-368.
- STOMMEL, J.R. AND SIMON, P.W. (1990): Multiple forms of invertase from *daucus carota* cell cultures. *Phytochemistry* (29): 2087-2089.
- STURM, A. (1999): Invertases. Primary structures, functions, and roles in plant development and sucrose partitioning. *Plant Physiol.* (121): 1-7.
- STURM, A.; LIENHARD, S.; SCHATT, S.; HARDEGGER, M. (1999): Tissuespecific expression of two genes for sucrose synthase in carrot (*Daucus carota* L.). *Plant Mol. Biol.* (39): 349-360.
- STURM, A. AND TANG, G.Q. (1999). The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. *Trends Plant Sci.* 4, 401-407.
- SUNNUCKS, P.; WILSON, A.C.C.; BEHEREGARAY, L.B.; ZENGER, K.; FRENCH, J.; TAYLOR, A.C. (2000): SSCP is not difficult: the application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. *Mol. Ecol.* (9): 1699-1710.

- SZABO, P. E. AND MANN, J. R. (1995): Allele-specific expression and total expression levels of imprinted genes during early mouse development: implications for imprinting mechanisms. *Genes Develop.* (9): 3097-3108.
- TABERLET, P.; FRIFFIN, S.; GOOSSENS, B.; QUESTIAU, S.; MANCEAU V. (1996): Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Res.* (24): 3189-3194
- TANG G.-Q.; LÜSCHER, M. AND STURM, A. (1999): Antisense Repression of Vacuolar and Cell Wall Invertase in Transgenic Carrot Alters Early Plant Development and Sucrose Partitioning. *The Plant Cell* (11): 177-189.
- TANG X.; RUFFNER, H.P.; SCHOLES, J.D.; ROLFE, S.A. (1996): Purification and characterisation of soluble invertases from leaves of *Arabidopsis thaliana*. *Planta* (198): 17-23.
- TANKSLEY, S.D.; GANAL, M.W.; PRINCE, J.P.; DE VICENTE, M.C.; BONIERBALE, M.W.; BROUN, P.; FULTON, T.M.; GIOVANNONI, J.J.; GRANDILLO, S.; MARTIN, G.B., MESSEGUER, R.; MILLER, J.C.; MILLER, L.; PATERSON, A.H.; PINEDA, O; RÖDER, M.S.; WING, R.A.; WU, W.; YOUNG, N.D. (1992): High-density molecular linkage maps of the tomato and potato genomes. *Genetics* (132):1141- 1160.
- TANKSLEY, S.D. (1993): Mapping polygenes. *Annu. Rev. Genet.* (27): 205-233.
- TAUBENBERGER, E.; HOFFMANN-BENNING, S.; FLEISCHER-NÖTER, H.; WILLMITZER, L.; FISAHN, J. (1999): Impact of invertase over-expression on cell size, starch granule formation and cell wall properties during tuber development in potatoes with modified carbon allocation patterns. *J. Exp. Bot.* (50): 477-486.
- TETLOW, I.J.; WAIT, R.; LU, Z.; AKKASAENG, R.; BOWSHER, C.G.; ESPOSITO, S.; KOSAR-HASHEMI, B.; MORELL, M-K.; EMES, M.J. (2004a): Protein phosphorylation in amyloplasts regulates starch branching enzyme activity and protein-protein interactions. *Plant Cell* (16): 694-708.

- TETLOW I. J.; MORELL M. K.; EMES M. J. (2004b): Recent developments in understanding the regulation of starch metabolism in higher plants. *J. Exp. Bot.* (55): 2131-2145.
- TETLOW, I.J.; BEISEL, G.K.; CAMERON, S.; MAKHMOUDOVA, A.; LIU, F.; BRESOLIN, N.S.; WAIT, R.; MORELL, M.K.; EMES, M.J. (2008): Analysis of protein complexes in wheat amyloplasts reveals functional interactions among starch biosynthetic enzymes. *Plant Physiol.* (146): 1878-1891.
- TYMOWSKA-LALANNE, Z. AND KREIS, M. (1998): The plant invertases: physiology, biochemistry and molecular biology. *Adv. Bot. Res.* (28): 71-117.
- VAN BUSKIRK, H.A. & THOMASHOW, M.F. (2006): *Arabidopsis* transcription factors regulating cold acclimation. *Physiol. Plant* (126): 72-80.
- VARGAS, W.; CUMINO, A.; SALERNO, G.L. (2003): Cyanobacterial alkaline/neutral invertases. Origin of sucrose hydrolysis in the plant cytosol? *Planta* (216): 951-960.
- VEITIA, R.A.; BOTTANI, S.; BIRCHLER, J.A. (2008): Cellular reactions to gene dosage imbalance: genomic, transcriptomic and proteomic effects. *Trends Genet.* (24): 390-397.
- WASSON, J.; SKOLNICK, G.; LOVE-GREGORY, L.; PERMUTT, A. (2002): Assessing allele frequencies of single nucleotides polymorphisms in DNA pools by PyrosequencingTM technology. *BioTechniques* (342): 1144- 1150.
- WEBER, H., BORISJUK, L., HEIM, U., BUCHNER, P. AND WOBUS, U. (1995): Seed coat-associated invertases of fava bean control both unloading and storage functions: Cloning of cDNAs and cell type-specific expression. *Plant Cell* (7), 1835- 1846.
- WEIL, M.; KRAUSGRILL, S.; SCHUSTER, A. AND RAUSCH T. (1994): A 17-kDa *Nicotiana tabacum* cellwall peptide acts as an in vitro inhibitor of the cell-wall isoform of acid invertase. *Planta* (193): 438- 445.
- WINTER, H. AND HUBER, S.C. (2000): Regulation of sucrose metabolism in higher plants: localization and regulation of activity of key enzymes. *Crit. Rev. Biochem. Mol. Biol.* (35): 253-289.

ZHANG C.; LIU J.; XIE C. H.: cDNA cloning and anti-sense repression of invertase in potato unpublished; accession AY341425 (<http://www.ncbi.nlm.nih.gov>)

ZHANG, D.; MISCHKE, S.; GOENAGA, R.; HEMEIDA, A.A.; SAUNDERS, J-A. (2006): Accuracy and Reliability of High-Throughput Microsatellite Genotyping for Cacao Clone Identification. *Crop Sci. J.* (46): 2084-2092

ZHOU, D.; MATTOO, A.; LI, N.; IMASEKI, H.; SOLOMOS, T. (1994): Complete nucleotide sequence of potato tuber acid invertase cDNA. *Plant Physiol.* (106): 397-398.

ZRENNER, R.; SALANOUBAT M.; WILLMITZER L. AND SONNEWALD, U. (1995) Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants (*Solanum tuberosum* L.). *Plant J.* (7): 97-107.

ZRENNER, R.; SCHÜLER, K.; SONNEWALD, U. (1996): Soluble acid invertase determines the hexose-to-sucrose ration in cold-stored potato tubers. *Planta* (198): 246-252.

Appendix

- A2** Map of the yeast expression vector 112 A1 NE.
- A3** Description of the amino acid exchanges using the alignment program Multalin version 5.4.1 (<http://bioinfo.genetoul.fr>).
- A3.1.1** Alignment of full-length amino acid sequences of *Pain-1* 'Satina' alleles.
- A3.1.2** Alignment of full-length amino acid sequences of *Pain-1* 'Diana' alleles.
- A3.1.3** Alignment of full-length amino acid sequences of *Pain-1* 'Theresa' alleles.
- A3.1.4** Alignment of full-length nucleotide sequences from SNPs variants of the *Pain-1* allele *Pain_SA*.
- A3.1.5** Alignment of full-length nucleotide sequences from SNPs variants of the *Pain-1* allele *Pain_SN*.
- A3.1.6** Alignment of full-length nucleotide sequences from SNPs variants of the *Pain-1* allele *Pain_DA*.
- A3.1.7** Alignment of full-length nucleotide sequences from SNPs variants of the *Pain-1* allele *Pain_DN1*.
- A3.1.8** Alignment of full-length nucleotide sequences from SNPs variants of the *Pain-1* allele *Pain_DN2*.
- A3.1.9** Alignment of full-length nucleotide sequences from SNPs variants of the *Pain-1* allele *Pain_TN1*.
- A3.1.10** Alignment of full-length nucleotide sequences from SNPs variants of the *Pain-1* allele *Pain_TN2*.
- A3.1.11** Alignment of full-length *Pain-1* nucleotide sequences obtained from all tetraploid genotypes.
- A3.1.12** Alignment of full-length amino acid sequences of *Pain-1* P18 alleles.
- A3.1.13** Alignment of full-length amino acid sequences of *Pain-1* P40 alleles.
- A3.1.14** Alignment of full-length amino acid sequences of *Pain-1* P54 alleles.
- A3.1.15** Alignment of full-length nucleotide sequences from SNPs variants of the *Pain-1* allele *Pain_P18N1*.
- A3.1.16** Alignment of full-length nucleotide sequences from SNPs variants of the *Pain-1* allele *Pain_P18N2*.
- A3.1.17** Alignment of full-length nucleotide sequences from SNPs variants of the *Pain-1* allele *Pain_P40N1*.

-
- A3.1.18** Alignment of full-length nucleotide sequences from SNPs variants of the *Pain-1* allele *Pain_P40N2*.
- A3.1.19** Alignment of full-length nucleotide sequences from SNPs variants of the *Pain-1* allele *Pain_P54N*.
- A3.1.20** Alignment of full-length *Pain-1* nucleotide sequences obtained from all diploid genotypes.
- A3.1.21** Alignment of full-length *Pain-1* nucleotide sequences obtained from all tetraploid and diploid genotypes.
- A3.1.22** Full-length alignment of nucleotide sequences of *Pain-1* alleles from ‘Satina’, ‘Diana’, and ‘Theresa’ to identify allele specific SNPs for assigning cloned alleles to associated SSCP fragments.
- A3.1.23** Amino acid alignment of *Pain-1* BAC insert sequences and the P40 alleles *Pain_P40N1* and *Pain_P40N2*.
- A3.1.24** Alignment of the genomic *Pain-1* BAC insert sequence with the *Pain-1* cDNA sequence to identify the exon- and intron structure of the *Pain-1* gene.
- A3.1.25** Alignment of full-length nucleotide sequences from SNPs variants of the *Pain-1* allele *Pain_SN*.
- A3.2** Exception of alleles, from which only one full-length sequence was obtained.
- A3.2.1** Alignment of full-length amino acid sequences of *invGE* ‘Satina’ alleles.
- A3.2.2** Alignment of full-length amino acid sequences of *invGE* ‘Diana’ alleles.
- A3.2.3** Alignment of full-length amino acid sequences of *invGE* ‘Theresa’ alleles.
- A3.2.4** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_SA*.
- A3.2.5** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_SNI*.
- A3.2.6** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_SN3*.
- A3.2.7** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_DA*.
- A3.2.8** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_DNI*.
- A3.2.9** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_TA*.

-
- A3.2.10** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_TN1*.
- A3.2.11** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_TN2*.
- A3.2.12** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_TN3*.
- A3.2.13** Alignment of full-length *invGE* nucleotide sequences obtained from all tetraploid genotypes.
- A3.2.14** Alignment of full-length amino acid sequences of *invGE* P18 alleles.
- A3.2.15** Alignment of full-length amino acid sequences of *invGE* P40 alleles.
- A3.2.16** Alignment of full-length amino acid sequences of *invGE* P54 alleles.
- A3.2.17** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_P18N1*.
- A3.2.18** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_P18N2*.
- A3.2.19** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_P40N1*.
- A3.2.20** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_P40N2*.
- A3.2.21** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_P54N1*.
- A3.2.22** Alignment of full-length nucleotide sequences from SNPs variants of the *invGE* allele *E_P54N2*.
- A3.2.23** Alignment of full-length *invGE* nucleotide sequences obtained from all diploid genotypes.
- A3.2.24** Alignment of full-length *invGE* nucleotide sequences obtained from all tetraploid and diploid genotypes.
- A3.2.25** Alignment of full-length amino acid sequences of *invGF* 'Satina' alleles.
- A3.2.26** Alignment of full-length amino acid sequences of *invGF* 'Diana' alleles.
- A3.2.27** Alignment of full-length amino acid sequences of *invGF* 'Theresa' alleles.
- A3.2.28** Alignment of full-length nucleotide sequences from SNPs variants of the *invGF* allele *F_SN1*.
- A3.2.29** Alignment of full-length nucleotide sequences from SNPs variants of the *invGF* allele *F_SN2*.

-
- A3.2.30** Alignment of full-length nucleotide sequences from SNPs variants of the *invGF* allele *F_SN3*.
- A3.2.31** Alignment of full-length nucleotide sequences from SNPs variants of the *invGF* allele *F_SN4*.
- A3.2.32** Alignment of full-length nucleotide sequences from SNPs variants of the *invGF* allele *F_DN2*.
- A3.2.33** Alignment of full-length nucleotide sequences from SNPs variants of the *invGF* allele *F_TN1*.
- A3.2.34** Alignment of full-length nucleotide sequences from SNPs variants of the *invGF* allele *F_TN2*.
- A3.2.35** Alignment of full-length *invGF* nucleotide sequences obtained from all tetraploid genotypes.
- A3.2.36** Alignment of full-length amino acid sequences of *invGF* P18 alleles.
- A3.2.37** Alignment of full-length amino acid sequences of *invGF* P40 alleles.
- A3.2.38** Alignment of full-length amino acid sequences of *invGF* P54 alleles.
- A3.2.39** Alignment of full-length nucleotide sequences from SNPs variants of the *invGF* allele *F_P18N*.
- A3.2.40** Alignment of full-length nucleotide sequences from SNPs variants of the *invGF* allele *F_P40N2*.
- A3.2.41** Alignment of full-length nucleotide sequences from SNPs variants of the *invGF* allele *F_P54N1*.
- A3.2.42** Alignment of full-length nucleotide sequences from SNPs variants of the *invGF* allele *F_P54N2*.
- A3.2.43** Alignment of full-length *invGF* nucleotide sequences obtained from all diploid genotypes.
- A3.2.44** Alignment of full-length *invGF* nucleotide sequences obtained from all tetraploid and diploid genotypes.
- A3.2.45** Alignment of full-length amino acid sequences of the *invGE* allele *E_SA* and *invGF* allele *F_SN4*.
- A3.3** Exception of alleles, from which only one full-length sequence was obtained.
- A3.3.1** Alignment of full-length amino acid sequences of *pCD111* ‘Satina’ alleles.
- A3.3.2** Alignment of full-length amino acid sequences of *pCD111* ‘Theresa’ alleles

-
- A3.3.3** Alignment of full-length nucleotide sequences from SNPs variants of the *pCD111* allele *CD111_S1*.
- A3.3.4** Alignment of full-length nucleotide sequences from SNPs variants of the *pCD111* allele *CD111_S2*.
- A3.3.5** Alignment of full-length nucleotide sequences from SNPs variants of the *pCD111* allele *CD111_T2*.
- A3.3.6** Alignment of full-length *pCD111* nucleotide sequences obtained from all tetraploid genotypes.
- A3.3.7** Alignment of full-length amino acid sequences of *pCD111* P54 alleles.
- A3.3.8** Alignment of full-length nucleotide sequences from SNPs variants of the *pCD111* allele *CD111_P54_1*.
- A3.3.9** Alignment of full-length *pCD111* nucleotide sequences obtained from all diploid genotypes.
- A3.3.10** Alignment of full-length *pCD111* nucleotide sequences obtained from all tetraploid and diploid genotypes.
- A3.3.11** Alignment of full-length amino acid sequences of *pCD141* ‘Satina’ alleles.
- A3.3.12** Alignment of full-length amino acid sequences of *pCD141* ‘Diana’ alleles.
- A3.3.13** Alignment of full-length amino acid sequences of *pCD141* ‘Theresa’ alleles.
- A3.3.14** Alignment of full-length nucleotide sequences from SNPs variants of the *pCD141* allele *CD141_S2*.
- A3.3.15** Alignment of full-length nucleotide sequences from SNPs variants of the *pCD141* allele *CD141_S3*.
- A3.3.16** Alignment of full-length nucleotide sequences from SNPs variants of the *pCD141* allele *CD141_D1*.
- A3.3.17** Alignment of full-length nucleotide sequences from SNPs variants of the *pCD141* allele *CD141_T1*.
- A3.3.18** Alignment of full-length nucleotide sequences from SNPs variants of the *pCD141* allele *CD141_T2*.
- A3.3.19** Alignment of full-length *pCD141* nucleotide sequences obtained from all tetraploid genotypes.
- A3.3.20** Alignment of full-length amino acid sequences of *pCD141* P18 alleles.
- A3.3.21** Alignment of full-length amino acid sequences of *pCD141* P40 alleles.
- A3.3.22** Alignment of full-length amino acid sequences of *pCD141* P54 alleles.

-
- A3.3.23** Alignment of full-length nucleotide sequences from SNPs variants of the *pCD141* allele *CD141_P18_1*.
- A3.3.24** Alignment of full-length nucleotide sequences from SNPs variants of the *pCD141* allele *CD141_P18_2*.
- A3.3.25** Alignment of full-length nucleotide sequences from SNPs variants of the *pCD141* allele *CD141_P40_1*.
- A3.3.26** Alignment of full-length nucleotide sequences from SNPs variants of the *pCD141* allele *CD141_P54_1*.
- A3.3.27** Alignment of full-length nucleotide sequences from SNPs variants of the *pCD141* allele *CD141_P54_2*.
- A3.3.28** Alignment of full-length *pCD141* nucleotide sequences obtained from all diploid genotypes.
- A3.3.29** Alignment of full-length *pCD141* nucleotide sequences obtained from all tetraploid and diploid genotypes.
- A3.3.30** Amino acid alignment of the *pCD111* BAC insert sequence and the P40 allele *CD111_P40_1*.
- A3.3.31** Amino acid alignment of the *pCD141* BAC insert sequence and the P40 allele *CD141_P40_1*.
- A3.3.32** Alignment of the genomic *pCD111* BAC insert sequence with the P40 *pCD111* cDNA sequence to identify the exon- and intron structure of the *pCD111* gene.
- A3.3.33** Alignment of the genomic *pCD141* BAC insert sequence with the P40 *pCD141* cDNA sequence to identify the exon- and intron structure of the *pCD141* gene.

Acknowledgements

Most importantly I want to thank PD Dr. Christiane Gebhardt for the great opportunity to work in her group at a topic even my grandmother understood the importance of. Thanks to Dr. Christiane Gebhardt for the supervision of this dissertation, for many discussions in the course of this challenging and dynamic project, for always having an open door to discuss results or disperse worries regarding the results, and for an excellent support during the writing process.

Also thanks to Prof. Maarten Koornneef for the chance to work in his department.

Many thanks to my present and past colleagues at the MPIZ: Dr. Claude Urbany, Dr. Agim Ballvora, Dr. Ute Achenbach, Birgit Walkemeier, Gabor Gyetvai, Lena Schreiber, Anna Camila Nader Njeto, Dr. Damaris Odeny, Dr. Matthias Fischer, Dr. Li Li, Tatjana von Frey, Sandra Mäurer, Charlotte Bulich, Christine Säger, Merle Noschinski, Markus Kuckenberg, Lysann Schmidt. Most of them contribute to get over difficulties, helped me with my work and had always an open ear when things needed to be discussed. Besides this, I appreciate the nice atmosphere in the group, especially with the other PhD students. Gabor Gyetvai always tried to discuss things regarding my work and find solutions – even though there were none.

I want to address special thanks to Dr. Claude Urbany, who helped me tremendously during my PhD work. Besides being a contact person regarding the protein work, he always tried to put results in a better light than they appeared in the first moment and, therefore, helped me moving on in the process of my work. Claude also spent “some” hours reading my PhD work to polish up my writing. His careful reading, detailed corrections and thorough comments led to remarkable improvements of this work. I am also grateful to him because of his never ending chocolate supply, for showing me that there is much more in life than failed PCRs and for his understanding me in a way, which is really unique.

Also special thanks to Birgit Walkemeier who helped me a lot with her technical assistance. She was always willingly to help me with screening BAC libraries, sequencing of clones and many other things.

I am also grateful to Dr. Ute Achenbach who helped me a lot with reading my PhD work and besides being very critical regarding my English style, she also was able to encourage me. Her careful reading and useful comments improved this work noticeably.

Also thanks to the collaborators of this work: Dr. Alisdair Fernie, Dr. Adriano Nunes-Nesi and Pawel Durek (MPIMP/Golm). Dr. Alisdair Fernie and Dr. Adriano Nunes-Nesi gave me the possibility to perform the biochemical analysis in the laboratory in Golm. Pawel Durek modelled the invertase alleles and, therefore, special thanks to him. He was always open for my new ideas, for more and more allele models, and spared me with too many details about things I never heard anything before.

Thanks to Dr. Benjamin Stich (MPIZ/Köln) for calculation of the significance levels of the biochemical parameters.

Thanks to the ADIS service group, who really sequenced a lot of invertase alleles for me. Special thanks to Iris Bürst, who took so many times special care of my samples.

Thanks to my former colleagues Dr. Silke Schulze und Dr. Melanie Bartsch for so many nice lunch breaks, also for useful work discussions, scientific support and being there when things went wrong and I needed a good word to get balanced again.

Finally, I thank my family: my husband Michael, who was always there when melancholy thoughts and doubts were overwhelming leading me in way to believe in myself again. His endless patience was often working to full capacity but nevertheless always present. He also ensured that normal life went on, that our fridge was filled, the car was fuelled and that more dishes were inside the cupboard than outside. He also took affectionately care of our daughter and was able to dress up all the knights in the right way. Besides all this he also helped me bringing my work in a nice shape, formatting results of three years work. My daughter Berenike helped me with her naive point of view not understanding why things of ‘the adults’ are so important when the sun is reflecting itself in a water drop. My mother Petra was always there for me even if not physically present. We spent lots of hours at the phone convincing me that everything in life has its sense even if it sometimes takes a while until it becomes revealed. My mother in law Sabine supported us directly with her willingness taking care of our daughter when stress occupied me and my husband.

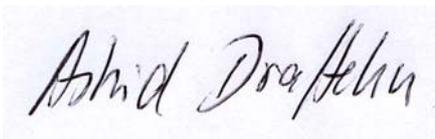
Die vorliegende Arbeit wurde am Max-Planck-Institut für Züchtungsforschung in Köln durchgeführt.

ERKLÄRUNG

Köln, 2009

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit – einschließlich Tabellen, Karten und Abbildungen – die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde.

Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von PD Dr. Christiane Gebhardt betreut worden.



Astrid Draffehn

Lebenslauf

Persönliche Daten

Name Astrid Martina Draffehn, geb. Fettin
 Geburtsdatum 27.06.1978
 Geburtsort Dessau
 Staatsangehörigkeit deutsch
 Familienstand verheiratet
 Kinder eine Tochter, geb. am 10.01.2004

Schulbildung

09/1985 – 06/1988 Polytechnische Oberschule „Friedrich Polling“, Dessau
 09/1988 – 06/1991 Polytechnische Oberschule „Ernst Thälmann“, Roßlau
 09/1991 – 07-1997 Goethe-Gymnasium, Roßlau
 04.07.1994 Abschluss: Abitur

Studium

09/1997 – 07/2000 Diplomstudiengang Biologie an der
Martin-Luther-Universität Halle-Wittenberg
 Abschluss: Vordiplom

08/2000 – 08/2001 Auslandsstudienjahr an der
University of Texas at El Paso, USA

09/2001 – 11/2003 Fortsetzung Diplomstudiengang Biologie an der
Martin-Luther-Universität Halle-Wittenberg
 Diplomprüfungen im Hauptfach Pflanzenphysiologie, in den
 Nebenfächern Genetik, Immunologie, Biochemie

26.11.2003 Abschluss: Diplom-Biologin

09/2005 – 06/2009 Promotion am
Max-Planck-Institut für Züchtungsforschung Köln (MPIZ) in der
 Abteilung Pflanzenzüchtung und Genetik, AG Christiane Gebhardt

Erweiternd zum Studium

08/2000 – 02/2001 Studium im Wintersemester an der *University of Texas at El Paso, USA*
 Kurse: Immunologie (Kurs für Master-Studiengang)
 Parasitologie (Kurs für Doktoranden)
 Desert Ecology
 English

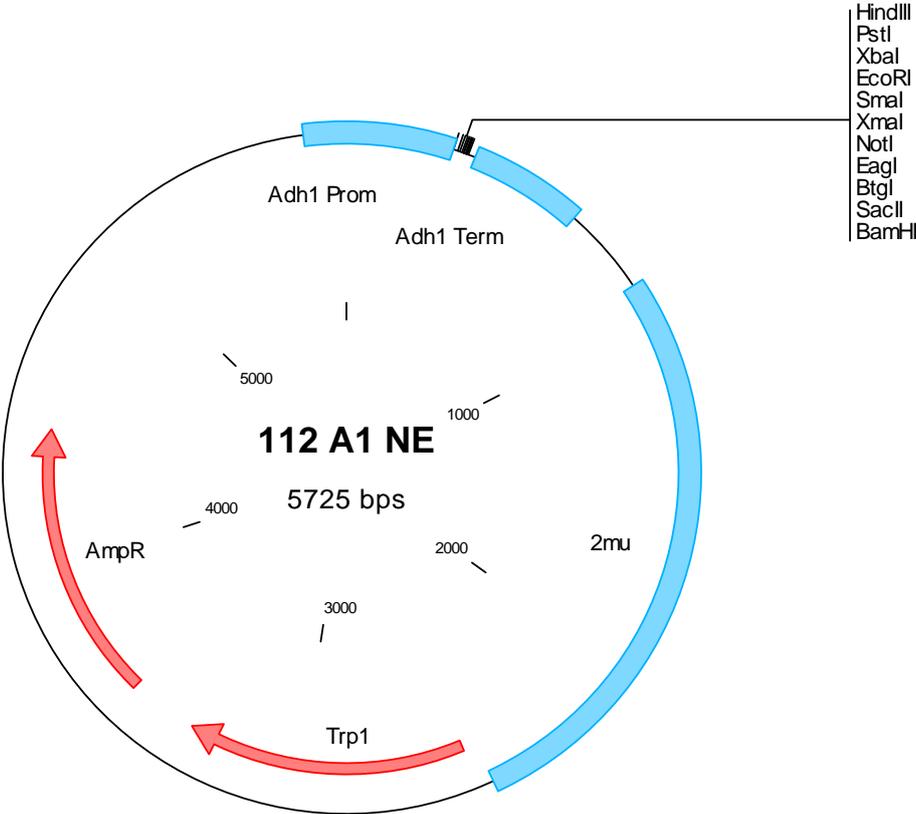
01/2001 – 08/2001 Praktisches Arbeiten in der Forschungsgruppe von Dr. Robert Webb,
University of Texas at El Paso, USA
 (in Deutschland Anerkennung als Forschungsgruppenpraktikum)
 Haupttätigkeit: Klonierung von bekannten und putativen
 Metallothionein-Sequenzen aus Cyanobakterien in *E.coli*,
 Überexpression der Metallothioneine, anschließende Protein-
 aufreinigung sowie 3D-Protein-Strukturanalyse

Fachbezogene Arbeiten und Praktika

- 11/1999 – 03/2000 Studentische Hilfskraft an der *Martin-Luther-Universität Halle-Wittenberg* in der AG Prof. Dr. Ralf-Bernd Klösgen
Haupttätigkeit: Isolierung von Chloroplasten aus *Arabidopsis thaliana* und Spinat, Fluoreszenzmikroskopie
- 08/2000 – 02/2001 Studium im Wintersemester an der *University of Texas at El Paso*, USA
Kurse: Immunologie (Kurs für Master-Studiengang)
Parasitologie (Kurs für Doktoranden)
Desert Ecology
English
- 01/2001 – 08/2001 Praktisches Arbeiten in der Forschungsgruppe von Dr. Robert Webb, *University of Texas at El Paso*, USA
(in Deutschland Anerkennung als Forschungsgruppenpraktikum)
Haupttätigkeit: Klonierung von bekannten und putativen Metallothionein-Sequenzen aus Cyanobakterien in *E.coli*, Überexpression der Metallothioneine, anschließende Proteinaufreinigung sowie 3D-Protein-Struktur-Analyse
- 02/2002 – 03/2002 Belegarbeit, Thema: „Amplifizierung von Metallothionein-Transkripten aus seneszenten Gersten-RNA mit Hilfe aus bekannten Metallothionein-Sequenzen abgeleiteter Primer“ an der *Martin-Luther-Universität Halle-Wittenberg* in der AG Prof. Dr. Klaus Humbeck
- 03/2003 – 11/2003 Diplomarbeit, Thema: „Expression heterologer *psbA*-Gene in *Clamydomonas reinhardtii*“ an der *Martin-Luther-Universität Halle-Wittenberg* in der AG Prof. Dr. Udo Johanningmeier
- 11/2003 Verteidigung Diplomarbeit

Appendix A2

Map of the yeast expression vector 112 A1 NE.



Appendix A3

For aligning the amino acid and nucleotide sequences of the obtained alleles the following software was used:

Multalin version 5.4.1 (<http://bioinfo.genetoul.fr>)

Copyright I.N.R.A. France 1989, 1991, 1994, 1996

Multiple sequence alignment with hierarchical clustering

F. CORPET, 1988, Nucl. Acids Res., 16 (22), 10881-10890

Different matrixes were used for comparing the allelic sequences.

For all alignments except A3.1.24, A3.2.45, A3.3.32, and A3.3.33 the following parameters were used:

Symbol comparison table: **blosum62**
Gap weight: 12
Gap length weight: 2
Consensus levels: high=90% low=50%
Consensus symbols:
! is anyone of IV
\$ is anyone of LM
% is anyone of FY
is anyone of NDQEBZ

For aligning the sequences of A3.1.24, A3.2.45, A3.3.32, and A3.3.33 the following matrix was used:

Symbol comparison table: **dayhoff**
Gap weight: 5
Gap length weight: 0
Consensus levels: high=90% low=50%
Consensus symbols:
\$ is anyone of LM
% is anyone of FY
is anyone of BDENQZ

1 10 20 30 40 50 60 70 80 90 100 110 120 130
Pain_SN MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSPAPPSRGVSGQVSDKTFRDVYNASHVSYAWSNAMLSMQRATAYHFQPQKNMNDPNGPLYHKG
Pain_SN1 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSPAPPSRGVSGQVSDKTFRDVYNASHVSYAWSNAMLSMQRATAYHFQPQKNMNDPNGPLYHKG
Pain_SN2 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSPAPPSRGVSGQVSDKTFRDVYNASHVSYAWSNAMLSMQRATAYHFQPQKNMNDPNGPLYHKG
Pain_SN3 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSPAPPSRGVSGQVSDKTFRDVYNASHVSYAWSNAMLSMQRATAYHFQPQKNMNDPNGPLYHKG
Pain_SN4 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSPAPPSRGVSGQVSDKTFRDVYNASHVSYAWSNAMLSMQRATAYHFQPQKNMNDPNGPLYHKG
Pain_SA MATQYHSSYDPENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSPAPPSRGVSGQVSDKTFRDVYNASHVSYAWSNAMLSMQRATAYHFQPQKNMNDPNGPLYHKG
Pain_SA1 MATQYHSSYDPENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSPAPPSRGVSGQVSDKTFRDVYNASHVSYAWSNAMLSMQRATAYHFQPQKNMNDPNGPLYHKG
Pain_SA3 MATQYHSSYDPENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSPAPPSRGVSGQVSDKTFRDVYNASHVSYAWSNAMLSMQRATAYHFQPQKNMNDPNGPLYHKG
Pain_SA2 MATQYHSSYDPENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSPAPPSRGVSGQVSDKTFRDVYNASHVSYAWSNAMLSMQRATAYHFQPQKNMNDPNGPLYHKG
Consensus MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSPAPPSRGVSGQVSDKTFRDVYNASHVSYAWSNAMLSMQRATAYHFQPQKNMNDPNGPLYHKG

131 140 150 160 170 180 190 200 210 220 230 240 250 260
Pain_SN MYHLFYQYNPDSAIMGNITWGHAVSKDLIHWLYLPFAIVPQWYDINGVWTSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSPLLLDWVKYKGNPVLVPPPGIGVKDFRDPTTAWTGPQNGQWLL
Pain_SN1 MYHLFYQYNPDSAIMGNITWGHAVSKDLIHWLYLPFAIVPQWYDINGVWTSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSPLLLDWVKYKGNPVLVPPPGIGVKDFRDPTTAWTGPQNGQWLL
Pain_SN2 MYHLFYQYNPDSAIMGNITWGHAVSKDLIHWLYLPFAIVPQWYDINGVWTSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSPLLLDWVKYKGNPVLVPPPGIGVKDFRDPTTAWTGPQNGQWLL
Pain_SN3 MYHLFYQYNPDSAIMGNITWGHAVSKDLIHWLYLPFAIVPQWYDINGVWTSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSPLLLDWVKYKGNPVLVPPPGIGVKDFRDPTTAWTGPQNGQWLL
Pain_SN4 MYHLFYQYNPDSAIMGNITWGHAVSKDLIHWLYLPFAIVPQWYDINGVWTSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSPLLLDWVKYKGNPVLVPPPGIGVKDFRDPTTAWTGPQNGQWLL
Pain_SA MYHLFYQYNPDSAIMGNITWGHAVSKDLIHWLYLPFAIVPQWYDINGVWTSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSPLLLDWVKYKGNPVLVPPPGIGIKDFRDPTTAWTGPQNGQWLL
Pain_SA1 MYHLFYQYNPDSAIMGNITWGHAVSKDLIHWLYLPFAIVPQWYDINGVWTSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSPLLLDWVKYKGNPVLVPPPGIGIKDFRDPTTAWTGPQNGQWLL
Pain_SA3 MYHLFYQYNPDSAIMGNITWGHAVSKDLIHWLYLPFAIVPQWYDINGVWTSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSPLLLDWVKYKGNPVLVPPPGIGIKDFRDPTTAWTGPQNGQWLL
Pain_SA2 MYHLFYQYNPDSAIMGNITWGHAVSKDLIHWLYLPFAIVPQWYDINGVWTSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSPLLLDWVKYKGNPVLVPPPGIGIKDFRDPTTAWTGPQNGQWLL
Consensus MYHLFYQYNPDSAIMGNITWGHAVSKDLIHWLYLPFAIVPQWYDINGVWTSATILPDGQIMMLYTGDTDDYVQVQNLAYPTNLSPLLLDWVKYKGNPVLVPPPGIGIKDFRDPTTAWTGPQNGQWLL

261 270 280 290 300 310 320 330 340 350 360 370 380 390
Pain_SN TIGSKIGKTGIALVYETSNFTSFKLLDEVLAHVPGTGMWECVDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYYASKTFYDPKKQRRVWLG
Pain_SN1 TIGSKIGKTGIALVYETSNFTSFKLLDEVLAHVPGTGMWECVDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYYASKTFYDPKKQRRVWLG
Pain_SN2 TIGSKIGKTGIALVYETSNFTSFKLLDEVLAHVPGTGMWECVDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYYASKTFYDPKKQRRVWLG
Pain_SN3 TIGSKIGKTGIALVYETSNFTSFKLLDEVLAHVPGTGMWECVDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYYASKTFYDPKKQRRVWLG
Pain_SN4 TIGSKIGKTGIALVYETSNFTSFKLLDEVLAHVPGTGMWECVDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYYASKTFYDPKKQRRVWLG
Pain_SA TIGSKIGKTGIALVYETSNFTSFKLLDEVLAHVPGTGMWECVDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYYASKTFYDPKKQRRVWLG
Pain_SA1 TIGSKIGKTGIALVYETSNFTSFKLLDEVLAHVPGTGMWECVDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYYASKTFYDPKKQRRVWLG
Pain_SA3 TIGSKIGKTGIALVYETSNFTSFKLLDEVLAHVPGTGMWECVDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYYASKTFYDPKKQRRVWLG
Pain_SA2 TIGSKIGKTGIALVYETSNFTSFKLLDEVLAHVPGTGMWECVDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYYASKTFYDPKKQRRVWLG
Consensus TIGSKIGKTGIALVYETSNFTSFKLLDEVLAHVPGTGMWECVDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYYASKTFYDPKKQRRVWLG

391 400 410 420 430 440 450 460 470 480 490 500 510 520
Pain_SN HIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAELDIEASFVYDKVALQGIIEADHVGFSCSTSGGAASRGILGPFVGVVVIADQTLSELT
Pain_SN1 HIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAELDIEASFVYDKVALQGIIEADHVGFSCSTSGGAASRGILGPFVGVVVIADQTLSELT
Pain_SN2 HIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAELDIEASFVYDKVALQGIIEADHVGFSCSTSGGAASRGILGPFVGVVVIADQTLSELT
Pain_SN3 HIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAELDIEASFVYDKVALQGIIEADHVGFSCSTSGGAASRGILGPFVGVVVIADQTLSELT
Pain_SN4 HIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAELDIEASFVYDKVALQGIIEADHVGFSCSTSGGAASRGILGPFVGVVVIADQTLSELT
Pain_SA HIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAELDIEASFVYDKVALQGIIEADHVGFSCSTSGGAASRGILGPFVGVVVIADQTLSELT
Pain_SA1 HIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAELDIEASFVYDKVALQGIIEADHVGFSCSTSGGAASRGILGPFVGVVVIADQTLSELT
Pain_SA3 HIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAELDIEASFVYDKVALQGIIEADHVGFSCSTSGGAASRGILGPFVGVVVIADQTLSELT
Pain_SA2 HIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAELDIEASFVYDKVALQGIIEADHVGFSCSTSGGAASRGILGPFVGVVVIADQTLSELT
Consensus HIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAELDIEASFVYDKVALQGIIEADHVGFSCSTSGGAASRGILGPFVGVVVIADQTLSELT

521 530 540 550 560 570 580 590 600 610 620 630 639
Pain_SN PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYVYSSVPVLDGEKHSRLLVDHSIYESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIMSLESANIQSFPLQDL
Pain_SN1 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYVYSSVPVLDGEKHSRLLVDHSIYESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIMSLESANIQSFPLQDL
Pain_SN2 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYVYSSVPVLDGEKHSRLLVDHSIYESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIMSLESANIQSFPLQDL
Pain_SN3 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYVYSSVPVLDGEKHSRLLVDHSIYESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIMSLESANIQSFPLQDL
Pain_SN4 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYVYSSVPVLDGEKHSRLLVDHSIYESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIMSLESANIQSFPLQDL
Pain_SA PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYVYSSVPVLDGEKHSRLLVDHSIYESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIMSLESANIRSFPLQDL
Pain_SA1 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYVYSSVPVLDGEKHSRLLVDHSIYESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIMSLESANIRSFPLQDL
Pain_SA3 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYVYSSVPVLDGEKHSRLLVDHSIYESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIMSLESANIRSFPLQDL
Pain_SA2 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYVYSSVPVLDGEKHSRLLVDHSIYESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIMSLESANIRSFPLQDL
Consensus PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYVYSSVPVLDGEKHSRLLVDHSIYESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIMSLESANIQSFPLQDL

1 10 20 30 40 50 60 70 80 90 100 110 120 130
Pain_TN1 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSRAPPSPRGVYSGVSDKTFRDVYNASHVSYAMSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_TN1_1 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSRAPPSPRGVYSGVSDKTFRDVYNASHVSYAMSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_TN1_2 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSRAPPSPRGVYSGVSDKTFRDVYNASHVSYAMSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_TN1_3 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSRAPPSPRGVYSGVSDKTFRDVYNASHVSYAMSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_TN2 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSRAPPSPRGVYSGVSDKTFRDVYNASHVSYAMSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_TN2_1 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSRAPPSPRGVYSGVSDKTFRDVYNASHVSYAMSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_TN2_2 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSRAPPSPRGVYSGVSDKTFRDVYNASHVSYAMSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_TN2_3 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSRAPPSPRGVYSGVSDKTFRDVYNASHVSYAMSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Consensus MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNNQSPDLQSNRSRAPPSPRGVYSGVSDKTFRDVYNASHVSYAMSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG

131 140 150 160 170 180 190 200 210 220 230 240 250 260
Pain_TN1 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPTNLSDPDLLDWVKYKGNPVLVPPPGLGKDFRDPTTAWTGPQNGQWLL
Pain_TN1_1 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPTNLSDPDLLDWVKYKGNPVLVPPPGLGKDFRDPTTAWTGPQNGQWLL
Pain_TN1_2 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPTNLSDPDLLDWVKYKGNPVLVPPPGLGKDFRDPTTAWTGPQNGQWLL
Pain_TN1_3 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPTNLSDPDLLDWVKYKGNPVLVPPPGLGKDFRDPTTAWTGPQNGQWLL
Pain_TN2 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPTNLSDPDLLDWVKYKGNPVLVPPPGLGKDFRDPTTAWTGPQNGQWLL
Pain_TN2_1 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPTNLSDPDLLDWVKYKGNPVLVPPPGLGKDFRDPTTAWTGPQNGQWLL
Pain_TN2_2 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPTNLSDPDLLDWVKYKGNPVLVPPPGLGKDFRDPTTAWTGPQNGQWLL
Pain_TN2_3 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPTNLSDPDLLDWVKYKGNPVLVPPPGLGKDFRDPTTAWTGPQNGQWLL
Consensus WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPTNLSDPDLLDWVKYKGNPVLVPPPGLGKDFRDPTTAWTGPQNGQWLL

261 270 280 290 300 310 320 330 340 350 360 370 380 390
Pain_TN1 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_TN1_1 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_TN1_2 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_TN1_3 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_TN2 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_TN2_1 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_TN2_2 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_TN2_3 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Consensus TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG

391 400 410 420 430 440 450 460 470 480 490 500 510 520
Pain_TN1 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIASFEYDKVALQGIEADHYGFSCSTSGGAASRGILGPFVYVVIADQTLSELT
Pain_TN1_1 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIASFEYDKVALQGIEADHYGFSCSTSGGAASRGILGPFVYVVIADQTLSELT
Pain_TN1_2 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIASFEYDKVALQGIEADHYGFSCSTSGGAASRGILGPFVYVVIADQTLSELT
Pain_TN1_3 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIASFEYDKVALQGIEADHYGFSCSTSGGAASRGILGPFVYVVIADQTLSELT
Pain_TN2 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIASFEYDKVALQGIEADHYGFSCSTSGGAASRGILGPFVYVVIADQTLSELT
Pain_TN2_1 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIASFEYDKVALQGIEADHYGFSCSTSGGAASRGILGPFVYVVIADQTLSELT
Pain_TN2_2 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIASFEYDKVALQGIEADHYGFSCSTSGGAASRGILGPFVYVVIADQTLSELT
Pain_TN2_3 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIASFEYDKVALQGIEADHYGFSCSTSGGAASRGILGPFVYVVIADQTLSELT
Consensus WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAAELDIASFEYDKVALQGIEADHYGFSCSTSGGAASRGILGPFVYVVIADQTLSELT

521 530 540 550 560 570 580 590 600 610 620 630 639
Pain_TN1 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIWSLESANIQSFPLQDL
Pain_TN1_1 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIWSLESANIQSFPLQDL
Pain_TN1_2 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIWSLESANIQSFPLQDL
Pain_TN1_3 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIWSLESANIQSFPLQDL
Pain_TN2 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIWSLESANIQSFPLQDL
Pain_TN2_1 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIWSLESANIQSFPLQDL
Pain_TN2_2 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIWSLESANIQSFPLQDL
Pain_TN2_3 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIWSLESANIQSFPLQDL
Consensus PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGSSVTASVKIWSLESANIQSFPLQDL

1 10 20 30 40 50 60 70 80 90 100 110 120 130
Pain_SN ATGGCCACGCGAGTACCATTCCAGTTATGACCTGGAAACTCCGCCTCCATTACACATTCCTCCCGGATCAACCAGATTCCGGCCACCAGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCTCTT
Pain_SN2 ATGGCCACGCGAGTACCATTCCAGTTATGACCTGGAAACTCCGCCTCCATTACACATTCCTCCCGGACCAACCAGATTCCGGCCACCAGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCTCTT
Pain_SN1 ATGGCCACGCGAGTACCATTCCAGTTATGACCTGGAAACTCCGCCTCCATTACACATTCCTCCCGGATCAACCAGATTCCGGCCACCAGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCTCTT
Pain_SN3 ATGGCCACGCGAGTACCATTCCAGTTATGACCTGGAAACTCCGCCTCCATTACACATTCCTCCCGGATCAACCAGATTCCGGCCACCAGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCTCTT
Pain_SN4 ATGGCCACGCGAGTACCATTCCAGTTATGACCTGGAAACTCCGCCTCCATTACACATTCCTCCCGGATCAACCAGATTCCGGCCACCAGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCTCTT
Consensus ATGGCCACGCGAGTACCATTCCAGTTATGACCTGGAAACTCCGCCTCCATTACACATTCCTCCCGGATCAACCAGATTCCGGCCACCAGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCTCTT

131 140 150 160 170 180 190 200 210 220 230 240 250 260
Pain_SN TCCTTTTGTCTTCTGTAGCCTCTTTCCGATCCTCAACACCAATCACCAGGACTGCAGAGTACTCCCGTTCCGCGGCCGCCGCTCAGAGGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_SN2 TCCTTTTGTCTTCTGTAGCCTCTTTCCGATCCTCAACACCAATCACCAGGACTGCAGAGTACTCCCGTTCCGCGGCCGCCGCTCAGAGGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_SN1 TCCTTTTGTCTTCTGTAGCCTCTTTCCGATCCTCAACACCAATCACCAGGACTGCAGAGTACTCCCGTTCCGCGGCCGCCGCTCAGAGGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_SN3 TCCTTTTGTCTTCTGTAGCCTCTTTCCGATCCTCAACACCAATCACCAGGACTGCAGAGTACTCCCGTTCCGCGGCCGCCGCTCAGAGGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_SN4 TCCTTTTGTCTTCTGTAGCCTCTTTCCGATCCTCAACACCAATCACCAGGACTGCAGAGTACTCCCGTTCCGCGGCCGCCGCTCAGAGGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Consensus TCCTTTTGTCTTCTGTAGCCTCTTTCCGATCCTCAACACCAATCACCAGGACTGCAGAGTACTCCCGTTCCGCGGCCGCCGCTCAGAGGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
Pain_SN TGTGCTCAATGCTAGTCACGTTTCTTATGCGTGGTCCAAATGCTATGCTTAGCTGGCAAGAAGTACTTACCATTTCARCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACRAGGGA
Pain_SN2 TGTGCTCAATGCTAGTCACGTTTCTTATGCGTGGTCCAAATGCTATGCTTAGCTGGCAAGAAGTACTTACCATTTCARCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACRAGGGA
Pain_SN1 TGTGCTCAATGCTAGTCACGTTTCTTATGCGTGGTCCAAATGCTATGCTTAGCTGGCAAGAAGTACTTACCATTTCARCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACRAGGGA
Pain_SN3 TGTGCTCAATGCTAGTCACGTTTCTTATGCGTGGTCCAAATGCTATGCTTAGCTGGCAAGAAGTACTTACCATTTCARCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACRAGGGA
Pain_SN4 TGTGCTCAATGCTAGTCACGTTTCTTATGCGTGGTCCAAATGCTATGCTTAGCTGGCAAGAAGTACTTACCATTTCARCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACRAGGGA
Consensus TGTGCTCAATGCTAGTCACGTTTCTTATGCGTGGTCCAAATGCTATGCTTAGCTGGCAAGAAGTACTTACCATTTCARCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACRAGGGA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
Pain_SN TGGTATCATCTTTTTATCAATCAATCCAGATTACGCTATTGGGGAAATACATGCGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCCATGGTTCCTGATCAATGGT
Pain_SN2 TGGTATCATCTTTTTATCAATCAATCCAGATTACGCTATTGGGGAAATACATGCGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCCATGGTTCCTGATCAATGGT
Pain_SN1 TGGTATCATCTTTTTATCAATCAATCCAGATTACGCTATTGGGGAAATACATGCGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCCATGGTTCCTGATCAATGGT
Pain_SN3 TGGTATCATCTTTTTATCAATCAATCCAGATTACGCTATTGGGGAAATACATGCGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCCATGGTTCCTGATCAATGGT
Pain_SN4 TGGTATCATCTTTTTATCAATCAATCCAGATTACGCTATTGGGGAAATACATGCGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCCATGGTTCCTGATCAATGGT
Consensus TGGTATCATCTTTTTATCAATCAATCCAGATTACGCTATTGGGGAAATACATGCGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCCATGGTTCCTGATCAATGGT

521 530 540 550 560 570 580 590 600 610 620 630 640 650
Pain_SN ACGATATAAACGGTGTCTGGACTGGGTCGCTACCATCTACCCGATGGTCAAGTATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGGTACCCCACTTATCTGA
Pain_SN2 ACGATATAAACGGTGTCTGGACTGGGTCGCTACCATCTACCCGATGGTCAAGTATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGGTACCCCACTTATCTGA
Pain_SN1 ACGATATAAACGGTGTCTGGACTGGGTCGCTACCATCTACCCGATGGTCAAGTATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGGTACCCCACTTATCTGA
Pain_SN3 ACGATATAAACGGTGTCTGGACTGGGTCGCTACCATCTACCCGATGGTCAAGTATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGGTACCCCACTTATCTGA
Pain_SN4 ACGATATAAACGGTGTCTGGACTGGGTCGCTACCATCTACCCGATGGTCAAGTATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGGTACCCCACTTATCTGA
Consensus ACGATATAAACGGTGTCTGGACTGGGTCGCTACCATCTACCCGATGGTCAAGTATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGGTACCCCACTTATCTGA

651 660 670 680 690 700 710 720 730 740 750 760 770 780
Pain_SN TCCTCTCTTCTAGACTGGGTCAGTCAAAAGGCCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Pain_SN2 TCCTCTCTTCTAGACTGGGTCAGTCAAAAGGCCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Pain_SN1 TCCTCTCTTCTAGACTGGGTCAGTCAAAAGGCCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Pain_SN3 TCCTCTCTTCTAGACTGGGTCAGTCAAAAGGCCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Pain_SN4 TCCTCTCTTCTAGACTGGGTCAGTCAAAAGGCCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Consensus TCCTCTCTTCTAGACTGGGTCAGTCAAAAGGCCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
Pain_SN ACARTCGGGTCTAAGATTGGTAAACGGGATTGCACCTGTTTATGAAACTTCCAACTCCACAGCTTAAAGCTATTGGATGAGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT
Pain_SN2 ACARTCGGGTCTAAGATTGGTAAACGGGATTGCACCTGTTTATGAAACTTCCAACTCCACAGCTTAAAGCTATTGGATGAGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT
Pain_SN1 ACARTCGGGTCTAAGATTGGTAAACGGGATTGCACCTGTTTATGAAACTTCCAACTCCACAGCTTAAAGCTATTGGATGAGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT
Pain_SN3 ACARTCGGGTCTAAGATTGGTAAACGGGATTGCACCTGTTTATGAAACTTCCAACTCCACAGCTTAAAGCTATTGGATGAGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT
Pain_SN4 ACARTCGGGTCTAAGATTGGTAAACGGGATTGCACCTGTTTATGAAACTTCCAACTCCACAGCTTAAAGCTATTGGATGAGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT
Consensus ACARTCGGGTCTAAGATTGGTAAACGGGATTGCACCTGTTTATGAAACTTCCAACTCCACAGCTTAAAGCTATTGGATGAGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
Pain_SN TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_SN2 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_SN1 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_SN3 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_SN4 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Consensus TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
Pain_SN GACAAAGAAACAATGGACACCCGATACCCGGATTGGATTGTGGATTGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGGAACACAGAGAGTACTGTGGGGA
Pain_SN2 GACAAAGAAACAATGGACACCCGATACCCGGATTGGATTGTGGATTGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGGAACACAGAGAGTACTGTGGGGA
Pain_SN1 GACAAAGAAACAATGGACACCCGATACCCGGATTGGATTGTGGATTGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGGAACACAGAGAGTACTGTGGGGA
Pain_SN3 GACAAAGAAACAATGGACACCCGATACCCGGATTGGATTGTGGATTGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGGAACACAGAGAGTACTGTGGGGA
Pain_SN4 GACAAAGAAACAATGGACACCCGATACCCGGATTGGATTGTGGATTGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGGAACACAGAGAGTACTGTGGGGA
Consensus GACAAAGAAACAATGGACACCCGATACCCGGATTGGATTGTGGATTGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGGAACACAGAGAGTACTGTGGGGA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
Pain_SN TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCCTTACGACRAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGARAGAA
Pain_SN2 TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCCTTACGACRAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGARAGAA
Pain_SN1 TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCCTTACGACRAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGARAGAA
Pain_SN3 TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCCTTACGACRAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGARAGAA
Pain_SN4 TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCCTTACGACRAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGARAGAA
Consensus TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCCTTACGACRAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGARAGAA

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
Pain_SN TTGAAGCTTAAAGAGCGGGTATCCTATTGTTAAGCAAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGGCTCATTGAGTGGCAAAAGTCGC
Pain_SN2 TTGAAGCTTAAAGAGCGGGTATCCTATTGTTAAGCAAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGGCTCATTGAGTGGCAAAAGTCGC
Pain_SN1 TTGAAGCTTAAAGAGCGGGTATCCTATTGTTAAGCAAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGGCTCATTGAGTGGCAAAAGTCGC
Pain_SN3 TTGAAGCTTAAAGAGCGGGTATCCTATTGTTAAGCAAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGGCTCATTGAGTGGCAAAAGTCGC
Pain_SN4 TTGAAGCTTAAAGAGCGGGTATCCTATTGTTAAGCAAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGGCTCATTGAGTGGCAAAAGTCGC
Consensus TTGAAGCTTAAAGAGCGGGTATCCTATTGTTAAGCAAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGGCTCATTGAGTGGCAAAAGTCGC

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
Pain_SN GCTCCAGGGAATATTGAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTAATTGCTGATCAACGCTATCTGAGCTAACG
Pain_SN2 GCTCCAGGGAATATTGAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTAATTGCTGATCAACGCTATCTGAGCTAACG
Pain_SN1 GCTCCAGGGAATATTGAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTAATTGCTGATCAACGCTATCTGAGCTAACG
Pain_SN3 GCTCCAGGGAATATTGAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTAATTGCTGATCAACGCTATCTGAGCTAACG
Pain_SN4 GCTCCAGGGAATATTGAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTAATTGCTGATCAACGCTATCTGAGCTAACG
Consensus GCTCCAGGGAATATTGAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTAATTGCTGATCAACGCTATCTGAGCTAACG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
Pain_SN CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAAAACAGATCTCAGAGGCTCCGGGAGTTGCTAARACAGTTTATGGTGTTCAGTACCCGTTGG
Pain_SN2 CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAAAACAGATCTCAGAGGCTCCGGGAGTTGCTAARACAGTTTATGGTGTTCAGTACCCGTTGG
Pain_SN1 CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAAAACAGATCTCAGAGGCTCCGGGAGTTGCTAARACAGTTTATGGTGTTCAGTACCCGTTGG
Pain_SN3 CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAAAACAGATCTCAGAGGCTCCGGGAGTTGCTAARACAGTTTATGGTGTTCAGTACCCGTTGG
Pain_SN4 CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAAAACAGATCTCAGAGGCTCCGGGAGTTGCTAARACAGTTTATGGTGTTCAGTACCCGTTGG
Consensus CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAAAACAGATCTCAGAGGCTCCGGGAGTTGCTAARACAGTTTATGGTGTTCAGTACCCGTTGG

1691 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800 1810 1820
Pain_SN ACGGTGAAAAACATTCCGATGAGATTATGGTGGACCACTCAATTGTGGAGAGCTTTGCTCAGGGAGGAGAACAGTCAACATCCGGAATTTACCCAACAAAGGCAAGTGAATGGAGCAGCAGCTCTT
Pain_SN2 ACGGTGAAAAACATTCCGATGAGATTATGGTGGACCACTCAATTGTGGAGAGCTTTGCTCAGGGAGGAGAACAGTCAACATCCGGAATTTACCCAACAAAGGCAAGTGAATGGAGCAGCAGCTCTT
Pain_SN1 ACGGTGAAAAACATTCCGATGAGATTATGGTGGACCACTCAATTGTGGAGAGCTTTGCTCAGGGAGGAGAACAGTCAACATCCGGAATTTACCCAACAAAGGCAAGTGAATGGAGCAGCAGCTCTT
Pain_SN3 ACGGTGAAAAACATTCCGATGAGATTATGGTGGACCACTCAATTGTGGAGAGCTTTGCTCAGGGAGGAGAACAGTCAACATCCGGAATTTACCCAACAAAGGCAAGTGAATGGAGCAGCAGCTCTT
Pain_SN4 ACGGTGAAAAACATTCCGATGAGATTATGGTGGACCACTCAATTGTGGAGAGCTTTGCTCAGGGAGGAGAACAGTCAACATCCGGAATTTACCCAACAAAGGCAAGTGAATGGAGCAGCAGCTCTT
Consensus ACGGTGAAAAACATTCCGATGAGATTATGGTGGACCACTCAATTGTGGAGAGCTTTGCTCAGGGAGGAGAACAGTCAACATCCGGAATTTACCCAACAAAGGCAAGTGAATGGAGCAGCAGCTCTT

1821 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
Pain_SN CGTTTTCAACAATGCCACAGGGTCTAGCGTGAATGCTCCGTCAGAGTTGGTCACTTGAAGTGGCTAATATTCAATCTTCCCTTGCAGAGCTTGTA
Pain_SN2 CGTTTTCAACAATGCCACAGGGTCTAGCGTGAATGCTCCGTCAGAGTTGGTCACTTGAAGTGGCTAATATTCAATCTTCCCTTGCAGAGCTTGTA
Pain_SN1 CGTTTTCAACAATGCCACAGGGTCTAGCGTGAATGCTCCGTCAGAGTTGGTCACTTGAAGTGGCTAATATTCAATCTTCCCTTGCAGAGCTTGTA
Pain_SN3 CGTTTTCAACAATGCCACAGGGTCTAGCGTGAATGCTCCGTCAGAGTTGGTCACTTGAAGTGGCTAATATTCAATCTTCCCTTGCAGAGCTTGTA
Pain_SN4 CGTTTTCAACAATGCCACAGGGTCTAGCGTGAATGCTCCGTCAGAGTTGGTCACTTGAAGTGGCTAATATTCAATCTTCCCTTGCAGAGCTTGTA
Consensus CGTTTTCAACAATGCCACAGGGTCTAGCGTGAATGCTCCGTCAGAGTTGGTCACTTGAAGTGGCTAATATTCAATCTTCCCTTGCAGAGCTTGTA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
Pain_DA ATGGCCACGCGAGTACCATTCCAGTTATGACCCGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACCCGATTCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Pain_DA2 ATGGCCACGCGAGTACCATTCCAGTTATGACCCGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACCCGATTCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Pain_DA1 ATGGCCACGCGAGTACCATTCCAGTTATGACCCGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACCCGATTCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Consensus ATGGCCACGCGAGTACCATTCCAGTTATGACCCGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACCCGATTCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT

131 140 150 160 170 180 190 200 210 220 230 240 250 260
Pain_DA TCCTTTTGTCTTCTGTAGCCTTCTTTCCGATCTCAACAACCAAGTCAACCGGACTTGACAGAGTAACTCCCGTTCCGCGGCGCCGCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_DA2 TCCTTTTGTCTTCTGTAGCCTTCTTTCCGATCTCAACAACCAAGTCAACCGGACTTGACAGAGTAACTCCCGTTCCGCGGCGCCGCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_DA1 TCCTTTTGTCTTCTGTAGCCTTCTTTCCGATCTCAACAACCAAGTCAACCGGACTTGACAGAGTAACTCCCGTTCCGCGGCGCCGCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Consensus TCCTTTTGTCTTCTGTAGCCTTCTTTCCGATCTCAACAACCAAGTCAACCGGACTTGACAGAGTAACTCCCGTTCCGCGGCGCCGCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
Pain_DA TGTGTCATGCTAGTACATTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA
Pain_DA2 TGTGTCATGCTAGTACATTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA
Pain_DA1 TGTGTCATGCTAGTACATTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA
Consensus TGTGTCATGCTAGTACATTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
Pain_DA TGGTATCATCTTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTACTTGGCTTTTGGCATGGTTCCTGATCAATGGT
Pain_DA2 TGGTATCATCTTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTACTTGGCTTTTGGCATGGTTCCTGATCAATGGT
Pain_DA1 TGGTATCATCTTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTACTTGGCTTTTGGCATGGTTCCTGATCAATGGT
Consensus TGGTATCATCTTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTACTTGGCTTTTGGCATGGTTCCTGATCAATGGT

521 530 540 550 560 570 580 590 600 610 620 630 640 650
Pain_DA ACGATATTACGGTGTCTGGACTGGGTCCGCCACCATCCTACCCGATGGTCAAGTATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGC AAAATCTTGGCTACCCCACTTATCTGA
Pain_DA2 ACGATATTACGGTGTCTGGACTGGGTCCGCCACCATCCTACCCGATGGTCAAGTATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGC AAAATCTTGGCTACCCCACTTATCTGA
Pain_DA1 ACGATATTACGGTGTCTGGACTGGGTCCGCCACCATCCTACCCGATGGTCAAGTATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGC AAAATCTTGGCTACCCCACTTATCTGA
Consensus ACGATATTACGGTGTCTGGACTGGGTCCGCCACCATCCTACCCGATGGTCAAGTATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGC AAAATCTTGGCTACCCCACTTATCTGA

651 660 670 680 690 700 710 720 730 740 750 760 770 780
Pain_DA TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCRAACCCGGTCTGGTTCTCCACCCGGCATGGTATCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Pain_DA2 TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCRAACCCGGTCTGGTTCTCCACCCGGCATGGTATCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Pain_DA1 TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCRAACCCGGTCTGGTTCTCCACCCGGCATGGTATCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Consensus TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCRAACCCGGTCTGGTTCTCCACCCGGCATGGTATCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
Pain_DA ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGCGCTTGTATTGAAACTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_DA2 ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGCGCTTGTATTGAAACTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_DA1 ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGCGCTTGTATTGAAACTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Consensus ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGCGCTTGTATTGAAACTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
Pain_DA TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATAACGGCCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_DA2 TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATAACGGCCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_DA1 TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATAACGGCCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Consensus TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATAACGGCCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
Pain_DA GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Pain_DA2 GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Pain_DA1 GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Consensus GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTATGACCCGAGAAACACGAGAGTACTGTGGGGA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
Pain_DA TGGATTGGGGAACCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGTCTTACGACAAAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Pain_DA2 TGGATTGGGGAACCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGTCTTACGACAAAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Pain_DA1 TGGATTGGGGAACCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGTCTTACGACAAAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Consensus TGGATTGGGGAACCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGTCTTACGACAAAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
Pain_DA TTGAAGCTTAAGAGTGGGTGATCCTATTGTTAAGCAAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGTGCAGAGTTGGATATAGAAGCCTCATTGAGTGGACAAGTTCGC
Pain_DA2 TTGAAGCTTAAGAGTGGGTGATCCTATTGTTAAGCAAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGTGCAGAGTTGGATATAGAAGCCTCATTGAGTGGACAAGTTCGC
Pain_DA1 TTGAAGCTTAAGAGTGGGTGATCCTATTGTTAAGCAAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGTGCAGAGTTGGATATAGAAGCCTCATTGAGTGGACAAGTTCGC
Consensus TTGAAGCTTAAGAGTGGGTGATCCTATTGTTAAGCAAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGTGCAGAGTTGGATATAGAAGCCTCATTGAGTGGACAAGTTCGC

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
Pain_DA GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTAATTGCTGATCAAAGCTATCTGAGCTAACG
Pain_DA2 GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTAATTGCTGATCAAAGCTATCTGAGCTAACG
Pain_DA1 GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTAATTGCTGATCAAAGCTATCTGAGCTAACG
Consensus GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTAATTGCTGATCAAAGCTATCTGAGCTAACG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
Pain_DA CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGTGCAGCTGAGACTCACTTCTGTGCTGATCAAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAAACAAGTTTATGGTAGTTCAGTACCCGTGTTAG
Pain_DA2 CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGTGCAGCTGAGACTCACTTCTGTGCTGATCAAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAAACAAGTTTATGGTAGTTCAGTACCCGTGTTAG
Pain_DA1 CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGTGCAGCTGAGACTCACTTCTGTGCTGATCAAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAAACAAGTTTATGGTAGTTCAGTACCCGTGTTAG
Consensus CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGTGCAGCTGAGACTCACTTCTGTGCTGATCAAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAAACAAGTTTATGGTAGTTCAGTACCCGTGTTAG

1691 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800 1810 1820
Pain_DA ACGGTGAAAAACATTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCCAAGGAGGAGAACAGTCAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT
Pain_DA2 ACGGTGAAAAACATTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCCAAGGAGGAGAACAGTCAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT
Pain_DA1 ACGGTGAAAAACATTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCCAAGGAGGAGAACAGTCAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT
Consensus ACGGTGAAAAACATTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCCAAGGAGGAGAACAGTCAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT

1821 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
Pain_DA CGTTTTCAACAATGCCACAGGGGCTAGCGTGACTGCTTCCGTCAGATTGGTCACTTGAGTCGGCTAATATTCGATCCTTCCCTTGCAGACTTGTA
Pain_DA2 CGTTTTCAACAATGCCACAGGGGCTAGCGTGACTGCTTCCGTCAGATTGGTCACTTGAGTCGGCTAATATTCGATCCTTCCCTTGCAGACTTGTA
Pain_DA1 CGTTTTCAACAATGCCACAGGGGCTAGCGTGACTGCTTCCGTCAGATTGGTCACTTGAGTCGGCTAATATTCGATCCTTCCCTTGCAGACTTGTA
Consensus CGTTTTCAACAATGCCACAGGGGCTAGCGTGACTGCTTCCGTCAGATTGGTCACTTGAGTCGGCTAATATTCGATCCTTCCCTTGCAGACTTGTA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
Pain_TN2 ATGGCCACGCAGTACCATTCCAGTTATGACCCGGAAACTCCGCTCCATTACACATTCCTCCCGGATCAACCGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Pain_TN2_1 ATGGCCACGCAGTACCATTCCAGTTATGACCCGGAAACTCCGCTCCATTACACATTCCTCCCGGATCAACCGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Pain_TN2_2 ATGGCCACGCAGTACCATTCCAGTTATGACCCGGAAACTCCGCTCCATTACACATTCCTCCCGGATCAACCGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Pain_TN2_3 ATGGCCACGCAGTACCATTCCAGTTATGACCCGGAAACTCCGCTCCATTACACATTCCTCCCGGATCAACCGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Consensus ATGGCCACGCAGTACCATTCCAGTTATGACCCGGAAACTCCGCTCCATTACACATTCCTCCCGGATCAACCGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT

131 140 150 160 170 180 190 200 210 220 230 240 250 260
Pain_TN2 TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACACCAAGTCACCGGACTTGACAGAGTAACTCCCGTTCGCCGGCCGCCGCTCAGAGGTTTCTCAGGGAGTCCCGATAGACTTTTCGAGA
Pain_TN2_1 TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACACCAAGTCACCGGACTTGACAGAGTAACTCCCGTTCGCCGGCCGCCGCTCAGAGGTTTCTCAGGGAGTCCCGATAGACTTTTCGAGA
Pain_TN2_2 TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACACCAAGTCACCGGACTTGACAGAGTAACTCCCGTTCGCCGGCCGCCGCTCAGAGGTTTCTCAGGGAGTCCCGATAGACTTTTCGAGA
Pain_TN2_3 TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACACCAAGTCACCGGACTTGACAGAGTAACTCCCGTTCGCCGGCCGCCGCTCAGAGGTTTCTCAGGGAGTCCCGATAGACTTTTCGAGA
Consensus TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACACCAAGTCACCGGACTTGACAGAGTAACTCCCGTTCGCCGGCCGCCGCTCAGAGGTTTCTCAGGGAGTCCCGATAGACTTTTCGAGA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
Pain_TN2 TGTGTCATGCTAGTACGTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGA
Pain_TN2_1 TGTGTCATGCTAGTACGTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGA
Pain_TN2_2 TGTGTCATGCTAGTACGTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGA
Pain_TN2_3 TGTGTCATGCTAGTACGTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGA
Consensus TGTGTCATGCTAGTACGTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
Pain_TN2 TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT
Pain_TN2_1 TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT
Pain_TN2_2 TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT
Pain_TN2_3 TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT
Consensus TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT

521 530 540 550 560 570 580 590 600 610 620 630 640 650
Pain_TN2 ACGATATTAACGGTGTCTGGACTGGGTCCGCTACCATCCTACCCGATGGTCAGATCATGATGCTTTATACCGGTGACACTGATGATTATGTGCAAGTGCAAAATCTTGCCTACCCACCACTTATCTGA
Pain_TN2_1 ACGATATTAACGGTGTCTGGACTGGGTCCGCTACCATCCTACCCGATGGTCAGATCATGATGCTTTATACCGGTGACACTGATGATTATGTGCAAGTGCAAAATCTTGCCTACCCACCACTTATCTGA
Pain_TN2_2 ACGATATTAACGGTGTCTGGACTGGGTCCGCTACCATCCTACCCGATGGTCAGATCATGATGCTTTATACCGGTGACACTGATGATTATGTGCAAGTGCAAAATCTTGCCTACCCACCACTTATCTGA
Pain_TN2_3 ACGATATTAACGGTGTCTGGACTGGGTCCGCTACCATCCTACCCGATGGTCAGATCATGATGCTTTATACCGGTGACACTGATGATTATGTGCAAGTGCAAAATCTTGCCTACCCACCACTTATCTGA
Consensus ACGATATTAACGGTGTCTGGACTGGGTCCGCTACCATCCTACCCGATGGTCAGATCATGATGCTTTATACCGGTGACACTGATGATTATGTGCAAGTGCAAAATCTTGCCTACCCACCACTTATCTGA

651 660 670 680 690 700 710 720 730 740 750 760 770 780
Pain_TN2 TCCTCTCCTTCTAGACTGGGTCAAGTACAAGGGCAACCCGGTCTGGTTCCTCCACCcGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGACCCCAAAATGGGCAATGGCTCTTA
Pain_TN2_1 TCCTCTCCTTCTAGACTGGGTCAAGTACAAGGGCAACCCGGTCTGGTTCCTCCACCcGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGACCCCAAAATGGGCAATGGCTCTTA
Pain_TN2_2 TCCTCTCCTTCTAGACTGGGTCAAGTACAAGGGCAACCCGGTCTGGTTCCTCCACCcGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGACCCCAAAATGGGCAATGGCTCTTA
Pain_TN2_3 TCCTCTCCTTCTAGACTGGGTCAAGTACAAGGGCAACCCGGTCTGGTTCCTCCACCcGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGACCCCAAAATGGGCAATGGCTCTTA
Consensus TCCTCTCCTTCTAGACTGGGTCAAGTACAAGGGCAACCCGGTCTGGTTCCTCCACCcGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGACCCCAAAATGGGCAATGGCTCTTA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
Pain_TN2 ACAATCGGGTCTAAGATTGGTAAACCGGATTGCACTTGTATGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_TN2_1 ACAATCGGGTCTAAGATTGGTAAACCGGATTGCACTTGTATGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_TN2_2 ACAATCGGGTCTAAGATTGGTAAACCGGATTGCACTTGTATGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_TN2_3 ACAATCGGGTCTAAGATTGGTAAACCGGATTGCACTTGTATGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Consensus ACAATCGGGTCTAAGATTGGTAAACCGGATTGCACTTGTATGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
Pain_TN2 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATACCGGCCGGGTGTAAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_TN2_1 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATACCGGCCGGGTGTAAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_TN2_2 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATACCGGCCGGGTGTAAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_TN2_3 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATACCGGCCGGGTGTAAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Consensus TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATACCGGCCGGGTGTAAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
Pain_TN2 GCAAGAACAATGGACACCCGATAAGCCGGATTGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Pain_TN2_1 GCAAGAACAATGGACACCCGATAAGCCGGATTGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Pain_TN2_2 GCAAGAACAATGGACACCCGATAAGCCGGATTGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Pain_TN2_3 GCAAGAACAATGGACACCCGATAAGCCGGATTGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Consensus GCAAGAACAATGGACACCCGATAAGCCGGATTGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGAAACACGAGAGTACTGTGGGGA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
Pain_TN2 TGGATTGGGGAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGGACAGTGTCTTACGACAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Pain_TN2_1 TGGATTGGGGAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGGACAGTGTCTTACGACAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Pain_TN2_2 TGGATTGGGGAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGGACAGTGTCTTACGACAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Pain_TN2_3 TGGATTGGGGAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGGACAGTGTCTTACGACAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Consensus TGGATTGGGGAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGGACAGTGTCTTACGACAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
Pain_TN2 TTGAAGCTTAAAGCGGGTATCCTATTGTTAAGCAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCTCATTGAGTGGACAAAGTCCG
Pain_TN2_1 TTGAAGCTTAAAGCGGGTATCCTATTGTTAAGCAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCTCATTGAGTGGACAAAGTCCG
Pain_TN2_2 TTGAAGCTTAAAGCGGGTATCCTATTGTTAAGCAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCTCATTGAGTGGACAAAGTCCG
Pain_TN2_3 TTGAAGCTTAAAGCGGGTATCCTATTGTTAAGCAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCTCATTGAGTGGACAAAGTCCG
Consensus TTGAAGCTTAAAGCGGGTATCCTATTGTTAAGCAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCTCATTGAGTGGACAAAGTCCG

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
Pain_TN2 GCTCCAGGGAAATATTGAAGCAGATCATGTAGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGATGATCAACGCTATCTGAGCTAACG
Pain_TN2_1 GCTCCAGGGAAATATTGAAGCAGATCATGTAGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGATGATCAACGCTATCTGAGCTAACG
Pain_TN2_2 GCTCCAGGGAAATATTGAAGCAGATCATGTAGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGATGATCAACGCTATCTGAGCTAACG
Pain_TN2_3 GCTCCAGGGAAATATTGAAGCAGATCATGTAGATTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGATGATCAACGCTATCTGAGCTAACG
Consensus GCTCCaGGGAAATATTGAAGCAGATCATGTAGgTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGATGATCAACGCTATCTGAGCTAACG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
Pain_TN2 CCAGTTTACTTCTTCAATTTCTAAGGAGCTGATGGTCGAGCTGAGACTCACTTCTGTGCTGATCAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAARCGAGTTTATGGTAGTTCAGTACCCGTTGG
Pain_TN2_1 CCAGTTTACTTCTTCAATTTCTAAGGAGCTGATGGTCGAGCTGAGACTCACTTCTGTGCTGATCAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAARCGAGTTTATGGTAGTTCAGTACCCGTTGG
Pain_TN2_2 CCAGTTTACTTCTTCAATTTCTAAGGAGCTGATGGTCGAGCTGAGACTCACTTCTGTGCTGATCAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAARCGAGTTTATGGTAGTTCAGTACCCGTTGG
Pain_TN2_3 CCAGTTTACTTCTTCAATTTCTAAGGAGCTGATGGTCGAGCTGAGACTCACTTCTGTGCTGATCAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAARCGAGTTTATGGTAGTTCAGTACCCGTTGG
Consensus CCAGTTTACTTCTTCAATTTCTAAGGAGCTGATGGTCGAGCTGAGACTCACTTCTGTGCTGATCAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAARCGAGTTTATGGTAGTTCAGTACCCGTTGG

1691 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800 1810 1820
Pain_TN2 ACGGTGAAAAACATTGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCTCAGGAGGAGAGACAGTCATACATCGCGAATTTACCCARCAAGGCAAGTGAATGGAGCAGCAGCTCTT
Pain_TN2_1 ACGGTGAAAAACATTGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCTCAGGAGGAGAGACAGTCATACATCGCGAATTTACCCARCAAGGCAAGTGAATGGAGCAGCAGCTCTT
Pain_TN2_2 ACGGTGAAAAACATTGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCTCAGGAGGAGAGACAGTCATACATCGCGAATTTACCCARCAAGGCAAGTGAATGGAGCAGCAGCTCTT
Pain_TN2_3 ACGGTGAAAAACATTGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCTCARGGAGGAGAGACAGTCATACATCGCGAATTTACCCARCAAGGCAAGTGAATGGAGCAGCAGCTCTT
Consensus ACGGTGAAAAACATTGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCTCARGGAGGAGAGACAGTCATACATCGCGAATTTACCCARCAAGGCAAGTGAATGGAGCAGCAGCTCTT

1821 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
Pain_TN2 CGTTTTCAATGATGCCACAGGGGCTAGCGTGACTGCCTCCGTCAGATTGGTCACTTGAGTCGGCTAATATTCGATCCTTCCCTTGCAAGACTTGTA
Pain_TN2_1 CGTTTTCAACATGATGCCACAGGGGCTAGCGTGACTGCCTCCGTCAGATTGGTCACTTGAGTCGGCTAATATTCGATCCTTCCCTTGCAAGACTTGTA
Pain_TN2_2 CGTTTTCAACATGATGCCACAGGGGCTAGCGTGACTGCCTCCGTCAGATTGGTCACTTGAGTCGGCTAATATTCGATCCTTCCCTTGCAAGACTTGTA
Pain_TN2_3 CGTTTTCAACATGATGCCACAGGGGCTAGCGTGACTGCCTCCGTCAGATTGGTCACTTGAGTCGGCTAATATTCGATCCTTCCCTTGCAAGACTTGTA
Consensus CGTTTTCAACaATGATGCCACAGGGGCTAGCGTGACTGCCTCCGTCAGATTGGTCACTTGAGTCGGCTAATATTCGATCCTTCCCTTGCAAGACTTGTA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
Pain_P18N1 MATQYHSSYDPENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNMQSPDLQSNRSRSPAPPSRGVYSGVSDKTFRDVYNASHISYAWNSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_P18N1_1 MATQYHSSYDPENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNMQSPDLQSNRSRSPAPPSRGVYSGVSDKTFRDVYNASHISYAWNSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_P18N1_2 MATQYHSSYDPENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNMQSPDLQSNRSRSPAPPSRGVYSGVSDKTFRDVYNASHISYAWNSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_P18N2 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNMQSPDLQSNRSRSPAPPSRGVYSGVSDKTFRDVYNASHVSYAWNSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_P18N2_1 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNMQSPDLQSNRSRSPAPPSRGVYSGVSDKTFRDVYNASHVSYAWNSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_P18N2_3 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNMQSPDLQSNRSRSPAPPSRGVYSGVSDKTFRDVYNASHVSYAWNSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_P18N2_2 MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNMQSPDLQSNRSRSPAPPSRGVYSGVSDKTFRDVYNASHVSYAWNSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Consensus MATQYHSSYDLENSASHYTFLLPDQPDGHRKSLKIISGIFLSSFLLLVAFFPILNMQSPDLQSNRSRSPAPPSRGVYSGVSDKTFRDVYNASHISYAWNSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG

131 140 150 160 170 180 190 200 210 220 230 240 250 260
Pain_P18N1 WYHLFYQYNPDSAINGNITWGHAYSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIMHLYTGDYDDYVQVQNLAYPTNLSDPDLLDWV KYKGNPVLVPPP GIGIKDFRDPTTAWTGPQNGQWLL
Pain_P18N1_1 WYHLFYQYNPDSAINGNITWGHAYSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIMHLYTGDYDDYVQVQNLAYPTNLSDPDLLDWV KYKGNPVLVPPP GIGIKDFRDPTTAWTGPQNGQWLL
Pain_P18N1_2 WYHLFYQYNPDSAINGNITWGHAYSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIMHLYTGDYDDYVQVQNLAYPTNLSDPDLLDWV KYKGNPVLVPPP GIGIKDFRDPTTAWTGPQNGQWLL
Pain_P18N2 WYHLFYQYNPDSAINGNITWGHAYSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIMHLYTGDYDDYVQVQNLAYPTNLSDPDLLDWV KYKGNPVLVPPP GIGIKDFRDPTTAWTGPQNGQWLL
Pain_P18N2_1 WYHLFYQYNPDSAINGNITWGHAYSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIMHLYTGDYDDYVQVQNLAYPTNLSDPDLLDWV KYKGNPVLVPPP GIGIKDFRDPTTAWTGPQNGQWLL
Pain_P18N2_3 WYHLFYQYNPDSAINGNITWGHAYSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIMHLYTGDYDDYVQVQNLAYPTNLSDPDLLDWV KYKGNPVLVPPP GIGIKDFRDPTTAWTGPQNGQWLL
Pain_P18N2_2 WYHLFYQYNPDSAINGNITWGHAYSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIMHLYTGDYDDYVQVQNLAYPTNLSDPDLLDWV KYKGNPVLVPPP GIGIKDFRDPTTAWTGPQNGQWLL
Consensus WYHLFYQYNPDSAINGNITWGHAYSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIMHLYTGDYDDYVQVQNLAYPTNLSDPDLLDWV KYKGNPVLVPPP GIGIKDFRDPTTAWTGPQNGQWLL

261 270 280 290 300 310 320 330 340 350 360 370 380 390
Pain_P18N1 TIGSKIGKTGIALVYETSNTSFKLLDEVLHAYPGTGMWECYDFYPVSTTEKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDL TKNKATPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_P18N1_1 TIGSKIGKTGIALVYETSNTSFKLLDEVLHAYPGTGMWECYDFYPVSTTEKTNGLDTSYNGPGVKRVLKASLDDNKQDHYAIGTYDL TKNKATPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_P18N1_2 TIGSTIGKTGIALVYETSNTSFKLLDEVLHAYPGTGMWECYDFYPVSTTEKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDL TKNKATPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_P18N2 TIGSKIGKTGIALVYETSNTSFKLLDEVLHAYPGTGMWECYDFYPVSTTEKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDL TKNKATPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_P18N2_1 TIGSKIGKTGIALVYETSNTSFKLLDEVLHAYPGTGMWECYDFYPVSTTEKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDL TKNKATPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_P18N2_3 TIGSKIGKTGIALVYETSNTSFKLLDEVLHAYPGTGMWECYDFYPVSTTEKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTHDL TKNKATPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_P18N2_2 TIGSKIGKTGIALVYETSNTSFKLLDEVLHAYPGTGMWECYDFYPVSTTEKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDL TKNKATPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Consensus TIGSKIGKTGIALVYETSNTSFKLLDEVLHAYPGTGMWECYDFYPVSTTEKTNGLDTSYNGPGVKHVLKASLDDNKQDHYAIGTYDL TKNKATPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG

391 400 410 420 430 440 450 460 470 480 490 500 510 520
Pain_P18N1 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRVGDPIVKQVNLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFSCSTSGGAASRGILGPFVYVYIADQKLSLSELT
Pain_P18N1_1 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRVGDPIVKQVNLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFSCSTSGGAASRGILGPFVYVYIADQKLSLSELT
Pain_P18N1_2 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRVGDPIVKQVNLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFSCSTSGGAASRGILGPFVYVYIADQKLSLSELT
Pain_P18N2 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFSCSTSGGAASRGILGPFVYVYIADQTLSELT
Pain_P18N2_1 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFSCSTSGGAASRGILGPFVYVYIADQTLSELT
Pain_P18N2_3 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFSCSTSGGAASRGILGPFVYVYIADQTLSELT
Pain_P18N2_2 WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFSCSTSGGAASRGILGPFVYVYIADQTLSELT
Consensus WIGETDSESADLQKGWASVQSIPTVLYDKKTGTHLLQWPVEEIESLRAGDPIVKQVNLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFSCSTSGGAASRGILGPFVYVYIADQTLSELT

521 530 540 550 560 570 580 590 600 610 620 630 639
Pain_P18N1 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAYNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL
Pain_P18N1_1 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAYNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL
Pain_P18N1_2 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAYNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL
Pain_P18N2 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAYNGAARLFVFNATGSSVTASVKIWSLESANIQSFPLQDL
Pain_P18N2_1 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAYNGAARLFVFNATGSSVTASVKIWSLESANIQSFPLQDL
Pain_P18N2_3 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAYNGAARLFVFNATGSSVTASVKIWSLESANIQSFPLQDL
Pain_P18N2_2 PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAYNGAARLFVFNATGSSVTASVKIWSLESANIQSFPLQDL
Consensus PYYFYISKGADGRAETHFCADQTRSSEAPGVAKQVYGGSSVPVLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAYNGAARLFVFNATGSSVTASVKIWSLESANIQSFPLQDL

1 10 20 30 40 50 60 70 80 90 100 110 120 130
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
Pain_P40N1 MATQYHSSYDPENSASHYTFLLPDQHDSGHRKSIKIISGIFLSSLLLLSLVFFPILNNQSPDLQSNASHPAPPSRGVYSGVSDKTFRDVYNASHVSYAWSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_P40N1_1 MATQYHSSYDPENSASHYTFLLPDQHDSGHRKSIKIISGIFLSSLLLLSLVFFPILNNQSPDLQSNASHPAPPSRGVYSGVSDKTFRDVYNASHVSYAWSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_P40N1_2 MATQYHSSYDPENSASHYTFLLPDQHDSGHRKSIKIISGIFLSSLLLLSLVFFPILNNQSPDLQSNASHPAPPSRGVYSGVSDKTFRDVYNASHVSYAWSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_P40N1_4 MATQYHSSYDPENSASHYTFLLPDQHDSGHRKSIKIISGIFLSSLLLLSLVFFPILNNQSPDLQSNASHPAPPSRGVYSGVSDKTFRDVYNASHVSYAWSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_P40N1_3 MATQYHSSYDPENSASHYTFLLPDQHDSGHRKSIKIISGIFLSSLLLLSLVFFPILNNQSPDLQSNASHPAPPSRGVYSGVSDKTFRDVYNASHVSYAWSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_P40N2 MATQYHSSYDPENSASHYTFLLPDQHDSGHRKSLKIISGIFLSSLLLLSLVFFPILNNQSPDLQSNARSPAPPSRGVYSGVSDKTFRDVYNASHVSYAWSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_P40N2_1 MATQYHSSYDPENSASHYTFLLPDQHDSGHRKSLKIISGIFLSSLLLLSLVFFPILNNQSPDLQSNARSPAPPSRGVYSGVSDKTFRDVYNASHVSYAWSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Pain_P40N2_2 MATQYHSSYDPENSASHYTFLLPDQHDSGHRKSLKIISGIFLSSLLLLSLVFFPILNNQSPDLQSNARSPAPPSRGVYSGVSDKTFRDVYNASHVSYAWSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG
Consensus MATQYHSSYDPENSASHYTFLLPDQHDSGHRKSIKIISGIFLSSLLLLSLVFFPILNNQSPDLQSNASHPAPPSRGVYSGVSDKTFRDVYNASHVSYAWSNAHLSMQRTAYHFQPQKNMNDPNGPLYHKG

131 140 150 160 170 180 190 200 210 220 230 240 250 260
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
Pain_P40N1 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPANLSDPLLLDWVKYKGNPVLVPPPGIGVKDFRDPTTAWTGPQNGQWLL
Pain_P40N1_1 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPANLSDPLLLDWVKYKGNPVLVPPPGIGVKDFRDPTTAWTGPQNGQWLL
Pain_P40N1_2 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPANLSDPLLLDWVKYKGNPVLVPPPGIGVKDFRDPTTAWTGPQNGQWLL
Pain_P40N1_4 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPANLSDPLLLDWVKYKGNPVLVPPPGIGVKDFRDPTTAWTGPQNGQWLL
Pain_P40N1_3 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPANLSDPLLLDWVKYKGNPVLVPPPGIGVKDFRDPTTAWTGPQNGQWLL
Pain_P40N2 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPANLSDPLLLDWVKYKGNPVLVPPPGIGVKDFRDPTTAWTGPQNGQWLL
Pain_P40N2_1 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPANLSDPLLLDWVKYKGNPVLVPPPGIGVKDFRDPTTAWTGPQNGQWLL
Pain_P40N2_2 WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPANLSDPLLLDWVKYKGNPVLVPPPGIGVKDFRDPTTAWTGPQNGQWLL
Consensus WYHLFYQYNPDSAINGNITWGHAYVSKDLIHWLYLPFAMVPDQWYDINGVHTGSATILPDGQIHMLYTGDTDDYVQVQNLAYPANLSDPLLLDWVKYKGNPVLVPPPGIGVKDFRDPTTAWTGPQNGQWLL

261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
Pain_P40N1 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHYLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_P40N1_1 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHYLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_P40N1_2 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHYLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_P40N1_4 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHYLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_P40N1_3 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHYLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_P40N2 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHYLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_P40N2_1 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHYLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Pain_P40N2_2 TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHYLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG
Consensus TIGSKIGKTGIALVYETSNTFSFKLLDEVLHAYPGTGMWECYDFYPVSTKTNGLDTSYNGPGVKHYLKASLDDNKQDHYAIGTYDLTKNKWTPDNPELDCGIGLKL DYGKYASKTFYDPKKQRRVLWG

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
Pain_P40N1 WIGETDSESADLQKGWASVQSIPTVLYDKKTRTHLLQWPVEEIESLRAGDPIVKQANLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFCSTSGGAASRGILGPFVYVVIADQTLSELT
Pain_P40N1_1 WIGETDSESADLQKGWASVQSIPTVLYDKKTRTHLLQWPVEEIESLRAGDPIVKQANLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFCSTSGGAASRGILGPFVYVVIADQTLSELT
Pain_P40N1_2 WIGETDSESADLQKGWASVQSIPTVLYDKKTRTHLLQWPVEEIESLRAGDPIVKQANLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFCSTSGGAASRGILGPFVYVVIADQTLSELT
Pain_P40N1_4 WIGETDSESADLQKGWASVQSIPTVLYDKKTRTHLLQWPVEEIESLRAGDPIVKQANLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFCSTSGGAASRGILGPFVYVVIADQTLSELT
Pain_P40N1_3 WIGETDSESADLQKGWASVQSIPTVLYDKKTRTHLLQWPVEEIESLRAGDPIVKQANLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFCSTSGGAASRGILGPFVYVVIADQTLSELT
Pain_P40N2 WIGETDSESADLQKGWASVQSIPTVLYDKKTRTHLLQWPVEEIESLRAGDPIVKQANLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFCSTSGGAASRGILGPFVYVVIADQTLSELT
Pain_P40N2_1 WIGETDSESADLQKGWASVQSIPTVLYDKKTRTHLLQWPVEEIESLRAGDPIVKQANLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFCSTSGGAASRGILGPFVYVVIADQTLSELT
Pain_P40N2_2 WIGETDSESADLQKGWASVQSIPTVLYDKKTRTHLLQWPVEEIESLRAGDPIVKQANLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFCSTSGGAASRGILGPFVYVVIADQTLSELT
Consensus WIGETDSESADLQKGWASVQSIPTVLYDKKTRTHLLQWPVEEIESLRAGDPIVKQANLQPGSIELLHVDSAEELDIASFEYDKVALQGIIEADHYGFCSTSGGAASRGILGPFVYVVIADQTLSELT

521 530 540 550 560 570 580 590 600 610 620 630 639
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
Pain_P40N1 PYYFYISKGADGRAQTHFCADQTRSSVAPGVAKQVYVYSSVPLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL
Pain_P40N1_1 PYYFYISKGADGRAQTHFCADQTRSSVAPGVAKQVYVYSSVPLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL
Pain_P40N1_2 PYYFYISKGADGRAQTHFCADQTRSSVAPGVAKQVYVYSSVPLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL
Pain_P40N1_4 PYYFYISKGADGRAQTHFCADQTRSSVAPGVAKQVYVYSSVPLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL
Pain_P40N1_3 PYYFYISKGADGRAQTHFCADQTRSSVAPGVAKQVYVYSSVPLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL
Pain_P40N2 PYYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYVYSSVPLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL
Pain_P40N2_1 PYYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYVYSSVPLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL
Pain_P40N2_2 PYYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYVYSSVPLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL
Consensus PYYFYISKGADGRAETHFCADQTRSSVAPGVAKQVYVYSSVPLDGEKHSRLLVDHSIVESFAQGGRTVITSRIYPTKAVNGAARLFVFNATGASVTASVKIWSLESANIRSFPLQDL

1 10 20 30 40 50 60 70 80 90 100 110 120 130
Pain_P18N1 ATGGCCACGCAGTACCATTCCAGTTATGACCCGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACCCGATTCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Pain_P18N1_2 ATGGCCACGCAGTACCATTCCAGTTATGACCCGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACCCGATTCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Pain_P18N1_1 ATGGCCACGCAGTACCATTCCAGTTATGACCCGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACCCGATTCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Consensus ATGGCCACGCAGTACCATTCCAGTTATGACCCGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACCCGATTCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT

131 140 150 160 170 180 190 200 210 220 230 240 250 260
Pain_P18N1 TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAaCAACCAAGTCAACCGACTTGCAAGAGTAACTCCCGTTCCGCGGCGCCGCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_P18N1_2 TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAaCAACCAAGTCAACCGACTTGCAAGAGTAACTCCCGTTCCGCGGCGCCGCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_P18N1_1 TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAaCAACCAAGTCAACCGACTTGCAAGAGTAACTCCCGTTCCGCGGCGCCGCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Consensus TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAaCAACCAAGTCAACCGACTTGCAAGAGTAACTCCCGTTCCGCGGCGCCGCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
Pain_P18N1 TGTGTCATGCTAGTCAATTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA
Pain_P18N1_2 TGTGTCATGCTAGTCAATTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA
Pain_P18N1_1 TGTGTCATGCTAGTCAATTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA
Consensus TGTGTCATGCTAGTCAATTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
Pain_P18N1 TGGTATCATCTTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTTACTTGCCTTTGGCATGGTTCCTGATCAATGGT
Pain_P18N1_2 TGGTATCATCTTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTTACTTGCCTTTGGCATGGTTCCTGATCAATGGT
Pain_P18N1_1 TGGTATCATCTTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTTACTTGCCTTTGGCATGGTTCCTGATCAATGGT
Consensus TGGTATCATCTTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTTACTTGCCTTTGGCATGGTTCCTGATCAATGGT

521 530 540 550 560 570 580 590 600 610 620 630 640 650
Pain_P18N1 ACGATATTACGGTGTCTGGACTGGGTCCGCCACCATCCTACCCGATGGTCAAGTCAATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGCCTACCCACCACTTATCTGA
Pain_P18N1_2 ACGATATTACGGTGTCTGGACTGGGTCCGCCACCATCCTACCCGATGGTCAAGTCAATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGCCTACCCACCACTTATCTGA
Pain_P18N1_1 ACGATATTACGGTGTCTGGACTGGGTCCGCCACCATCCTACCCGATGGTCAAGTCAATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGCCTACCCACCACTTATCTGA
Consensus ACGATATTACGGTGTCTGGACTGGGTCCGCCACCATCCTACCCGATGGTCAAGTCAATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGCCTACCCACCACTTATCTGA

651 660 670 680 690 700 710 720 730 740 750 760 770 780
Pain_P18N1 TCCTCTCCTTCTAGACTGGGTCAAGTACAAGGCCAACCCGGTCTGGTTCTCCACCCGGCATTGGTATCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Pain_P18N1_2 TCCTCTCCTTCTAGACTGGGTCAAGTACAAGGCCAACCCGGTCTGGTTCTCCACCCGGCATTGGTATCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Pain_P18N1_1 TCCTCTCCTTCTAGACTGGGTCAAGTACAAGGCCAACCCGGTCTGGTTCTCCACCCGGCATTGGTATCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Consensus TCCTCTCCTTCTAGACTGGGTCAAGTACAAGGCCAACCCGGTCTGGTTCTCCACCCGGCATTGGTATCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
Pain_P18N1 ACAATCGGGTCTAaGATTGGTAAACCGGTATTGCACTTGTATTGAACTTCCAACTTCAAAaGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT
Pain_P18N1_2 ACAATCGGGTCTAaGATTGGTAAACCGGTATTGCACTTGTATTGAACTTCCAACTTCAAAaGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT
Pain_P18N1_1 ACAATCGGGTCTAaGATTGGTAAACCGGTATTGCACTTGTATTGAACTTCCAACTTCAAAaGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT
Consensus ACAATCGGGTCTAaGATTGGTAAACCGGTATTGCACTTGTATTGAACTTCCAACTTCAAAaGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
Pain_P18N1 TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATAACGGCCCGGGTGAAGCaTGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_P18N1_2 TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATAACGGCCCGGGTGAAGCaTGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_P18N1_1 TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATAACGGCCCGGGTGAAGCaTGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Consensus TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATAACGGCCCGGGTGAAGCaTGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
Pain_P18N1 GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Pain_P18N1_2 GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Pain_P18N1_1 GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Consensus GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTATGACCCGAGAAACACGAGAGTACTGTGGGGA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
Pain_P18N1 TGGATTGGGGAACCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCTTTACGACAAAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Pain_P18N1_2 TGGATTGGGGAACCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCTTTACGACAAAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Pain_P18N1_1 TGGATTGGGGAACCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCTTTACGACAAAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Consensus TGGATTGGGGAACCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCTTTACGACAAAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
Pain_P18N1 TTGAAGCTTAAGAGTGGGTGATCCTATTGTTAAGCAAGTCAATCTTCAACCAAGTTCAATTGAGCTACTCCATGTTGACTCAGTGCAGAGTTGGATATAGAAGCCTATTGAGTGGACAAGTTCGC
Pain_P18N1_2 TTGAAGCTTAAGAGTGGGTGATCCTATTGTTAAGCAAGTCAATCTTCAACCAAGTTCAATTGAGCTACTCCATGTTGACTCAGTGCAGAGTTGGATATAGAAGCCTATTGAGTGGACAAGTTCGC
Pain_P18N1_1 TTGAAGCTTAAGAGTGGGTGATCCTATTGTTAAGCAAGTCAATCTTCAACCAAGTTCAATTGAGCTACTCCATGTTGACTCAGTGCAGAGTTGGATATAGAAGCCTATTGAGTGGACAAGTTCGC
Consensus TTGAAGCTTAAGAGTGGGTGATCCTATTGTTAAGCAAGTCAATCTTCAACCAAGTTCAATTGAGCTACTCCATGTTGACTCAGTGCAGAGTTGGATATAGAAGCCTATTGAGTGGACAAGTTCGC

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
Pain_P18N1 GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTAATTGCTGATCAAAGCTATCTGAGCTAACG
Pain_P18N1_2 GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTAATTGCTGATCAAAGCTATCTGAGCTAACG
Pain_P18N1_1 GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTAATTGCTGATCAAAGCTATCTGAGCTAACG
Consensus GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTAATTGCTGATCAAAGCTATCTGAGCTAACG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
Pain_P18N1 CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGTCAAGCTGAGACTCACTTCTGTGCTGATCAAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAAACAAGTTTATGGTAGTTCAGTACCCGTGTTAG
Pain_P18N1_2 CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGTCAAGCTGAGACTCACTTCTGTGCTGATCAAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAAACAAGTTTATGGTAGTTCAGTACCCGTGTTAG
Pain_P18N1_1 CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGTCAAGCTGAGACTCACTTCTGTGCTGATCAAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAAACAAGTTTATGGTAGTTCAGTACCCGTGTTAG
Consensus CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGTCAAGCTGAGACTCACTTCTGTGCTGATCAAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAAACAAGTTTATGGTAGTTCAGTACCCGTGTTAG

1691 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800 1810 1820
Pain_P18N1 ACGGTGAaAAACATTTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCCAAGGAGGAGAACAGTCAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT
Pain_P18N1_2 ACGGTGAaAAACATTTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCCAAGGAGGAGAACAGTCAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT
Pain_P18N1_1 ACGGTGAaAAACATTTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCCAAGGAGGAGAACAGTCAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT
Consensus ACGGTGAaAAACATTTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCCAAGGAGGAGAACAGTCAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT

1821 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
Pain_P18N1 CGTTTTCAACAATGCCACAGGGGCTAGCGTGAAGTCTTCCGTCAGATTGGTCACTTGAGTCCGCTAATATTCGATCCTTCCCTTGCAAGACTTGTAa
Pain_P18N1_2 CGTTTTCAACAATGCCACAGGGGCTAGCGTGAAGTCTTCCGTCAGATTGGTCACTTGAGTCCGCTAATATTCGATCCTTCCCTTGCAAGACTTGTAa
Pain_P18N1_1 CGTTTTCAACAATGCCACAGGGGCTAGCGTGAAGTCTTCCGTCAGATTGGTCACTTGAGTCCGCTAATATTCGATCCTTCCCTTGCAAGACTTGTAa
Consensus CGTTTTCAACAATGCCACAGGGGCTAGCGTGAAGTCTTCCGTCAGATTGGTCACTTGAGTCCGCTAATATTCGATCCTTCCCTTGCAAGACTTGTAa

1 10 20 30 40 50 60 70 80 90 100 110 120 130
Pain_P18N2 ATGGCCACGCAGTACCATTCCAGTTATGACCTGGAAACTCCGCTCCATTACACATTCCTCCCGGATCAACCGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCT
Pain_P18N2_1 ATGGCCACGCAGTACCATTCCAGTTATGACCTGGAAACTCCGCTCCATTACACATTCCTCCCGGATCAACCGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCT
Pain_P18N2_3 ATGGCCACGCAGTACCATTCCAGTTATGACCTGGAAACTCCGCTCCATTACACATTCCTCCCGGATCAACCGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCT
Pain_P18N2_2 ATGGCCACGCAGTACCATTCCAGTTATGACCTGGAAACTCCGCTCCATTACACATTCCTCCCGGATCAACCGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCT
Consensus ATGGCCACGCAGTACCATTCCAGTTATGACCTGGAAACTCCGCTCCATTACACATTCCTCCCGGATCAACCGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCT

131 140 150 160 170 180 190 200 210 220 230 240 250 260
Pain_P18N2 TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACCAACCAATCACCGGACTTGCAGAGTAACTCCCGTTCGCCGGCCGCCCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_P18N2_1 TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACCAACCAATCACCGGACTTGCAGAGTAACTCCCGTTCGCCGGCCGCCCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_P18N2_3 TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACCAACCAATCACCGGACTTGCAGAGTAACTCCCGTTCGCCGGCCGCCCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_P18N2_2 TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACCAACCAATCACCGGACTTGCAGAGTAACTCCCGTTCGCCGGCCGCCCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Consensus TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACCAACCAATCACCGGACTTGCAGAGTAACTCCCGTTCGCCGGCCGCCCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
Pain_P18N2 TGTGTCATGCTAGTACGTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGA
Pain_P18N2_1 TGTGTCATGCTAGTACGTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGA
Pain_P18N2_3 TGTGTCATGCTAGTACGTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGA
Pain_P18N2_2 TGTGTCATGCTAGTACGTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGA
Consensus TGTGTCATGCTAGTACGTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
Pain_P18N2 TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT
Pain_P18N2_1 TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT
Pain_P18N2_3 TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT
Pain_P18N2_2 TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT
Consensus TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT

521 530 540 550 560 570 580 590 600 610 620 630 640 650
Pain_P18N2 ACGATATAAACGGTGTCTGGACTGGGTCGCTACCATCTACCCGATGGTCAGATCATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGCGTACCCCACTTATCTGA
Pain_P18N2_1 ACGATATAAACGGTGTCTGGACTGGGTCGCTACCATCTACCCGATGGTCAGATCATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGCGTACCCCACTTATCTGA
Pain_P18N2_3 ACGATATAAACGGTGTCTGGACTGGGTCGCTACCATCTACCCGATGGTCAGATCATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGCGTACCCCACTTATCTGA
Pain_P18N2_2 ACGATATAAACGGTGTCTGGACTGGGTCGCTACCATCTACCCGATGGTCAGATCATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGCGTACCCCACTTATCTGA
Consensus ACGATATAAACGGTGTCTGGACTGGGTCGCTACCATCTACCCGATGGTCAGATCATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGCAAAATCTTGCGTACCCCACTTATCTGA

651 660 670 680 690 700 710 720 730 740 750 760 770 780
Pain_P18N2 TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGACCCAAATGGGCAATGGCTTTTA
Pain_P18N2_1 TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGACCCAAATGGGCAATGGCTTTTA
Pain_P18N2_3 TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGACCCAAATGGGCAATGGCTTTTA
Pain_P18N2_2 TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGACCCAAATGGGCAATGGCTTTTA
Consensus TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGACCCAAATGGGCAATGGCTTTTA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
Pain_P18N2 ACAATCGGGTCTAAGATTGGTAAACCGGATTGCACTTGTATGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_P18N2_1 ACAATCGGGTCTAAGATTGGTAAACCGGATTGCACTTGTATGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_P18N2_3 ACAATCGGGTCTAAGATTGGTAAACCGGATTGCACTTGTATGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_P18N2_2 ACAATCGGGTCTAAGATTGGTAAACCGGATTGCACTTGTATGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Consensus ACAATCGGGTCTAAGATTGGTAAACCGGATTGCACTTGTATGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
Pain_P18N2 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATACCGGCCGGGTGTAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGATGACTTT
Pain_P18N2_1 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATACCGGCCGGGTGTAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGATGACTTT
Pain_P18N2_3 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATACCGGCCGGGTGTAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGATGACTTT
Pain_P18N2_2 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATACCGGCCGGGTGTAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGATGACTTT
Consensus TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATACCGGCCGGGTGTAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGATGACTTT

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
Pain_P18N2 GCAAAAGAAATTAATGGACACCCGATAACCCGGAATTGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGAAACACGAGAGTACTGTGGGA
Pain_P18N2_1 GCAAAAGAAATTAATGGACACCCGATAACCCGGAATTGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGAAACACGAGAGTACTGTGGGA
Pain_P18N2_3 GCAAAAGAAATTAATGGACACCCGATAACCCGGAATTGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGAAACACGAGAGTACTGTGGGA
Pain_P18N2_2 GCAAAAGAAATTAATGGACACCCGATAACCCGGAATTGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGAAACACGAGAGTACTGTGGGA
Consensus GCAAAAGAAATTAATGGACACCCGATAACCCGGAATTGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGAAACACGAGAGTACTGTGGGA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
Pain_P18N2 TGGATTGGGGAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGGACAGTGTCTTACGACAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Pain_P18N2_1 TGGATTGGGGAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGGACAGTGTCTTACGACAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Pain_P18N2_3 TGGATTGGGGAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGGACAGTGTCTTACGACAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Pain_P18N2_2 TGGATTGGGGAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGGACAGTGTCTTACGACAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Consensus TGGATTGGGGAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCAGGGACAGTGTCTTACGACAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
Pain_P18N2 TTGAAGCTTAAAGCGGGTATCCTATTGTTAAGCAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCTCATTGAGTGGACAAAGTCCG
Pain_P18N2_1 TTGAAGCTTAAAGCGGGTATCCTATTGTTAAGCAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCTCATTGAGTGGACAAAGTCCG
Pain_P18N2_3 TTGAAGCTTAAAGCGGGTATCCTATTGTTAAGCAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCTCATTGAGTGGACAAAGTCCG
Pain_P18N2_2 TTGAAGCTTAAAGCGGGTATCCTATTGTTAAGCAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCTCATTGAGTGGACAAAGTCCG
Consensus TTGAAGCTTAAAGCGGGTATCCTATTGTTAAGCAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCTCATTGAGTGGACAAAGTCCG

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
Pain_P18N2 GCTCCAGGGAATTAATGAGCAGATCATGTAGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGATGATCAACGCTATCTGAGCTAACG
Pain_P18N2_1 GCTCCAGGGAATTAATGAGCAGATCATGTAGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGATGATCAACGCTATCTGAGCTAACG
Pain_P18N2_3 GCTCCAGGGAATTAATGAGCAGATCATGTAGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGATGATCAACGCTATCTGAGCTAACG
Pain_P18N2_2 GCTCCAGGGAATTAATGAGCAGATCATGTAGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGATGATCAACGCTATCTGAGCTAACG
Consensus GCTCCAGGGAATTAATGAGCAGATCATGTAGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGATGATCAACGCTATCTGAGCTAACG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
Pain_P18N2 CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAACCCAGATCCTCAGAGGCTCCGGGAGTTGCTAARCAAGTTTATGGTAGTTCAGTACCCGTTGG
Pain_P18N2_1 CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAACCCAGATCCTCAGAGGCTCCGGGAGTTGCTAARCAAGTTTATGGTAGTTCAGTACCCGTTGG
Pain_P18N2_3 CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAACCCAGATCCTCAGAGGCTCCGGGAGTTGCTAARCAAGTTTATGGTAGTTCAGTACCCGTTGG
Pain_P18N2_2 CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAACCCAGATCCTCAGAGGCTCCGGGAGTTGCTAARCAAGTTTATGGTAGTTCAGTACCCGTTGG
Consensus CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAACCCAGATCCTCAGAGGCTCCGGGAGTTGCTAARCAAGTTTATGGTAGTTCAGTACCCGTTGG

1691 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800 1810 1820
Pain_P18N2 ACGGTGAAAACATTCCGATGAGATTATGGTGGACCACTCAATTGTGGAGAGCTTGTCTCAGGAGGAGAGACAGTCAATACATCGCGAATTTACCCARCAAGGCAAGTGAATGGAGCAGCAGCTCTT
Pain_P18N2_1 ACGGTGAAAACATTCCGATGAGATTATGGTGGACCACTCAATTGTGGAGAGCTTGTCTCAGGAGGAGAGACAGTCAATACATCGCGAATTTACCCARCAAGGCAAGTGAATGGAGCAGCAGCTCTT
Pain_P18N2_3 ACGGTGAAAACATTCCGATGAGATTATGGTGGACCACTCAATTGTGGAGAGCTTGTCTCAGGAGGAGAGACAGTCAATACATCGCGAATTTACCCARCAAGGCAAGTGAATGGAGCAGCAGCTCTT
Pain_P18N2_2 ACGGTGAAAACATTCCGATGAGATTATGGTGGACCACTCAATTGTGGAGAGCTTGTCTCAGGAGGAGAGACAGTCAATACATCGCGAATTTACCCARCAAGGCAAGTGAATGGAGCAGCAGCTCTT
Consensus ACGGTGAAAACATTCCGATGAGATTATGGTGGACCACTCAATTGTGGAGAGCTTGTCTCAGGAGGAGAGACAGTCAATACATCGCGAATTTACCCARCAAGGCAAGTGAATGGAGCAGCAGCTCTT

1821 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
Pain_P18N2 CGTTTTCAACATGCCACAGGGTCTAGCGTGACTGCCTCCGTCAGATTGGTCACTTGAAGTCGGCTAATATTCATCCTTCCCTTGCAGAGCTTGTAA
Pain_P18N2_1 CGTTTTCAACATGCCACAGGGTCTAGCGTGACTGCCTCCGTCAGATTGGTCACTTGAAGTCGGCTAATATTCATCCTTCCCTTGCAGAGCTTGTAA
Pain_P18N2_3 CGTTTTCAACATGCCACAGGGTCTAGCGTGACTGCCTCCGTCAGATTGGTCACTTGAAGTCGGCTAATATTCATCCTTCCCTTGCAGAGCTTGTAA
Pain_P18N2_2 CGTTTTCAACATGCCACAGGGTCTAGCGTGACTGCCTCCGTCAGATTGGTCACTTGAAGTCGGCTAATATTCATCCTTCCCTTGCAGAGCTTGTAA
Consensus CGTTTTCAACATGCCACAGGGTCTAGCGTGACTGCCTCCGTCAGATTGGTCACTTGAAGTCGGCTAATATTCATCCTTCCCTTGCAGAGCTTGTAA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
Pain_P40N2 ATGGCCACGCGAGTACCCTCAAGTTATGACCCGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACACGATTCCGGCCACCGGAATCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTC
Pain_P40N2_1 ATGGCCACGCGAGTACCCTCAAGTTATGACCCGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACACGATTCCGGCCACCGGAATCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTC
Pain_P40N2_2 ATGGCCACGCGAGTACCCTCAAGTTATGACCCGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACACGATTCCGGCCACCGGAATCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTC
Consensus ATGGCCACGCGAGTACCCTCAAGTTATGACCCGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACACGATTCCGGCCACCGGAATCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTC

131 140 150 160 170 180 190 200 210 220 230 240 250 260
Pain_P40N2 TCCTTTTGCTTTCTTAGTCTTCTTCCGATCTCAACAACCAAGTCAACCGGACTTGCAAGTACGCCCGTTCCGGCCGCGCCCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_P40N2_1 TCCTTTTGCTTTCTTAGTCTTCTTCCGATCTCAACAACCAAGTCAACCGGACTTGCAAGTACGCCCGTTCCGGCCGCGCCCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_P40N2_2 TCCTTTTGCTTTCTTAGTCTTCTTCCGATCTCAACAACCAAGTCAACCGGACTTGCAAGTACGCCCGTTCCGGCCGCGCCCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Consensus TCCTTTTGCTTTCTTAGTCTTCTTCCGATCTCAACAACCAAGTCAACCGGACTTGCAAGTACGCCCGTTCCGGCCGCGCCCGTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
Pain_P40N2 TGTCGTCATGCTAGTACGTTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCTTACGGTCCATTGTACCACAGGGGA
Pain_P40N2_1 TGTCGTCATGCTAGTACGTTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCTTACGGTCCATTGTACCACAGGGGA
Pain_P40N2_2 TGTCGTCATGCTAGTACGTTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCTTACGGTCCATTGTACCACAGGGGA
Consensus TGTCGTCATGCTAGTACGTTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCTTACGGTCCATTGTACCACAGGGGA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
Pain_P40N2 TGGTATCATCTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTACTTGGCTTTTGGCATGGTTCCTGATCAATGGT
Pain_P40N2_1 TGGTATCATCTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTACTTGGCTTTTGGCATGGTTCCTGATCAATGGT
Pain_P40N2_2 TGGTATCATCTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTACTTGGCTTTTGGCATGGTTCCTGATCAATGGT
Consensus TGGTATCATCTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTACTTGGCTTTTGGCATGGTTCCTGATCAATGGT

521 530 540 550 560 570 580 590 600 610 620 630 640 650
Pain_P40N2 ACGATATTACGGGGTCTGGACTGGGTCCGCTACCATCCTACCCGATGGTCAAGTATGATGCTTTATACTGGTGACACTGATGATTATGTGCAAGTGC AAAATCTTGGCTACCCCGCAACTTATCTGA
Pain_P40N2_1 ACGATATTACGGGGTCTGGACTGGGTCCGCTACCATCCTACCCGATGGTCAAGTATGATGCTTTATACTGGTGACACTGATGATTATGTGCAAGTGC AAAATCTTGGCTACCCCGCAACTTATCTGA
Pain_P40N2_2 ACGATATTACGGGGTCTGGACTGGGTCCGCTACCATCCTACCCGATGGTCAAGTATGATGCTTTATACTGGTGACACTGATGATTATGTGCAAGTGC AAAATCTTGGCTACCCCGCAACTTATCTGA
Consensus ACGATATTACGGGGTCTGGACTGGGTCCGCTACCATCCTACCCGATGGTCAAGTATGATGCTTTATACTGGTGACACTGATGATTATGTGCAAGTGC AAAATCTTGGCTACCCCGCAACTTATCTGA

651 660 670 680 690 700 710 720 730 740 750 760 770 780
Pain_P40N2 TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCRAACCCGGTCTGGTTCTCCACCCGGCATGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Pain_P40N2_1 TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCRAACCCGGTCTGGTTCTCCACCCGGCATGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Pain_P40N2_2 TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCRAACCCGGTCTGGTTCTCCACCCGGCATGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Consensus TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCRAACCCGGTCTGGTTCTCCACCCGGCATGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
Pain_P40N2 ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGCACTTGTATGAACTTCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_P40N2_1 ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGCACTTGTATGAACTTCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_P40N2_2 ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGCACTTGTATGAACTTCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Consensus ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGCACTTGTATGAACTTCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
Pain_P40N2 TTTACCCGGTATCGACCGAAAAACAACCGGGTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_P40N2_1 TTTACCCGGTATCGACCGAAAAACAACCGGGTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_P40N2_2 TTTACCCGGTATCGACCGAAAAACAACCGGGTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Consensus TTTACCCGGTATCGACCGAAAAACAACCGGGTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
Pain_P40N2 GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTTTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Pain_P40N2_1 GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTTTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Pain_P40N2_2 GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTTTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Consensus GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTTTATGACCCGAGAAACACGAGAGTACTGTGGGGA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
Pain_P40N2 TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCTTTACGACAGAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Pain_P40N2_1 TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCTTTACGACAGAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Pain_P40N2_2 TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCTTTACGACAGAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Consensus TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCTTTACGACAGAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
Pain_P40N2 TTGAAGCTTAAGAGCGGGTGTCTTATTGTTAAGCAAGCCATCTTCAACCAGGTTTCGATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAAGCCTCATTTGAGTGGACAAAGTCGC
Pain_P40N2_1 TTGAAGCTTAAGAGCGGGTGTCTTATTGTTAAGCAAGCCATCTTCAACCAGGTTTCGATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAAGCCTCATTTGAGTGGACAAAGTCGC
Pain_P40N2_2 TTGAAGCTTAAGAGCGGGTGTCTTATTGTTAAGCAAGCCATCTTCAACCAGGTTTCGATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAAGCCTCATTTGAGTGGACAAAGTCGC
Consensus TTGAAGCTTAAGAGCGGGTGTCTTATTGTTAAGCAAGCCATCTTCAACCAGGTTTCGATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAAGCCTCATTTGAGTGGACAAAGTCGC

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
Pain_P40N2 GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTATCGTTGTAATTGCTGATCAACCGTATCTGAGCTAACG
Pain_P40N2_1 GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTATCGTTGTAATTGCTGATCAACCGTATCTGAGCTAACG
Pain_P40N2_2 GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTATCGTTGTAATTGCTGATCAACCGTATCTGAGCTAACG
Consensus GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTATCGTTGTAATTGCTGATCAACCGTATCTGAGCTAACG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
Pain_P40N2 CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGTGCAGCTGAGACTCACTTCTGTGCTGATCAACTAGATCCTCAGTGGCTCCGGGAGTTGCTAACAAGTTTATGGTAGTTCAGTACCGGTGTTGG
Pain_P40N2_1 CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGTGCAGCTGAGACTCACTTCTGTGCTGATCAACTAGATCCTCAGTGGCTCCGGGAGTTGCTAACAAGTTTATGGTAGTTCAGTACCGGTGTTGG
Pain_P40N2_2 CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGTGCAGCTGAGACTCACTTCTGTGCTGATCAACTAGATCCTCAGTGGCTCCGGGAGTTGCTAACAAGTTTATGGTAGTTCAGTACCGGTGTTGG
Consensus CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGTGCAGCTGAGACTCACTTCTGTGCTGATCAACTAGATCCTCAGTGGCTCCGGGAGTTGCTAACAAGTTTATGGTAGTTCAGTACCGGTGTTGG

1691 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800 1810 1820
Pain_P40N2 ACGGTGAAAAACATTCGATGAGATTATTGGTGGACCACTCAATTTGGGAGAGCTTTGCTCAAGGAGGAGAACAGTCATAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT
Pain_P40N2_1 ACGGTGAAAAACATTCGATGAGATTATTGGTGGACCACTCAATTTGGGAGAGCTTTGCTCAAGGAGGAGAACAGTCATAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT
Pain_P40N2_2 ACGGTGAAAAACATTCGATGAGATTATTGGTGGACCACTCAATTTGGGAGAGCTTTGCTCAAGGAGGAGAACAGTCATAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT
Consensus ACGGTGAAAAACATTCGATGAGATTATTGGTGGACCACTCAATTTGGGAGAGCTTTGCTCAAGGAGGAGAACAGTCATAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT

1821 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
Pain_P40N2 CGTTTTCAACAATGCCACAGGGGCTAGCGTGACTGCCTCCGTCAGATTGGTCACTTGAGTCCGGCTAATATTCGATCCTTCCCTTGCAAGACTTGTA
Pain_P40N2_1 CGTTTTCAACAATGCCACAGGGGCTAGCGTGACTGCCTCCGTCAGATTGGTCACTTGAGTCCGGCTAATATTCGATCCTTCCCTTGCAAGACTTGTA
Pain_P40N2_2 CGTTTTCAACAATGCCACAGGGGCTAGCGTGACTGCCTCCGTCAGATTGGTCACTTGAGTCCGGCTAATATTCGATCCTTCCCTTGCAAGACTTGTA
Consensus CGTTTTCAACAATGCCACAGGGGCTAGCGTGACTGCCTCCGTCAGATTGGTCACTTGAGTCCGGCTAATATTCGATCCTTCCCTTGCAAGACTTGTA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
Pain_P40N1 ATGGCCACGCGAGTACCACCTCAAGTTATGACCCGGAARACTCCGCCTCCATTACACATTCCTCCCGGATCAACACGATTCCGGCCACCAGGAAATCCATTAAARATCATCTCCGGCATTTTCTCTCTCTC
Pain_P40N2 ATGGCCACGCGAGTACCACCTCAAGTTATGACCCGGAARACTCCGCCTCCATTACACATTCCTCCCGGATCAACACGATTCCGGCCACCAGGAAATCCATTAAARATCATCTCCGGCATTTTCTCTCTCTC
Pain_P18N2 ATGGCCACGCGAGTACCATTCCAGTTATGACCTGGAAARACTCCGCCTCCATTACACATTCCTCCCGGATCAACACGATTCCGGCCACCAGGAAATCCATTAAARATCATCTCCGGCATTTTCTCTCTCTT
Pain_P54N ATGGCCACGCGAGTACCATTCCAGTTATGACCTGGAAARACTCCGCCTCCATTACACATTCCTCCCGGATCAACACGATTCCGGCCACCAGGAAATCCATTAAARATCATCTCCGGCATTTTCTCTCTCTT
Pain_P18N1 ATGGCCACGCGAGTACCATTCCAGTTATGACCCGGAARACTCCGCCTCCATTACACATTCCTCCCGGATCAACACGATTCCGGCCACCAGGAAATCCATTAAARATCATCTCCGGCATTTTCTCTCTCTT
Consensus ATGGCCACGCGAGTACCACCTCAAGTTATGACCCGGAARACTCCGCCTCCATTACACATTCCTCCCGGATCAACACGATTCCGGCCACCAGGAAATCCATTAAARATCATCTCCGGCATTTTCTCTCTCTC

131 140 150 160 170 180 190 200 210 220 230 240 250 260
Pain_P40N1 TCCTTTTGTCTTTCTTGTAGTCTTCTTTCCGATCCTCAACACCAAGTCAACCGGACTTGCAAGTARACGCCATTTCGCCGGCCGCCCGCTCAGAGGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_P40N2 TCCTTTTGTCTTTCTTGTAGTCTTCTTTCCGATCCTCAACACCAAGTCAACCGGACTTGCAAGTARACGCCATTTCGCCGGCCGCCCGCTCAGAGGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_P18N2 TCCTTTTGTCTTTCTTGTAGTCTTCTTTCCGATCCTCAACACCAATCAACCGGACTTGCAAGTARACTCCCGTTCCGCCGGCCGCCCGCTCAGAGGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_P54N TCCTTTTGTCTTTCTTGTAGTCTTCTTTCCGATCCTCAACACCAATCAACCGGACTTGCAAGTARACTCCCGTTCCGCCGGCCGCCCGCTCAGAGGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_P18N1 TCCTTTTGTCTTTCTTGTAGTCTTCTTTCCGATCCTCAACACCAAGTCAACCGGACTTGCAAGTARACTCCCGTTCCGCCGGCCGCCCGCTCAGAGGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Consensus TCCTTTTGTCTTTCTTGTAGTCTTCTTTCCGATCCTCAACACCAAGTCAACCGGACTTGCAAGTARACTCCCGTTCCGCCGGCCGCCCGCTCAGAGGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
Pain_P40N1 TGTGCTCAATGCTAGTACGCTTTCTTATGCGTGGTCCAAATGCTATGCTTAGCTGGCAAGAAGTCTTACCATTTCARCTCAAAAAATTTGGATGACGATCCTAATGGTCCATTGTACCACRAGGGA
Pain_P40N2 TGTGCTCAATGCTAGTACGCTTTCTTATGCGTGGTCCAAATGCTATGCTTAGCTGGCAAGAAGTCTTACCATTTCARCTCAAAAAATTTGGATGACGATCCTAATGGTCCATTGTACCACRAGGGA
Pain_P18N2 TGTGCTCAATGCTAGTACGCTTTCTTATGCGTGGTCCAAATGCTATGCTTAGCTGGCAAGAAGTCTTACCATTTCARCTCAAAAAATTTGGATGACGATCCTAATGGTCCATTGTACCACRAGGGA
Pain_P54N TGTGCTCAATGCTAGTACGCTTTCTTATGCGTGGTCCAAATGCTATGCTTAGCTGGCAAGAAGTCTTACCATTTCARCTCAAAAAATTTGGATGACGATCCTAATGGTCCATTGTACCACRAGGGA
Pain_P18N1 TGTGCTCAATGCTAGTACGCTTTCTTATGCGTGGTCCAAATGCTATGCTTAGCTGGCAAGAAGTCTTACCATTTCARCTCAAAAAATTTGGATGACGATCCTAATGGTCCATTGTACCACRAGGGA
Consensus TGTGCTCAATGCTAGTACGCTTTCTTATGCGTGGTCCAAATGCTATGCTTAGCTGGCAAGAAGTCTTACCATTTCARCTCAAAAAATTTGGATGACGATCCTAATGGTCCATTGTACCACRAGGGA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
Pain_P40N1 TGGTATCATCTTTTTATCAATACAATCCAGATTCAAGCTATTGGGGAAATATACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCCATGGTTCCTGATCAATGGT
Pain_P40N2 TGGTATCATCTTTTTATCAATACAATCCAGATTCAAGCTATTGGGGAAATATACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCCATGGTTCCTGATCAATGGT
Pain_P18N2 TGGTATCATCTTTTTATCAATACAATCCAGATTCAAGCTATTGGGGAAATATACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCCATGGTTCCTGATCAATGGT
Pain_P54N TGGTATCATCTTTTTATCAATACAATCCAGATTCAAGCTATTGGGGAAATATACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCCATGGTTCCTGATCAATGGT
Pain_P18N1 TGGTATCATCTTTTTATCAATACAATCCAGATTCAAGCTATTGGGGAAATATACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCCATGGTTCCTGATCAATGGT
Consensus TGGTATCATCTTTTTATCAATACAATCCAGATTCAAGCTATTGGGGAAATATACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCCATGGTTCCTGATCAATGGT

521 530 540 550 560 570 580 590 600 610 620 630 640 650
Pain_P40N1 ACGATATTACGGTGTCTGGACTGGGTCCGCTACCATCTACCCGATGGTCAGATCATGATGCTTTATACGGTGACACTGATGATTATGTGCAAGTGCAAAATCTTGGTACCCCGCAACTTATCTGA
Pain_P40N2 ACGATATTACGGGTCTGGACTGGGTCCGCTACCATCTACCCGATGGTCAGATCATGATGCTTTATACGGTGACACTGATGATTATGTGCAAGTGCAAAATCTTGGTACCCCGCAACTTATCTGA
Pain_P18N2 ACGATATTACGGTGTCTGGACTGGGTCCGCTACCATCTACCCGATGGTCAGATCATGATGCTTTATACGGTGACACTGATGATTATGTGCAAGTGCAAAATCTTGGTACCCCGCAACTTATCTGA
Pain_P54N ACGATATTACGGTGTCTGGACTGGGTCCGCTACCATCTACCCGATGGTCAGATCATGATGCTTTATACGGTGACACTGATGATTATGTGCAAGTGCAAAATCTTGGTACCCCGCAACTTATCTGA
Pain_P18N1 ACGATATTACGGTGTCTGGACTGGGTCCGCTACCATCTACCCGATGGTCAGATCATGATGCTTTATACGGTGACACTGATGATTATGTGCAAGTGCAAAATCTTGGTACCCCGCAACTTATCTGA
Consensus ACGATATTACGGTGTCTGGACTGGGTCCGCTACCATCTACCCGATGGTCAGATCATGATGCTTTATACGGTGACACTGATGATTATGTGCAAGTGCAAAATCTTGGTACCCCGCAACTTATCTGA

651 660 670 680 690 700 710 720 730 740 750 760 770 780
Pain_P40N1 TCCTCTCTTCTAGACTGGGTCAAGTACAAGGCCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCCAAAATGGGCAATGGCTTTTA
Pain_P40N2 TCCTCTCTTCTAGACTGGGTCAAGTACAAGGCCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCCAAAATGGGCAATGGCTTTTA
Pain_P18N2 TCCTCTCTTCTAGACTGGGTCAAGTACAAGGCCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCCAAAATGGGCAATGGCTTTTA
Pain_P54N TCCTCTCTTCTAGACTGGGTCAAGTACAAGGCCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCCAAAATGGGCAATGGCTTTTA
Pain_P18N1 TCCTCTCTTCTAGACTGGGTCAAGTACAAGGCCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCCAAAATGGGCAATGGCTTTTA
Consensus TCCTCTCTTCTAGACTGGGTCAAGTACAAGGCCAACCCGGTCTGGTTCCTCCACCCGGCATTGGTGTCAAGGACTTTAGAGACCCGACCCTGCTTGGACCGGACCCCAAAATGGGCAATGGCTTTTA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
Pain_P40N1 ACAATCGGGTCTAAGATTGGTAAACGGGATTGCACCTGTTTATGAARCTTCAACTTCACAGCTTTAACTATTGGATGAGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT
Pain_P40N2 ACAATCGGGTCTAAGATTGGTAAACGGGATTGCACCTGTTTATGAARCTTCAACTTCACAGCTTTAACTATTGGATGAGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT
Pain_P18N2 ACAATCGGGTCTAAGATTGGTAAACGGGATTGCACCTGTTTATGAARCTTCAACTTCACAGCTTTAACTATTGGATGAGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT
Pain_P54N ACAATCGGGTCTAAGATTGGTAAACGGGATTGCACCTGTTTATGAARCTTCAACTTCACAGCTTTAACTATTGGATGAGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT
Pain_P18N1 ACAATCGGGTCTAAGATTGGTAAACGGGATTGCACCTGTTTATGAARCTTCAACTTCACAGCTTTAACTATTGGATGAGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT
Consensus ACAATCGGGTCTAAGATTGGTAAACGGGATTGCACCTGTTTATGAARCTTCAACTTCACAGCTTTAACTATTGGATGAGTGCATGCGGTTCCGGGTACGGGTATGTGGAGTGTGTGGACT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
Pain_P40N1 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_P40N2 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_P18N2 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_P54N TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_P18N1 TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Consensus TTTACCCGGTATCGACTGAAAAACAACCGGTTGGACACATCATATAACGGCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
Pain_P40N1 GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGGAACACACGAGAGTCTGTGGGGA
Pain_P40N2 GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGGAACACACGAGAGTCTGTGGGGA
Pain_P18N2 GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGGAACACACGAGAGTCTGTGGGGA
Pain_P54N GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGGAACACACGAGAGTCTGTGGGGA
Pain_P18N1 GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGGAACACACGAGAGTCTGTGGGGA
Consensus GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTATGACCCGAGGAACACACGAGAGTCTGTGGGGA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
Pain_P40N1 TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATTCARGGACAGTGCCTTACGACRAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGARAGAA
Pain_P40N2 TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATTCARGGACAGTGCCTTACGACRAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGARAGAA
Pain_P18N2 TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATTCARGGACAGTGCCTTACGACRAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGARAGAA
Pain_P54N TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATTCARGGACAGTGCCTTACGACRAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGARAGAA
Pain_P18N1 TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATTCARGGACAGTGCCTTACGACRAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGARAGAA
Consensus TGGATTGGGGAARCTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATTCARGGACAGTGCCTTACGACRAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGARAGAA

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
Pain_P40N1 TTGAAGCTTAAGAGCGGGTATCCTATTGTTAAGCAGCTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCCTCATTGAGTGGCAAGTCCG
Pain_P40N2 TTGAAGCTTAAGAGCGGGTATCCTATTGTTAAGCAGCTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCCTCATTGAGTGGCAAGTCCG
Pain_P18N2 TTGAAGCTTAAGAGCGGGTATCCTATTGTTAAGCAGCTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCCTCATTGAGTGGCAAGTCCG
Pain_P54N TTGAAGCTTAAGAGCGGGTATCCTATTGTTAAGCAGCTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCCTCATTGAGTGGCAAGTCCG
Pain_P18N1 TTGAAGCTTAAGAGCGGGTATCCTATTGTTAAGCAGCTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCCTCATTGAGTGGCAAGTCCG
Consensus TTGAAGCTTAAGAGCGGGTATCCTATTGTTAAGCAGCTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCCTCATTGAGTGGCAAGTCCG

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
Pain_P40N1 GCTCCAGGGAATATTGAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTGTAATTGCTGATCAACCGCTATCTGAGCTAACG
Pain_P40N2 GCTCCAGGGAATATTGAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTGTAATTGCTGATCAACCGCTATCTGAGCTAACG
Pain_P18N2 GCTCCAGGGAATATTGAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTGTAATTGCTGATCAACCGCTATCTGAGCTAACG
Pain_P54N GCTCCAGGGAATATTGAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTGTAATTGCTGATCAACCGCTATCTGAGCTAACG
Pain_P18N1 GCTCCAGGGAATATTGAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTGTAATTGCTGATCAACCGCTATCTGAGCTAACG
Consensus GCTCCAGGGAATATTGAGCAGATCATGTAGGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTGTAATTGCTGATCAACCGCTATCTGAGCTAACG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
Pain_P40N1 CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGTTCGAGCTCAGACTCACTTCTGTGCTGATCAAACTAGATCTCAGTGGCTCCGGGAGTTGCTAARACAGTTTATGGTAGTTCAGTACCCTGTTGG
Pain_P40N2 CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGTTCGAGCTCAGACTCACTTCTGTGCTGATCAAACTAGATCTCAGTGGCTCCGGGAGTTGCTAARACAGTTTATGGTAGTTCAGTACCCTGTTGG
Pain_P18N2 CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGTTCGAGCTCAGACTCACTTCTGTGCTGATCAAACTAGATCTCAGTGGCTCCGGGAGTTGCTAARACAGTTTATGGTAGTTCAGTACCCTGTTGG
Pain_P54N CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGTTCGAGCTCAGACTCACTTCTGTGCTGATCAAACTAGATCTCAGTGGCTCCGGGAGTTGCTAARACAGTTTATGGTAGTTCAGTACCCTGTTGG
Pain_P18N1 CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGTTCGAGCTCAGACTCACTTCTGTGCTGATCAAACTAGATCTCAGTGGCTCCGGGAGTTGCTAARACAGTTTATGGTAGTTCAGTACCCTGTTGG
Consensus CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGTTCGAGCTCAGACTCACTTCTGTGCTGATCAAACTAGATCTCAGTGGCTCCGGGAGTTGCTAARACAGTTTATGGTAGTTCAGTACCCTGTTGG

1691 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800 1810 1820
Pain_P40N1 ACGGTGAARAACATTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGGCTTTGCTCAGGGAGGAGAACAGTCAACATCCGAAATTTACCCACRAGGACAGTGAATGGAGCAGCAGCTCTT
Pain_P40N2 ACGGTGAARAACATTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGGCTTTGCTCAGGGAGGAGAACAGTCAACATCCGAAATTTACCCACRAGGACAGTGAATGGAGCAGCAGCTCTT
Pain_P18N2 ACGGTGAARAACATTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGGCTTTGCTCAGGGAGGAGAACAGTCAACATCCGAAATTTACCCACRAGGACAGTGAATGGAGCAGCAGCTCTT
Pain_P54N ACGGTGAARAACATTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGGCTTTGCTCAGGGAGGAGAACAGTCAACATCCGAAATTTACCCACRAGGACAGTGAATGGAGCAGCAGCTCTT
Pain_P18N1 ACGGTGAARAACATTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGGCTTTGCTCAGGGAGGAGAACAGTCAACATCCGAAATTTACCCACRAGGACAGTGAATGGAGCAGCAGCTCTT
Consensus ACGGTGAARAACATTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGGCTTTGCTCAGGGAGGAGAACAGTCAACATCCGAAATTTACCCACRAGGACAGTGAATGGAGCAGCAGCTCTT

1821 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
Pain_P40N1 CGTTTTCAACAATGCCACAGGGGCTAGCGTGACTGCCCTCCGTCAGAGTTGGTCACTTGAGTCGGCTAATATTGATCCTTCCCTTGCAAGACTTGTA
Pain_P40N2 CGTTTTCAACAATGCCACAGGGGCTAGCGTGACTGCCCTCCGTCAGAGTTGGTCACTTGAGTCGGCTAATATTGATCCTTCCCTTGCAAGACTTGTA
Pain_P18N2 CGTTTTCAACAATGCCACAGGGGCTAGCGTGACTGCCCTCCGTCAGAGTTGGTCACTTGAGTCGGCTAATATTGATCCTTCCCTTGCAAGACTTGTA
Pain_P54N CGTTTTCAACAATGCCACAGGGGCTAGCGTGACTGCCCTCCGTCAGAGTTGGTCACTTGAGTCGGCTAATATTGATCCTTCCCTTGCAAGACTTGTA
Pain_P18N1 CGTTTTCAACAATGCCACAGGGGCTAGCGTGACTGCCCTCCGTCAGAGTTGGTCACTTGAGTCGGCTAATATTGATCCTTCCCTTGCAAGACTTGTA
Consensus CGTTTTCAACAATGCCACAGGGGCTAGCGTGACTGCCCTCCGTCAGAGTTGGTCACTTGAGTCGGCTAATATTGATCCTTCCCTTGCAAGACTTGTA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
Pain_DA ATGGCCACGCAGTACCATTCCAGTTATGACCCGGAARACTCCGCTCCATTACACATTCTCCCGGATCAACCcGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Pain_SA ATGGCCACGCAGTACCATTCCAGTTATGACCCGGAARACTCCGCTCCATTACACATTCTCCCGGATCAACCcGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Pain_TN2 ATGGCCACGCAGTACCATTCCAGTTATGACCCGGAARACTCCGCTCCATTACACATTCTCCCGGATCAACCcGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Pain_TN1 ATGGCCACGCAGTACCATTCCAGTTATGACCCGGAARACTCCGCTCCATTACACATTCTCCCGGATCAACCcGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Consensus ATGGCCACGCAGTACCATTCCAGTTATGACCCGGAARACTCCGCTCCATTACACATTCTCCCGGATCAACCcGATTCCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT

131 140 150 160 170 180 190 200 210 220 230 240 250 260
Pain_DA TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACCAACCAgTCACCcGGACTTGCAGAGTAACTCCCGTTCGCCGGCCGCCcCGCTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_SA TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACCAACCAgTCACCcGGACTTGCAGAGTAACTCCCGTTCGCCGGCCGCCcCGCTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_TN2 TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACCAACCAgTCACCcGGACTTGCAGAGTAACTCCCGTTCGCCGGCCGCCcCGCTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_TN1 TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACCAACCAgTCACCcGGACTTGCAGAGTAACTCCCGTTCGCCGGCCGCCcCGCTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Consensus TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACCAACCAgTCACCcGGACTTGCAGAGTAACTCCCGTTCGCCGGCCGCCcCGCTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
Pain_DA TGTCGTCATGCTAGTCACgTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA
Pain_SA TGTCGTCATGCTAGTCACgTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA
Pain_TN2 TGTCGTCATGCTAGTCACgTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA
Pain_TN1 TGTCGTCATGCTAGTCACgTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA
Consensus TGTCGTCATGCTAGTCACgTTTCTTATGCGTGGTCCATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
Pain_DA TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT
Pain_SA TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT
Pain_TN2 TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT
Pain_TN1 TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT
Consensus TGGTATCATCTTTTTATCAATACATCCAGATTACGATTTTGGGGAATATACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTCTACTTGCCTTTTGCATGGTCTCTGATCAATGGT

521 530 540 550 560 570 580 590 600 610 620 630 640 650
Pain_DA ACGATATAACGGTGTCTGGACTGGGTCCGCcACCATCCTACCcGATGGTcAGATCATGATGCTTTATACCcGGTGCACCTGATGATTATGTcCAAGTGC AAAATCTTGCCTACCCcCAACTTATCTGA
Pain_SA ACGATATAACGGTGTCTGGACTGGGTCCGCcACCATCCTACCcGATGGTcAGATCATGATGCTTTATACCcGGTGCACCTGATGATTATGTcCAAGTGC AAAATCTTGCCTACCCcCAACTTATCTGA
Pain_TN2 ACGATATAACGGTGTCTGGACTGGGTCCGCcACCATCCTACCcGATGGTcAGATCATGATGCTTTATACCcGGTGCACCTGATGATTATGTcCAAGTGC AAAATCTTGCCTACCCcCAACTTATCTGA
Pain_TN1 ACGATATAACGGTGTCTGGACTGGGTCCGCcACCATCCTACCcGATGGTcAGATCATGATGCTTTATACCcGGTGCACCTGATGATTATGTcCAAGTGC AAAATCTTGCCTACCCcCAACTTATCTGA
Consensus ACGATATAACGGTGTCTGGACTGGGTCCGCcACCATCCTACCcGATGGTcAGATCATGATGCTTTATACCcGGTGCACCTGATGATTATGTcCAAGTGC AAAATCTTGCCTACCCcCAACTTATCTGA

651 660 670 680 690 700 710 720 730 740 750 760 770 780
Pain_DA TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCACCCcGGTCTGGTTCCTCCACCcGGCATTGGTcTCAAGGACTTTAGAGACCcGACcACTGCTTGGACCcGGACCCcAAAATGGGCAATGGCTTTTA
Pain_SA TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCACCCcGGTCTGGTTCCTCCACCcGGCATTGGTcTCAAGGACTTTAGAGACCcGACcACTGCTTGGACCcGGACCCcAAAATGGGCAATGGCTTTTA
Pain_TN2 TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCACCCcGGTCTGGTTCCTCCACCcGGCATTGGTcTCAAGGACTTTAGAGACCcGACcACTGCTTGGACCcGGACCCcAAAATGGGCAATGGCTTTTA
Pain_TN1 TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCACCCcGGTCTGGTTCCTCCACCcGGCATTGGTcTCAAGGACTTTAGAGACCcGACcACTGCTTGGACCcGGACCCcAAAATGGGCAATGGCTTTTA
Consensus TCCTCTCCTTCTAGACTGGGTCAAGTACAAAGGCACCCcGGTCTGGTTCCTCCACCcGGCATTGGTcTCAAGGACTTTAGAGACCcGACcACTGCTTGGACCcGGACCCcAAAATGGGCAATGGCTTTTA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
Pain_DA ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGcGCTTGTATTGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_SA ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGcGCTTGTATTGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_TN2 ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGcGCTTGTATTGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_TN1 ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGcGCTTGTATTGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Consensus ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGcGCTTGTATTGAARCTTCCAACTTCAACAGCTTTAAGCTATTGGATGAAGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
Pain_DA TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATACCGCCcGGGTGTAAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_SA TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATACCGCCcGGGTGTAAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_TN2 TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATACCGCCcGGGTGTAAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_TN1 TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATACCGCCcGGGTGTAAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Consensus TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATACCGCCcGGGTGTAAAGCATGTGTTAAAGCAGTTTAGATGACAAATAGCAAGATCACTATGCTATTGGGACGTATGACTT

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
Pain_DA GCAARAAGACAATGGACACCCGATAACCCcGGAAATGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTTATGACCCcGARGAARACAGAGAGTACTGTGGGGA
Pain_SA GCAARAAGACAATGGACACCCGATAACCCcGGAAATGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTTATGACCCcGARGAARACAGAGAGTACTGTGGGGA
Pain_TN2 GCAARAAGACAATGGACACCCGATAACCCcGGAAATGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTTATGACCCcGARGAARACAGAGAGTACTGTGGGGA
Pain_TN1 GCAARAAGACAATGGACACCCGATAACCCcGGAAATGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTTATGACCCcGARGAARACAGAGAGTACTGTGGGGA
Consensus GCAARAAGACAATGGACACCCGATAACCCcGGAAATGGATTGTGGAAATGGGTTGAAGCTGGATTATGGGAATATTATGCATCAAGACATTTTATGACCCcGARGAARACAGAGAGTACTGTGGGGA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
Pain_DA TGGATTGGGGAAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCcAGGACAGTGCCTTACGACAGAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGGAAA
Pain_SA TGGATTGGGGAAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCcAGGACAGTGCCTTACGACAGAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGGAAA
Pain_TN2 TGGATTGGGGAAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCcAGGACAGTGCCTTACGACAGAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGGAAA
Pain_TN1 TGGATTGGGGAAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCcAGGACAGTGCCTTACGACAGAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGGAAA
Consensus TGGATTGGGGAAACTGATAGTGAATCTGCTGACCTGCAGAGGGATGGGCATCTGTACAGAGTATCCcAGGACAGTGCCTTACGACAGAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGGAAA

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
Pain_DA TTGAARCTTAAAGAGTGGGTGATCCTATTGTTAAGCAGTCAATCTTCAACCAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAAGCCTCATTGAGTGGACAAAGTCCG
Pain_SA TTGAARCTTAAAGAGTGGGTGATCCTATTGTTAAGCAGTCAATCTTCAACCAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAAGCCTCATTGAGTGGACAAAGTCCG
Pain_TN2 TTGAARCTTAAAGAGTGGGTGATCCTATTGTTAAGCAGTCAATCTTCAACCAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAAGCCTCATTGAGTGGACAAAGTCCG
Pain_TN1 TTGAARCTTAAAGAGTGGGTGATCCTATTGTTAAGCAGTCAATCTTCAACCAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAAGCCTCATTGAGTGGACAAAGTCCG
Consensus TTGAARCTTAAAGAGTGGGTGATCCTATTGTTAAGCAGTCAATCTTCAACCAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAAGCCTCATTGAGTGGACAAAGTCCG

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
Pain_DA GCTCCAGGGAATAATTGAAGCAGATCATGTAGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGTAATTGCTGATCAAAAGCTATCTGAGCTAACG
Pain_SA GCTCCAGGGAATAATTGAAGCAGATCATGTAGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGTAATTGCTGATCAAAAGCTATCTGAGCTAACG
Pain_TN2 GCTCCAGGGAATAATTGAAGCAGATCATGTAGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGTAATTGCTGATCAAAAGCTATCTGAGCTAACG
Pain_TN1 GCTCCAGGGAATAATTGAAGCAGATCATGTAGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGTAATTGCTGATCAAAAGCTATCTGAGCTAACG
Consensus GCTCCAGGGAATAATTGAAGCAGATCATGTAGTTTCAGCTGCTCTACTAGTGGAGGTGCTGCTAGCAGAGGCAATTTGGGACCATTGGTGTCTGTTGTAATTGCTGATCAAAAGCTATCTGAGCTAACG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
Pain_DA CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGTcGAGCTGAGACTCACTTCTGTGCTGATCAAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAAACcAGTTTATGGTAGTTCAGTACCCGTTGg
Pain_SA CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGTcGAGCTGAGACTCACTTCTGTGCTGATCAAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAAACcAGTTTATGGTAGTTCAGTACCCGTTGg
Pain_TN2 CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGTcGAGCTGAGACTCACTTCTGTGCTGATCAAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAAACcAGTTTATGGTAGTTCAGTACCCGTTGg
Pain_TN1 CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGTcGAGCTGAGACTCACTTCTGTGCTGATCAAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAAACcAGTTTATGGTAGTTCAGTACCCGTTGg
Consensus CCAGTTTACTTCTACATTTCTAARAGGAGCTGATGGTcGAGCTGAGACTCACTTCTGTGCTGATCAAACTAGATCCTCAGAGGCTCCGGGAGTTGCTAAACcAGTTTATGGTAGTTCAGTACCCGTTGg

1691 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800 1810 1820
Pain_DA ACGGTGAAAAACATTCCGATGAGATTATTGGTGGACCCTCAATTGTGGAGAGCTTTGCCcARAGGAGGAGACAGTCAATACATCGCGAATTTACCCARCAAGGCGAGTGAATGGAGCAGCAGCTCTT
Pain_SA ACGGTGAAAAACATTCCGATGAGATTATTGGTGGACCCTCAATTGTGGAGAGCTTTGCTcARAGGAGGAGACAGTCAATACATCGCGAATTTACCCARCAAGGCGAGTGAATGGAGCAGCAGCTCTT
Pain_TN2 ACGGTGAAAAACATTCCGATGAGATTATTGGTGGACCCTCAATTGTGGAGAGCTTTGCTcARAGGAGGAGACAGTCAATACATCGCGAATTTACCCARCAAGGCGAGTGAATGGAGCAGCAGCTCTT
Pain_TN1 ACGGTGAAAAACATTCCGATGAGATTATTGGTGGACCCTCAATTGTGGAGAGCTTTGCTcARAGGAGGAGACAGTCAATACATCGCGAATTTACCCARCAAGGCGAGTGAATGGAGCAGCAGCTCTT
Consensus ACGGTGAAAAACATTCCGATGAGATTATTGGTGGACCCTCAATTGTGGAGAGCTTTGCTcARAGGAGGAGACAGTCAATACATCGCGAATTTACCCARCAAGGCGAGTGAATGGAGCAGCAGCTCTT

1821 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
Pain_DA CGTTTTCAACcAATGCCACAGGGGcTAGCGTGACTGCTTCCGTCAGATTGGTCACTTGAAGTCGGCTAATATTCcGATCCTTCCCCTTGCAAGACTTGTA
Pain_SA CGTTTTCAACcAATGCCACAGGGGcTAGCGTGACTGCTTCCGTCAGATTGGTCACTTGAAGTCGGCTAATATTCcGATCCTTCCCCTTGCAAGACTTGTA
Pain_TN2 CGTTTTCAATcAATGCCACAGGGGcTAGCGTGACTGCTTCCGTCAGATTGGTCACTTGAAGTCGGCTAATATTCcGATCCTTCCCCTTGCAAGACTTGTA
Pain_TN1 CGTTTTCAACcAATGCCACAGGGGcTAGCGTGACTGCTTCCGTCAGATTGGTCACTTGAAGTCGGCTAATATTCcGATCCTTCCCCTTGCAAGACTTGTA
Consensus CGTTTTCAACcAATGCCACAGGGGcTAGCGTGACTGCTTCCGTCAGATTGGTCACTTGAAGTCGGCTAATATTCcGATCCTTCCCCTTGCAAGACTTGTA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
Pain_SN ATGGCCACGCAGTACCATTCCAGTTATGACCTGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACCTGATTCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Pain_SN** ATGGCCACGCAGTACCATTCCAGTTATGACCTGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACCTGATTCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Pain_SN* ATGGCCACGCAGTACCATTCCAGTTATGACCTGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACCTGATTCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT
Consensus ATGGCCACGCAGTACCATTCCAGTTATGACCTGGAAACTCCGCCTCCATTACACATTCTCCCGGATCAACCTGATTCGGCCACCGGAGTCCCTTAAATCATCTCCGGCATTTCCTCTCCTCTT

131 140 150 160 170 180 190 200 210 220 230 240 250 260
Pain_SN TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACAACCAATCACCGGACTTGACAGGTAACCTCCGTTCCGGCCGCGCCGCTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_SN** TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACAACCAATCACCGGACTTGACAGGTAACCTCCGTTCCGGCCGCGCCGCTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Pain_SN* TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACAACCAATCACCGGACTTGACAGGTAACCTCCGTTCCGGCCGCGCCGCTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA
Consensus TCCTTTTGTCTTCTGTAGCCTTCTTCCGATCCTCAACAACCAATCACCGGACTTGACAGGTAACCTCCGTTCCGGCCGCGCCGCTCAAGAGGTGTTTCTCAGGGAGTCTCCGATAGACTTTTCGAGA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
Pain_SN TGTGTCATGCTAGTACGTTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA
Pain_SN** TGTGTCATGCTAGTACGTTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA
Pain_SN* TGTGTCATGCTAGTACGTTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA
Consensus TGTGTCATGCTAGTACGTTTCTTATGCGTGGTCCAATGCTATGCTTAGCTGGCAAGAACTGCTTACCATTTTCAACCTCAAAAAATGGATGACGATCCTAATGGTCCATTGTACCACAGGGGA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
Pain_SN TGGTATCATCTTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTACTTGGCTTTTGGCATGGTTCCTGATCAATGGT
Pain_SN** TGGTATCATCTTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTACTTGGCTTTTGGCATGGTTCCTGATCAATGGT
Pain_SN* TGGTATCATCTTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTACTTGGCTTTTGGCATGGTTCCTGATCAATGGT
Consensus TGGTATCATCTTTTTATCAATACATCCAGATTCAGCTATTTGGGGAATATCACATGGGGCCATGCCGATCCAGGACTTGATCCACTGGCTACTTGGCTTTTGGCATGGTTCCTGATCAATGGT

521 530 540 550 560 570 580 590 600 610 620 630 640 650
Pain_SN ACGATATAACGGTGTCTGGACTGGGTCCGCTACCATCCTACCCGATGGTCAAGTATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGC AAAATCTTGGCTACCCCACTTATCTGA
Pain_SN** ACGATATAACGGTGTCTGGACTGGGTCCGCTACCATCCTACCCGATGGTCAAGTATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGC AAAATCTTGGCTACCCCACTTATCTGA
Pain_SN* ACGATATAACGGTGTCTGGACTGGGTCCGCTACCATCCTACCCGATGGTCAAGTATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGC AAAATCTTGGCTACCCCACTTATCTGA
Consensus ACGATATAACGGTGTCTGGACTGGGTCCGCTACCATCCTACCCGATGGTCAAGTATGATGCTTTATACCGGTGACACTGATGATTATGTACAGTGC AAAATCTTGGCTACCCCACTTATCTGA

651 660 670 680 690 700 710 720 730 740 750 760 770 780
Pain_SN TCCTCTCCTTCTAGACTGGGTCAAGTACAAGGCCAACCCGGTCTGGTTCTCCACCCGGCATGGTGTCAAGGACTTTAGAGACCCGACACTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Pain_SN** TCCTCTCCTTCTAGACTGGGTCAAGTACAAGGCCAACCCGGTCTGGTTCTCCACCCGGCATGGTGTCAAGGACTTTAGAGACCCGACACTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Pain_SN* TCCTCTCCTTCTAGACTGGGTCAAGTACAAGGCCAACCCGGTCTGGTTCTCCACCCGGCATGGTGTCAAGGACTTTAGAGACCCGACACTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA
Consensus TCCTCTCCTTCTAGACTGGGTCAAGTACAAGGCCAACCCGGTCTGGTTCTCCACCCGGCATGGTGTCAAGGACTTTAGAGACCCGACACTGCTTGGACCGGACCCAAATGGGCAATGGCTTTTA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
Pain_SN ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGCACTTGTATGAACTTCCACTTCCACAGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_SN** ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGCACTTGTATGAACTTCCACTTCCACAGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Pain_SN* ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGCACTTGTATGAACTTCCACTTCCACAGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT
Consensus ACAATCGGGTCTAAGATTGGTAAACCGGGTATTGCACTTGTATGAACTTCCACTTCCACAGCTTTAAGCTATTGGATGAAGTGTGCATGCGGTTCCGGGTACGGGTATGTGGGAGTGTGTGGACT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
Pain_SN TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATAACGGCCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_SN** TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATAACGGCCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Pain_SN* TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATAACGGCCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACATAGCAAGATCACTATGCTATTGGGACGTATGACTT
Consensus TTTACCCGGTATCGACTGAAAAACAACCGGGTGGACACATCATATAACGGCCCGGGTGAAGCATGTGTTAAAGCAAGTTAGATGACATAGCAAGATCACTATGCTATTGGGACGTATGACTT

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
Pain_SN GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTTTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Pain_SN** GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTTTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Pain_SN* GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTTTATGACCCGAGAAACACGAGAGTACTGTGGGGA
Consensus GACAAAGAACAAATGGACACCCGATACCCGGAAATGGATTGTGGAAATGGGTGAAGCTGGATTATGGGAATATTATGCATCAAGAGCATTTTATGACCCGAGAAACACGAGAGTACTGTGGGGA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
Pain_SN TGGATTGGGGAACCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCTTTACGACAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Pain_SN** TGGATTGGGGAACCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCTTTACGACAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Pain_SN* TGGATTGGGGAACCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCTTTACGACAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA
Consensus TGGATTGGGGAACCTGATAGTGAATCTGCTGACCTGCAGAGGGGATGGGCATCTGTACAGAGTATCCAGGACAGTGCTTTACGACAGAGACAGGGACACATCTACTTCAGTGGCCAGTTGAGAAA

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
Pain_SN TTGAAGCTTAAGAGCGGGTGTCTTATTGTTAAGCAAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCTCATTGAGTGGACAAAGTCGC
Pain_SN** TTGAAGCTTAAGAGCGGGTGTCTTATTGTTAAGCAAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCTCATTGAGTGGACAAAGTCGC
Pain_SN* TTGAAGCTTAAGAGCGGGTGTCTTATTGTTAAGCAAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCTCATTGAGTGGACAAAGTCGC
Consensus TTGAAGCTTAAGAGCGGGTGTCTTATTGTTAAGCAAGTCAATCTTCAACAGGTTCAATTGAGCTACTCCATGTTGACTCAGCTGCAGAGTTGGATATAGAGCCTCATTGAGTGGACAAAGTCGC

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
Pain_SN GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTAATTGCTGATCAACCGTATCTGAGCTAACG
Pain_SN** GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTAATTGCTGATCAACCGTATCTGAGCTAACG
Pain_SN* GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTAATTGCTGATCAACCGTATCTGAGCTAACG
Consensus GCTCCAGGGAATTAATGAAGCAGATCATGTAGGTTTCAGCTGCTACTAGTGGAGGTGCTGCTAGCAGAGGCATTTGGGACCATTGGTGTCTGTTAATTGCTGATCAACCGTATCTGAGCTAACG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
Pain_SN CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAACCCAGATCCTCAGAGGCTCCGGGAGTTGCTAACAAGTTTATGGTAGTTCAGTACCCGTGTTGG
Pain_SN** CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAACCCAGATCCTCAGAGGCTCCGGGAGTTGCTAACAAGTTTATGGTAGTTCAGTACCCGTGTTGG
Pain_SN* CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAACCCAGATCCTCAGAGGCTCCGGGAGTTGCTAACAAGTTTATGGTAGTTCAGTACCCGTGTTGG
Consensus CCAGTTTACTTCTACATTTCTAAGGAGCTGATGGCCGAGCTGAGACTCACTTCTGTGCTGATCAACCCAGATCCTCAGAGGCTCCGGGAGTTGCTAACAAGTTTATGGTAGTTCAGTACCCGTGTTGG

1691 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800 1810 1820
Pain_SN ACGGTGAAAAACATTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCTCAGGAGGAGAGACAGTCATAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT
Pain_SN** ACGGTGAAAAACATTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCTCAGGAGGAGAGACAGTCATAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT
Pain_SN* ACGGTGAAAAACATTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCTCAGGAGGAGAGACAGTCATAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT
Consensus ACGGTGAAAAACATTCGATGAGATTATTGGTGGACCACTCAATTGTGGAGAGCTTTGCTCAGGAGGAGAGACAGTCATAACATCGCGAATTTACCAACAAGGCAAGTGAATGGAGCAGCAGACTCTT

1821 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
Pain_SN CGTTTTCAACAATGCCACAGGGTCTAGCGTACTGCTCCGTCAGATTGGTCACTTGAGTCGGCTAATATTCARTCCTTCCCTTGCAGACTTGTA
Pain_SN** CGTTTTCAACAATGCCACAGGGTCTAGCGTACTGCTCCGTCAGATTGGTCACTTGAGTCGGCTAATATTCARTCCTTCCCTTGCAGACTTGTA
Pain_SN* CGTTTTCAACAATGCCACAGGGTCTAGCGTACTGCTCCGTCAGATTGGTCACTTGAGTCGGCTAATATTCARTCCTTCCCTTGCAGACTTGTA
Consensus CGTTTTCAACAATGCCACAGGGTCTAGCGTACTGCTCCGTCAGATTGGTCACTTGAGTCGGCTAATATTCARTCCTTCCCTTGCAGACTTGTA

Appendix 3.2

A sequence was defined as an allele when it was found twice in two independent PCRs. Additionally, the consensus sequence of all alleles found in one genotype was used for allele definition when variable sequence polymorphisms occurred.

In the following Table, *invGE* and *invGF* alleles are listed, which do not correspond to this definition because only one full-length sequence was obtained. Nevertheless, several independent PCR amplifications gave rise to sequences that after partial sequencing indicated the existence of the alleles as mentioned above, clearly implying their existence. These additional sequences exhibited frame shifts or other modifications (e.g. missing or modified start and stop codons) and, therefore, were not completely sequenced. Furthermore, pyrosequencing analysis was carried out for allele specific SNPs demonstrating the presence of the latter SNPs in the corresponding genotype at genomic level.

Table A3.2.1: Overview of fully and partially sequenced *invGE* and *invGF* alleles.

Gene	Full-length allele	Additional partial sequences	Pyrosequencing assay
<i>invGE</i>	<i>E_SN2</i>	No additional sequences	Section 3.2.2.1.1, Figure 3.2.24
	<i>E_DN2</i>	No additional sequences	Section 3.2.2.1.1, Figure 3.2.24
<i>invGF</i>	<i>F_DN1</i>	2	Section 3.2.2.1.3, Figure 3.2.26
	<i>F_TN2</i>	2	Section 3.2.2.1.3, Figure 3.2.26
	<i>F_P40N1</i>	2	Section 3.2.2.1.4, Figure 3.2.27

1 10 20 30 40 50 60 70 80 90 100 110 120 130
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
E_SA MELFMKSSSLWGLEIYLF^CFFI^VLSNIN^KV^FGSHNV^FLDLQSSSAISVKNV^HRT^GFHFQPPK^HWINDPNAPHYYNGVYHLFYQYNPKGSVMGNIYVAHSVSK^DLINWIHLEPAIYPSKKFDKYGAWSGSA
E_SA3 MELFMKSSSLWGLEIYLF^CFFI^VLSNIN^KV^FGSHNV^FLDLQSSSAISVKNV^HRT^GFHFQPPK^HWINDPNAPHYYNGVYHLFYQYNPKGSVMGNIYVAHSVSK^DLINWIHLEPAIYPSKKFDKYGAWSGSA
E_SA2 MELFMKSSSLWGLEIYLF^CFFI^VLSNIN^KV^FGSHNV^FLDLQSSSAISVKNV^HRT^GFHFQPPK^HWINDPNAPHYYNGVYHLFYQYNPKGSVMGNIYVAHSVSK^DLINWIHLEPAIYPSKKFDKYGAWSGSA
E_SA1 MELFMKSSSLWGLEIYLF^CFFI^VLSNIN^KV^FGSHNV^FLDLQSSSAISVKNV^HRT^GFHFQPPK^HWINDPNAPHYYNGVYHLFYQYNPKGSVMGNIYVAHSVSK^DLINWIHLEPAIYPSKKFDKYGAWSGSA
E_SN1 MELFMKSSSLWGLEIYLF^CLFI^VLSNIN^GV^FASHNIF^LDLQSSSAISVKNV^HRT^GFHFQPPK^HWINDPNAPHYYNGVYHLFYQYNPKGSVMGNIYVAHSVSK^DLINWIHLEPAIYPSKKFDKYGAWSGSA
E_SN1_1 MELFMKSSSLWGLEIYLF^CLFI^VLSNIN^GV^FASHNIF^LDLQSSSAISVKNV^HRT^GFHFQPPK^HWINDPNAPHYYNGVYHLFYQYNPKGSVMGNIYVAHSVSK^DLINWIHLEPAIYPSKKFDKYGAWSGSA
E_SN2 MELFMKSSSLWGLEIYLF^CLFI^VLSNIN^GV^FASHNIF^LDLQSSSAISVKNV^HRT^GFHFQPPK^HWINDPNAPHYYNGVYHLFYQYNPKGSVMGNIYVAHSVSK^DLINWIHLEPAIYPSKKFDKYGAWSGSA
E_SN3 MELFMKSSSLWGLEIYLF^CFFI^VLSNIN^KV^FASHNIF^LDLQSSSAISVKNV^HRT^SFHFQPPK^YWINDPNAPHYYNGVYHLFYQYNPKGSVMGNIYVAHSVSK^DLINWIHLEPAIYPSKKFDKYGAWSGSA
E_SN3_1 MELFMKSSSLWGLEIYLF^CFFI^VLSNIN^KV^FASHNIF^LDLQSSSAISVKNV^HRT^SFHFQPPK^YWINDPNAPHYYNGVYHLFYQYNPKGSVMGNIYVAHSVSK^DLINWIHLEPAIYPSKKFDKYGAWSGSA
E_SN3_2 MELFMKSSSLWGLEIYLF^CFFI^VLSNIN^KV^FASHNIF^LDLQSSSAISVKNV^HRT^SFHFQPPK^YWINDPNAPHYYNGVYHLFYQYNPKGSVMGNIYVAHSVSK^DLINWIHLEPAIYPSKKFDKYGAWSGSA
Consensus MELFMKSSSLWGLEIYLF^CFFI^VLSNIN^kV^faSHN!FLDLQSSSAISVKNV^HRT^gFHFQPPK^hWINDPNAPHYYNGVYHLFYQYNPKGSVMGNIYVAHSVSK^dLINWIHLEPAIYPSKKFDKYGAWSGSA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
E_SA TILPNNKPVILYTG^VVDSD^HTQVQNYAIPANLSDPFLRKWYKPN^NNPLIIPD^NSINKTKFRDPTTAWMGV^DGVWRIYIGSHR^KKHRGMALLYRSRDFIKW^YKAQHPLHSSPHTGNWECDFFPVSL^NNTNG
E_SA3 TILPNNKPVILYTG^VVDSD^HTQVQNYAIPANLSDPFLRKWYKPN^NNPLIIPD^NSINKTKFRDPTTAWMGV^DGVWRIYIGSHR^KKHRGMALLYRGRDFIKW^YKAQHPLHSSPHTGNWECDFFPVSL^NNTNG
E_SA2 TILPNNKPVIXYTGV^VSDSD^HTQVQNYAIPANLSDPFLRKWYKPN^NNPLIIPD^NSINKTKFRDPTTAWMGV^DGVWRIYIGSHR^KKHRGMALLYRSRDFIKW^YKAQHPLHSSPHTGNWECDFFPVSL^NNTNG
E_SA1 TILPNNKPVILYTG^VVDSD^HTQVQNYAIPANLSDPFLRKWYKPN^NNPLIIPD^NSINKTKFRDPTTAWMGV^DGVWRIYIGSHR^KKHRGMALLYRSRDFIKW^YKAQHPLHSSPHTGNWECDFFPVSL^NNTNG
E_SN1 TILPNNKPVILYTG^VVDSD^HSQVQNYAIPANLSDPFLRKWIKPN^NNPLIIPD^NSINKTKFRDPTTAWMG^QDGLWRIYIGSHR^KKHRGMALLYRSRDFIKW^AKAQHPLHSSPHTGNWECDFFPVSL^KNTNG
E_SN1_1 TILPNNKPVILYTG^VVDSD^HSQVQNYAIPANLSDPFLRKWIKPN^NNPLIIPD^NSINKTKFRDPTTAWMG^QDGLWRIYIGSHR^KKHRGMALLYRSRDFIKW^AKAQHPLHSSPHTGNWECDFFPVSL^KNTNG
E_SN2 TILPNNKPIILYTG^VVDSD^HSQVQNYAIPANLSDPFLRKWIKPN^NNPLIIPD^NSINKTKFRDPTTAWMG^QDGLWRIYIGSHR^NKHRGMALLYRSRDFIKW^TKAQHPLHSSPHTGNWECDFFPVSL^KNTNG
E_SN3 TILPNNKPVILYTG^VVDSD^HSQVQNYAIPANLSDPFLRKWIKPN^NNPLIIPD^NSINKTKFRDPTTAWMG^QDGLWRIYIGSHR^KKHRGMALLYRSRDFIKW^AKAQHPLHSSPHTGNWECDFFPVSL^KNTNG
E_SN3_1 TILPNNKPVILYTG^VVDSD^HSQVQNYAIPANLSDPFLRKWIKPN^NNPLIIPD^NSINKTKFRDPTTAWMG^QDGLWRIYIGSHR^KKHRGMALLYRSRDFIKW^AKAQHPLHSSPHTGNWECDFFPVSL^KNTNG
E_SN3_2 TILPNNKPVILYTG^VVDSD^HSQVQNYAIPANLSDPFLRKWIKPN^NNPLIIPD^NSINKTKFRDPTTAWMG^QDGLWRIYIGSHR^KKHRGMALLYRSRDFIKW^AKAQHPLHSSPHTGNWECDFFPVSL^KNTNG
Consensus TILPNNKPVILYTG^VVDSD^sQVQNYAIPANLSDPFLRKW!KPN^NNPLI!PD^NSINKTKFRDPTTAWMG^qDGLWRIYIGSHR^kKHRGMALLYRSRDFIKW[.]KAQHPLHSSPHTGNWECDFFPVSL^kNTNG

261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
E_SA LDASYRGK-NV^KYVLKNSLDV^NRF^DYTTIGHYD^TRKDRYIPD^NNSIDG^CKGLRLDYGNFYASKSFYD^PTKNRRIYV^GWGTNESD^VLPDDEIKKGWAGIQAI^PRKYWLDHSGKQLIQMPIEELET^LRKQKI-
E_SA3 LDASYRGK-NV^KYVLKNSLDV^NRF^DYTTIGHYD^TRKDRYIPD^NNSIDG^CKGLRLDYGNLYASKSFYD^PTKNRRIYV^GWGTNESD^VLPDDEIKKGWAGIQAI^PRKYWLDHSGKQLIQMPIEELET^LRKQKI-
E_SA2 LDASYRGK-NV^KYVLKNSLDV^NRF^DYTTIGHYD^TRKDRYIPD^NNSIDG^CKGLRLDYGNFYASKSFYD^PTKNRRIYV^GWGTNESD^VLPDDEIKKGWAGIQAI^PRKYWLDHSGKQLIQMPIEELET^LRKQKI-
E_SA1 LDASYRGK-NV^KYVLKNSLDV^NRF^DYTTIGHYD^TRKDRYIPD^NNSIDG^CKGLRLDYGNFYASKSFYD^PTKNRRIYV^GWGTNESD^VLPDDEIKKGWAGIQAI^PRKYWLDHSGKQLIQMPIEELET^LRKQKI-
E_SN1 LDASYRGK^KK^KY^KH^VLKNSLDV^NRF^EYTTIGHYD^TKDRYIPD^NNSIDG^SKGLRLDYGNFYASKSFYD^PMKNRRIYV^GWGTNESD^VLPDDEIKKGWAGIQAI^PRKYWLD^PSGKQLIQMPIEELET^LRKQKI-
E_SN1_1 LDASYRGK^KK^KY^KH^VLKNSLDV^NRF^EYTTIGHYD^TKDRYIPD^NNSIDG^SKGLRLDYGNFYASKSFYD^PMKNRRIYV^GWGTNESD^VLPDDEIKKGWAGIQAI^PRKYWLD^PSGKQLIQMPIEELET^LRKQKI-
E_SN2 LDASYRGK^KN^VK^HY^VLKNSLDV^NRF^EYTTIGHYD^TKDRYIPD^NNSIDG^SKGLRLDYGNFYASKSFYD^PMKNRRIYV^GWGTNESD^VLPDDEIKKGWAGIQAI^PRKYWLD^PSGKQLIQMPIEELET^LRKQKI-
E_SN3 LDASYRGK-NV^KH^VLKNSLDV^NRF^DYTTIGHYD^TKDRYIPD^NNSIDG^SKGLRLDYGNFYASKSFYD^PMKNRRIYV^GWGTNESD^VLPDDEIKKGWAGIQAI^PRKYWLD^PSGKQLIQMPIEELET^LRKQKI-
E_SN3_1 LDASYRGK-NV^KH^VLKNSLDV^NRF^DYTTIGHYD^TKDRYIPD^NNSIDG^SKGLRLDYGNFYASKSFYD^PMKNRRIYV^GWGTNESD^VLPDDEIKKGWAGIQAI^PRKYWLD^PSGKQLIQMPIEELET^LRKQKI-
E_SN3_2 LDASYRGK-NV^KH^VLKNSLDV^NRF^SYTTIGHYD^TKDRYIPD^NNSIDG^SKGLRLDYGNFYASKSFYD^PMKNRRIYV^GWGTNESD^VLPDDEIKKGWAGIQAI^PRKYWLD^PSGKQLIQMPIEELET^LRKQKI-
Consensus LDASYRGK[.]_nV^kH^vY^vLKNSLDV^NRf[#]YTTIGHYD^Tk^kDRYIPD^NNSIDG^sKGLRLDYGNFYASKSFYD^pmKNRRIYV^GWGTNESD^VLPDDEIKKGWAGIQAI^PRKYWLD^pSGKQLIQMPIEELET^LRKQKI[.]

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
E_SA QLNNK^LSKGEMFEV^KGISASQ^SDI^EV^SFS^FSSLN^KAEQ^FDPN^WADL^YAQDYCAIKG^STIQGGLGPFGL^AT^LASKNLEEYTPVFFRV^FKAQK^NY^KYL^HCSDARRSTHRQNEAMYKPSFAGYVDV^LYD^MK
E_SA3 QLNNK^LSKGEMFEV^KGISASQ^SDI^EV^SFS^FSSLN^KAEQ^FDPN^WADL^YAQDYCAIKG^STIQGGLGPFGL^AT^LASKNLEEYTPVFFRV^FKAQK^NY^KYL^HCSDARRSTHRQNEAMYKPSFAGYVDV^LYD^MK
E_SA2 QSNNK^LSKGEMFEV^KGISASQ^SDI^EV^SFS^FSSLN^KAEQ^FDPN^WADL^YAQDYCAIKG^STIQGGLGPFGL^AT^LASKNLEEYTPVFFRV^FKAQK^NY^KYL^HCSDARRSTHRQNEAMYKPSFAGYVDV^LYD^MK
E_SA1 QLNNK^LSKGEMFEV^KGISASQ^ADI^EV^SFS^FSSLN^KAEQ^FDPN^WADL^YAQDYCAIKG^STIQGGLGPFGL^AT^LASKNLEEYTPVFFRV^FKAQK^NY^KYL^HCSDARRSTHRQNEAMYKPSFAGYVDV^LYD^MK
E_SN1 QLNNK^LSKGEMFEV^KGISASQ^ADI^EV^SFS^FSSLN^EAEQ^FDPN^WADL^YAQDYCAIKG^STIQGGLGPFGL^AT^LASKNLEEYTPVFFRV^FKAQK^NY^KYL^HCSDARRSTHRQNEAMYKPSFAGYVDV^LYD^MK
E_SN1_1 QLNNK^LSKGEMFEV^KGISASQ^ADI^EV^SFS^FSSLN^EAEQ^FDPN^WADL^YAQDYCAIKG^STIQGGLGPFGL^AT^LASKNLEEYTPVFFRV^FKAQK^NY^KYL^HCSDARRSTHRQNEAMYKPSFAGYVDV^LYD^MK
E_SN2 QLNNK^LSKGEMFEV^KGISASQ^ADI^EV^SFS^FSSLN^KAEQ^FDPN^WADL^YAQDYCAIKG^STIQGGLGPFGL^VT^LASKNLEEYTPVFFRV^FKAQK^NY^KYL^HCSDARRSTHRQNEAMYKPSFAGYVDV^LYD^TK
E_SN3 QLNNK^LSKGEMFEV^EGISASQ^ADI^EV^SFS^FSSLN^KAEQ^FDPN^WADL^YAQDYCAIKG^STIQGGLGPFGL^AT^VASKNLEEYTPVFFRV^FKAQK^NY^KYL^HCSDARRSTHRQNEAMYKPSFAGYVDV^LYD^MK
E_SN3_1 QLNNK^LSKGEMFEV^EGISASQ^ADI^EV^SFS^FSSLN^KAEQ^FDPN^WADL^YAQDYCAIKG^STIQGGLGPFGL^AT^VASKNLEEYTPVFFRV^FKAQK^NY^KYL^HCSDARRSTHRQNEAMYKPSFAGYVDV^LYD^MK
E_SN3_2 QLNNK^LSKGEMFEV^EGISASQ^ADI^EV^SFS^FSSLN^KAEQ^FDPN^WADL^YAQDYCAIKG^STIQGGLGPFGL^AT^VASKNLEEYTPVFFRV^FKAQK^NY^KYL^HCSDARRSTHRQNEAMYKPSFAGYVDV^LYD^MK
Consensus QLNNK^LSKGEMFEV^kGISASQ^aDI^EV^SFS^fSSLN^kAEQ^FDPN^wADL^YAQDYCAIKG^STIQGGLGPFGL^at^LASKNLEEYTPVFFRV^FKAQK^NY^K!YL^HCSDARRSTHRQNEAMYKPSFAGYVDV^LYD^mK

521 530 540 550 560 570 580 586
|-----|-----|-----|-----|-----|-----|-----|
E_SA KLSLRSLIDNSV^VESFGAGGK^CITSRVYPTLAIH^NNAHLFVFNNGSETIT^IETLNAWSHDV^PKMH
E_SA3 KLSLRSLIDNSV^VESFGAGGK^CITSRVYPTLAIH^NNAHLFVFNNGSETIT^IETLNAWSHDV^PKMH
E_SA2 KLSLRSLIDNSV^VESFGAGGK^CITSRVYPTLAIH^NNAHLFVFNNGSETIT^IETLNAWSHDV^PKMH
E_SA1 KLSLRSLIDNSV^VESFGAGGK^CITSRVYPTLAIH^NNAHLFVFNNGSETIT^IETLNAWSHDV^PKMH
E_SN1 KLSLRSLIDNSV^VESFGAGGK^CITSRVYPTLAIH^NNAHLFVFNNGSETIT^IETLNAWSHDV^PKMH
E_SN1_1 KLSLRSLIDNSV^VESFGAGGK^CITSRVYPTLAIH^NNAHLFVFNNGSETIT^IETLNAWSHDV^PKMH
E_SN2 KLSLRSLIDNSV^VESFGAGGK^CITSRVYPTLAIH^NNAHLFVFNNGSETIT^IETLNAWSHDV^PKMH
E_SN3 KLSLRSLIDNSV^VESFGAGGK^CITSRVYPTLAIH^NNAHLFVFNNGSETIT^IETLNAWSHDV^PKMH
E_SN3_1 KLSLRSLIDNSV^VESFGAGGK^CITSRVYPTLAIH^NNAHLFVFNNGSETIT^IETLNAWSHDV^PKMH
E_SN3_2 KLSLRSLIDNSV^VESFGAGGK^CITSRVYPTLAIH^NNAHLFVFNNGSETIT^IETLNAWSHDV^PKMH
Consensus KLSLRSLIDNSV^VESFGAGGK^CITSRVYPTLAIH[#]NAHLFVFNNGSETIT^IETLNAWSHDV^PKMH

1 10 20 30 40 50 60 70 80 90 100 110 120 130
E_DA MELFMKSSSLWGLEIYLFCLFFIYLSNINKVFGSHNVFLDLQSSSAISVKNVHRTGFHFQPPKHWINDPNAPHYYNGVYHLFYQYNPKGSVWGNIYVAHSVSKDLINWHLLEPAIYPSKKFDKYGAWSGSA
E_DA2 MELFMKSSSLWGLEIYLFCLFFIYLSNINKVFGSHNVFLDLQSSSAISVKNVHRTGFHFQPPKHWINDPNAPHYYNGVYHLFYQYNPKGSVWGNIYVAHSVSKDLINWHLLEPAIYPSKKFDKYGAWSGSA
E_DA1 MELFMKSSSLWGLEIYLFCLFFIYLSNINKVFGSHNVFLDLQSSSAISVKNVHRTGFHFQPPKHWINDPNAPHYYNGVYHLFYQYNPKGSVWGNIYVAHSVSKDLINWHLLEPAIYPSKKFDKYGAWSGSA
E_DN1 MELFMKSSSLWGLEIYLFCLFFIYLSNINKVFGSHNVFLDLQSSSAISVKNVHRTGFHFQPPKHWINDPNAPHYYNGVYHLFYQYNPKGSVWGNIYVAHSVSKDLINWHLLEPAIYPSKKFDKYGAWSGSA
E_DN1_3 MELFMKSSSLWGLEIYLFCLFFIYLSNINKVFGSHNVFLDLQSSSAISVKNVHRTGFHFQPPKHWINDPNAPHYYNGVYHLFYQYNPKGSVWGNIYVAHSVSKDLINWHLLEPAIYPSKKFDKYGAWSGSA
E_DN1_2 MELFMKSSSLWGLEIYLFCLFFIYLSNINKVFGSHNVFLDLQSSSAISVKNVHRTGFHFQPPKHWINDPNAPHYYNGVYHLFYQYNPKGSVWGNIYVAHSVSKDLINWHLLEPAIYPSKKFDKYGAWSGSA
E_DN1_1 MELFMKSSSLWGLEIYLFCLFFIYLSNINKVFGSHNVFLDLQSSSAISVKNVHRTGFHFQPPKHWINDPNAPHYYNGVYHLFYQYNPKGSVWGNIYVAHSVSKDLINWHLLEPAIYPSKKFDKYGAWSGSA
E_DN2 MELFMKSSSLWGLEIYLFCLFFIYLSNINKVFGSHNVFLDLQSSSAISVKNVHRTGFHFQPPKHWINDPNAPHYYNGVYHLFYQYNPKGSVWGNIYVAHSVSKDLINWHLLEPAIYPSKKFDKYGAWSGSA
Consensus MELFMKSSSLWGLEIYLFCLFFIYLSNINKVFGSHNVFLDLQSSSAISVKNVHRTGFHFQPPKHWINDPNAPHYYNGVYHLFYQYNPKGSVWGNIYVAHSVSKDLINWHLLEPAIYPSKKFDKYGAWSGSA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
E_DA TILPNNKPVILYTGVDSDHTQVQNYAIPANLSDPFLRKWVKPNNPLIIPDNSINKTKFRDPTTAWMGVDGVMRIVIGSMRKHRGMALLYRSRDFIKWVKAQHPLHSSPHTGNWECPDFFPVSLNNTNG
E_DA2 TILPNNKPVILYTGVDSDHTQVQNYAIPANLSDPFLRKWVKPNNPLIIPDNSINKTKFRDPTTAWMGVDGVMRIVIGSMRKHRGMALLYRSRDFIKWVKAQHPLHSSPHTGNWECPDFFPVSLNNTNG
E_DA1 TILPNNKPVILYTGVDSDHTQVQNYAIPANLSDPFLRKWVKPNNPLIIPDNSINKTKFRDPTTAWMGVDGVMRIVIGSMRKHRGMALLYRSRDFIKWVKAQHPLHSSPHTGNWECPDFFPVSLNNTNG
E_DN1 TILPNNKPVILYTGVDSDHTQVQNYAIPANLSDPFLRKWVKPNNPLIIPDNSINKTKFRDPTTAWMGVDGVMRIVIGSMRKHRGMALLYRSRDFIKWVKAQHPLHSSPHTGNWECPDFFPVSLNNTNG
E_DN1_3 TILPNNKPVILYTGVDSDHTQVQNYAIPANLSDPFLRKWVKPNNPLIIPDNSINKTKFRDPTTAWMGVDGVMRIVIGSMRKHRGMALLYRSRDFIKWVKAQHPLHSSPHTGNWECPDFFPVSLNNTNG
E_DN1_2 TILPNNKPVILYTGVDSDHTQVQNYAIPANLSDPFLRKWVKPNNPLIIPDNSINKTKFRDPTTAWMGVDGVMRIVIGSMRKHRGMALLYRSRDFIKWVKAQHPLHSSPHTGNWECPDFFPVSLNNTNG
E_DN1_1 TILPNNKPVILYTGVDSDHTQVQNYAIPANLSDPFLRKWVKPNNPLIIPDNSINKTKFRDPTTAWMGVDGVMRIVIGSMRKHRGMALLYRSRDFIKWVKAQHPLHSSPHTGNWECPDFFPVSLNNTNG
E_DN2 TILPNNKPVILYTGVDSDHTQVQNYAIPANLSDPFLRKWVKPNNPLIIPDNSINKTKFRDPTTAWMGVDGVMRIVIGSMRKHRGMALLYRSRDFIKWVKAQHPLHSSPHTGNWECPDFFPVSLNNTNG
Consensus TILPNNKPVILYTGVDSDHTQVQNYAIPANLSDPFLRKWVKPNNPLIIPDNSINKTKFRDPTTAWMGVDGVMRIVIGSMRKHRGMALLYRSRDFIKWVKAQHPLHSSPHTGNWECPDFFPVSLNNTNG

261 270 280 290 300 310 320 330 340 350 360 370 380 390
E_DA LDASYRGK-NVKYVLKNSLDYNRFDYTTIGMYDTRKDRYIPDNNSIDGCKGLRLDYGNFYASKSFYDPTKNRRIYVWGTNESDVLPPDEIKKGWAGIQAIPRKVWLDHSGKQLIQWPIEELETLRKQKI-
E_DA2 LDASYRGK-NVKYVLKNSLDYNRFDYTTIGMYDTRKDRYIPDNNSIDGCKGLRLDYGNFYASKSFYDPTKNRRIYVWGTNESDVLPPDEIKKGWAGIQAIPRKVWLDHSGKQLIQWPIEELETLRKQKI-
E_DA1 LDASYRGK-NVKYVLKNSLDYNRFDYTTIGMYDTRKDRYIPDNNSIDGCKGLRLDYGNFYASKSFYDPTKNRRIYVWGTNESDVLPPDEIKKGWAGIQAIPRKVWLDHSGKQLIQWPIEELETLRKQKI-
E_DN1 LDASYRGK-NVKYVLKNSLDYNRFEYTTIGMYDTRKDRYIPDNNSIDGCKGLRLDYGNFYASKSFYDPTKNRRIYVWGTNESDVLPPDEIKKGWAGIQAIPRKVWLDHSGKQLIQWPIEELETLRKQKI
E_DN1_3 LDASYRGK-NVKYVLKNSLDYNRFEYTTIGMYDTRKDRYIPDNNSIDGCKGLRLDYGNFYASKSFYDPTKNRRIYVWGTNESDVLPPDEIKKGWAGIQAIPRKVWLDHSGKQLIQWPIEELETLRKQKI
E_DN1_2 LDASYRGK-NVKYVLKNSLDYNRFEYTTIGMYDTRKDRYIPDNNSIDGCKGLRLDYGNFYASKSFYDPTKNRRIYVWGTNESDVLPPDEIKKGWAGIQAIPRKVWLDHSGKQLIQWPIEELETLRKQKI
E_DN1_1 LDASYRGK-NVKYVLKNSLDYNRFEYTTIGMYDTRKDRYIPDNNSIDGCKGLRLDYGNFYASKSFYDPTKNRRIYVWGTNESDVLPPDEIKKGWAGIQAIPRKVWLDHSGKQLIQWPIEELETLRKQKI
E_DN2 LDASYRGK-NVKYVLKNSLDYNRFEYTTIGMYDTRKDRYIPDNNSIDGCKGLRLDYGNFYASKSFYDPTKNRRIYVWGTNESDVLPPDEIKKGWAGIQAIPRKVWLDHSGKQLIQWPIEELETLRKQKI
Consensus LDASYRGK-NVKYVLKNSLDYNRFEYTTIGMYDTRKDRYIPDNNSIDGCKGLRLDYGNFYASKSFYDPTKNRRIYVWGTNESDVLPPDEIKKGWAGIQAIPRKVWLDHSGKQLIQWPIEELETLRKQKI

391 400 410 420 430 440 450 460 470 480 490 500 510 520
E_DA QLNKKLSKGFMEYKGISASQSDIEVSFSFSSLNKAQFDPNWADLYAQDYCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLHCSARRSTHRQNEAMYKPSFAGYVDVLDVDMK
E_DA2 QLNKKLSKGFMEYKGISASQSDIEVSFSFSSLNKAQFDPNWADLYAQDYCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLHCSARRSTHRQNEAMYKPSFAGYVDVLDVDMK
E_DA1 QLNKKLSKGFMEYKGISASQSDIEVSFSFSSLNKAQFDPNWADLYAQDYCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLHCSARRSTHRQNEAMYKPSFAGYVDVLDVDMK
E_DN1 QLNKKLSKGFMEYKGISASQSDIEVSFSFSSLNKAQFDPNWADLYAQDYCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLHCSARRSTHRQNEAMYKPSFAGYVDVLDVDMK
E_DN1_3 QLNKKLSKGFMEYKGISASQSDIEVSFSFSSLNKAQFDPNWADLYAQDYCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLHCSARRSTHRQNEAMYKPSFAGYVDVLDVDMK
E_DN1_2 QLNKKLSKGFMEYKGISASQSDIEVSFSFSSLNKAQFDPNWADLYAQDYCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLHCSARRSTHRQNEAMYKPSFAGYVDVLDVDMK
E_DN1_1 QLNKKLSKGFMEYKGISASQSDIEVSFSFSSLNKAQFDPNWADLYAQDYCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLHCSARRSTHRQNEAMYKPSFAGYVDVLDVDMK
E_DN2 QLNKKLSKGFMEYKGISASQSDIEVSFSFSSLNKAQFDPNWADLYAQDYCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLHCSARRSTHRQNEAMYKPSFAGYVDVLDVDMK
Consensus QLNKKLSKGFMEYKGISASQSDIEVSFSFSSLNKAQFDPNWADLYAQDYCAIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLHCSARRSTHRQNEAMYKPSFAGYVDVLDVDMK

521 530 540 550 560 570 580 586
E_DA KLSLRSIDNSVYESFGAGGKTCITSRVYPTLAIHNAHLVFNNGSETITITETLNAWSMDVPKMH
E_DA2 KLSLRSIDNSVYESFGAGGKTCITSRVYPTLAIHNAHLVFNNGSETITITETLNAWSMDVPKMH
E_DA1 KLSLRSIDNSVYESFGAGGKTCITSRVYPTLAIHNAHLVFNNGSETITITETLNAWSMDVPKMH
E_DN1 KLSLRSIDNSVYESFGAGGKTCITSRVYPTLAIHNAHLVFNNGSETITITETLNAWSMDVPKMH
E_DN1_3 KLSLRSIDNSVYESFGAGGKTCITSRVYPTLAIHNAHLVFNNGSETITITETLNAWSMDVPKMH
E_DN1_2 KLSLRSIDNSVYESFGAGGKTCITSRVYPTLAIHNAHLVFNNGSETITITETLNAWSMDVPKMH
E_DN1_1 KLSLRSIDNSVYESFGAGGKTCITSRVYPTLAIHNAHLVFNNGSETITITETLNAWSMDVPKMH
E_DN2 KLSLRSIDNSVYESFGAGGKTCITSRVYPTLAIHNAHLVFNNGSETITITETLNAWSMDVPKMH
Consensus KLSLRSIDNSVYESFGAGGKTCITSRVYPTLAIHNAHLVFNNGSETITITETLNAWSMDVPKMH

1 10 20 30 40 50 60 70 80 90 100 110 120 130
E_SA ATGGAATTATTTATGAAAGCTCTCTCTTTGGGGTTAGAAATTTATTTATTTTGGCTCTTATAGTTTATCAACATTAAATAGGTGTTGGTTCATATGTTTTTTGGACTTGCATCTTCAA
E_SA2 ATGGAATTATTTATGAAAGCTCTCTCTTTGGGGTTAGAAATTTATTTATTTTGGCTCTTATAGTTTATCAACATTAAATAGGTGTTGGTTCATATGTTTTTTGGACTTGCATCTTCAA
E_SA3 ATGGAATTATTTATGAAAGCTCTCTCTTTGGGGTTAGAAATTTATTTATTTTGGCTCTTATAGTTTATCAACATTAAATAGGTGTTGGTTCATATGTTTTTTGGACTTGCATCTTCAA
E_SA1 ATGGAATTATTTATGAAAGCTCTCTCTTTGGGGTTAGAAATTTATTTATTTTGGCTCTTATAGTTTATCAACATTAAATAGGTGTTGGTTCATATGTTTTTTGGACTTGCATCTTCAA
Consensus ATGGAATTATTTATGAAAGCTCTCTCTTTGGGGTTAGAAATTTATTTATTTTGGCTCTTATAGTTTATCAACATTAAATAGGTGTTGGTTCATATGTTTTTTGGACTTGCATCTTCAA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
E_SA GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAACATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
E_SA2 GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAACATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
E_SA3 GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAACATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
E_SA1 GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAACATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
Consensus GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAACATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
E_SA AGGATCAGTATGGGGTAAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGGATAAATTGGATCCATTAGAACCCGCAATTTACCCATCCAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCC
E_SA2 AGGATCAGTATGGGGTAAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGGATAAATTGGATCCATTAGAACCCGCAATTTACCCATCCAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCC
E_SA3 AGGATCAGTATGGGGTAAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGGATAAATTGGATCCATTAGAACCCGCAATTTACCCATCCAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCC
E_SA1 AGGATCAGTATGGGGTAAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGGATAAATTGGATCCATTAGAACCCGCAATTTACCCATCCAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCC
Consensus AGGATCAGTATGGGGTAAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGGATAAATTGGATCCATTAGAACCCGCAATTTACCCATCCAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCC

391 400 410 420 430 440 450 460 470 480 490 500 510 520
E_SA ACTATTCTACCTAATAACAGCCTGTTATTTATACACTGGAGTAGTAGATTCTCATGATCTCAAGTTCAAACCTATGCCATCCAGCTAATTTGCTGATCCGTTTCTTCGTAATGGGTCAAACCCA
E_SA2 ACTATTCTACCTAATAACAGCCTGTTATTTATACACTGGAGTAGTAGATTCTCATGATCTCAAGTTCAAACCTATGCCATCCAGCTAATTTGCTGATCCGTTTCTTCGTAATGGGTCAAACCCA
E_SA3 ACTATTCTACCTAATAACAGCCTGTTATTTATACACTGGAGTAGTAGATTCTCATGATCTCAAGTTCAAACCTATGCCATCCAGCTAATTTGCTGATCCGTTTCTTCGTAATGGGTCAAACCCA
E_SA1 ACTATTCTACCTAATAACAGCCTGTTATTTATACACTGGAGTAGTAGATTCTCATGATCTCAAGTTCAAACCTATGCCATCCAGCTAATTTGCTGATCCGTTTCTTCGTAATGGGTCAAACCCA
Consensus ACTATTCTACCTAATAACAGCCTGTTATTTATACACTGGAGTAGTAGATTCTCATGATCTCAAGTTCAAACCTATGCCATCCAGCTAATTTGCTGATCCGTTTCTTCGTAATGGGTCAAACCCA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
E_SA ATACAACCCGTTGATTATCTGCAATAGCATCAACAACCAAAATTTCTGATCCACACCCGCTGGATGGGTGTAGATGGGGTTGGAGGATTGTAATAGGAGTATGAGAAACATAGAGGGAT
E_SA2 ATACAACCCGTTGATTATCTGCAATAGCATCAACAACCAAAATTTCTGATCCACACCCGCTGGATGGGTGTAGATGGGGTTGGAGGATTGTAATAGGAGTATGAGAAACATAGAGGGAT
E_SA3 ATACAACCCGTTGATTATCTGCAATAGCATCAACAACCAAAATTTCTGATCCACACCCGCTGGATGGGTGTAGATGGGGTTGGAGGATTGTAATAGGAGTATGAGAAACATAGAGGGAT
E_SA1 ATACAACCCGTTGATTATCTGCAATAGCATCAACAACCAAAATTTCTGATCCACACCCGCTGGATGGGTGTAGATGGGGTTGGAGGATTGTAATAGGAGTATGAGAAACATAGAGGGAT
Consensus ATACAACCCGTTGATTATCTGCAATAGCATCAACAACCAAAATTTCTGATCCACACCCGCTGGATGGGTGTAGATGGGGTTGGAGGATTGTAATAGGAGTATGAGAAACATAGAGGGAT

651 660 670 680 690 700 710 720 730 740 750 760 770 780
E_SA GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGTCAAAGCCACACCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGCCTGATTTTTCCCTGTATCATTAAATAACTAATGGT
E_SA2 GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGTCAAAGCCACACCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGCCTGATTTTTCCCTGTATCATTAAATAACTAATGGT
E_SA3 GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGTCAAAGCCACACCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGCCTGATTTTTCCCTGTATCATTAAATAACTAATGGT
E_SA1 GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGTCAAAGCCACACCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGCCTGATTTTTCCCTGTATCATTAAATAACTAATGGT
Consensus GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGTCAAAGCCACACCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGCCTGATTTTTCCCTGTATCATTAAATAACTAATGGT

781 790 800 810 820 830 840 850 860 870 880 890 900 910
E_SA TTAGATGCATCGTATCGCGAAAAATGTCAAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTGATTACTACACTATTGGCATGTATGACACCAGAAAGATAGGTACATTCTGATAACATT
E_SA2 TTAGATGCATCGTATCGCGAAAAATGTCAAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTGATTACTACACTATTGGCATGTATGACACCAGAAAGATAGGTACATTCTGATAACATT
E_SA3 TTAGATGCATCGTATCGCGAAAAATGTCAAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTGATTACTACACTATTGGCATGTATGACACCAGAAAGATAGGTACATTCTGATAACATT
E_SA1 TTAGATGCATCGTATCGCGAAAAATGTCAAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTGATTACTACACTATTGGCATGTATGACACCAGAAAGATAGGTACATTCTGATAACATT
Consensus TTAGATGCATCGTATCGCGAAAAATGTCAAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTGATTACTACACTATTGGCATGTATGACACCAGAAAGATAGGTACATTCTGATAACATT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
E_SA CTATCGATGGTTGTAAGGGATTGAGGCTTGATTATGGGAATTTCTATGCATCTAATCATTATGATCCTACTAAGAAATCGAAGAAATGTTGTTGGGGTTGGACCAATGAATCAGATGTTTTACCTGACGA
E_SA2 CTATCGATGGTTGTAAGGGATTGAGGCTTGATTATGGGAATTTCTATGCATCTAATCATTATGATCCTACTAAGAAATCGAAGAAATGTTGTTGGGGTTGGACCAATGAATCAGATGTTTTACCTGACGA
E_SA3 CTATCGATGGTTGTAAGGGATTGAGGCTTGATTATGGGAATTTCTATGCATCTAATCATTATGATCCTACTAAGAAATCGAAGAAATGTTGTTGGGGTTGGACCAATGAATCAGATGTTTTACCTGACGA
E_SA1 CTATCGATGGTTGTAAGGGATTGAGGCTTGATTATGGGAATTTCTATGCATCTAATCATTATGATCCTACTAAGAAATCGAAGAAATGTTGTTGGGGTTGGACCAATGAATCAGATGTTTTACCTGACGA
Consensus CTATCGATGGTTGTAAGGGATTGAGGCTTGATTATGGGAATTTCTATGCATCTAATCATTATGATCCTACTAAGAAATCGAAGAAATGTTGTTGGGGTTGGACCAATGAATCAGATGTTTTACCTGACGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
E_SA TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGAAAAGTATGGCTCGATCATAGTGGTAACCAATTGATTCATGGCTATTGAGAAATAGAAACCTAAGAAACAAAAGATCCAATG
E_SA2 TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGAAAAGTATGGCTCGATCATAGTGGTAACCAATTGATTCATGGCTATTGAGAAATAGAAACCTAAGAAACAAAAGATCCAATG
E_SA3 TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGAAAAGTATGGCTCGATCATAGTGGTAACCAATTGATTCATGGCTATTGAGAAATAGAAACCTAAGAAACAAAAGATCCAATG
E_SA1 TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGAAAAGTATGGCTCGATCATAGTGGTAACCAATTGATTCATGGCTATTGAGAAATAGAAACCTAAGAAACAAAAGATCCAATG
Consensus TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGAAAAGTATGGCTCGATCATAGTGGTAACCAATTGATTCATGGCTATTGAGAAATAGAAACCTAAGAAACAAAAGATCCAATG

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
E_SA AATAACAGAGTTGAGCAGGGGAGAAATGTTGAAAGTTAAGGAATCTCAGCATCACAGTCTGATATTGAAGTGTCAATTTCTTTTTCAGTTTGAACAAAGCTGAACAATTTGATCCTAATGGGCTG
E_SA2 AATAACAGAGTTGAGCAGGGGAGAAATGTTGAAAGTTAAGGAATCTCAGCATCACAGTCTGATATTGAAGTGTCAATTTCTTTTTCAGTTTGAACAAAGCTGAACAATTTGATCCTAATGGGCTG
E_SA3 AATAACAGAGTTGAGCAGGGGAGAAATGTTGAAAGTTAAGGAATCTCAGCATCACAGTCTGATATTGAAGTGTCAATTTCTTTTTCAGTTTGAACAAAGCTGAACAATTTGATCCTAATGGGCTG
E_SA1 AATAACAGAGTTGAGCAGGGGAGAAATGTTGAAAGTTAAGGAATCTCAGCATCACAGTCTGATATTGAAGTGTCAATTTCTTTTTCAGTTTGAACAAAGCTGAACAATTTGATCCTAATGGGCTG
Consensus AATAACAGAGTTGAGCAGGGGAGAAATGTTGAAAGTTAAGGAATCTCAGCATCACAGTCTGATATTGAAGTGTCAATTTCTTTTTCAGTTTGAACAAAGCTGAACAATTTGATCCTAATGGGCTG

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
E_SA ACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAAACCTTGAAGAAATATACACCTGTTTTCTTTTCGAGTGTTTAA
E_SA2 ACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAAACCTTGAAGAAATATACACCTGTTTTCTTTTCGAGTGTTTAA
E_SA3 ACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAAACCTTGAAGAAATATACACCTGTTTTCTTTTCGAGTGTTTAA
E_SA1 ACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAAACCTTGAAGAAATATACACCTGTTTTCTTTTCGAGTGTTTAA
Consensus ACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAAACCTTGAAGAAATATACACCTGTTTTCTTTTCGAGTGTTTAA

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
E_SA GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACATGAGAGGTTA
E_SA2 GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACATGAGAGGTTA
E_SA3 GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACATGAGAGGTTA
E_SA1 GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACATGAGAGGTTA
Consensus GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACATGAGAGGTTA

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
E_SA TCTCTTAGGAGTTTGGTGGTAACTCAGTAGTGGAGAGTTTGGTGTGGAGGAAAACATGCATAACATCGAGGGTGTATCCACATTAGCAATTCATGATAATGCACATTTATTTGCTTCAATATG
E_SA2 TCTCTTAGGAGTTTGGTGGTAACTCAGTAGTGGAGAGTTTGGTGTGGAGGAAAACATGCATAACATCGAGGGTGTATCCACATTAGCAATTCATGATAATGCACATTTATTTGCTTCAATATG
E_SA3 TCTCTTAGGAGTTTGGTGGTAACTCAGTAGTGGAGAGTTTGGTGTGGAGGAAAACATGCATAACATCGAGGGTGTATCCACATTAGCAATTCATGATAATGCACATTTATTTGCTTCAATATG
E_SA1 TCTCTTAGGAGTTTGGTGGTAACTCAGTAGTGGAGAGTTTGGTGTGGAGGAAAACATGCATAACATCGAGGGTGTATCCACATTAGCAATTCATGATAATGCACATTTATTTGCTTCAATATG
Consensus TCTCTTAGGAGTTTGGTGGTAACTCAGTAGTGGAGAGTTTGGTGTGGAGGAAAACATGCATAACATCGAGGGTGTATCCACATTAGCAATTCATGATAATGCACATTTATTTGCTTCAATATG

1691 1700 1710 1720 1730 1740 1750 1755
E_SA GATCTGAGACAAATCACAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACCTAA
E_SA2 GATCTGAGACAAATCACAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACCTAA
E_SA3 GATCTGAGACAAATCACAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACCTAA
E_SA1 GATCTGAGACAAATCACAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACCTAA
Consensus GATCTGAGACAAATCACAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACCTAA

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
E_SN1	-----													
E_SN1_1	ATGGAATTATTTATGAAAAGCTCTTCTCTTTGGGGTTTAGAAATTTATTTATTTTGCCCTCTTACAGTTTTATCAAACATTAATGGGGTGGTTGCTTCTCATATATTTTTTTGGACTTGCARTCTTCAA													
Consensus	ATGGAATTATTTATGAAAAGCTCTTCTCTTTGGGGTTTAGAAATTTATTTATTTTGCCCTCTTACAGTTTTATCAAACATTAATGGGGTGGTTGCTTCTCATATATTTTTTTGGACTTGCARTCTTCAA													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
E_SN1	-----													
E_SN1_1	GTGCTATTAGTGTCAAGAATGTTTCATAGAACTGGTTTTCAATTTCAACCTCCTAACATTGGATTAAATGACCCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA													
Consensus	GTGCTATTAGTGTCAAGAATGTTTCATAGAACTGGTTTTCAATTTCAACCTCCTAACATTGGATTAAATGACCCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
E_SN1	-----													
E_SN1_1	AGGATCAGTATGGGGCAATATTGTTGGGCTCATTCAAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAAATTTGACAAATATGGTGCTGGTCCGGGTCAGCA													
Consensus	AGGATCAGTATGGGGCAATATTGTTGGGCTCATTCAAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAAATTTGACAAATATGGTGCTGGTCCGGGTCAGCA													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
E_SN1	-----													
E_SN1_1	ACTATTCTACCAATAACAAGCCCGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAAGTTCAAAATTATGCARTCCCGGCTAACTTGTCTGATCCATTTCTTCGTAATGGATCAACCTA													
Consensus	ACTATTCTACCAATAACAAGCCCGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAAGTTCAAAATTATGCARTCCCGGCTAACTTGTCTGATCCATTTCTTCGTAATGGATCAACCTA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
E_SN1	-----													
E_SN1_1	ATAACAACCCGTTGATTGTTCTGACAATAGCATCAACAAACCAAAATTTTCGTGATCCACACCCGATGGATGGGCCAAGATGGGCTTTGGAGAATTGTAATAGGAAGTATGAGAAACATAGAGGGAT													
Consensus	ATAACAACCCGTTGATTGTTCTGACAATAGCATCAACAAACCAAAATTTTCGTGATCCACACCCGATGGATGGGCCAAGATGGGCTTTGGAGAATTGTAATAGGAAGTATGAGAAACATAGAGGGAT													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
E_SN1	-----													
E_SN1_1	GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGCCCAACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGAATGTCCTGATTTTTCCCTGTATCATTAAAAAATACTAATGGC													
Consensus	GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGCCCAACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGAATGTCCTGATTTTTCCCTGTATCATTAAAAAATACTAATGGC													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
E_SN1	-----													
E_SN1_1	TTAGATGCATCGTATCGCGGAAAAAAGTCAACATGTCCTTAAGAATAGCCTTGATGTTAATAGGTTTGAGTATTACACTATTGGTATGTATGACACCAAAAAAGATAGGTACATTCTGATAACA													
Consensus	TTAGATGCATCGTATCGCGGAAAAAAGTCAACATGTCCTTAAGAATAGCCTTGATGTTAATAGGTTTGAGTATTACACTATTGGTATGTATGACACCAAAAAAGATAGGTACATTCTGATAACA													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
E_SN1	-----													
E_SN1_1	ATTCTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAAATCATTCTATGACCCTATGAGAATCGAAGAATTGTATGGGGTTGGACAATGAATCAGATGTTTTACCTGA													
Consensus	ATTCTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAAATCATTCTATGACCCTATGAGAATCGAAGAATTGTATGGGGTTGGACAATGAATCAGATGTTTTACCTGA													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
E_SN1	-----													
E_SN1_1	CGATGAATTAAGAAGGATGGGCTGGTATTCAAGCTATTCCGCGTAAGTATGGCTCGACCCTAGTGGTAACCAATTGATTCAATGGCCTATTGAGAATTAGAACCCTTGAGAAGCAAAAGATTCAA													
Consensus	CGATGAATTAAGAAGGATGGGCTGGTATTCAAGCTATTCCGCGTAAGTATGGCTCGACCCTAGTGGTAACCAATTGATTCAATGGCCTATTGAGAATTAGAACCCTTGAGAAGCAAAAGATTCAA													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
E_SN1	-----													
E_SN1_1	TTGAACAACAAGAGTTGAGCAAGGGAGAAATGTTTGAAGTTAAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTCTGTTCTTTTTCAAGTTTGACGAGGGCCGAACAATTTGATCCTAATGGG													
Consensus	TTGAACAACAAGAGTTGAGCAAGGGAGAAATGTTTGAAGTTAAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTCTGTTCTTTTTCAAGTTTGACGAGGGCCGAACAATTTGATCCTAATGGG													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
E_SN1	-----													
E_SN1_1	CCGACCTTTATGCCAAGATGTTTGTGCCATTAAAGGTTTCGACTATCCAAGGTGGGCTTGGGCCATTTGGGCTTGCAGACATTAGCTTCTAAAACTTGGAGAATACACACCTGTTTTCTTTCGAGTGT													
Consensus	CCGACCTTTATGCCAAGATGTTTGTGCCATTAAAGGTTTCGACTATCCAAGGTGGGCTTGGGCCATTTGGGCTTGCAGACATTAGCTTCTAAAACTTGGAGAATACACACCTGTTTTCTTTCGAGTGT													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
E_SN1	-----													
E_SN1_1	TAAGGCTCAAAGAATTATAAGATTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACATGAGAAG													
Consensus	TAAGGCTCAAAGAATTATAAGATTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACATGAGAAG													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
E_SN1	-----													
E_SN1_1	TTATCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTCAATAATGCACATTTATTTGTCTTCAACA													
Consensus	TTATCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTCAATAATGCACATTTATTTGTCTTCAACA													
	1691	1700	1710	1720	1730	1740	1750	1758						
E_SN1	-----													
E_SN1_1	ATGGATCTGAGACAATCACAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACCTAA													
Consensus	ATGGATCTGAGACAATCACAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACCTAA													

1 10 20 30 40 50 60 70 80 90 100 110 120 130
E_SN3 ATGGAATTATTTATGAAAAGCTCTTCTCTTTGGGGTTTAGAAATTTATTTATTTTGCCTCTTCATAGTTTATCAACATTAAATAGGTGTTGCTTCTCATATATTTTTTTGGACTTGCATCTTCAA
E_SN3_1 ATGGAATTATTTATGAAAAGCTCTTCTCTTTGGGGTTTAGAAATTTATTTATTTTGCCTCTTCATAGTTTATCAACATTAAATAGGTGTTGCTTCTCATATATTTTTTTGGACTTGCATCTTCAA
E_SN3_2 ATGGAATTATTTATGAAAAGCTCTTCTCTTTGGGGTTTAGAAATTTATTTATTTTGCCTCTTCATAGTTTATCAACATTAAATAGGTGTTGCTTCTCATATATTTTTTTGGACTTGCATCTTCAA
Consensus ATGGAATTATTTATGAAAAGCTCTTCTCTTTGGGGTTTAGAAATTTATTTATTTTGCCTCTTCATAGTTTATCAACATTAAATAGGTGTTGCTTCTCATATATTTTTTTGGACTTGCATCTTCAA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
E_SN3 GTGCAATTAGTGTCAAGAAATGTTTCATAGAACTAGTTTTCATTTTCACCTCCTAAATATTGGATTAAATGACCCATGACCCAAATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA
E_SN3_1 GTGCAATTAGTGTCAAGAAATGTTTCATAGAACTAGTTTTCATTTTCACCTCCTAAATATTGGATTAAATGACCCATGACCCAAATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA
E_SN3_2 GTGCAATTAGTGTCAAGAAATGTTTCATAGAACTAGTTTTCATTTTCACCTCCTAAATATTGGATTAAATGACCCATGACCCAAATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA
Consensus GTGCAATTAGTGTCAAGAAATGTTTCATAGAACTAGTTTTCATTTTCACCTCCTAAATATTGGATTAAATGACCCATGACCCAAATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
E_SN3 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTCTGGTCCGGGTCAGCA
E_SN3_1 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTCTGGTCCGGGTCAGCA
E_SN3_2 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTCTGGTCCGGGTCAGCA
Consensus AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTCTGGTCCGGGTCAGCA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
E_SN3 ACTATTCTACCTAATAACAAACCTGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAGGTTTCAAGATTATGCAATCCCGGCTAAGTTGTCTGATCCATTTCTTCGTAATGGATCAACCTA
E_SN3_1 ACTATTCTACCTAATAACAAACCTGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAGGTTTCAAGATTATGCAATCCCGGCTAAGTTGTCTGATCCATTTCTTCGTAATGGATCAACCTA
E_SN3_2 ACTATTCTACCTAATAACAAACCTGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAGGTTTCAAGATTATGCAATCCCGGCTAAGTTGTCTGATCCATTTCTTCGTAATGGATCAACCTA
Consensus ACTATTCTACCTAATAACAAACCTGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAGGTTTCAAGATTATGCAATCCCGGCTAAGTTGTCTGATCCATTTCTTCGTAATGGATCAACCTA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
E_SN3 ATAACAACCCGTTGATTGTACCTGACAAATAGCATCAACAACCAAAATTTTCGTGATCCACAACCCGTTGGATGGCCAGAGATGGGCTTTGGAGATTGTAATAGGAAGTATGAGAAACATAGAGGGAT
E_SN3_1 ATAACAACCCGTTGATTGTACCTGACAAATAGCATCAACAACCAAAATTTTCGTGATCCACAACCCGTTGGATGGCCAGAGATGGGCTTTGGAGATTGTAATAGGAAGTATGAGAAACATAGAGGGAT
E_SN3_2 ATAACAACCCGTTGATTGTACCTGACAAATAGCATCAACAACCAAAATTTTCGTGATCCACAACCCGTTGGATGGCCAGAGATGGGCTTTGGAGATTGTAATAGGAAGTATGAGAAACATAGAGGGAT
Consensus ATAACAACCCGTTGATTGTACCTGACAAATAGCATCAACAACCAAAATTTTCGTGATCCACAACCCGTTGGATGGCCAGAGATGGGCTTTGGAGATTGTAATAGGAAGTATGAGAAACATAGAGGGAT

651 660 670 680 690 700 710 720 730 740 750 760 770 780
E_SN3 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTTTCTGTATCATTAAAAATACTAATGGC
E_SN3_1 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTTTCTGTATCATTAAAAATACTAATGGC
E_SN3_2 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTTTCTGTATCATTAAAAATACTAATGGC
Consensus GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTTTCTGTATCATTAAAAATACTAATGGC

781 790 800 810 820 830 840 850 860 870 880 890 900 910
E_SN3 TTAGATGCATCGTATCGCGAAAAATGTCAACATGTCCTTAAGAAATAGCCTTGATGTTAATAGGTCTGATTATTACACTATTGGTATGTATGACCCAAAAAGATAGGTACATTCTGATACAATT
E_SN3_1 TTAGATGCATCGTATCGCGAAAAATGTCAACATGTCCTTAAGAAATAGCCTTGATGTTAATAGGTCTGATTATTACACTATTGGTATGTATGACCCAAAAAGATAGGTACATTCTGATACAATT
E_SN3_2 TTAGATGCATCGTATCGCGAAAAATGTCAACATGTCCTTAAGAAATAGCCTTGATGTTAATAGGTCTGATTATTACACTATTGGTATGTATGACCCAAAAAGATAGGTACATTCTGATACAATT
Consensus TTAGATGCATCGTATCGCGAAAAATGTCAACATGTCCTTAAGAAATAGCCTTGATGTTAATAGGTCTGATTATTACACTATTGGTATGTATGACCCAAAAAGATAGGTACATTCTGATACAATT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
E_SN3 CTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAATCATTATTTATGACCCATGAGAAATCGAAGATTGTATGGGGTTGGACAAATGAATCAGATGTTTTACCTGACGA
E_SN3_1 CTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAATCATTATTTATGACCCATGAGAAATCGAAGATTGTATGGGGTTGGACAAATGAATCAGATGTTTTACCTGACGA
E_SN3_2 CTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAATCATTATTTATGACCCATGAGAAATCGAAGATTGTATGGGGTTGGACAAATGAATCAGATGTTTTACCTGACGA
Consensus CTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAATCATTATTTATGACCCATGAGAAATCGAAGATTGTATGGGGTTGGACAAATGAATCAGATGTTTTACCTGACGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
E_SN3 TGAATTAAAGAAAGGATGGGCAGGAATTCAGCTATTCCACGTAAGATATGGCTCGACCCATGAGCAAAATGAAGCAATGATTCATGGCCTATTGAGAAATAGAACATTAGAAAGCAAAAGATTCAATTG
E_SN3_1 TGAATTAAAGAAAGGATGGGCAGGAATTCAGCTATTCCACGTAAGATATGGCTCGACCCATGAGCAAAATGAAGCAATGATTCATGGCCTATTGAGAAATAGAACATTAGAAAGCAAAAGATTCAATTG
E_SN3_2 TGAATTAAAGAAAGGATGGGCAGGAATTCAGCTATTCCACGTAAGATATGGCTCGACCCATGAGCAAAATGAAGCAATGATTCATGGCCTATTGAGAAATAGAACATTAGAAAGCAAAAGATTCAATTG
Consensus TGAATTAAAGAAAGGATGGGCAGGAATTCAGCTATTCCACGTAAGATATGGCTCGACCCATGAGCAAAATGAAGCAATGATTCATGGCCTATTGAGAAATAGAACATTAGAAAGCAAAAGATTCAATTG

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
E_SN3 AACAAACAGAGTTGAGCAAGGGAGAAATGTTTGAAGTTGAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTCTTCTCTTTTTCAAGTTTGAACAAAGGCCGACAAATTTGATCCTAATGGGCTG
E_SN3_1 AACAAACAGAGTTGAGCAAGGGAGAAATGTTTGAAGTTGAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTCTTCTCTTTTTCAAGTTTGAACAAAGGCCGACAAATTTGATCCTAATGGGCTG
E_SN3_2 AACAAACAGAGTTGAGCAAGGGAGAAATGTTTGAAGTTGAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTCTTCTCTTTTTCAAGTTTGAACAAAGGCCGACAAATTTGATCCTAATGGGCTG
Consensus AACAAACAGAGTTGAGCAAGGGAGAAATGTTTGAAGTTGAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTCTTCTCTTTTTCAAGTTTGAACAAAGGCCGACAAATTTGATCCTAATGGGCTG

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
E_SN3 ACCTTTATGCCAAGATGTTTGTGCCATTAAGGGTTCGACTATCCAAGGTGGGCTTGACCATTGGGCTTGCACAGTAGCTTCTAAGAACTTGGAGAAATACACACCTGTTTTCTTTTCGAGTGTTTAA
E_SN3_1 ACCTTTATGCCAAGATGTTTGTGCCATTAAGGGTTCGACTATCCAAGGTGGGCTTGACCATTGGGCTTGCACAGTAGCTTCTAAGAACTTGGAGAAATACACACCTGTTTTCTTTTCGAGTGTTTAA
E_SN3_2 ACCTTTATGCCAAGATGTTTGTGCCATTAAGGGTTCGACTATCCAAGGTGGGCTTGACCATTGGGCTTGCACAGTAGCTTCTAAGAACTTGGAGAAATACACACCTGTTTTCTTTTCGAGTGTTTAA
Consensus ACCTTTATGCCAAGATGTTTGTGCCATTAAGGGTTCGACTATCCAAGGTGGGCTTGACCATTGGGCTTGCACAGTAGCTTCTAAGAACTTGGAGAAATACACACCTGTTTTCTTTTCGAGTGTTTAA

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
E_SN3 GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAGAGGTTA
E_SN3_1 GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAGAGGTTA
E_SN3_2 GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAGAGGTTA
Consensus GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAGAGGTTA

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
E_SN3 TCTCTTAGGAGTTTATTGATAACTCAGTAGTGGAGAGTTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTCAATTAATGCACATTTATTTGTCTTCAATAATG
E_SN3_1 TCTCTTAGGAGTTTATTGATAACTCAGTAGTGGAGAGTTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTCAATTAATGCACATTTATTTGTCTTCAATAATG
E_SN3_2 TCTCTTAGGAGTTTATTGATAACTCAGTAGTGGAGAGTTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTCAATTAATGCACATTTATTTGTCTTCAATAATG
Consensus TCTCTTAGGAGTTTATTGATAACTCAGTAGTGGAGAGTTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTCAATTAATGCACATTTATTTGTCTTCAATAATG

1691 1700 1710 1720 1730 1740 1750 1755
E_SN3 GATCTGAGACAAATCAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
E_SN3_1 GATCTGAGACAAATCAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
E_SN3_2 GATCTGAGACAAATCAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
Consensus GATCTGAGACAAATCAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
E_DA ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTAGAAATTTATTTATTTTGCCTCTTTATAGTTTATCAACATTAAATAGGTGTTGGTTCTCATATGTTTTTTGGACTTGCATCTTCAA
E_DA2 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTAGAAATTTATTTATTTTGCCTCTTTATAGTTTATCAACATTAAATAGGTGTTGGTTCTCATATGTTTTTTGGACTTGCATCTTCAA
E_DA1 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTAGAAATTTATTTATTTTGCCTCTTTATAGTTTATCAACATTAAATAGGTGTTGGTTCTCATATGTTTTTTGGACTTGCATCTTCAA
Consensus ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTAGAAATTTATTTATTTTGCCTCTTTATAGTTTATCAACATTAAATAGGTGTTGGTTCTCATATGTTTTTTGGACTTGCATCTTCAA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
E_DA GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAAACATTGGATTAAATGACCCATATGCACCAATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA
E_DA2 GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAAACATTGGATTAAATGACCCATATGCACCAATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA
E_DA1 GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAAACATTGGATTAAATGACCCATATGCACCAATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA
Consensus GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAAACATTGGATTAAATGACCCATATGCACCAATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
E_DA AGGATCAGTATGGGGTAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTACCCATCCAAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCC
E_DA2 AGGATCAGTATGGGGTAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTACCCATCCAAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCC
E_DA1 AGGATCAGTATGGGGTAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTACCCATCCAAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCC
Consensus AGGATCAGTATGGGGTAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTACCCATCCAAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCC

391 400 410 420 430 440 450 460 470 480 490 500 510 520
E_DA ACTATTCTACCTAATAACAAGCCTGTTATTTATACACTGGAGTAGTAGATTCTCATGATACTCAAGTTCAAAACATATGCCATCCAGCTAAGTGTCTGATCCGTTTCTTCGTAATGGGTCARACCCA
E_DA2 ACTATTCTACCTAATAACAAGCCTGTTATTTATACACTGGAGTAGTAGATTCTCATGATACTCAAGTTCAAAACATATGCCATCCAGCTAAGTGTCTGATCCGTTTCTTCGTAATGGGTCARACCCA
E_DA1 ACTATTCTACCTAATAACAAGCCTGTTATTTATACACTGGAGTAGTAGATTCTCATGATACTCAAGTTCAAAACATATGCCATCCAGCTAAGTGTCTGATCCGTTTCTTCGTAATGGGTCARACCCA
Consensus ACTATTCTACCTAATAACAAGCCTGTTATTTATACACTGGAGTAGTAGATTCTCATGATACTCAAGTTCAAAACATATGCCATCCAGCTAAGTGTCTGATCCGTTTCTTCGTAATGGGTCARACCCA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
E_DA ATAACAACCCGTTGATTATTCCTGACAAATAGCATCAACAARACCAATTTTCGTGATCCACAACCCGTTGGATGGGTGTAGATGGGGTTGGAGGATTGTAATAGGAAGTATGAGAAACATAGAGGGAT
E_DA2 ATAACAACCCGTTGATTATTCCTGACAAATAGCATCAACAARACCAATTTTCGTGATCCACAACCCGTTGGATGGGTGTAGATGGGGTTGGAGGATTGTAATAGGAAGTATGAGAAACATAGAGGGAT
E_DA1 ATAACAACCCGTTGATTATTCCTGACAAATAGCATCAACAARACCAATTTTCGTGATCCACAACCCGTTGGATGGGTGTAGATGGGGTTGGAGGATTGTAATAGGAAGTATGAGAAACATAGAGGGAT
Consensus ATAACAACCCGTTGATTATTCCTGACAAATAGCATCAACAARACCAATTTTCGTGATCCACAACCCGTTGGATGGGTGTAGATGGGGTTGGAGGATTGTAATAGGAAGTATGAGAAACATAGAGGGAT

651 660 670 680 690 700 710 720 730 740 750 760 770 780
E_DA GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGTCAAGGCCAACACCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTTCCCTGTATCATTAAATAACTAATGGT
E_DA2 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGTCAAGGCCAACACCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTTCCCTGTATCATTAAATAACTAATGGT
E_DA1 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGTCAAGGCCAACACCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTTCCCTGTATCATTAAATAACTAATGGT
Consensus GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGTCAAGGCCAACACCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTTCCCTGTATCATTAAATAACTAATGGT

781 790 800 810 820 830 840 850 860 870 880 890 900 910
E_DA TTAGATGCATCGTATCGCGAAAAAATGTCAAAATATGCTCTTAAGAAATAGCCTTGATGTTAATAGGTTTGATTACTACACTATTGGCATGTATGACACCAGAAAGATAGGTACATTCTGATACAATT
E_DA2 TTAGATGCATCGTATCGCGAAAAAATGTCAAAATATGCTCTTAAGAAATAGCCTTGATGTTAATAGGTTTGATTACTACACTATTGGCATGTATGACACCAGAAAGATAGGTACATTCTGATACAATT
E_DA1 TTAGATGCATCGTATCGCGAAAAAATGTCAAAATATGCTCTTAAGAAATAGCCTTGATGTTAATAGGTTTGATTACTACACTATTGGCATGTATGACACCAGAAAGATAGGTACATTCTGATACAATT
Consensus TTAGATGCATCGTATCGCGAAAAAATGTCAAAATATGCTCTTAAGAAATAGCCTTGATGTTAATAGGTTTGATTACTACACTATTGGCATGTATGACACCAGAAAGATAGGTACATTCTGATACAATT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
E_DA CTATCGATGGTTGTAAGGGATTGAGGCTTGATTATGGGAATTTCTATGCATCTAATCATTATTTATGATCCTACTAAGAAATCGAAGAAATGTGTGGGGTTGGACCAATGAATCAGATGTTTTACCTGACGA
E_DA2 CTATCGATGGTTGTAAGGGATTGAGGCTTGATTATGGGAATTTCTATGCATCTAATCATTATTTATGATCCTACTAAGAAATCGAAGAAATGTGTGGGGTTGGACCAATGAATCAGATGTTTTACCTGACGA
E_DA1 CTATCGATGGTTGTAAGGGATTGAGGCTTGATTATGGGAATTTCTATGCATCTAATCATTATTTATGATCCTACTAAGAAATCGAAGAAATGTGTGGGGTTGGACCAATGAATCAGATGTTTTACCTGACGA
Consensus CTATCGATGGTTGTAAGGGATTGAGGCTTGATTATGGGAATTTCTATGCATCTAATCATTATTTATGATCCTACTAAGAAATCGAAGAAATGTGTGGGGTTGGACCAATGAATCAGATGTTTTACCTGACGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
E_DA TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGAAAAGTATGGCTCGATCATAGTGGTAAACAATTGATTCATGGCCTATTGAAGAAATAGAAACCTTAGAAAACAAAGATCCAAATG
E_DA2 TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGAAAAGTATGGCTCGATCATAGTGGTAAACAATTGATTCATGGCCTATTGAAGAAATAGAAACCTTAGAAAACAAAGATCCAAATG
E_DA1 TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGAAAAGTATGGCTCGATCATAGTGGTAAACAATTGATTCATGGCCTATTGAAGAAATAGAAACCTTAGAAAACAAAGATCCAAATG
Consensus TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGAAAAGTATGGCTCGATCATAGTGGTAAACAATTGATTCATGGCCTATTGAAGAAATAGAAACCTTAGAAAACAAAGATCCAAATG

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
E_DA AATAACAAGAGTTGAGCAAGGGAGAAATGTTTGAAGTTAAGGAATCTCAGCATCACAGTCTGATATTGAAGTGTCAATTTCTTTTTCAAGTTTGACAAAGCTGACAAATTTGATCCTAATGGGCTG
E_DA2 AATAACAAGAGTTGAGCAAGGGAGAAATGTTTGAAGTTAAGGAATCTCAGCATCACAGTCTGATATTGAAGTGTCAATTTCTTTTTCAAGTTTGACAAAGCTGACAAATTTGATCCTAATGGGCTG
E_DA1 AATAACAAGAGTTGAGCAAGGGAGAAATGTTTGAAGTTAAGGAATCTCAGCATCACAGTCTGATATTGAAGTGTCAATTTCTTTTTCAAGTTTGACAAAGCTGACAAATTTGATCCTAATGGGCTG
Consensus AATAACAAGAGTTGAGCAAGGGAGAAATGTTTGAAGTTAAGGAATCTCAGCATCACAGTCTGATATTGAAGTGTCAATTTCTTTTTCAAGTTTGACAAAGCTGACAAATTTGATCCTAATGGGCTG

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
E_DA ACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAAGGGTGGGCTTGACCATTGGGCTTGCAACATTAGCTTCTAAAACCTTGGAGAAATATACACCTGTTTTCTTTTCGAGTGTTTAA
E_DA2 ACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAAGGGTGGGCTTGACCATTGGGCTTGCAACATTAGCTTCTAAAACCTTGGAGAAATATACACCTGTTTTCTTTTCGAGTGTTTAA
E_DA1 ACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAAGGGTGGGCTTGACCATTGGGCTTGCAACATTAGCTTCTAAAACCTTGGAGAAATATACACCTGTTTTCTTTTCGAGTGTTTAA
Consensus ACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAAGGGTGGGCTTGACCATTGGGCTTGCAACATTAGCTTCTAAAACCTTGGAGAAATATACACCTGTTTTCTTTTCGAGTGTTTAA

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
E_DA GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAAGAGTTA
E_DA2 GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAAGAGTTA
E_DA1 GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAAGAGTTA
Consensus GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAAGAGTTA

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
E_DA TCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTTGGTGTGGAGGAAAACATGCATAACATCGAGGGTGTATCCAACATTAGCAATTCATGATAATGCACATTTATTTGTCTTCAATAATG
E_DA2 TCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTTGGTGTGGAGGAAAACATGCATAACATCGAGGGTGTATCCAACATTAGCAATTCATGATAATGCACATTTATTTGTCTTCAATAATG
E_DA1 TCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTTGGTGTGGAGGAAAACATGCATAACATCGAGGGTGTATCCAACATTAGCAATTCATGATAATGCACATTTATTTGTCTTCAATAATG
Consensus TCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTTGGTGTGGAGGAAAACATGCATAACATCGAGGGTGTATCCAACATTAGCAATTCATGATAATGCACATTTATTTGTCTTCAATAATG

1691 1700 1710 1720 1730 1740 1750 1755
E_DA GATCTGAGACAAATCAAAATGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
E_DA2 GATCTGAGACAAATCAAAATGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
E_DA1 GATCTGAGACAAATCAAAATGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
Consensus GATCTGAGACAAATCAAAATGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
E_DN1 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCCTCTTATAGTTTATCAACATTAAATGGGGTGGTGGCTTCTCATATATTTTTGGACTTGCATCTTCAA
E_DN1_1 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCCTCTTATAGTTTATCAACATTAAATGGGGTGGTGGCTTCTCATATATTTTTGGACTTGCATCTTCAA
E_DN1_3 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCCTCTTATAGTTTATCAACATTAAATGGGGTGGTGGCTTCTCATATATTTTTGGACTTGCATCTTCAA
E_DN1_2 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCCTCTTATAGTTTATCAACATTAAATGGGGTGGTGGCTTCTCATATATTTTTGGACTTGCATCTTCAA
Consensus ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCCTCTTATAGTTTATCAACATTAAATGGGGTGGTGGCTTCTCATATATTTTTGGACTTGCATCTTCAA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
E_DN1 GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCAACCTCTAARCAATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
E_DN1_1 GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCAACCTCTAARCAATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
E_DN1_3 GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCAACCTCTAARCAATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
E_DN1_2 GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCAACCTCTAARCAATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
Consensus GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCAACCTCTAARCAATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
E_DN1 AGGATCAGTATGGGGCAATATTGTTGGGCTCATTGCTCTCAAAAGCTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCA
E_DN1_1 AGGATCAGTATGGGGCAATATTGTTGGGCTCATTGCTCTCAAAAGCTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCA
E_DN1_3 AGGATCAGTATGGGGCAATATTGTTGGGCTCATTGCTCTCAAAAGCTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCA
E_DN1_2 AGGATCAGTATGGGGCAATATTGTTGGGCTCATTGCTCTCAAAAGCTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCA
Consensus AGGATCAGTATGGGGCAATATTGTTGGGCTCATTGCTCTCAAAAGCTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
E_DN1 ACTATTCTACCAATAACAGCCGTTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTAAGTTCAAATTTATGCATCCCGGCTAATTTGCTGATCCATTTCTTCGTAATGGATCAACCTA
E_DN1_1 ACTATTCTACCAATAACAGCCGTTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTAAGTTCAAATTTATGCATCCCGGCTAATTTGCTGATCCATTTCTTCGTAATGGATCAACCTA
E_DN1_3 ACTATTCTACCAATAACAGCCGTTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTAAGTTCAAATTTATGCATCCCGGCTAATTTGCTGATCCATTTCTTCGTAATGGATCAACCTA
E_DN1_2 ACTATTCTACCAATAACAGCCGTTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTAAGTTCAAATTTATGCATCCCGGCTAATTTGCTGATCCATTTCTTCGTAATGGATCAACCTA
Consensus ACTATTCTACCAATAACAGCCGTTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTAAGTTCAAATTTATGCATCCCGGCTAATTTGCTGATCCATTTCTTCGTAATGGATCAACCTA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
E_DN1 ATACAACCCGTTGATTGTACTGCAATAGCATCAACAARACCAATTTCTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTGTAATAGGAGTATGAGAAACATAGAGGGAT
E_DN1_1 ATACAACCCGTTGATTGTACTGCAATAGCATCAACAARACCAATTTCTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTGTAATAGGAGTATGAGAAACATAGAGGGAT
E_DN1_3 ATACAACCCGTTGATTGTACTGCAATAGCATCAACAARACCAATTTCTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTGTAATAGGAGTATGAGAAACATAGAGGGAT
E_DN1_2 ATACAACCCGTTGATTGTACTGCAATAGCATCAACAARACCAATTTCTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTGTAATAGGAGTATGAGAAACATAGAGGGAT
Consensus ATACAACCCGTTGATTGTACTGCAATAGCATCAACAARACCAATTTCTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTGTAATAGGAGTATGAGAAACATAGAGGGAT

651 660 670 680 690 700 710 720 730 740 750 760 770 780
E_DN1 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGCCACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGATGTCTGATTTTTCTGTATCATTAAAAATCAATGGC
E_DN1_1 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGCCACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGATGTCTGATTTTTCTGTATCATTAAAAATCAATGGC
E_DN1_3 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGCCACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGATGTCTGATTTTTCTGTATCATTAAAAATCAATGGC
E_DN1_2 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGCCACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGATGTCTGATTTTTCTGTATCATTAAAAATCAATGGC
Consensus GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGCCACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGATGTCTGATTTTTCTGTATCATTAAAAATCAATGGC

781 790 800 810 820 830 840 850 860 870 880 890 900 910
E_DN1 TTAGATGCATCGTATCGCGAAAAATGTCAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTTGGATATTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGATAACATT
E_DN1_1 TTAGATGCATCGTATCGCGAAAAATGTCAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTTGGATATTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGATAACATT
E_DN1_3 TTAGATGCATCGTATCGCGAAAAATGTCAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTTGGATATTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGATAACATT
E_DN1_2 TTAGATGCATCGTATCGCGAAAAATGTCAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTTGGATATTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGATAACATT
Consensus TTAGATGCATCGTATCGCGAAAAATGTCAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTTGGATATTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGATAACATT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
E_DN1 CTATCGATGGTTCGAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGAAATGATGGGGTTGGACAATGAATCAGATGTTTACCTGACGA
E_DN1_1 CTATCGATGGTTCGAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGAAATGATGGGGTTGGACAATGAATCAGATGTTTACCTGACGA
E_DN1_3 CTATCGATGGTTCGAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGAAATGATGGGGTTGGACAATGAATCAGATGTTTACCTGACGA
E_DN1_2 CTATCGATGGTTCGAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGAAATGATGGGGTTGGACAATGAATCAGATGTTTACCTGACGA
Consensus CTATCGATGGTTCGAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGAAATGATGGGGTTGGACAATGAATCAGATGTTTACCTGACGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
E_DN1 TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGTAAAGTATGGCTCGACCCTAGTGGTAARCACTGATTCATGGCTATTGAGAAATAGAACATTAAAGAAAGCAAAAGATTATTCAA
E_DN1_1 TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGTAAAGTATGGCTCGACCCTAGTGGTAARCACTGATTCATGGCTATTGAGAAATAGAACATTAAAGAAAGCAAAAGATTATTCAA
E_DN1_3 TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGTAAAGTATGGCTCGACCCTAGTGGTAARCACTGATTCATGGCTATTGAGAAATAGAACATTAAAGAAAGCAAAAGATTATTCAA
E_DN1_2 TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGTAAAGTATGGCTCGACCCTAGTGGTAARCACTGATTCATGGCTATTGAGAAATAGAACATTAAAGAAAGCAAAAGATTATTCAA
Consensus TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGTAAAGTATGGCTCGACCCTAGTGGTAARCACTGATTCATGGCTATTGAGAAATAGAACATTAAAGAAAGCAAAAGATTATTCAA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
E_DN1 TTGAACAACAGAAAGTTGAGCAAGGGAGAATGTTTGAAGTTAAGGAAATCTCAGCATCACAGGCTGATATTGAAGTGTCTGTTCTTTTTTCAGTTTGAACAAGGCCGACAAATTTGATCCTAATGGG
E_DN1_1 TTGAACAACAGAAAGTTGAGCAAGGGAGAATGTTTGAAGTTAAGGAAATCTCAGCATCACAGGCTGATATTGAAGTGTCTGTTCTTTTTTCAGTTTGAACAAGGCCGACAAATTTGATCCTAATGGG
E_DN1_3 TTGAACAACAGAAAGTTGAGCAAGGGAGAATGTTTGAAGTTAAGGAAATCTCAGCATCACAGGCTGATATTGAAGTGTCTGTTCTTTTTTCAGTTTGAACAAGGCCGACAAATTTGATCCTAATGGG
E_DN1_2 TTGAACAACAGAAAGTTGAGCAAGGGAGAATGTTTGAAGTTAAGGAAATCTCAGCATCACAGGCTGATATTGAAGTGTCTGTTCTTTTTTCAGTTTGAACAAGGCCGACAAATTTGATCCTAATGGG
Consensus TTGAACAACAGAAAGTTGAGCAAGGGAGAATGTTTGAAGTTAAGGAAATCTCAGCATCACAGGCTGATATTGAAGTGTCTGTTCTTTTTTCAGTTTGAACAAGGCCGACAAATTTGATCCTAATGGG

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
E_DN1 CCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTGACTATCCAAAGGTGGGCTTGGACCATTGGGCTTGTGACATTAGCTTCTAAAACTGGAGAAATACACACCTGTTTCTTTTCGAGTGT
E_DN1_1 CCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTGACTATCCAAAGGTGGGCTTGGACCATTGGGCTTGTGACATTAGCTTCTAAAACTGGAGAAATACACACCTGTTTCTTTTCGAGTGT
E_DN1_3 CCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTGACTATCCAAAGGTGGGCTTGGACCATTGGGCTTGTGACATTAGCTTCTAAAACTGGAGAAATACACACCTGTTTCTTTTCGAGTGT
E_DN1_2 CCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTGACTATCCAAAGGTGGGCTTGGACCATTGGGCTTGTGACATTAGCTTCTAAAACTGGAGAAATACACACCTGTTTCTTTTCGAGTGT
Consensus CCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTGACTATCCAAAGGTGGGCTTGGACCATTGGGCTTGTGACATTAGCTTCTAAAACTGGAGAAATACACACCTGTTTCTTTTCGAGTGT

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
E_DN1 TAAGGCTCAAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAGCAATGTACAGCCCTATTGCTGGATATGTAGATGTAGATTAGTAGACACGAGAGAG
E_DN1_1 TAAGGCTCAAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAGCAATGTACAGCCCTATTGCTGGATATGTAGATGTAGATTAGTAGACACGAGAGAG
E_DN1_3 TAAGGCTCAAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAGCAATGTACAGCCCTATTGCTGGATATGTAGATGTAGATTAGTAGACACGAGAGAG
E_DN1_2 TAAGGCTCAAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAGCAATGTACAGCCCTATTGCTGGATATGTAGATGTAGATTAGTAGACACGAGAGAG
Consensus TAAGGCTCAAAAGAAATTAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAGCAATGTACAGCCCTATTGCTGGATATGTAGATGTAGATTAGTAGACACGAGAGAG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
E_DN1 TTATCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTACAAATATGCACATTTATTTGTCTTCATA
E_DN1_1 TTATCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTACAAATATGCACATTTATTTGTCTTCATA
E_DN1_3 TTATCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTACAAATATGCACATTTATTTGTCTTCATA
E_DN1_2 TTATCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTACAAATATGCACATTTATTTGTCTTCATA
Consensus TTATCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTACAAATATGCACATTTATTTGTCTTCATA

1691 1700 1710 1720 1730 1740 1750 1758
E_DN1 ATGGATCTGAGCAATCAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
E_DN1_1 ATGGATCTGAGCAATCAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
E_DN1_3 ATGGATCTGAGCAATCAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
E_DN1_2 ATGGATCTGAGCAATCAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
Consensus ATGGATCTGAGCAATCAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
E_TN2	-----													
E_TN2_1	ATGGAATTATTTATGAAAAGCTCTTCTCTTTGGGGTTTAGAATTTATTTATTTGGCTCTTATAGTTTATCAACATTAAATGGGGTGTGGCTTCTCATATATTTTTGGACTTGCATCTTCAA													
Consensus	ATGGAATTATTTATGAAAAGCTCTTCTCTTTGGGGTTTAGAATTTATTTATTTGGCTCTTATAGTTTATCAACATTAAATGGGGTGTGGCTTCTCATATATTTTTGGACTTGCATCTTCAA													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
E_TN2	-----													
E_TN2_1	GTGCTATTAGTGTCAAGAATGTTTCATAGAACTGGTTTTCAATTTCAACCTCCTAATATTGGATTAAATGACCCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATACATCCAA													
Consensus	GTGCTATTAGTGTCAAGAATGTTTCATAGAACTGGTTTTCAATTTCAACCTCCTAATATTGGATTAAATGACCCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATACATCCAA													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
E_TN2	-----													
E_TN2_1	AGGATCAGTATGGGGCAATATTGTTGGGCTCATTCAAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTGGTCCGGGTGAGCA													
Consensus	AGGATCAGTATGGGGCAATATTGTTGGGCTCATTCAAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTGGTCCGGGTGAGCA													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
E_TN2	-----													
E_TN2_1	ACTATTCTACCAATAACAAGCCCGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAAGTTCAAAATTATGCAATCCCGGCTAACTTGTCTGATCCATTTCTTCGTAATGGATCAACCTA													
Consensus	ACTATTCTACCAATAACAAGCCCGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAAGTTCAAAATTATGCAATCCCGGCTAACTTGTCTGATCCATTTCTTCGTAATGGATCAACCTA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
E_TN2	-----													
E_TN2_1	ATAACAACCCGTTGATTGTACCTGACAATAGCATCAACAAACCAAAATTTTCGTGATCCACACCCGATGGATGGGCCAAGATGGGCTTTGGAGAATTGTAATAGGAAGTATGAGAAACATAGAGGGAT													
Consensus	ATAACAACCCGTTGATTGTACCTGACAATAGCATCAACAAACCAAAATTTTCGTGATCCACACCCGATGGATGGGCCAAGATGGGCTTTGGAGAATTGTAATAGGAAGTATGAGAAACATAGAGGGAT													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
E_TN2	-----													
E_TN2_1	GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGCCCAACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGAATGTCTGATTTTTTCTGTATCATTAAAAATACAATGGC													
Consensus	GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGCCCAACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGAATGTCTGATTTTTTCTGTATCATTAAAAATACAATGGC													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
E_TN2	-----													
E_TN2_1	TTAGATGCATCGTATCGCGGAAAAATGTCAATATGTCCTTAAGAATAGCCTTGATGTTAATAGGTTTGGAGTATTACACTATTGGTATGTATGACACCAAAAAAGATAGGTACATTCTGATAACAATT													
Consensus	TTAGATGCATCGTATCGCGGAAAAATGTCAATATGTCCTTAAGAATAGCCTTGATGTTAATAGGTTTGGAGTATTACACTATTGGTATGTATGACACCAAAAAAGATAGGTACATTCTGATAACAATT													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
E_TN2	-----													
E_TN2_1	CTATCGATGGTTCGAAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAAATCATTCTATGACCCTATGAGAATCGAGAATTGTATGGGGTGGACAATGAATCAGATGTTTACCTGACGA													
Consensus	CTATCGATGGTTCGAAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAAATCATTCTATGACCCTATGAGAATCGAGAATTGTATGGGGTGGACAATGAATCAGATGTTTACCTGACGA													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
E_TN2	-----													
E_TN2_1	TGAATTAAGAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAGTATGGCTCGACCCTAGTGGTAACAACTGATTCATGGCCTATTGAGAATTAGAACATTAGAAGGCAAAAGATTATTCAA													
Consensus	TGAATTAAGAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAGTATGGCTCGACCCTAGTGGTAACAACTGATTCATGGCCTATTGAGAATTAGAACATTAGAAGGCAAAAGATTATTCAA													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
E_TN2	-----													
E_TN2_1	TTGAACAACAAGAGTTGAGCAAGGGAGAAATGTTTGAAGTTAAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTCTGTTCTTTTTCAAGTTTGACACAGGCCGACAAATTTGATCCTAATGGG													
Consensus	TTGAACAACAAGAGTTGAGCAAGGGAGAAATGTTTGAAGTTAAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTCTGTTCTTTTTCAAGTTTGACACAGGCCGACAAATTTGATCCTAATGGG													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
E_TN2	-----													
E_TN2_1	CCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAAGGTGGGCTTGGACCATTTGGGCTTGTGACATTAGCTTCTAAAACTTGGAGAATACACACCTGTTTTCTTTCGAGTGT													
Consensus	CCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAAGGTGGGCTTGGACCATTTGGGCTTGTGACATTAGCTTCTAAAACTTGGAGAATACACACCTGTTTTCTTTCGAGTGT													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
E_TN2	-----													
E_TN2_1	TAAGGCTCAAAGAATTATAAGTTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACACGAGAGAG													
Consensus	TAAGGCTCAAAGAATTATAAGTTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACACGAGAGAG													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
E_TN2	-----													
E_TN2_1	TTATCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTCAATAATGCACATTTATTTGTCTTCAATA													
Consensus	TTATCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTCAATAATGCACATTTATTTGTCTTCAATA													
	1691	1700	1710	1720	1730	1740	1750	1758						
E_TN2	-----													
E_TN2_1	ATGGATCTGAGACAATCACAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACCTAA													
Consensus	ATGGATCTGAGACAATCACAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACCTAA													

1 10 20 30 40 50 60 70 80 90 100 110 120 130
E_TN3 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCTCTTATAGTTTTATCAACATTAAATGGGGTGGTTGCTTCTCATATATTTTTGGACTTGCATCTTCAA
E_TN3_1 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCTCTTATAGTTTTATCAACATTAAATGGGGTGGTTGCTTCTCATATATTTTTGGACTTGCATCTTCAA
E_TN3_2 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCTCTTATAGTTTTATCAACATTAAATGGGGTGGTTGCTTCTCATATATTTTTGGACTTGCATCTTCAA
E_TN3_3 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCTCTTATAGTTTTATCAACATTAAATGGGGTGGTTGCTTCTCATATATTTTTGGACTTGCATCTTCAA
Consensus ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCTCTTATAGTTTTATCAACATTAAATGGGGTGGTTGCTTCTCATATATTTTTGGACTTGCATCTTCAA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
E_TN3 GTCCATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAACATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
E_TN3_1 GTCCATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAACATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
E_TN3_2 GTCCATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAACATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
E_TN3_3 GTCCATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAACATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
Consensus GTCCATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAACATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
E_TN3 AGGATCAGTATGGGGTAAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGTCCAGCA
E_TN3_1 AGGATCAGTATGGGGTAAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGTCCAGCA
E_TN3_2 AGGATCAGTATGGGGTAAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGTCCAGCA
E_TN3_3 AGGATCAGTATGGGGTAAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGTCCAGCA
Consensus AGGATCAGTATGGGGTAAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGTCCAGCA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
E_TN3 ACTATTCTACCAATAACAACTGTTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTCAAGTTCAAATTTATGCATCCCGGCTAATTTGCTGATCCATTTCTTCGTAATGGATCAACCTA
E_TN3_1 ACTATTCTACCAATAACAACTGTTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTCAAGTTCAAATTTATGCATCCCGGCTAATTTGCTGATCCATTTCTTCGTAATGGATCAACCTA
E_TN3_2 ACTATTCTACCAATAACAACTGTTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTCAAGTTCAAATTTATGCATCCCGGCTAATTTGCTGATCCATTTCTTCGTAATGGATCAACCTA
E_TN3_3 ACTATTCTACCAATAACAACTGTTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTCAAGTTCAAATTTATGCATCCCGGCTAATTTGCTGATCCATTTCTTCGTAATGGATCAACCTA
Consensus ACTATTCTACCAATAACAACTGTTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTCAAGTTCAAATTTATGCATCCCGGCTAATTTGCTGATCCATTTCTTCGTAATGGATCAACCTA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
E_TN3 ATACAACCCGTTGATTGTTCTGCAATAGCATCAACAACCAAAATTTCTGACCAACACCCGATGGATGGCCAGATGGGCTTTGGAGAATTGTAATAGGAGTATGAGAAACATAGAGGGAT
E_TN3_1 ATACAACCCGTTGATTGTTCTGCAATAGCATCAACAACCAAAATTTCTGACCAACACCCGATGGATGGCCAGATGGGCTTTGGAGAATTGTAATAGGAGTATGAGAAACATAGAGGGAT
E_TN3_2 ATACAACCCGTTGATTGTTCTGCAATAGCATCAACAACCAAAATTTCTGACCAACACCCGATGGATGGCCAGATGGGCTTTGGAGAATTGTAATAGGAGTATGAGAAACATAGAGGGAT
E_TN3_3 ATACAACCCGTTGATTGTTCTGCAATAGCATCAACAACCAAAATTTCTGACCAACACCCGATGGATGGCCAGATGGGCTTTGGAGAATTGTAATAGGAGTATGAGAAACATAGAGGGAT
Consensus ATACAACCCGTTGATTGTTCTGCAATAGCATCAACAACCAAAATTTCTGACCAACACCCGATGGATGGCCAGATGGGCTTTGGAGAATTGTAATAGGAGTATGAGAAACATAGAGGGAT

651 660 670 680 690 700 710 720 730 740 750 760 770 780
E_TN3 GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGCCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGCCTGATTTTTCTCTGTATCATTAAAAATACTAATGGC
E_TN3_1 GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGCCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGCCTGATTTTTCTCTGTATCATTAAAAATACTAATGGC
E_TN3_2 GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGCCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGCCTGATTTTTCTCTGTATCATTAAAAATACTAATGGC
E_TN3_3 GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGCCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGCCTGATTTTTCTCTGTATCATTAAAAATACTAATGGC
Consensus GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGCCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGCCTGATTTTTCTCTGTATCATTAAAAATACTAATGGC

781 790 800 810 820 830 840 850 860 870 880 890 900 910
E_TN3 TTAGATGCATCGTATCGCGAAAAATGTCAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTGAGTATTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGTATAATTT
E_TN3_1 TTAGATGCATCGTATCGCGAAAAATGTCAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTGAGTATTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGTATAATTT
E_TN3_2 TTAGATGCATCGTATCGCGAAAAATGTCAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTGAGTATTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGTATAATTT
E_TN3_3 TTAGATGCATCGTATCGCGAAAAATGTCAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTGAGTATTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGTATAATTT
Consensus TTAGATGCATCGTATCGCGAAAAATGTCAATATGCTCTAAGAAATAGCCTTGATGTTAATAGGTTGAGTATTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGTATAATTT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
E_TN3 CTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGAAATGATGGGGTTGGACAATGAATCAGATGTTTTACCTGACGA
E_TN3_1 CTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGAAATGATGGGGTTGGACAATGAATCAGATGTTTTACCTGACGA
E_TN3_2 CTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGAAATGATGGGGTTGGACAATGAATCAGATGTTTTACCTGACGA
E_TN3_3 CTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGAAATGATGGGGTTGGACAATGAATCAGATGTTTTACCTGACGA
Consensus CTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGAAATGATGGGGTTGGACAATGAATCAGATGTTTTACCTGACGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
E_TN3 TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGTAAAGTATGGCTCGACCCTAGTGGTAAACAAATGATTCAATGGCTATTGAGAAATAGAACATTAAAGAAAGCAAAGATTCAATTG
E_TN3_1 TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGTAAAGTATGGCTCGACCCTAGTGGTAAACAAATGATTCAATGGCTATTGAGAAATAGAACATTAAAGAAAGCAAAGATTCAATTG
E_TN3_2 TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGTAAAGTATGGCTCGACCCTAGTGGTAAACAAATGATTCAATGGCTATTGAGAAATAGAACATTAAAGAAAGCAAAGATTCAATTG
E_TN3_3 TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGTAAAGTATGGCTCGACCCTAGTGGTAAACAAATGATTCAATGGCTATTGAGAAATAGAACATTAAAGAAAGCAAAGATTCAATTG
Consensus TGAATTAAGAAAGGATGGGCTGGAAATTCAGCTATTCCGCGTAAAGTATGGCTCGACCCTAGTGGTAAACAAATGATTCAATGGCTATTGAGAAATAGAACATTAAAGAAAGCAAAGATTCAATTG

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
E_TN3 AACAAAGAGAGTTGAGCAGGGGAGAAATGTTGAAATTAAGGAAATCTCAGCATCACAGGCTGATATTGAAAGTGTCTCTCTTTTTCAGTTTGAACAGGGCCGACAAATTTGATCCTAATGGGCCG
E_TN3_1 AACAAAGAGAGTTGAGCAGGGGAGAAATGTTGAAATTAAGGAAATCTCAGCATCACAGGCTGATATTGAAAGTGTCTCTCTTTTTCAGTTTGAACAGGGCCGACAAATTTGATCCTAATGGGCCG
E_TN3_2 AACAAAGAGAGTTGAGCAGGGGAGAAATGTTGAAATTAAGGAAATCTCAGCATCACAGGCTGATATTGAAAGTGTCTCTCTTTTTCAGTTTGAACAGGGCCGACAAATTTGATCCTAATGGGCCG
E_TN3_3 AACAAAGAGAGTTGAGCAGGGGAGAAATGTTGAAATTAAGGAAATCTCAGCATCACAGGCTGATATTGAAAGTGTCTCTCTTTTTCAGTTTGAACAGGGCCGACAAATTTGATCCTAATGGGCCG
Consensus AACAAAGAGAGTTGAGCAGGGGAGAAATGTTGAAATTAAGGAAATCTCAGCATCACAGGCTGATATTGAAAGTGTCTCTCTTTTTCAGTTTGAACAGGGCCGACAAATTTGATCCTAATGGGCCG

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
E_TN3 ACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGGACCATTGGGCTTGTGACATTAGCTTCTAAAACCTTGAAGAAATACACACCTGTTTTCTTTCAAGTGTTTAA
E_TN3_1 ACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGGACCATTGGGCTTGTGACATTAGCTTCTAAAACCTTGAAGAAATACACACCTGTTTTCTTTCAAGTGTTTAA
E_TN3_2 ACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGGACCATTGGGCTTGTGACATTAGCTTCTAAAACCTTGAAGAAATACACACCTGTTTTCTTTCAAGTGTTTAA
E_TN3_3 ACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGGACCATTGGGCTTGTGACATTAGCTTCTAAAACCTTGAAGAAATACACACCTGTTTTCTTTCAAGTGTTTAA
Consensus ACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGGACCATTGGGCTTGTGACATTAGCTTCTAAAACCTTGAAGAAATACACACCTGTTTTCTTTCAAGTGTTTAA

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
E_TN3 GGCTCAAAGAAATTATAGGTTCTCATGTGCTCAGATGCGAGAAGATCACCATGAGACAAATGAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACATGAAAGGTTA
E_TN3_1 GGCTCAAAGAAATTATAGGTTCTCATGTGCTCAGATGCGAGAAGATCACCATGAGACAAATGAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACATGAAAGGTTA
E_TN3_2 GGCTCAAAGAAATTATAGGTTCTCATGTGCTCAGATGCGAGAAGATCACCATGAGACAAATGAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACATGAAAGGTTA
E_TN3_3 GGCTCAAAGAAATTATAGGTTCTCATGTGCTCAGATGCGAGAAGATCACCATGAGACAAATGAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACATGAAAGGTTA
Consensus GGCTCAAAGAAATTATAGGTTCTCATGTGCTCAGATGCGAGAAGATCACCATGAGACAAATGAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACATGAAAGGTTA

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
E_TN3 TCTCTTAGGAGTTTGGTGGTAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTACAAATATGCACATTTATTTGCTTCAATATG
E_TN3_1 TCTCTTAGGAGTTTGGTGGTAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTACAAATATGCACATTTATTTGCTTCAATATG
E_TN3_2 TCTCTTAGGAGTTTGGTGGTAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTACAAATATGCACATTTATTTGCTTCAATATG
E_TN3_3 TCTCTTAGGAGTTTGGTGGTAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTACAAATATGCACATTTATTTGCTTCAATATG
Consensus TCTCTTAGGAGTTTGGTGGTAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTACAAATATGCACATTTATTTGCTTCAATATG

1691 1700 1710 1720 1730 1740 1755
E_TN3 GATCTGAGACAAATCACAATTGAGACTCTTAAATGCTTGGAGCATGGATGTACCTAAGATGCACAA
E_TN3_1 GATCTGAGACAAATCACAATTGAGACTCTTAAATGCTTGGAGCATGGATGTACCTAAGATGCACAA
E_TN3_2 GATCTGAGACAAATCACAATTGAGACTCTTAAATGCTTGGAGCATGGATGTACCTAAGATGCACAA
E_TN3_3 GATCTGAGACAAATCACAATTGAGACTCTTAAATGCTTGGAGCATGGATGTACCTAAGATGCACAA
Consensus GATCTGAGACAAATCACAATTGAGACTCTTAAATGCTTGGAGCATGGATGTACCTAAGATGCACAA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
E_P18N1 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCCTCTTATAGTTTATCAACATTAAATGGGGTGGTGGCTTCTCATATATATTTTGGACTTGCATCTTCAA
E_P18N1_1 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCCTCTTATAGTTTATCAACATTAAATGGGGTGGTGGCTTCTCATATATATTTTGGACTTGCATCTTCAA
E_P18N1_3 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCCTCTTATAGTTTATCAACATTAAATGGGGTGGTGGCTTCTCATATATATTTTGGACTTGCATCTTCAA
E_P18N1_2 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCCTCTTATAGTTTATCAACATTAAATGGGGTGGTGGCTTCTCATATATATTTTGGACTTGCATCTTCAA
Consensus ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTAGAAATTTATTTATTTTGCCTCTTATAGTTTATCAACATTAAATGGGGTGGTGGCTTCTCATATATATTTTGGACTTGCATCTTCAA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
E_P18N1 GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCAACCTCTAACCATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
E_P18N1_1 GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCAACCTCTAACCATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
E_P18N1_3 GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCAACCTCTAACCATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
E_P18N1_2 GTGCCATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCAACCTCTAACCATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA
Consensus GTGCTATTAGTGTCAAGAAATGTTTCATAGAACTGGTTTTCATTTTCAACCTCTAACCATTGGATTAAATGACCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATCAATCCAA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
E_P18N1 AGGATCAGTATGGGGCAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCA
E_P18N1_1 AGGATCAGTATGGGGCAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCA
E_P18N1_3 AGGATCAGTATGGGGCAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCA
E_P18N1_2 AGGATCAGTATGGGGCAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCA
Consensus AGGATCAGTATGGGGCAATATTGTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGCCTGGTCCGGGTCAGCA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
E_P18N1 ACTATTCTACCAATAACAAACCCATTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTAAGTTCAAAATTTATGCATCCCGGCTAAGTGTCTGATCCATTTCTTCGTAATGGATCAACCTA
E_P18N1_1 ACTATTCTACCAATAACAAACCCATTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTAAGTTCAAAATTTATGCATCCCGGCTAAGTGTCTGATCCATTTCTTCGTAATGGATCAACCTA
E_P18N1_3 ACTATTCTACCAATAACAAACCCATTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTAAGTTCAAAATTTATGCATCCCGGCTAAGTGTCTGATCCATTTCTTCGTAATGGATCAACCTA
E_P18N1_2 ACTATTCTACCAATAACAAACCCATTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTAAGTTCAAAATTTATGCATCCCGGCTAAGTGTCTGATCCATTTCTTCGTAATGGATCAACCTA
Consensus ACTATTCTACCAATAACAAACCCATTATCTTATACACCGGAGTAGTAGATCCCATGATTCCTAAGTTCAAAATTTATGCATCCCGGCTAAGTGTCTGATCCATTTCTTCGTAATGGATCAACCTA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
E_P18N1 ATACAACCCATTGATTGTACCTGACAAATAGCATCAACAACCAAAATTTCTGTATCCACACCCGATGGATGGGCCAAGATGGGCTTTGGAGAAATGTAATAGGAGTATGGAACCATAGAGGGAT
E_P18N1_1 ATACAACCCATTGATTGTACCTGACAAATAGCATCAACAACCAAAATTTCTGTATCCACACCCGATGGATGGGCCAAGATGGGCTTTGGAGAAATGTAATAGGAGTATGGAACCATAGAGGGAT
E_P18N1_3 ATACAACCCATTGATTGTACCTGACAAATAGCATCAACAACCAAAATTTCTGTATCCACACCCGATGGATGGGCCAAGATGGGCTTTGGAGAAATGTAATAGGAGTATGGAACCATAGAGGGAT
E_P18N1_2 ATACAACCCATTGATTGTACCTGACAAATAGCATCAACAACCAAAATTTCTGTATCCACACCCGATGGATGGGCCAAGATGGGCTTTGGAGAAATGTAATAGGAGTATGGAACCATAGAGGGAT
Consensus ATACAACCCATTGATTGTACCTGACAAATAGCATCAACAACCAAAATTTCTGTATCCACACCCGATGGATGGGCCAAGATGGGCTTTGGAGAAATGTAATAGGAGTATGGAACCATAGAGGGAT

651 660 670 680 690 700 710 720 730 740 750 760 770 780
E_P18N1 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGACCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGAATGTCTGATTTTTCCCTGTATCATTAAAAATACTAATGGC
E_P18N1_1 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGACCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGAATGTCTGATTTTTCCCTGTATCATTAAAAATACTAATGGC
E_P18N1_3 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGACCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGAATGTCTGATTTTTCCCTGTATCATTAAAAATACTAATGGC
E_P18N1_2 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGACCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGAATGTCTGATTTTTCCCTGTATCATTAAAAATACTAATGGC
Consensus GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGACCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGAATGTCTGATTTTTCCCTGTATCATTAAAAATACTAATGGC

781 790 800 810 820 830 840 850 860 870 880 890 900 910
E_P18N1 TTAGATGCATCGTATCGCGAAAAAATGTCAACATGTCTTAGAATAGCCTTGATGTTAATAGGTTTGGATTTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGATACCA
E_P18N1_1 TTAGATGCATCGTATCGCGAAAAAATGTCAACATGTCTTAGAATAGCCTTGATGTTAATAGGTTTGGATTTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGATACCA
E_P18N1_3 TTAGATGCATCGTATCGCGAAAAAATGTCAACATGTCTTAGAATAGCCTTGATGTTAATAGGTTTGGATTTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGATACCA
E_P18N1_2 TTAGATGCATCGTATCGCGAAAAAATGTCAACATGTCTTAGAATAGCCTTGATGTTAATAGGTTTGGATTTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGATACCA
Consensus TTAGATGCATCGTATCGCGAAAAAATGTCAACATGTCTTAGAATAGCCTTGATGTTAATAGGTTTGGATTTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTGATACCA

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
E_P18N1 ATTCTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGATTGTATGGGGTTGGACAAATGAATCAGATGTTTTACCTGA
E_P18N1_1 ATTCTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGATTGTATGGGGTTGGACAAATGAATCAGATGTTTTACCTGA
E_P18N1_3 ATTCTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGATTGTATGGGGTTGGACAAATGAATCAGATGTTTTACCTGA
E_P18N1_2 ATTCTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGATTGTATGGGGTTGGACAAATGAATCAGATGTTTTACCTGA
Consensus ATTCTATCGATGGTTCGAGGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCAATCATTCTATGACCTATGAGAAATCGAAGATTGTATGGGGTTGGACAAATGAATCAGATGTTTTACCTGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
E_P18N1 CGATGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCGCGTAAGATGGCTCGACCTAGTGGTAACAATTGATTCATGGCCTATTGAGAAATAGAAACATTAGAAAGCAAAAGATTATT
E_P18N1_1 CGATGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCGCGTAAGATGGCTCGACCTAGTGGTAACAATTGATTCATGGCCTATTGAGAAATAGAAACATTAGAAAGCAAAAGATTATT
E_P18N1_3 CGATGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCGCGTAAGATGGCTCGACCTAGTGGTAACAATTGATTCATGGCCTATTGAGAAATAGAAACATTAGAAAGCAAAAGATTATT
E_P18N1_2 CGATGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCGCGTAAGATGGCTCGACCTAGTGGTAACAATTGATTCATGGCCTATTGAGAAATAGAAACATTAGAAAGCAAAAGATTATT
Consensus CGATGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCGCGTAAGATGGCTCGACCTAGTGGTAACAATTGATTCATGGCCTATTGAGAAATAGAAACATTAGAAAGCAAAAGATTATT

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
E_P18N1 CAATTGAACACCAAGAGTTGAGCAGGGGAGAATGTTGAGTAAAGGAATCTCAGCATCACAGGCTGATATTGAGTGTCTTCTCTTTTCAAGTTTAAACAGGGCCGACAAATTTGATCCTAAT
E_P18N1_1 CAATTGAACACCAAGAGTTGAGCAGGGGAGAATGTTGAGTAAAGGAATCTCAGCATCACAGGCTGATATTGAGTGTCTTCTCTTTTCAAGTTTAAACAGGGCCGACAAATTTGATCCTAAT
E_P18N1_3 CAATTGAACACCAAGAGTTGAGCAGGGGAGAATGTTGAGTAAAGGAATCTCAGCATCACAGGCTGATATTGAGTGTCTTCTCTTTTCAAGTTTAAACAGGGCCGACAAATTTGATCCTAAT
E_P18N1_2 CAATTGAACACCAAGAGTTGAGCAGGGGAGAATGTTGAGTAAAGGAATCTCAGCATCACAGGCTGATATTGAGTGTCTTCTCTTTTCAAGTTTAAACAGGGCCGACAAATTTGATCCTAAT
Consensus CAATTGAACACCAAGAGTTGAGCAGGGGAGAATGTTGAGTAAAGGAATCTCAGCATCACAGGCTGATATTGAGTGTCTTCTCTTTTCAAGTTTAAACAGGGCCGACAAATTTGATCCTAAT

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
E_P18N1 GGGCCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGACCATTGGGCTTGTGACATTAGCTTCTAAAACCTTGGAGAAATACACACCTGTTTCTTTCGAGT
E_P18N1_1 GGGCCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGACCATTGGGCTTGTGACATTAGCTTCTAAAACCTTGGAGAAATACACACCTGTTTCTTTCGAGT
E_P18N1_3 GGGCCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGACCATTGGGCTTGTGACATTAGCTTCTAAAACCTTGGAGAAATACACACCTGTTTCTTTCGAGT
E_P18N1_2 GGGCCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGACCATTGGGCTTGTGACATTAGCTTCTAAAACCTTGGAGAAATACACACCTGTTTCTTTCGAGT
Consensus GGGCCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAGGTGGGCTTGACCATTGGGCTTGTGACATTAGCTTCTAAAACCTTGGAGAAATACACACCTGTTTCTTTCGAGT

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
E_P18N1 GTTAAAGGCTCAAAGAAATATAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAAATGAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACACGAG
E_P18N1_1 GTTAAAGGCTCAAAGAAATATAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAAATGAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACACGAG
E_P18N1_3 GTTAAAGGCTCAAAGAAATATAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAAATGAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACACGAG
E_P18N1_2 GTTAAAGGCTCAAAGAAATATAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAAATGAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACACGAG
Consensus GTTAAAGGCTCAAAGAAATATAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAAATGAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACACGAG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
E_P18N1 AAGTTATCTCTTAGGAGTTTGAATGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTTCATATAATGCACATTTATTTGCTTCA
E_P18N1_1 AAGTTATCTCTTAGGAGTTTGAATGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTTCATATAATGCACATTTATTTGCTTCA
E_P18N1_3 AAGTTATCTCTTAGGAGTTTGAATGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTTCATATAATGCACATTTATTTGCTTCA
E_P18N1_2 AAGTTATCTCTTAGGAGTTTGAATGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTTCATATAATGCACATTTATTTGCTTCA
Consensus AAGTTATCTCTTAGGAGTTTGAATGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTTCATATAATGCACATTTATTTGCTTCA

1691 1700 1710 1720 1730 1740 1750 1761
E_P18N1 AATATGGATCTGAGACAAATCACAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
E_P18N1_1 AATATGGATCTGAGACAAATCACAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
E_P18N1_3 AATATGGATCTGAGACAAATCACAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
E_P18N1_2 AATATGGATCTGAGACAAATCACAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
Consensus AATATGGATCTGAGACAAATCACAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
E_P18N2 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTGAAGATTTATTTATTTTGCCCTTTATAGTTTTATCAACATTAAATGGGGTGTGCTTCTCATATATTTTTTTGGACTTGCARTCTTCA
E_P18N2_1 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTGAAGATTTATTTATTTTGCCCTTTATAGTTTTATCAACATTAAATGGGGTGTGCTTCTCATATATTTTTTTGGACTTGCARTCTTCA
E_P18N2_3 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTGAAGATTTATTTATTTTGCCCTTTATAGTTTTATCAACATTAAATGGGGTGTGCTTCTCATATATTTTTTTGGACTTGCARTCTTCA
E_P18N2_4 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTGAAGATTTATTTATTTTGCCCTTTATAGTTTTATCAACATTAAATGGGGTGTGCTTCTCATATATTTTTTTGGACTTGCARTCTTCA
E_P18N2_2 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTGAAGATTTATTTATTTTGCCCTTTATAGTTTTATCAACATTAAATGGGGTGTGCTTCTCATATATTTTTTTGGACTTGCARTCTTCA
Consensus ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTGAAGATTTATTTATTTTGCCCTTTATAGTTTTATCAACATTAAATGGGGTGTGCTTCTCATATATTTTTTTGGACTTGCARTCTTCA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
E_P18N2 GTGCTATTAGTGTCAAGATGTTTCATAGAACTGGTTTCATTTTCAACCTCCTAATATTGGATTAAATGACCCATGACCCATGATTATATGAGTATATCATTATTCTATCAATACATCCAA
E_P18N2_1 GTGCTATTAGTGTCAAGATGTTTCATAGAACTGGTTTCATTTTCAACCTCCTAATATTGGATTAAATGACCCATGACCCATGATTATATGAGTATATCATTATTCTATCAATACATCCAA
E_P18N2_3 GTGCTATTAGTGTCAAGATGTTTCATAGAACTGGTTTCATTTTCAACCTCCTAATATTGGATTAAATGACCCATGACCCATGATTATATGAGTATATCATTATTCTATCAATACATCCAA
E_P18N2_4 GTGCTATTAGTGTCAAGATGTTTCATAGAACTGGTTTCATTTTCAACCTCCTAATATTGGATTAAATGACCCATGACCCATGATTATATGAGTATATCATTATTCTATCAATACATCCAA
E_P18N2_2 GTGCTATTAGTGTCAAGATGTTTCATAGAACTGGTTTCATTTTCAACCTCCTAATATTGGATTAAATGACCCATGACCCATGATTATATGAGTATATCATTATTCTATCAATACATCCAA
Consensus GTGCTATTAGTGTCAAGATGTTTCATAGAACTGGTTTCATTTTCAACCTCCTAATATTGGATTAAATGACCCATGACCCATGATTATATGAGTATATCATTATTCTATCAATACATCCAA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
E_P18N2 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTGCTCTCAAGACTTGATAAATGGATCCATTAGAACCCGCAATTTATCCATCAAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCA
E_P18N2_1 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTGCTCTCAAGACTTGATAAATGGATCCATTAGAACCCGCAATTTATCCATCAAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCA
E_P18N2_3 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTGCTCTCAAGACTTGATAAATGGATCCATTAGAACCCGCAATTTATCCATCAAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCA
E_P18N2_4 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTGCTCTCAAGACTTGATAAATGGATCCATTAGAACCCGCAATTTATCCATCAAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCA
E_P18N2_2 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTGCTCTCAAGACTTGATAAATGGATCCATTAGAACCCGCAATTTATCCATCAAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCA
Consensus AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTGCTCTCAAGACTTGATAAATGGATCCATTAGAACCCGCAATTTATCCATCAAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
E_P18N2 ACTATTCTACCAATAACAGCCCGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTTCTCAAGTTCAAATATGCAATCCCGGCTAAGTGTCTGATCCATTCTTCGTAATGGATCAACCTA
E_P18N2_1 ACTATTCTACCAATAACAGCCCGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTTCTCAAGTTCAAATATGCAATCCCGGCTAAGTGTCTGATCCATTCTTCGTAATGGATCAACCTA
E_P18N2_3 ACTATTCTACCAATAACAGCCCGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTTCTCAAGTTCAAATATGCAATCCCGGCTAAGTGTCTGATCCATTCTTCGTAATGGATCAACCTA
E_P18N2_4 ACTATTCTACCAATAACAGCCCGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTTCTCAAGTTCAAATATGCAATCCCGGCTAAGTGTCTGATCCATTCTTCGTAATGGATCAACCTA
E_P18N2_2 ACTATTCTACCAATAACAGCCCGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTTCTCAAGTTCAAATATGCAATCCCGGCTAAGTGTCTGATCCATTCTTCGTAATGGATCAACCTA
Consensus ACTATTCTACCAATAACAGCCCGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTTCTCAAGTTCAAATATGCAATCCCGGCTAAGTGTCTGATCCATTCTTCGTAATGGATCAACCTA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
E_P18N2 ATACCAACCCGTTGATTGTACCTGACATAGCATCAACAAACCAATTTCTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTGTAATAGGAGATGAGAAACATAGAGGGAT
E_P18N2_1 ATACCAACCCGTTGATTGTACCTGACATAGCATCAACAAACCAATTTCTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTGTAATAGGAGATGAGAAACATAGAGGGAT
E_P18N2_3 ATACCAACCCGTTGATTGTACCTGACATAGCATCAACAAACCAATTTCTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTGTAATAGGAGATGAGAAACATAGAGGGAT
E_P18N2_4 ATACCAACCCGTTGATTGTACCTGACATAGCATCAACAAACCAATTTCTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTGTAATAGGAGATGAGAAACATAGAGGGAT
E_P18N2_2 ATACCAACCCGTTGATTGTACCTGACATAGCATCAACAAACCAATTTCTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTGTAATAGGAGATGAGAAACATAGAGGGAT
Consensus ATACCAACCCGTTGATTGTACCTGACATAGCATCAACAAACCAATTTCTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTGTAATAGGAGATGAGAAACATAGAGGGAT

651 660 670 680 690 700 710 720 730 740 750 760 770 780
E_P18N2 GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGCCAAGCCCAACATCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGTCCTGATTTTTTCTGTATCATTAAAAATCAAAATGGC
E_P18N2_1 GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGCCAAGCCCAACATCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGTCCTGATTTTTTCTGTATCATTAAAAATCAAAATGGC
E_P18N2_3 GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGCCAAGCCCAACATCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGTCCTGATTTTTTCTGTATCATTAAAAATCAAAATGGC
E_P18N2_4 GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGCCAAGCCCAACATCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGTCCTGATTTTTTCTGTATCATTAAAAATCAAAATGGC
E_P18N2_2 GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGCCAAGCCCAACATCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGTCCTGATTTTTTCTGTATCATTAAAAATCAAAATGGC
Consensus GGCATTATTGTATAGAGTAGAGACTTCATTAATGGGCCAAGCCCAACATCCACTTCATTCATCTCCTCATACTGGAAATGGGAATGTCCTGATTTTTTCTGTATCATTAAAAATCAAAATGGC

781 790 800 810 820 830 840 850 860 870 880 890 900 910
E_P18N2 TTAGATGCATCGTATCGGGAAAAATGTCARATATGCTTTAGGAATAGCCTTGATGTTAATAGGTTTGGATTTACACTATTGGTATGATGACCCAAAAAGATAGGTACATTCTGATACCAAT
E_P18N2_1 TTAGATGCATCGTATCGGGAAAAATGTCARATATGCTTTAGGAATAGCCTTGATGTTAATAGGTTTGGATTTACACTATTGGTATGATGACCCAAAAAGATAGGTACATTCTGATACCAAT
E_P18N2_3 TTAGATGCATCGTATCGGGAAAAATGTCARATATGCTTTAGGAATAGCCTTGATGTTAATAGGTTTGGATTTACACTATTGGTATGATGACCCAAAAAGATAGGTACATTCTGATACCAAT
E_P18N2_4 TTAGATGCATCGTATCGGGAAAAATGTCARATATGCTTTAGGAATAGCCTTGATGTTAATAGGTTTGGATTTACACTATTGGTATGATGACCCAAAAAGATAGGTACATTCTGATACCAAT
E_P18N2_2 TTAGATGCATCGTATCGGGAAAAATGTCARATATGCTTTAGGAATAGCCTTGATGTTAATAGGTTTGGATTTACACTATTGGTATGATGACCCAAAAAGATAGGTACATTCTGATACCAAT
Consensus TTAGATGCATCGTATCGGGAAAAATGTCARATATGCTTTAGGAATAGCCTTGATGTTAATAGGTTTGGATTTACACTATTGGTATGATGACCCAAAAAGATAGGTACATTCTGATACCAAT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
E_P18N2 CTATCGATGGTTGAGGGGATTTGAGGCTTGACTATGGGAAATTTCTATGCATCTAATCATTCTATGACCCATGAGGATCGAGAATTTGATGGGGTTGGCAAAATGAATCAGATGTTTTACCTGACGA
E_P18N2_1 CTATCGATGGTTGAGGGGATTTGAGGCTTGACTATGGGAAATTTCTATGCATCTAATCATTCTATGACCCATGAGGATCGAGAATTTGATGGGGTTGGCAAAATGAATCAGATGTTTTACCTGACGA
E_P18N2_3 CTATCGATGGTTGAGGGGATTTGAGGCTTGACTATGGGAAATTTCTATGCATCTAATCATTCTATGACCCATGAGGATCGAGAATTTGATGGGGTTGGCAAAATGAATCAGATGTTTTACCTGACGA
E_P18N2_4 CTATCGATGGTTGAGGGGATTTGAGGCTTGACTATGGGAAATTTCTATGCATCTAATCATTCTATGACCCATGAGGATCGAGAATTTGATGGGGTTGGCAAAATGAATCAGATGTTTTACCTGACGA
E_P18N2_2 CTATCGATGGTTGAGGGGATTTGAGGCTTGACTATGGGAAATTTCTATGCATCTAATCATTCTATGACCCATGAGGATCGAGAATTTGATGGGGTTGGCAAAATGAATCAGATGTTTTACCTGACGA
Consensus CTATCGATGGTTGAGGGGATTTGAGGCTTGACTATGGGAAATTTCTATGCATCTAATCATTCTATGACCCATGAGGATCGAGAATTTGATGGGGTTGGCAAAATGAATCAGATGTTTTACCTGACGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
E_P18N2 TGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTGACCCTAGTGGTAAACACTGATTCATGGCTATTGAGGATTAAGAACATTAAGAAAGCAAAAGATTATCA
E_P18N2_1 TGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTGACCCTAGTGGTAAACACTGATTCATGGCTATTGAGGATTAAGAACATTAAGAAAGCAAAAGATTATCA
E_P18N2_3 TGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTGACCCTAGTGGTAAACACTGATTCATGGCTATTGAGGATTAAGAACATTAAGAAAGCAAAAGATTATCA
E_P18N2_4 TGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTGACCCTAGTGGTAAACACTGATTCATGGCTATTGAGGATTAAGAACATTAAGAAAGCAAAAGATTATCA
E_P18N2_2 TGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTGACCCTAGTGGTAAACACTGATTCATGGCTATTGAGGATTAAGAACATTAAGAAAGCAAAAGATTATCA
Consensus TGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTGACCCTAGTGGTAAACACTGATTCATGGCTATTGAGGATTAAGAACATTAAGAAAGCAAAAGATTATCA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
E_P18N2 TTGAACACRAGAGTTGAGCAGGGGAGAATGTTTGAAGTTAAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTGCTTCTTTTTCAAGTTTGAACAGGCCGACAAATTTGATCCTAATGGG
E_P18N2_1 TTGAACACRAGAGTTGAGCAGGGGAGAATGTTTGAAGTTAAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTGCTTCTTTTTCAAGTTTGAACAGGCCGACAAATTTGATCCTAATGGG
E_P18N2_3 TTGAACACRAGAGTTGAGCAGGGGAGAATGTTTGAAGTTAAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTGCTTCTTTTTCAAGTTTGAACAGGCCGACAAATTTGATCCTAATGGG
E_P18N2_4 TTGAACACRAGAGTTGAGCAGGGGAGAATGTTTGAAGTTAAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTGCTTCTTTTTCAAGTTTGAACAGGCCGACAAATTTGATCCTAATGGG
E_P18N2_2 TTGAACACRAGAGTTGAGCAGGGGAGAATGTTTGAAGTTAAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTGCTTCTTTTTCAAGTTTGAACAGGCCGACAAATTTGATCCTAATGGG
Consensus TTGAACACRAGAGTTGAGCAGGGGAGAATGTTTGAAGTTAAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTGCTTCTTTTTCAAGTTTGAACAGGCCGACAAATTTGATCCTAATGGG

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
E_P18N2 CCGACCTTTATGCCAAGATGTTTGTGCCAARAGGTTTCGACTATCCAGGTTGGGCTTGACCATTTGGGCTTGTGACATTAGCTTCTAAAACCTGGAGAAATACACACCTGTTTTCTTTCGAGTGT
E_P18N2_1 CCGACCTTTATGCCAAGATGTTTGTGCCAARAGGTTTCGACTATCCAGGTTGGGCTTGACCATTTGGGCTTGTGACATTAGCTTCTAAAACCTGGAGAAATACACACCTGTTTTCTTTCGAGTGT
E_P18N2_3 CCGACCTTTATGCCAAGATGTTTGTGCCAARAGGTTTCGACTATCCAGGTTGGGCTTGACCATTTGGGCTTGTGACATTAGCTTCTAAAACCTGGAGAAATACACACCTGTTTTCTTTCGAGTGT
E_P18N2_4 CCGACCTTTATGCCAAGATGTTTGTGCCAARAGGTTTCGACTATCCAGGTTGGGCTTGACCATTTGGGCTTGTGACATTAGCTTCTAAAACCTGGAGAAATACACACCTGTTTTCTTTCGAGTGT
E_P18N2_2 CCGACCTTTATGCCAAGATGTTTGTGCCAARAGGTTTCGACTATCCAGGTTGGGCTTGACCATTTGGGCTTGTGACATTAGCTTCTAAAACCTGGAGAAATACACACCTGTTTTCTTTCGAGTGT
Consensus CCGACCTTTATGCCAAGATGTTTGTGCCAARAGGTTTCGACTATCCAGGTTGGGCTTGACCATTTGGGCTTGTGACATTAGCTTCTAAAACCTGGAGAAATACACACCTGTTTTCTTTCGAGTGT

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
E_P18N2 TAAGGCTCAAAAGATTATAGGTTTCATGTGCTCAGATGCTAGAAGTCTACCATGAGACAAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACACGAGAG
E_P18N2_1 TAAGGCTCAAAAGATTATAGGTTTCATGTGCTCAGATGCTAGAAGTCTACCATGAGACAAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACACGAGAG
E_P18N2_3 TAAGGCTCAAAAGATTATAGGTTTCATGTGCTCAGATGCTAGAAGTCTACCATGAGACAAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACACGAGAG
E_P18N2_4 TAAGGCTCAAAAGATTATAGGTTTCATGTGCTCAGATGCTAGAAGTCTACCATGAGACAAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACACGAGAG
E_P18N2_2 TAAGGCTCAAAAGATTATAGGTTTCATGTGCTCAGATGCTAGAAGTCTACCATGAGACAAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACACGAGAG
Consensus TAAGGCTCAAAAGATTATAGGTTTCATGTGCTCAGATGCTAGAAGTCTACCATGAGACAAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACACGAGAG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
E_P18N2 TTATCTCTTAGGAGTTTGTATTGATAACTCAGTAGTGGAGAGTTTTGGTGTCTGGTGGAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTCTAATATGACATTTATTTGTCTTCAATA
E_P18N2_1 TTATCTCTTAGGAGTTTGTATTGATAACTCAGTAGTGGAGAGTTTTGGTGTCTGGTGGAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTCTAATATGACATTTATTTGTCTTCAATA
E_P18N2_3 TTATCTCTTAGGAGTTTGTATTGATAACTCAGTAGTGGAGAGTTTTGGTGTCTGGTGGAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTCTAATATGACATTTATTTGTCTTCAATA
E_P18N2_4 TTATCTCTTAGGAGTTTGTATTGATAACTCAGTAGTGGAGAGTTTTGGTGTCTGGTGGAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTCTAATATGACATTTATTTGTCTTCAATA
E_P18N2_2 TTATCTCTTAGGAGTTTGTATTGATAACTCAGTAGTGGAGAGTTTTGGTGTCTGGTGGAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTCTAATATGACATTTATTTGTCTTCAATA
Consensus TTATCTCTTAGGAGTTTGTATTGATAACTCAGTAGTGGAGAGTTTTGGTGTCTGGTGGAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTCTAATATGACATTTATTTGTCTTCAATA

1691 1700 1710 1720 1730 1740 1750 1758
E_P18N2 ATGGATCTGAGACARTCACARTTGAAGCTCTAATGCTTGGAGCATGGATGACCTAAGATGCACTAA
E_P18N2_1 ATGGATCTGAGACARTCACARTTGAAGCTCTAATGCTTGGAGCATGGATGACCTAAGATGCACTAA
E_P18N2_3 ATGGATCTGAGACARTCACARTTGAAGCTCTAATGCTTGGAGCATGGATGACCTAAGATGCACTAA
E_P18N2_4 ATGGATCTGAGACARTCACARTTGAAGCTCTAATGCTTGGAGCATGGATGACCTAAGATGCACTAA
E_P18N2_2 ATGGATCTGAGACARTCACARTTGAAGCTCTAATGCTTGGAGCATGGATGACCTAAGATGCACTAA
Consensus ATGGATCTGAGACARTCACARTTGAAGCTCTAATGCTTGGAGCATGGATGACCTAAGATGCACTAA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
E_P40N1 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTAGAAATTTATTTATTTTGCTCTTTATAGTTTATCAACATTAAATGGGGTGTGGCTTCTCATATATATTTTGGACTTGCATCTTCAA
E_P40N1_1 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTAGAAATTTATTTATTTTGCTCTTTATAGTTTATCAACATTAAATGGGGTGTGGCTTCTCATATATATTTTGGACTTGCATCTTCAA
E_P40N1_2 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTAGAAATTTATTTATTTTGCTCTTTATAGTTTATCAACATTAAATGGGGTGTGGCTTCTCATATATATTTTGGACTTGCATCTTCAA
Consensus ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTAGAAATTTATTTATTTTGCTCTTTATAGTTTATCAACATTAAATGGGGTGTGGCTTCTCATATATATTTTGGACTTGCATCTTCAA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
E_P40N1 GTGCTATTAGTGTCAAGAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAARACATTGGATTAAATGACCCATGACCCATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA
E_P40N1_1 GTGCTATTAGTGTCAAGAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAARACATTGGATTAAATGACCCATGACCCATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA
E_P40N1_2 GTGCTATTAGTGTCAAGAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAARACATTGGATTAAATGACCCATGACCCATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA
Consensus GTGCTATTAGTGTCAAGAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAARACATTGGATTAAATGACCCATGACCCATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
E_P40N1 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTGGTCCGGGTGAGCA
E_P40N1_1 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTGGTCCGGGTGAGCA
E_P40N1_2 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTGGTCCGGGTGAGCA
Consensus AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTGGTCCGGGTGAGCA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
E_P40N1 ACTATTCTACCAATAACAAACCCATTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAGTTCAAAATTATGCAATCCCGGCTAAGTGTCTGATCCATTTCTTCGTAATGGATCAACCTA
E_P40N1_1 ACTATTCTACCAATAACAAACCCATTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAGTTCAAAATTATGCAATCCCGGCTAAGTGTCTGATCCATTTCTTCGTAATGGATCAACCTA
E_P40N1_2 ACTATTCTACCAATAACAAACCCATTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAGTTCAAAATTATGCAATCCCGGCTAAGTGTCTGATCCATTTCTTCGTAATGGATCAACCTA
Consensus ACTATTCTACCAATAACAAACCCATTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAGTTCAAAATTATGCAATCCCGGCTAAGTGTCTGATCCATTTCTTCGTAATGGATCAACCTA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
E_P40N1 ATAACAACCCATTGATTGTACCTGACATAGCATCAACAACCAAAATTTTCGTGATCCACAACCCGATGGATGGCCAGAGTGGGCTTTGGAGATTGTAATAGGAGTATGAGAAACCATAGAGGGAT
E_P40N1_1 ATAACAACCCATTGATTGTACCTGACATAGCATCAACAACCAAAATTTTCGTGATCCACAACCCGATGGATGGCCAGAGTGGGCTTTGGAGATTGTAATAGGAGTATGAGAAACCATAGAGGGAT
E_P40N1_2 ATAACAACCCATTGATTGTACCTGACATAGCATCAACAACCAAAATTTTCGTGATCCACAACCCGATGGATGGCCAGAGTGGGCTTTGGAGATTGTAATAGGAGTATGAGAAACCATAGAGGGAT
Consensus ATAACAACCCATTGATTGTACCTGACATAGCATCAACAACCAAAATTTTCGTGATCCACAACCCGATGGATGGCCAGAGTGGGCTTTGGAGATTGTAATAGGAGTATGAGAAACCATAGAGGGAT

651 660 670 680 690 700 710 720 730 740 750 760 770 780
E_P40N1 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGACCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTCCCTGTATCATTAAAAATACTAATGGC
E_P40N1_1 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGACCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTCCCTGTATCATTAAAAATACTAATGGC
E_P40N1_2 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGACCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTCCCTGTATCATTAAAAATACTAATGGC
Consensus GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGACCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTCCCTGTATCATTAAAAATACTAATGGC

781 790 800 810 820 830 840 850 860 870 880 890 900 910
E_P40N1 TTAGATGCATCGTATCGCGAAAAAATGTCAACATGTCTTAAAGATAGCCTTGATGTTAATAGGTTTGGATATTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTTGATAACA
E_P40N1_1 TTAGATGCATCGTATCGCGAAAAAATGTCAACATGTCTTAAAGATAGCCTTGATGTTAATAGGTTTGGATATTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTTGATAACA
E_P40N1_2 TTAGATGCATCGTATCGCGAAAAAATGTCAACATGTCTTAAAGATAGCCTTGATGTTAATAGGTTTGGATATTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTTGATAACA
Consensus TTAGATGCATCGTATCGCGAAAAAATGTCAACATGTCTTAAAGATAGCCTTGATGTTAATAGGTTTGGATATTACACTATTGGTATGTATGACACCAAAAAGATAGGTACATTCTTGATAACA

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
E_P40N1 ATTCTATCGATGGTTCGAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAATCATTCTATGACCCATGAGGATCGAAGATTGTATGGGGTTGGACAATGAATCAGATGTTTTACCTGA
E_P40N1_1 ATTCTATCGATGGTTCGAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAATCATTCTATGACCCATGAGGATCGAAGATTGTATGGGGTTGGACAATGAATCAGATGTTTTACCTGA
E_P40N1_2 ATTCTATCGATGGTTCGAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAATCATTCTATGACCCATGAGGATCGAAGATTGTATGGGGTTGGACAATGAATCAGATGTTTTACCTGA
Consensus ATTCTATCGATGGTTCGAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAATCATTCTATGACCCATGAGGATCGAAGATTGTATGGGGTTGGACAATGAATCAGATGTTTTACCTGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
E_P40N1 CGATGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTCGACCCATAGTGGTAACAATTGATTCAATGGCTATTGAGGATTAAGAACATTAAAGAAAGCAAAGATTATT
E_P40N1_1 CGATGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTCGACCCATAGTGGTAACAATTGATTCAATGGCTATTGAGGATTAAGAACATTAAAGAAAGCAAAGATTATT
E_P40N1_2 CGATGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTCGACCCATAGTGGTAACAATTGATTCAATGGCTATTGAGGATTAAGAACATTAAAGAAAGCAAAGATTATT
Consensus CGATGAATTAAGAAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTCGACCCATAGTGGTAACAATTGATTCAATGGCTATTGAGGATTAAGAACATTAAAGAAAGCAAAGATTATT

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
E_P40N1 CAATTGAACACACAGGATTGAGCAGGGGAGAATGTTGAGGTTAAGGAATCTCAGCATCACAGGCTGATATTGAGGTGTCGTTCTCTTTTTCAGTTTAAACAGGCCGAACAATTTGATCCTAAT
E_P40N1_1 CAATTGAACACACAGGATTGAGCAGGGGAGAATGTTGAGGTTAAGGAATCTCAGCATCACAGGCTGATATTGAGGTGTCGTTCTCTTTTTCAGTTTAAACAGGCCGAACAATTTGATCCTAAT
E_P40N1_2 CAATTGAACACACAGGATTGAGCAGGGGAGAATGTTGAGGTTAAGGAATCTCAGCATCACAGGCTGATATTGAGGTGTCGTTCTCTTTTTCAGTTTAAACAGGCCGAACAATTTGATCCTAAT
Consensus CAATTGAACACACAGGATTGAGCAGGGGAGAATGTTGAGGTTAAGGAATCTCAGCATCACAGGCTGATATTGAGGTGTCGTTCTCTTTTTCAGTTTAAACAGGCCGAACAATTTGATCCTAAT

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
E_P40N1 GGGCCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGTTTCGACTATCCAGGTGGGCTTGGACCATTGGGCTTGTGACATTAGCTTCTAAAACTTGGAGAATACACACCTGTTTTCTTCGAGT
E_P40N1_1 GGGCCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGTTTCGACTATCCAGGTGGGCTTGGACCATTGGGCTTGTGACATTAGCTTCTAAAACTTGGAGAATACACACCTGTTTTCTTCGAGT
E_P40N1_2 GGGCCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGTTTCGACTATCCAGGTGGGCTTGGACCATTGGGCTTGTGACATTAGCTTCTAAAACTTGGAGAATACACACCTGTTTTCTTCGAGT
Consensus GGGCCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGTTTCGACTATCCAGGTGGGCTTGGACCATTGGGCTTGTGACATTAGCTTCTAAAACTTGGAGAATACACACCTGTTTTCTTCGAGT

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
E_P40N1 GTTTAAGGCTCAAAGAATTATAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACACGAG
E_P40N1_1 GTTTAAGGCTCAAAGAATTATAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACACGAG
E_P40N1_2 GTTTAAGGCTCAAAGAATTATAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACACGAG
Consensus GTTTAAGGCTCAAAGAATTATAAGGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACACGAG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
E_P40N1 AAGTTATCTCTTAGGAGTTTGGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTCAATAATGCACATTTATTTGTCTTCA
E_P40N1_1 AAGTTATCTCTTAGGAGTTTGGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTCAATAATGCACATTTATTTGTCTTCA
E_P40N1_2 AAGTTATCTCTTAGGAGTTTGGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTCAATAATGCACATTTATTTGTCTTCA
Consensus AAGTTATCTCTTAGGAGTTTGGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAAACATGCATAACATCGAGGGTGTATCCACGTTAGCGATTCAATAATGCACATTTATTTGTCTTCA

1691 1700 1710 1720 1730 1740 1750 1761
E_P40N1 ATAATGGATCTGAGACAATCAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
E_P40N1_1 ATAATGGATCTGAGACAATCAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
E_P40N1_2 ATAATGGATCTGAGACAATCAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA
Consensus ATAATGGATCTGAGACAATCAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACTAA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
E_P40N2 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTGAAGATTTATTTATTTTGGCTCTTTATAGTTTTATCAACATTAAATAGGTTGTTGCTTCTCATATATTTTTTTGGACTTGCATCTTCA
E_P40N2_4 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTGAAGATTTATTTATTTTGGCTCTTTATAGTTTTATCAACATTAAATAGGTTGTTGCTTCTCATATATTTTTTTGGACTTGCATCTTCA
E_P40N2_2 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTGAAGATTTATTTATTTTGGCTCTTTATAGTTTTATCAACATTAAATAGGTTGTTGCTTCTCATATATTTTTTTGGACTTGCATCTTCA
E_P40N2_1 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTGAAGATTTATTTATTTTGGCTCTTTATAGTTTTATCAACATTAAATAGGTTGTTGCTTCTCATATATTTTTTTGGACTTGCATCTTCA
E_P40N2_3 ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTGAAGATTTATTTATTTTGGCTCTTTATAGTTTTATCAACATTAAATAGGTTGTTGCTTCTCATATATTTTTTTGGACTTGCATCTTCA
Consensus ATGGAATTATTTATGAAAGCTCTTCTCTTTGGGGTTTGAAGATTTATTTATTTTGGCTCTTTATAGTTTTATCAACATTAAATAGGTTGTTGCTTCTCATATATTTTTTTGGACTTGCATCTTCA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
E_P40N2 GTGCTATTAGTGTCAAGATGTTTCATAGAACTGGTTTCATTTTCAACCTCTTAACATTGGATTARTGACCCATGACCCATGATTATATGAGTATATCATTATTCTATCATACACCCAA
E_P40N2_4 GTGCTATTAGTGTCAAGATGTTTCATAGAACTGGTTTCATTTTCAACCTCTTAACATTGGATTARTGACCCATGACCCATGATTATATGAGTATATCATTATTCTATCATACACCCAA
E_P40N2_2 GTGCTATTAGTGTCAAGATGTTTCATAGAACTGGTTTCATTTTCAACCTCTTAACATTGGATTARTGACCCATGACCCATGATTATATGAGTATATCATTATTCTATCATACACCCAA
E_P40N2_1 GTGCTATTAGTGTCAAGATGTTTCATAGAACTGGTTTCATTTTCAACCTCTTAACATTGGATTARTGACCCATGACCCATGATTATATGAGTATATCATTATTCTATCATACACCCAA
E_P40N2_3 GTGCTATTAGTGTCAAGATGTTTCATAGAACTGGTTTCATTTTCAACCTCTTAACATTGGATTARTGACCCATGACCCATGATTATATGAGTATATCATTATTCTATCATACACCCAA
Consensus GTGCTATTAGTGTCAAGATGTTTCATAGAACTGGTTTCATTTTCAACCTCTTAACATTGGATTARTGACCCATGACCCATGATTATATGAGTATATCATTATTCTATCATACACCCAA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
E_P40N2 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTGCTCTCAAGACTTGATAAATGGATCCATTAGAACCCGCAATTTATCCATCTAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCA
E_P40N2_4 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTGCTCTCAAGACTTGATAAATGGATCCATTAGAACCCGCAATTTATCCATCTAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCA
E_P40N2_2 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTGCTCTCAAGACTTGATAAATGGATCCATTAGAACCCGCAATTTATCCATCTAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCA
E_P40N2_1 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTGCTCTCAAGACTTGATAAATGGATCCATTAGAACCCGCAATTTATCCATCTAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCA
E_P40N2_3 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTGCTCTCAAGACTTGATAAATGGATCCATTAGAACCCGCAATTTATCCATCTAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCA
Consensus AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTGCTCTCAAGACTTGATAAATGGATCCATTAGAACCCGCAATTTATCCATCTAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
E_P40N2 ACTATTCTACCTAATAACAACCTGTTATCTTATATACCGGAGTAGTAGATCCCATGATTTCTCAGGTTTCAAGATTATGCAATCCCGGCTAATCTGTCTGATCCATTCTTCGTAATGGATCAACCTA
E_P40N2_4 ACTATTCTACCTAATAACAACCTGTTATCTTATATACCGGAGTAGTAGATCCCATGATTTCTCAGGTTTCAAGATTATGCAATCCCGGCTAATCTGTCTGATCCATTCTTCGTAATGGATCAACCTA
E_P40N2_2 ACTATTCTACCTAATAACAACCTGTTATCTTATATACCGGAGTAGTAGATCCCATGATTTCTCAGGTTTCAAGATTATGCAATCCCGGCTAATCTGTCTGATCCATTCTTCGTAATGGATCAACCTA
E_P40N2_1 ACTATTCTACCTAATAACAACCTGTTATCTTATATACCGGAGTAGTAGATCCCATGATTTCTCAGGTTTCAAGATTATGCAATCCCGGCTAATCTGTCTGATCCATTCTTCGTAATGGATCAACCTA
E_P40N2_3 ACTATTCTACCTAATAACAACCTGTTATCTTATATACCGGAGTAGTAGATCCCATGATTTCTCAGGTTTCAAGATTATGCAATCCCGGCTAATCTGTCTGATCCATTCTTCGTAATGGATCAACCTA
Consensus ACTATTCTACCTAATAACAACCTGTTATCTTATATACCGGAGTAGTAGATCCCATGATTTCTCAGGTTTCAAGATTATGCAATCCCGGCTAATCTGTCTGATCCATTCTTCGTAATGGATCAACCTA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
E_P40N2 ATACAACCCGTTGATTGTTCTGACATAGCATCAACAACCCGAATTCGTTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTATATAGGAGTATGAGAARACATAGAGGGAT
E_P40N2_4 ATACAACCCGTTGATTGTTCTGACATAGCATCAACAACCCGAATTCGTTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTATATAGGAGTATGAGAARACATAGAGGGAT
E_P40N2_2 ATACAACCCGTTGATTGTTCTGACATAGCATCAACAACCCGAATTCGTTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTATATAGGAGTATGAGAARACATAGAGGGAT
E_P40N2_1 ATACAACCCGTTGATTGTTCTGACATAGCATCAACAACCCGAATTCGTTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTATATAGGAGTATGAGAARACATAGAGGGAT
E_P40N2_3 ATACAACCCGTTGATTGTTCTGACATAGCATCAACAACCCGAATTCGTTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTATATAGGAGTATGAGAARACATAGAGGGAT
Consensus ATACAACCCGTTGATTGTTCTGACATAGCATCAACAACCCGAATTCGTTGATCCACACCCGATGGATGGCCAGATGGGCTTTGGAGATTATATAGGAGTATGAGAARACATAGAGGGAT

651 660 670 680 690 700 710 720 730 740 750 760 770 780
E_P40N2 GGCATTATTGTATAGAGTAGAGACTTCATTAAATGGGCCAAGCCACATCCACTTCATTTCATCTCCTCATACTGGAAATGGGAATGTCCTGATTTTTTCTGTATCATTAAAAAATACTAATGGC
E_P40N2_4 GGCATTATTGTATAGAGTAGAGACTTCATTAAATGGGCCAAGCCACATCCACTTCATTTCATCTCCTCATACTGGAAATGGGAATGTCCTGATTTTTTCTGTATCATTAAAAAATACTAATGGC
E_P40N2_2 GGCATTATTGTATAGAGTAGAGACTTCATTAAATGGGCCAAGCCACATCCACTTCATTTCATCTCCTCATACTGGAAATGGGAATGTCCTGATTTTTTCTGTATCATTAAAAAATACTAATGGC
E_P40N2_1 GGCATTATTGTATAGAGTAGAGACTTCATTAAATGGGCCAAGCCACATCCACTTCATTTCATCTCCTCATACTGGAAATGGGAATGTCCTGATTTTTTCTGTATCATTAAAAAATACTAATGGC
E_P40N2_3 GGCATTATTGTATAGAGTAGAGACTTCATTAAATGGGCCAAGCCACATCCACTTCATTTCATCTCCTCATACTGGAAATGGGAATGTCCTGATTTTTTCTGTATCATTAAAAAATACTAATGGC
Consensus GGCATTATTGTATAGAGTAGAGACTTCATTAAATGGGCCAAGCCACATCCACTTCATTTCATCTCCTCATACTGGAAATGGGAATGTCCTGATTTTTTCTGTATCATTAAAAAATACTAATGGC

781 790 800 810 820 830 840 850 860 870 880 890 900 910
E_P40N2 TTAGATGCATCGTATCGGGAAAAATGTCARACATGTCCTTARGAATAGCCTTGATGTTAATAGGTTTGATTATTACACTATTGGTATGATGACACCAAAAAGATAGGTACATTCTGATACCAAT
E_P40N2_4 TTAGATGCATCGTATCGGGAAAAATGTCARACATGTCCTTARGAATAGCCTTGATGTTAATAGGTTTGATTATTACACTATTGGTATGATGACACCAAAAAGATAGGTACATTCTGATACCAAT
E_P40N2_2 TTAGATGCATCGTATCGGGAAAAATGTCARACATGTCCTTARGAATAGCCTTGATGTTAATAGGTTTGATTATTACACTATTGGTATGATGACACCAAAAAGATAGGTACATTCTGATACCAAT
E_P40N2_1 TTAGATGCATCGTATCGGGAAAAATGTCARACATGTCCTTARGAATAGCCTTGATGTTAATAGGTTTGATTATTACACTATTGGTATGATGACACCAAAAAGATAGGTACATTCTGATACCAAT
E_P40N2_3 TTAGATGCATCGTATCGGGAAAAATGTCARACATGTCCTTARGAATAGCCTTGATGTTAATAGGTTTGATTATTACACTATTGGTATGATGACACCAAAAAGATAGGTACATTCTGATACCAAT
Consensus TTAGATGCATCGTATCGGGAAAAATGTCARACATGTCCTTARGAATAGCCTTGATGTTAATAGGTTTGATTATTACACTATTGGTATGATGACACCAAAAAGATAGGTACATTCTGATACCAAT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
E_P40N2 CTATCGATGGTTGAGGGGATTTGAGGCTTGACTATGGGAAATTTCTATGCATCTAATCATTATGACCCATGAGGATCGAGAATGTTATGGGGTTGGCAAAATGAATCAGATGTTTTACCTGACGA
E_P40N2_4 CTATCGATGGTTGAGGGGATTTGAGGCTTGACTATGGGAAATTTCTATGCATCTAATCATTATGACCCATGAGGATCGAGAATGTTATGGGGTTGGCAAAATGAATCAGATGTTTTACCTGACGA
E_P40N2_2 CTATCGATGGTTGAGGGGATTTGAGGCTTGACTATGGGAAATTTCTATGCATCTAATCATTATGACCCATGAGGATCGAGAATGTTATGGGGTTGGCAAAATGAATCAGATGTTTTACCTGACGA
E_P40N2_1 CTATCGATGGTTGAGGGGATTTGAGGCTTGACTATGGGAAATTTCTATGCATCTAATCATTATGACCCATGAGGATCGAGAATGTTATGGGGTTGGCAAAATGAATCAGATGTTTTACCTGACGA
E_P40N2_3 CTATCGATGGTTGAGGGGATTTGAGGCTTGACTATGGGAAATTTCTATGCATCTAATCATTATGACCCATGAGGATCGAGAATGTTATGGGGTTGGCAAAATGAATCAGATGTTTTACCTGACGA
Consensus CTATCGATGGTTGAGGGGATTTGAGGCTTGACTATGGGAAATTTCTATGCATCTAATCATTATGACCCATGAGGATCGAGAATGTTATGGGGTTGGCAAAATGAATCAGATGTTTTACCTGACGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
E_P40N2 TGAATTAAGAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTCGATCCTAGTGGTAAACATTTGATTCATAGGCTATTGAGGATTAAGAACATTAAGAAGCAAAAGATTCAATTG
E_P40N2_4 TGAATTAAGAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTCGATCCTAGTGGTAAACATTTGATTCATAGGCTATTGAGGATTAAGAACATTAAGAAGCAAAAGATTCAATTG
E_P40N2_2 TGAATTAAGAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTCGATCCTAGTGGTAAACATTTGATTCATAGGCTATTGAGGATTAAGAACATTAAGAAGCAAAAGATTCAATTG
E_P40N2_1 TGAATTAAGAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTCGATCCTAGTGGTAAACATTTGATTCATAGGCTATTGAGGATTAAGAACATTAAGAAGCAAAAGATTCAATTG
E_P40N2_3 TGAATTAAGAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTCGATCCTAGTGGTAAACATTTGATTCATAGGCTATTGAGGATTAAGAACATTAAGAAGCAAAAGATTCAATTG
Consensus TGAATTAAGAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAAGTATGGCTCGATCCTAGTGGTAAACATTTGATTCATAGGCTATTGAGGATTAAGAACATTAAGAAGCAAAAGATTCAATTG

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
E_P40N2 AACACACAGAGTTGAGCAGGGGAGAAATGTTGAGTTGAGGAAATTCAGCATCACAGGCTGATATTGAGTGTCTGTTCTCTTTTTCAGTTTGAACAGGCCGACCAATTTGATCCTAATGGGCCG
E_P40N2_4 AACACACAGAGTTGAGCAGGGGAGAAATGTTGAGTTGAGGAAATTCAGCATCACAGGCTGATATTGAGTGTCTGTTCTCTTTTTCAGTTTGAACAGGCCGACCAATTTGATCCTAATGGGCCG
E_P40N2_2 AACACACAGAGTTGAGCAGGGGAGAAATGTTGAGTTGAGGAAATTCAGCATCACAGGCTGATATTGAGTGTCTGTTCTCTTTTTCAGTTTGAACAGGCCGACCAATTTGATCCTAATGGGCCG
E_P40N2_1 AACACACAGAGTTGAGCAGGGGAGAAATGTTGAGTTGAGGAAATTCAGCATCACAGGCTGATATTGAGTGTCTGTTCTCTTTTTCAGTTTGAACAGGCCGACCAATTTGATCCTAATGGGCCG
E_P40N2_3 AACACACAGAGTTGAGCAGGGGAGAAATGTTGAGTTGAGGAAATTCAGCATCACAGGCTGATATTGAGTGTCTGTTCTCTTTTTCAGTTTGAACAGGCCGACCAATTTGATCCTAATGGGCCG
Consensus AACACACAGAGTTGAGCAGGGGAGAAATGTTGAGTTGAGGAAATTCAGCATCACAGGCTGATATTGAGTGTCTGTTCTCTTTTTCAGTTTGAACAGGCCGACCAATTTGATCCTAATGGGCCG

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
E_P40N2 ACCTTTATGCCAAGATGTTTGTGCCATTAGGGTTCCGACTATCCAGGTTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAGAACTTGGAGAAATACACACCTGTTTTCTTTCGAGTGTTTAA
E_P40N2_4 ACCTTTATGCCAAGATGTTTGTGCCATTAGGGTTCCGACTATCCAGGTTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAGAACTTGGAGAAATACACACCTGTTTTCTTTCGAGTGTTTAA
E_P40N2_2 ACCTTTATGCCAAGATGTTTGTGCCATTAGGGTTCCGACTATCCAGGTTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAGAACTTGGAGAAATACACACCTGTTTTCTTTCGAGTGTTTAA
E_P40N2_1 ACCTTTATGCCAAGATGTTTGTGCCATTAGGGTTCCGACTATCCAGGTTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAGAACTTGGAGAAATACACACCTGTTTTCTTTCGAGTGTTTAA
E_P40N2_3 ACCTTTATGCCAAGATGTTTGTGCCATTAGGGTTCCGACTATCCAGGTTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAGAACTTGGAGAAATACACACCTGTTTTCTTTCGAGTGTTTAA
Consensus ACCTTTATGCCAAGATGTTTGTGCCATTAGGGTTCCGACTATCCAGGTTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAGAACTTGGAGAAATACACACCTGTTTTCTTTCGAGTGTTTAA

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
E_P40N2 GGCTCAAAAGAAATTAAGGTTCTCCTATGCTCAGATGCTAGAGATCTACCATGAGACAAATGARGCAATGTACAGGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAGAGGTTA
E_P40N2_4 GGCTCAAAAGAAATTAAGGTTCTCCTATGCTCAGATGCTAGAGATCTACCATGAGACAAATGARGCAATGTACAGGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAGAGGTTA
E_P40N2_2 GGCTCAAAAGAAATTAAGGTTCTCCTATGCTCAGATGCTAGAGATCTACCATGAGACAAATGARGCAATGTACAGGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAGAGGTTA
E_P40N2_1 GGCTCAAAAGAAATTAAGGTTCTCCTATGCTCAGATGCTAGAGATCTACCATGAGACAAATGARGCAATGTACAGGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAGAGGTTA
E_P40N2_3 GGCTCAAAAGAAATTAAGGTTCTCCTATGCTCAGATGCTAGAGATCTACCATGAGACAAATGARGCAATGTACAGGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAGAGGTTA
Consensus GGCTCAAAAGAAATTAAGGTTCTCCTATGCTCAGATGCTAGAGATCTACCATGAGACAAATGARGCAATGTACAGGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAGAGGTTA

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
E_P40N2 TCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGATGGAAAACATGCATARCATCGAGGGTGTATCCACGTTAGCGATTTCATATAATGCACATTTATTTGTCTTCATTAATG
E_P40N2_4 TCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGATGGAAAACATGCATARCATCGAGGGTGTATCCACGTTAGCGATTTCATATAATGCACATTTATTTGTCTTCATTAATG
E_P40N2_2 TCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGATGGAAAACATGCATARCATCGAGGGTGTATCCACGTTAGCGATTTCATATAATGCACATTTATTTGTCTTCATTAATG
E_P40N2_1 TCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGATGGAAAACATGCATARCATCGAGGGTGTATCCACGTTAGCGATTTCATATAATGCACATTTATTTGTCTTCATTAATG
E_P40N2_3 TCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGATGGAAAACATGCATARCATCGAGGGTGTATCCACGTTAGCGATTTCATATAATGCACATTTATTTGTCTTCATTAATG
Consensus TCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTGGTGTGATGGAAAACATGCATARCATCGAGGGTGTATCCACGTTAGCGATTTCATATAATGCACATTTATTTGTCTTCATTAATG

1691 1700 1710 1720 1730 1740 1750 1755
E_P40N2 GATCTGAGCAATCATAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAGATGCACTAA
E_P40N2_4 GATCTGAGCAATCATAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAGATGCACTAA
E_P40N2_2 GATCTGAGCAATCATAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAGATGCACTAA
E_P40N2_1 GATCTGAGCAATCATAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAGATGCACTAA
E_P40N2_3 GATCTGAGCAATCATAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAGATGCACTAA
Consensus GATCTGAGCAATCATAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAGATGCACTAA

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
E_P54N1	-----													
E_P54N1_1	ATGGAATTATTTATGAAAAGCTCTTCTCTTTGGGGTTTAGAATTTATTTATTTGCTCTTTATAGTTTATCAACATTAATGGGGTGTGGCTTCTCATATATTTTTTTGGACTTGCATCTTCAA													
Consensus	ATGGAATTATTTATGAAAAGCTCTTCTCTTTGGGGTTTAGAATTTATTTATTTGCTCTTTATAGTTTATCAACATTAATGGGGTGTGGCTTCTCATATATTTTTTTGGACTTGCATCTTCAA													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
E_P54N1	-----													
E_P54N1_1	GTGCTATTAGTGTCAAGAATGTTTCATAGAACTGGTTTTCAATTTCAACCTCCTAACATTGGATTAAATGACCCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATACATCCAA													
Consensus	GTGCTATTAGTGTCAAGAATGTTTCATAGAACTGGTTTTCAATTTCAACCTCCTAACATTGGATTAAATGACCCTAATGCACCAATGTATTATAATGGAGTATATCATTATTCTATCAATACATCCAA													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
E_P54N1	-----													
E_P54N1_1	AGGATCAGTATGGGGCAATATTGTTGGGCTCATTCAAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCA													
Consensus	AGGATCAGTATGGGGCAATATTGTTGGGCTCATTCAAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTGGTCCGGGTCAGCA													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
E_P54N1	-----													
E_P54N1_1	ACTATTCTACCAATAACAAGCCCGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAAGTTCAAAATTATGCAATCCCGGCTAACTTGTCTGATCCATTTCTTCGTAATGGATCAACCTA													
Consensus	ACTATTCTACCAATAACAAGCCCGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAAGTTCAAAATTATGCAATCCCGGCTAACTTGTCTGATCCATTTCTTCGTAATGGATCAACCTA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
E_P54N1	-----													
E_P54N1_1	ATAACAACCCGTTGATTGTACCTGACAATAGCATCAACAAACCAAAATTCGTGATCCACACCCGATGGATGGGCCAAGATGGGCTTTGGAGAATTGTAATAGGAAGTATGAGAAACATAGAGGGAT													
Consensus	ATAACAACCCGTTGATTGTACCTGACAATAGCATCAACAAACCAAAATTCGTGATCCACACCCGATGGATGGGCCAAGATGGGCTTTGGAGAATTGTAATAGGAAGTATGAGAAACATAGAGGGAT													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
E_P54N1	-----													
E_P54N1_1	GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGCCCAACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGAATGTCCTGATTTTTTCTGTATCATTAAAAATACAATGGC													
Consensus	GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGCCCAACATCCACTTCATTCATCTCCTCATACTGGAAATTTGGGAATGTCCTGATTTTTTCTGTATCATTAAAAATACAATGGC													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
E_P54N1	-----													
E_P54N1_1	TTAGATGCATCGTATCGCGGAAAAATGTCAATATGTCCTTAAGAATAGCCTTGATGTTAATAGGTTTGAGTATTACACTATTGGTATGTATGACACCAAAAAAGATAGGTACATTCTGATAACAATT													
Consensus	TTAGATGCATCGTATCGCGGAAAAATGTCAATATGTCCTTAAGAATAGCCTTGATGTTAATAGGTTTGAGTATTACACTATTGGTATGTATGACACCAAAAAAGATAGGTACATTCTGATAACAATT													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
E_P54N1	-----													
E_P54N1_1	CTATCGATGGTTCGAAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAAATCATTCTATGACCCTATGAGAATCGAGAATTGTATGGGGTTGGACAATGAATCAGATGTTTTACCTGACGA													
Consensus	CTATCGATGGTTCGAAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAAATCATTCTATGACCCTATGAGAATCGAGAATTGTATGGGGTTGGACAATGAATCAGATGTTTTACCTGACGA													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
E_P54N1	-----													
E_P54N1_1	TGAATTAAGAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAGTATGGCTCGACCCTAGTGGTAACAACTGATTCATGGCCTATTGAGAATTAGAACATTAGAAGCAAAAGATTATTCAA													
Consensus	TGAATTAAGAAGGATGGGCTGGAATTCAGCTATTCCGCGTAAGTATGGCTCGACCCTAGTGGTAACAACTGATTCATGGCCTATTGAGAATTAGAACATTAGAAGCAAAAGATTATTCAA													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
E_P54N1	-----													
E_P54N1_1	TTGAACAACAAGAGTTGAGCAAGGGAGAAATGTTTGAAGTTAAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTCTGTTCTTTTTCAAGTTTGACACAGGCCGACAAATTTGATCCTAATGGG													
Consensus	TTGAACAACAAGAGTTGAGCAAGGGAGAAATGTTTGAAGTTAAGGAATCTCAGCATCACAGGCTGATATTGAAGTGTCTGTTCTTTTTCAAGTTTGACACAGGCCGACAAATTTGATCCTAATGGG													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
E_P54N1	-----													
E_P54N1_1	CCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAAGGTGGGCTTGGACCATTTGGGCTTGTGACATTAGCTTCTAAAACTTGGAGAaTACACACCTGTTTTCTTTCGAGTGT													
Consensus	CCGACCTTTATGCCAAGATGTTTGTGCCATAAAGGGTTCGACTATCCAAGGTGGGCTTGGACCATTTGGGCTTGTGACATTAGCTTCTAAAACTTGGAGAaTACACACCTGTTTTCTTTCGAGTGT													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
E_P54N1	-----													
E_P54N1_1	TAAGGCTCAAAGAATTATAAGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACACGAGAGAG													
Consensus	TAAGGCTCAAAGAATTATAAGTTCTCATGTGCTCAGATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTTAGTAGACACGAGAGAG													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
E_P54N1	-----													
E_P54N1_1	TTATCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTACAAATATGCACATTTATTTGTCTTCAATA													
Consensus	TTATCTCTTAGGAGTTTGATTGATAACTCAGTAGTGGAGAGTTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCACGTTAGCGATTACAAATATGCACATTTATTTGTCTTCAATA													
	1691	1700	1710	1720	1730	1740	1750	1758						
E_P54N1	-----													
E_P54N1_1	ATGGATCTGAGACAATCAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACCTAA													
Consensus	ATGGATCTGAGACAATCAATTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACCTAA													

1 10 20 30 40 50 60 70 80 90 100 110 120 130
|-----|
E_P54N2 ATGGAATTATTTATGAAAGCTCTTCTTTGGGGTTTAAAGATTTATTTATTTTGGCTTCTTTATAGTTTATCAACATTATTAAGGTGTTGCTTCTCATATATTTTTTTGGACTTGCATCTTCAA
E_P54N2_2 ATGGAATTATTTATGAAAGCTCTTCTTTGGGGTTTAAAGATTTATTTATTTTGGCTTCTTTATAGTTTATCAACATTATTAAGGTGTTGCTTCTCATATATTTTTTTGGACTTGCATCTTCAA
E_P54N2_1 ATGGAATTATTTATGAAAGCTCTTCTTTGGGGTTTAAAGATTTATTTATTTTGGCTTCTTTATAGTTTATCAACATTATTAAGGTGTTGCTTCTCATATATTTTTTTGGACTTGCATCTTCAA
Consensus ATGGAATTATTTATGAAAGCTCTTCTTTGGGGTTTAAAGATTTATTTATTTTGGCTTCTTTATAGTTTATCAACATTATTAAGGTGTTGCTTCTCATATATTTTTTTGGACTTGCATCTTCAA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
|-----|
E_P54N2 GTGCTATTAGTGTCAAGAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAAAATTGGATTAAATGACCCATGACCCATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA
E_P54N2_2 GTGCTATTAGTGTCAAGAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAAAATTGGATTAAATGACCCATGACCCATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA
E_P54N2_1 GTGCTATTAGTGTCAAGAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAAAATTGGATTAAATGACCCATGACCCATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA
Consensus GTGCTATTAGTGTCAAGAATGTTTCATAGAACTGGTTTTCATTTTCACCTCCTAAAATTGGATTAAATGACCCATGACCCATGATTATAATGGAGTATATCATTATTCTATCAATACATCCAAA

261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----|
E_P54N2 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTCTGGTCCGGGTGAGCA
E_P54N2_2 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTCTGGTCCGGGTGAGCA
E_P54N2_1 AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTCTGGTCCGGGTGAGCA
Consensus AGGATCAGTATGGGGCAATATTGTTTGGGCTCATTTCAGTCTCAAAGACTTGATAAATTGGATCCATTTAGAACCCGCAATTTATCCATCTAAAAATTTGACAAATATGGTGTCTGGTCCGGGTGAGCA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----|
E_P54N2 ACTATTCTACCTAATAACAAACCTGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAGGTTTCAAGATTATGCAATCCCGGCTAAGTTGTCTGATCCATTTCTTCGTAATGGATCAACCTA
E_P54N2_2 ACTATTCTACCTAATAACAAACCTGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAGGTTTCAAGATTATGCAATCCCGGCTAAGTTGTCTGATCCATTTCTTCGTAATGGATCAACCTA
E_P54N2_1 ACTATTCTACCTAATAACAAACCTGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAGGTTTCAAGATTATGCAATCCCGGCTAAGTTGTCTGATCCATTTCTTCGTAATGGATCAACCTA
Consensus ACTATTCTACCTAATAACAAACCTGTTATCTTATACACCGGAGTAGTAGATTCCCATGATTCTCAGGTTTCAAGATTATGCAATCCCGGCTAAGTTGTCTGATCCATTTCTTCGTAATGGATCAACCTA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
|-----|
E_P54N2 ATAACAACCCGTTGATTGTTTCTGCAATAGCATCAACAACCCGAAATTTCTGATCCACAACCCGATGGATGGCCAGAGTGGGCTTTGGAGATTGTAATAGGAGTATGAGAAACATAGGGGGAT
E_P54N2_2 ATAACAACCCGTTGATTGTTTCTGCAATAGCATCAACAACCCGAAATTTCTGATCCACAACCCGATGGATGGCCAGAGTGGGCTTTGGAGATTGTAATAGGAGTATGAGAAACATAGGGGGAT
E_P54N2_1 ATAACAACCCGTTGATTGTTTCTGCAATAGCATCAACAACCCGAAATTTCTGATCCACAACCCGATGGATGGCCAGAGTGGGCTTTGGAGATTGTAATAGGAGTATGAGAAACATAGGGGGAT
Consensus ATAACAACCCGTTGATTGTTTCTGCAATAGCATCAACAACCCGAAATTTCTGATCCACAACCCGATGGATGGCCAGAGTGGGCTTTGGAGATTGTAATAGGAGTATGAGAAACATAGGGGGAT

651 660 670 680 690 700 710 720 730 740 750 760 770 780
|-----|
E_P54N2 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTTCCCTGTATCATTAAAAATACTAATGGC
E_P54N2_2 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTTCCCTGTATCATTAAAAATACTAATGGC
E_P54N2_1 GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTTCCCTGTATCATTAAAAATACTAATGGC
Consensus GGCATTATTGTATAGAAGTAGAGACTTCATTAATGGGCCAAGGCCAACATCCACTTCATTCATCTCCTCATACTGGAATTTGGGATGTCTGATTTTTTCCCTGTATCATTAAAAATACTAATGGC

781 790 800 810 820 830 840 850 860 870 880 890 900 910
|-----|
E_P54N2 TTAGATGCATCGTATCGCGAAAAAATGTCAAACATGTCCTTAAGAAATAGCCTTGATGTTAATAGGTTTGGATTATTACACTATTGGTATGATGACCCAAAAAAGATAGGTACATTCTGATACAAAT
E_P54N2_2 TTAGATGCATCGTATCGCGAAAAAATGTCAAACATGTCCTTAAGAAATAGCCTTGATGTTAATAGGTTTGGATTATTACACTATTGGTATGATGACCCAAAAAAGATAGGTACATTCTGATACAAAT
E_P54N2_1 TTAGATGCATCGTATCGCGAAAAAATGTCAAACATGTCCTTAAGAAATAGCCTTGATGTTAATAGGTTTGGATTATTACACTATTGGTATGATGACCCAAAAAAGATAGGTACATTCTGATACAAAT
Consensus TTAGATGCATCGTATCGCGAAAAAATGTCAAACATGTCCTTAAGAAATAGCCTTGATGTTAATAGGTTTGGATTATTACACTATTGGTATGATGACCCAAAAAAGATAGGTACATTCTGATACAAAT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
|-----|
E_P54N2 CTATCGATGGTTCGAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAATCATTATTTATGACCCATGAGAAATCGAAGATTGATGGGGTTGGACAAATGAATCAGATGGTTTACCTGACGA
E_P54N2_2 CTATCGATGGTTCGAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAATCATTATTTATGACCCATGAGAAATCGAAGATTGATGGGGTTGGACAAATGAATCAGATGGTTTACCTGACGA
E_P54N2_1 CTATCGATGGTTCGAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAATCATTATTTATGACCCATGAGAAATCGAAGATTGATGGGGTTGGACAAATGAATCAGATGGTTTACCTGACGA
Consensus CTATCGATGGTTCGAGGGATTGAGGCTTGACTATGGGAATTTCTATGCATCTAATCATTATTTATGACCCATGAGAAATCGAAGATTGATGGGGTTGGACAAATGAATCAGATGGTTTACCTGACGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
|-----|
E_P54N2 TGAATTAGAAAGGATGGGCTGGAAATCAAGCTATTCCGCGTAAAGTATGGCTCGACCCATGTTGTTAAGCAATGATTCATGGCCTATTGAGAAATAGAACATTAGAAAGCAAAGATTCAATTG
E_P54N2_2 TGAATTAGAAAGGATGGGCTGGAAATCAAGCTATTCCGCGTAAAGTATGGCTCGACCCATGTTGTTAAGCAATGATTCATGGCCTATTGAGAAATAGAACATTAGAAAGCAAAGATTCAATTG
E_P54N2_1 TGAATTAGAAAGGATGGGCTGGAAATCAAGCTATTCCGCGTAAAGTATGGCTCGACCCATGTTGTTAAGCAATGATTCATGGCCTATTGAGAAATAGAACATTAGAAAGCAAAGATTCAATTG
Consensus TGAATTAGAAAGGATGGGCTGGAAATCAAGCTATTCCGCGTAAAGTATGGCTCGACCCATGTTGTTAAGCAATGATTCATGGCCTATTGAGAAATAGAACATTAGAAAGCAAAGATTCAATTG

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
|-----|
E_P54N2 AACAAACAGAGTTGAGCAAGGGAGAAATGTTGAGTTGAGGAATCTCAGCATCACAGGCTGATATTGAGTGTCTTCTCTTTTTCAAGTTTGAACAAAGGCCGAAACATTTGATCCTAATGGGCCG
E_P54N2_2 AACAAACAGAGTTGAGCAAGGGAGAAATGTTGAGTTGAGGAATCTCAGCATCACAGGCTGATATTGAGTGTCTTCTCTTTTTCAAGTTTGAACAAAGGCCGAAACATTTGATCCTAATGGGCCG
E_P54N2_1 AACAAACAGAGTTGAGCAAGGGAGAAATGTTGAGTTGAGGAATCTCAGCATCACAGGCTGATATTGAGTGTCTTCTCTTTTTCAAGTTTGAACAAAGGCCGAAACATTTGATCCTAATGGGCCG
Consensus AACAAACAGAGTTGAGCAAGGGAGAAATGTTGAGTTGAGGAATCTCAGCATCACAGGCTGATATTGAGTGTCTTCTCTTTTTCAAGTTTGAACAAAGGCCGAAACATTTGATCCTAATGGGCCG

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
|-----|
E_P54N2 ACCTTTATGCCAAGATGTTTGTGCCATTAAGGGTTCGACTATCCAAGGTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAGAACTTGGAGAAATACACACCTGTTTTCTTTTCGAGTGTTTAA
E_P54N2_2 ACCTTTATGCCAAGATGTTTGTGCCATTAAGGGTTCGACTATCCAAGGTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAGAACTTGGAGAAATACACACCTGTTTTCTTTTCGAGTGTTTAA
E_P54N2_1 ACCTTTATGCCAAGATGTTTGTGCCATTAAGGGTTCGACTATCCAAGGTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAGAACTTGGAGAAATACACACCTGTTTTCTTTTCGAGTGTTTAA
Consensus ACCTTTATGCCAAGATGTTTGTGCCATTAAGGGTTCGACTATCCAAGGTGGGCTTGGACCATTGGGCTTGCACATTAGCTTCTAAGAACTTGGAGAAATACACACCTGTTTTCTTTTCGAGTGTTTAA

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
|-----|
E_P54N2 GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAGAGTTA
E_P54N2_2 GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAGAGTTA
E_P54N2_1 GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAGAGTTA
Consensus GGCTCAAAGAAATTAAGGTTCTCATGTGCTCAATGCTAGAAGATCTACCATGAGACAAATGAAGCAATGTACAGCCCTCATTGCTGGATATGTAGATGTAGATTAGTAGACATGAGAGTTA

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
|-----|
E_P54N2 TCTCTTAGGAGTTTATTGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCAAATGTTAGCGATTCAATAATGCACATTTATTTGTCTTCAATAATG
E_P54N2_2 TCTCTTAGGAGTTTATTGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCAAATGTTAGCGATTCAATAATGCACATTTATTTGTCTTCAATAATG
E_P54N2_1 TCTCTTAGGAGTTTATTGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCAAATGTTAGCGATTCAATAATGCACATTTATTTGTCTTCAATAATG
Consensus TCTCTTAGGAGTTTATTGATAACTCAGTAGTGGAGAGTTTGGTGTGGTGGAAAAACATGCATACATCGAGGGTGTATCCAAATGTTAGCGATTCAATAATGCACATTTATTTGTCTTCAATAATG

1691 1700 1710 1720 1730 1740 1750 1755
|-----|
E_P54N2 GATCTGAGACAAATCAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACAA
E_P54N2_2 GATCTGAGACAAATCAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACAA
E_P54N2_1 GATCTGAGACAAATCAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACAA
Consensus GATCTGAGACAAATCAATTTGAGACTCTTAATGCTTGGAGCATGGATGTACCTAAGATGCACAA

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN1	MDYSSNSRWTLPVILVCFEIVLLSNVYVFASHKVF IHLQSQNAVNVHTVHRTGYHFQPEKHWINDPNAPHYFNGVYHLFYQYNPNGSVMGNIYWAHSVSKDLINWINLEPAIYPSKPFDDQFGTWSGSATI													
F_DN2	MDYSSNSRWALPVILVCFEIVLLSNVYVFASHKVF IHLQSQNAVNVQTVHRTGYHFQPEKHWINDPNAPHYFNGVYHLFYQYNPNGSVMGNIYWAHSVSKDLINWINLEPAIYPSKPFDDQFGTWSGSATI													
F_DN2_1	MDYSSNSRWALPVILVCFEIVLLSNVYVFASHKVF IHLQSQNAVNVQTVHRTGYHFQPEKHWINDPNAPHYFNGVYHLFYQYNPNGSVMGNIYWAHSVSKDLINWINLEPAIYPSKPFDDQFGTWSGSATI													
Consensus	MDYSSNSRWaLPVILVCFEIVLLSNVYVFASHKVF IHLQSQNAVNVqTVHRTGYHFQPEKHWINDPNAPHYFNGVYHLFYQYNPNGSVMGNIYWAHSVSKDLINWINLEPAIYPSKPFDDQFGTWSGSATI													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN1	LPGNKPVILYTGIVDANQTQVQNYAVPANISDPYLREWIKPDNNPLIYADASINKTKFRDPTTAWMGKDGHWRIYMGSLRKHSRGLAIMYRSKDFHKWYKAKHPLHSTNGTGNWECPDFFPVALKGTNGQ													
F_DN2	LPGNKPVILYTGIVDANQTQVQNYAIPANLSDPYLREWIKPDNNPLIYADASINKTKFRDPTTAWMGKDGHWRIYMGSLRKHSRGLAIMYRSKDFHKWYKAKHPLHSTNGTGNWECPDFFPVALKGTNGI													
F_DN2_1	LPGNKPVILYTGIVDANQTQVQNYAIPANLSDPYLREWIKPDNNPLIYADASINKTKFRDPTTAWMGKDGHWRIYMGSLRKHSRGLAIMYRSKDFHKWYKAKHPLHSTNGTGNWECPDFFPVALKGTNGI													
Consensus	LPGNKPVILYTGIVDANQTQVQNYA!PANLSDPYLREWIKPDNNPLIYADASINKTKFRDPTTAWMGKDGHWRIYMGSLRKHSRGLAIMYRSKDFHKWYKAKHPLHSTNGTGNWECPDFFPVALKGTNGi													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN1	DQYGEEHKYVLKNSHDLTRFEYITLGKYDTKKDRYIPDVGSIKGLRFDYGNFYASKSFYDPSKNRRVIWGSNESDIFPEDNAKGWAGIQLIPRKYHLDPGKQLIQMPVEELETLRQKVQLSNK													
F_DN2	DQYGEEYKYVLKNSHDLTRFEYITLGKYDTKKDRYIPDVGSIKGLRFDYGNFYASKTFYDPSKNRRVIWGSNESDIFPEDNAKGWAGIQLIPRKYHLDPGKQLVQMPVEELETLRQKVQLSNK													
F_DN2_1	DQYGEEYKYVLKNSHDLTRFEYITLGKYDTKKDRYIPDVGSIKGLRFDYGNFYASKTFYDPSKNRRVIWGSNESDIFPEDNAKGWAGIQLIPRKYHLDPGKQLVQMPVEELETLRQKVQLSNK													
Consensus	DQYGEEyKYVLKNSHDLTRFEYITLGKYDTKKDRY!PDVGSIKGLRFDYGNFYASKtFYDtSKNRRVIWGSNESDIFPEDNAKGWAGIQLIPRKYHLDPGKQL!QMPVEELETLRQKVQLSNK													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN1	KLNGEKVEYTGITPAQADVEYTFASFSLDKAESFDPSWTDMYAQDYVCGLKGADYQGGLGPFGLATLATENLEENTPVFFRYVFKAQQNYKVLLCSDAKRSTLKFNETHYKVSFAGFYVDVLDADKLSLRS													
F_DN2	KLNGEKVEYTGITPAQADVEYTFASFSLDKAESFDPSWTDMYAQDYVCGLKGADYQGGLGPFGLATLATENLEENTPVFFRYVFKAQQNYKVLLCSDAKRSTLKFNETHYKVSFAGFYVDVLDADKLSLRS													
F_DN2_1	KLNGEKVEYTGITPAQADVEYTFASFSLDKAESFDPSWTDMYAQDYVCGLKGADYQGGLGPFGLATLATENLEENTPVFFRYVFKAQQNYKVLLCSDAKRSTLKFNETHYKVSFAGFYVDVLDADKLSLRS													
Consensus	KLNGEKVEYTGITPAQADVEYTFASFSLDKAESFDPSWTDMYAQDYVCGLKGADYQGGLGPFGLATLATENLEENTPVFFRYVFKAQQNYKVLLCSDAKRSTLKFNETHYKVSFAGFYVDVLDADKLSLRS													
	521	530	540	550	560	570	581							
	-----+-----+-----+-----+-----+-----+-----													
F_DN1	LIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNGTEPITIETLDAWSMGKAKIQY													
F_DN2	LIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNGTEPITIETLDAWSMGKAKIQY													
F_DN2_1	LIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNGTEPITIETLDAWSMGKAKIQY													
Consensus	LIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNGTEPITIETLDAWSMGKAKIQY													

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_TN1	MDYSSNSRWALPVILVCFYVLLSNVYVFASHKVF IHLQSQNAVNVHTVHRTGYHFQPEKHWINDPNAPHYFNGVYHLFYQYNPNGSVMGNIYWAHSVSKDLINWINLEPAIYPSKPFDDQFGTWSGSATI													
F_TN1_1	MDYSSNSRWALPVILVCFYVLLSNVYVFASHKVF IHLQSQNAVNVHTVHRTGYHFQPEKHWINDPNAPHYFNGVYHLFYQYNPNGSVMGNIYWAHSVSKDLINWINLEPAIYPSKPFDDQFGTWSGSATI													
F_TN2	MDYSSNSRWALPVILVCFYVLLSNVYVFASHKVF IHLQSQNAVNVQTVHRTGYHFQPEKHWINDPNAPHYFNGVYHLFYQYNPNGSVMGNIYWAHSVSKDLINWINLEPAIYPSKPFDDQFGTWSGSATI													
Consensus	MDYSSNSRWALPVILVCFY !VLLSNVYVFASHKVF IHLQSQNAVNVhTVHRTGYHFQPEKHWINDPNAPHYFNGVYHLFYQYNPNGSVMGNIYWAHSVSKDLINWINLEPAIYPSKPFDDQFGTWSGSATI													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_TN1	LPGNKPVILYTGIVDANQTQVQNYAVPANI SDPYLREWIKPDNNPLIYADASINKTKFRDPTTAWMGKDGHWRIYMGSLRKHSRGLAIMYRSKDFHKWYKAKHPLHSTNGTGNWECDFFPVALKGTNGQ													
F_TN1_1	LPGNKPVILYTGIVDANQTQVQNYAVPANI SDPYLREWIKPDNNPLIYADASINKTKFRDPTTAWMGKDGHWRIYMGSLRKHSRGLAIMYRSKDFHKWYKAKHPLHSTNGTGNWECDFFPVALKGTNGQ													
F_TN2	LPGNKPVILYTGIVDANQTQVQNYAVPANLSDPHLREWIKPDNNPLIYADASINKTKFRDPTTAWMGKDGHWRIYMGSLRKHSRGLAIMYRSKDFHKWYKAKHPLHSTNGTGNWECDFFPVALKGTNGI													
Consensus	LPGNKPVILYTGIVDANQTQVQNYAVPANI SDPYLREWIKPDNNPLIYADASINKTKFRDPTTAWMGKDGHWRIYMGSLRKHSRGLAIMYRSKDFHKWYKAKHPLHSTNGTGNWECDFFPVALKGTNGq													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_TN1	DQYGEEHKYVLKNSMDL TRFEYITLGKYDTKKDRYIPDVGSIKGLRFDYGNFYASKSFYDPSKNRRVIWGSNESDIFPEDNAKGWAGIQLIPRKYHLDPGKQLIQMPVEELETLRQKVQLSNK													
F_TN1_1	DQYGEEHKYVLKNSMDL TRFEYITLGKYDTKKDRYIPDVGSIKGLRFDYGNFYASKSFYDPSKNRRVIWGSNESDIFPEDNAKGWAGIQLIPRKYHLDPGKQLIQMPVEELETLRQKVQLSNK													
F_TN2	DQYGEEYKNVYKNSMDL TRFEYITLGKYDTKKDRYIPDVGSIKGLRFDYGNFYASKTFYDPSKNRRVIWGSNESDIFPEDNAKGWAGIQLIPRKYHLDPGKQLIQMPVEELETLRQKVQLSNK													
Consensus	DQYGEEhKyVLKNSMDL TRFEYITLGKYDTKKDRY !PDVGSIKGLRFDYGNFYASKsFYDpSKNRRVIWGSNESDIFPEDNAKGWAGIQLIPRKYHLDPGKQLIQMPVEELETLRQKVQLSNK													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_TN1	KLNNGEKVEYTGITPAQADVEYTF SFASLDKAE SFDPSWTDMYAQDYCGLKGADYQGGLGPFGLATLATENLEENTPVFFRYVFKAQQNYKVLLCSDAKRSTLKFNETHYKVSFAGFYDVLADKKLSLRS													
F_TN1_1	KLNNGEKVEYTGITPAQADVEYTF SFASLDKAE SFDPSWTDMYAQDYCGLKGADYQGGLGPFGLATLATENLEENTPVFFRYVFKAQQNYKVLLCSDAKRSTLKFNETHYRVSFAGFYDVLADKKLSLRS													
F_TN2	KLNNGEKVEYTGITPAQADVEYTF SFASLDKAE LFDSSWTDMYAQDYCGLKGADYQGGLGPFGLATLATENLEENTPVFFRYVFKAQQNYKVLLCSDAKRSTLKFNETHYKVSFAGFYDVLADKKLSLRS													
Consensus	KLNNGEKVEYTGITPAQADVEYTF SFASLDKAE sFDpSWTDMYAQDYCGLKGADYQGGLGPFGLATLATENLEENTPVFFRYVFKAQQNYKVLLCSDAKRSTLKFNETHYkVSFAGFYDVLADKKLSLRS													
	521	530	540	550	560	570	581							
	-----+-----+-----+-----+-----+-----+-----													
F_TN1	LIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNGTEPITIETLDAWSMGKAKIQY													
F_TN1_1	LIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNGTEPITIETLDAWSMGKAKIQY													
F_TN2	LIDNSVIESFGAGGKTCITSRVYPTLAINDKAHLFAFNNGTEPITIETLDAWSMGKAKIQY													
Consensus	LIDNSVIESFGAGGKTCITSRVYPTLAIN#KAHLFAFNNGTEPITIETLDAWSMGKAKIQY													

1 10 20 30 40 50 60 70 80 90 100 110 120 130
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
F_SN1 ATGGATTATTCATCTAATTCCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGTCTTTTATAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCACAAAGTTTTATTTCATTGCAATCTCAAAATGCTG
F_SN1_1 ATGGATTATTCATCTAATTCCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGTCTTTTATAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCACAAAGTTTTATTTCATTGCAATCTCAAAATGCTG
F_SN1_2 ATGGATTATTCATCTAATTCCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGTCTTTTATAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCACAAAGTTTTATTTCATTGCAATCTCAAAATGCTG
Consensus ATGGATTATTCATCTAATTCCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGTCTTTTATAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCACAAAGTTTTATTTCATTGCAATCTCAAAATGCTG

131 140 150 160 170 180 190 200 210 220 230 240 250 260
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
F_SN1 TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGTATTTCAATGGAGTCTACCATCTATTCTACCAATACAACCAATGGGTC
F_SN1_1 TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGTATTTCAATGGAGTCTACCATCTATTCTACCAATACAACCAATGGGTC
F_SN1_2 TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGTATTTCAATGGAGTCTACCATCTATTCTACCAATACAACCAATGGGTC
Consensus TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGTATTTCAATGGAGTCTACCATCTATTCTACCAATACAACCAATGGGTC

261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
F_SN1 AGTATGGGGCAATATTGTTTGGGCTCATTTCAGTTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAARCCCTTTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT
F_SN1_1 AGTATGGGGCAATATTGTTTGGGCTCATTTCAGTTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAARCCCTTTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT
F_SN1_2 AGTATGGGGCAATATTGTTTGGGCTCATTTCAGTTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAARCCCTTTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT
Consensus AGTATGGGGCAATATTGTTTGGGCTCATTTCAGTTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAARCCCTTTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
F_SN1 CTACCTGGTAAACAGCCTGTTATCTTGACACTGGAAATAGTAGATGCCAACCAACTCAAGTTCAAACCTACGCGATCCAGCTAATCTGATCCATATCTCCGTGAATGGATCAAGCCTGATAACA
F_SN1_1 CTACCTGGTAAACAGCCTGTTATCTTGACACTGGAAATAGTAGATGCCAACCAACTCAAGTTCAAACCTACGCGATCCAGCTAATCTGATCCATATCTCCGTGAATGGATCAAGCCTGATAACA
F_SN1_2 CTACCTGGTAAACAGCCTGTTATCTTGACACTGGAAATAGTAGATGCCAACCAACTCAAGTTCAAACCTACGCGATCCAGCTAATCTGATCCATATCTCCGTGAATGGATCAAGCCTGATAACA
Consensus CTACCTGGTAAACAGCCTGTTATCTTGACACTGGAAATAGTAGATGCCAACCAACTCAAGTTCAAACCTACGCGATCCAGCTAATCTGATCCATATCTCCGTGAATGGATCAAGCCTGATAACA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
F_SN1 ATCCATTGATTGTAGCTGATGCTAGCATCAACAAGACCAATTTTCGTGATCCACACCCGATGGATGGGTAAGATGGACATTGGAGAATTGTGATGGGAAGTTTGAGGAACATAGTAGGGGTTAGC
F_SN1_1 ATCCATTGATTGTAGCTGATGCTAGCATCAACAAGACCAATTTTCGTGATCCACACCCGATGGATGGGTAAGATGGACATTGGAGAATTGTGATGGGAAGTTTGAGGAACATAGTAGGGGTTAGC
F_SN1_2 ATCCATTGATTGTAGCTGATGCTAGCATCAACAAGACCAATTTTCGTGATCCACACCCGATGGATGGGTAAGATGGACATTGGAGAATTGTGATGGGAAGTTTGAGGAACATAGTAGGGGTTAGC
Consensus ATCCATTGATTGTAGCTGATGCTAGCATCAACAAGACCAATTTTCGTGATCCACACCCGATGGATGGGTAAGATGGACATTGGAGAATTGTGATGGGAAGTTTGAGGAACATAGTAGGGGTTAGC

651 660 670 680 690 700 710 720 730 740 750 760 770 780
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
F_SN1 TATAATGTATAGAGCAAGATTTTCATGAATGGGTTAAGGCTAACACCCACTTCACTCAACTAATGGTACTGGAACTGGGAATGTCTGATTTTTCCCTGTTGCATTGAAGGAACTAATGGGATA
F_SN1_1 TATAATGTATAGAGCAAGATTTTCATGAATGGGTTAAGGCTAACACCCACTTCACTCAACTAATGGTACTGGAACTGGGAATGTCTGATTTTTCCCTGTTGCATTGAAGGAACTAATGGGATA
F_SN1_2 TATAATGTATAGAGCAAGATTTTCATGAATGGGTTAAGGCTAACACCCACTTCACTCAACTAATGGTACTGGAACTGGGAATGTCTGATTTTTCCCTGTTGCATTGAAGGAACTAATGGGATA
Consensus TATAATGTATAGAGCAAGATTTTCATGAATGGGTTAAGGCTAACACCCACTTCACTCAACTAATGGTACTGGAACTGGGAATGTCTGATTTTTCCCTGTTGCATTGAAGGAACTAATGGGATA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
F_SN1 GATCAATATGGTGAAGATATAAATATGTGCTTAAGAAATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAACTACGATACAAAAAGGATAGGTATGTTCCAGATGTTGGTCTATTGATA
F_SN1_1 GATCAATATGGTGAAGATATAAATATGTGCTTAAGAAATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAACTACGATACAAAAAGGATAGGTATGTTCCAGATGTTGGTCTATTGATA
F_SN1_2 GATCAATATGGTGAAGATATAAATATGTGCTTAAGAAATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAACTACGATACAAAAAGGATAGGTATGTTCCAGATGTTGGTCTATTGATA
Consensus GATCAATATGGTGAAGATATAAATATGTGCTTAAGAAATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAACTACGATACAAAAAGGATAGGTATGTTCCAGATGTTGGTCTATTGATA

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
F_SN1 GTTGGAAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC
F_SN1_1 GTTGGAAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC
F_SN1_2 GTTGGAAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC
Consensus GTTGGAAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
F_SN1 GAAAGGATGGGCTGGAAATTCATTTGATCCACGTAAGTATGGCTTGACCCAGTGGTAAACAATGGTTCAATGGCCTGTTGAGAAATTAGAACCCTAAGAACCAAAAGGTTCAATTGAGTAAACAA
F_SN1_1 GAAAGGATGGGCTGGAAATTCATTTGATCCACGTAAGTATGGCTTGACCCAGTGGTAAACAATGGTTCAATGGCCTGTTGAGAAATTAGAACCCTAAGAACCAAAAGGTTCAATTGAGTAAACAA
F_SN1_2 GAAAGGATGGGCTGGAAATTCATTTGATCCACGTAAGTATGGCTTGACCCAGTGGTAAACAATGGTTCAATGGCCTGTTGAGAAATTAGAACCCTAAGAACCAAAAGGTTCAATTGAGTAAACAA
Consensus GAAAGGATGGGCTGGAAATTCATTTGATCCACGTAAGTATGGCTTGACCCAGTGGTAAACAATGGTTCAATGGCCTGTTGAGAAATTAGAACCCTAAGAACCAAAAGGTTCAATTGAGTAAACAA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
F_SN1 AAATTGAACAATGGTGAAGAGGTTGAAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAAGTGAATTTCTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCAGTTGGACTGATATGTATG
F_SN1_1 AAATTGAACAATGGTGAAGAGGTTGAAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAAGTGAATTTCTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCAGTTGGACTGATATGTATG
F_SN1_2 AAATTGAACAATGGTGAAGAGGTTGAAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAAGTGAATTTCTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCAGTTGGACTGATATGTATG
Consensus AAATTGAACAATGGTGAAGAGGTTGAAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAAGTGAATTTCTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCAGTTGGACTGATATGTATG

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
F_SN1 CACAAGATGTTTGTGGACTCAAGGGTGCAGATGTACAAGGTGGGCTTGGCCATTTGGTCTTGTACATTGGCTACTGAAACTTAGAAGAAACACACCTGTTTTCTCCAGTTTTCAAGCACAACA
F_SN1_1 CACAAGATGTTTGTGGACTCAAGGGTGCAGATGTACAAGGTGGGCTTGGCCATTTGGTCTTGTACATTGGCTACTGAAACTTAGAAGAAACACACCTGTTTTCTCCAGTTTTCAAGCACAACA
F_SN1_2 CACAAGATGTTTGTGGACTCAAGGGTGCAGATGTACAAGGTGGGCTTGGCCATTTGGTCTTGTACATTGGCTACTGAAACTTAGAAGAAACACACCTGTTTTCTCCAGTTTTCAAGCACAACA
Consensus CACAAGATGTTTGTGGACTCAAGGGTGCAGATGTACAAGGTGGGCTTGGCCATTTGGTCTTGTACATTGGCTACTGAAACTTAGAAGAAACACACCTGTTTTCTCCAGTTTTCAAGCACAACA

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
F_SN1 AAATTACAAGGTTCTCTTGTGCTCTGACGCTAAAAGGTCACCTCTTAAGTTCATGAACAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCTGACAGAAATTGTCCTCAGAGC
F_SN1_1 AAATTACAAGGTTCTCTTGTGCTCTGACGCTAAAAGGTCACCTCTTAAGTTCATGAACAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCTGACAGAAATTGTCCTCAGAGC
F_SN1_2 AAATTACAAGGTTCTCTTGTGCTCTGACGCTAAAAGGTCACCTCTTAAGTTCATGAACAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCTGACAGAAATTGTCCTCAGAGC
Consensus AAATTACAAGGTTCTCTTGTGCTCTGACGCTAAAAGGTCACCTCTTAAGTTCATGAACAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCTGACAGAAATTGTCCTCAGAGC

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
F_SN1 TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTAAATGAGAAGGCACATTTATTTGCTTCAACACCGGAAGTGAAGCCAA
F_SN1_1 TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTAAATGAGAAGGCACATTTATTTGCTTCAACACCGGAAGTGAAGCCAA
F_SN1_2 TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTAAATGAGAAGGCACATTTATTTGCTTCAACACCGGAAGTGAAGCCAA
Consensus TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTAAATGAGAAGGCACATTTATTTGCTTCAACACCGGAAGTGAAGCCAA

1691 1700 1710 1720 1730 1740 1746
|-----|-----|-----|-----|-----|-----|
F_SN1 TCACAATTGAGACTTTGGATGCTAGGATGATGGGCAAGCTAAGATACAATATTGA
F_SN1_1 TCACAATTGAGACTTTGGATGCTAGGATGATGGGCAAGCTAAGATACAATATTGA
F_SN1_2 TCACAATTGAGACTTTGGATGCTAGGATGATGGGCAAGCTAAGATACAATATTGA
Consensus TCACAATTGAGACTTTGGATGCTAGGATGATGGGCAAGCTAAGATACAATATTGA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
F_SN2 ATGGATTATTCATCTAATTCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCACTTGCATCTCAAATGCTG
F_SN2_1 ATGGATTATTCATCTAATTCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCACTTGCATCTCAAATGCTG
F_SN2_3 ATGGATTATTCATCTAATTCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCACTTGCATCTCAAATGCTG
F_SN2_2 ATGGATTATTCATCTAATTCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCACTTGCATCTCAAATGCTG
Consensus ATGGATTATTCATCTAATTCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCACTTGCATCTCAAATGCTG

131 140 150 160 170 180 190 200 210 220 230 240 250 260
F_SN2 TAATGTTCCACTGTTTCATAGAACTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAAATTTACCATCTATTCTACCAATACACCCAAACGGGTC
F_SN2_1 TAATGTTCCACTGTTTCATAGAACTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAAATTTACCATCTATTCTACCAATACACCCAAACGGGTC
F_SN2_3 TAATGTTCCACTGTTTCATAGAACTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAAATTTACCATCTATTCTACCAATACACCCAAACGGGTC
F_SN2_2 TAATGTTCCACTGTTTCATAGAACTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAAATTTACCATCTATTCTACCAATACACCCAAACGGGTC
Consensus TAATGTTCCACTGTTTCATAGAACTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAAATTTACCATCTATTCTACCAATACACCCAAACGGGTC

261 270 280 290 300 310 320 330 340 350 360 370 380 390
F_SN2 AGTATGGGGCAACATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCTAACCCATTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT
F_SN2_1 AGTATGGGGCAACATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCTAACCCATTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT
F_SN2_3 AGTATGGGGCAACATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCTAACCCATTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT
F_SN2_2 AGTATGGGGCAACATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCTAACCCATTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT
Consensus AGTATGGGGCAACATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCTAACCCATTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT

391 400 410 420 430 440 450 460 470 480 490 500 510 520
F_SN2 CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAATTTACGCGATTCCAGCTAACGATCTGATCCTTATCTCCGTGAATGGATCAGCCTGATAACA
F_SN2_1 CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAATTTACGCGATTCCAGCTAACGATCTGATCCTTATCTCCGTGAATGGATCAGCCTGATAACA
F_SN2_3 CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAATTTACGCGATTCCAGCTAACGATCTGATCCTTATCTCCGTGAATGGATCAGCCTGATAACA
F_SN2_2 CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAATTTACGCGATTCCAGCTAACGATCTGATCCTTATCTCCGTGAATGGATCAGCCTGATAACA
Consensus CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAATTTACGCGATTCCAGCTAACGATCTGATCCTTATCTCCGTGAATGGATCAGCCTGATAACA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
F_SN2 ATCCATTGATTGTAGCTGATGATAGTATCAACAAACCAAAATTTCTGATCCAACTACTGCTGGATGGGTAAGATGGACATTGGAGAAATTTGATGGGAAGTTTGGAAACATAGTAGGGGTTTACG
F_SN2_1 ATCCATTGATTGTAGCTGATGATAGTATCAACAAACCAAAATTTCTGATCCAACTACTGCTGGATGGGTAAGATGGACATTGGAGAAATTTGATGGGAAGTTTGGAAACATAGTAGGGGTTTACG
F_SN2_3 ATCCATTGATTGTAGCTGATGATAGTATCAACAAACCAAAATTTCTGATCCAACTACTGCTGGATGGGTAAGATGGACATTGGAGAAATTTGATGGGAAGTTTGGAAACATAGTAGGGGTTTACG
F_SN2_2 ATCCATTGATTGTAGCTGATGATAGTATCAACAAACCAAAATTTCTGATCCAACTACTGCTGGATGGGTAAGATGGACATTGGAGAAATTTGATGGGAAGTTTGGAAACATAGTAGGGGTTTACG
Consensus ATCCATTGATTGTAGCTGATGATAGTATCAACAAACCAAAATTTCTGATCCAACTACTGCTGGATGGGTAAGATGGACATTGGAGAAATTTGATGGGAAGTTTGGAAACATAGTAGGGGTTTACG

651 660 670 680 690 700 710 720 730 740 750 760 770 780
F_SN2 AATAATGTATGAAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAATTTGGAAATGTCCTGATTTTTTCTGTTGCATTGAARAGGAATTAATGGGATA
F_SN2_1 AATAATGTATGAAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAATTTGGAAATGTCCTGATTTTTTCTGTTGCATTGAARAGGAATTAATGGGATA
F_SN2_3 AATAATGTATGAAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAATTTGGAAATGTCCTGATTTTTTCTGTTGCATTGAARAGGAATTAATGGGATA
F_SN2_2 AATAATGTATGAAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAATTTGGAAATGTCCTGATTTTTTCTGTTGCATTGAARAGGAATTAATGGGATA
Consensus AATAATGTATGAAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAATTTGGAAATGTCCTGATTTTTTCTGTTGCATTGAARAGGAATTAATGGGATA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
F_SN2 GATCAATATGGTGAAGAAATATAAATATGTGCTTAAAGAAATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTATGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTCTATTGATA
F_SN2_1 GATCAATATGGTGAAGAAATATAAATATGTGCTTAAAGAAATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTATGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTCTATTGATA
F_SN2_3 GATCAATATGGTGAAGAAATATAAATATGTGCTTAAAGAAATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTATGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTCTATTGATA
F_SN2_2 GATCAATATGGTGAAGAAATATAAATATGTGCTTAAAGAAATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTATGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTCTATTGATA
Consensus GATCAATATGGTGAAGAAATATAAATATGTGCTTAAAGAAATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTATGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTCTATTGATA

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
F_SN2 GTTGGAAAGGGATTGAGATTTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAAAGATGATAATGC
F_SN2_1 GTTGGAAAGGGATTGAGATTTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAAAGATGATAATGC
F_SN2_3 GTTGGAAAGGGATTGAGATTTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAAAGATGATAATGC
F_SN2_2 GTTGGAAAGGGATTGAGATTTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAAAGATGATAATGC
Consensus GTTGGAAAGGGATTGAGATTTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAAAGATGATAATGC

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
F_SN2 GAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGACCCAGTGGTAACCAATTGGTTCAGTGGCTGTTGAAAGATTAAGAACCTAAGAACCCAAAGGTTCAATTGAGTAACAAA
F_SN2_1 GAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGACCCAGTGGTAACCAATTGGTTCAGTGGCTGTTGAAAGATTAAGAACCTAAGAACCCAAAGGTTCAATTGAGTAACAAA
F_SN2_3 GAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGACCCAGTGGTAACCAATTGGTTCAGTGGCTGTTGAAAGATTAAGAACCTAAGAACCCAAAGGTTCAATTGAGTAACAAA
F_SN2_2 GAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGACCCAGTGGTAACCAATTGGTTCAGTGGCTGTTGAAAGATTAAGAACCTAAGAACCCAAAGGTTCAATTGAGTAACAAA
Consensus GAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGACCCAGTGGTAACCAATTGGTTCAGTGGCTGTTGAAAGATTAAGAACCTAAGAACCCAAAGGTTCAATTGAGTAACAAA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
F_SN2 AAATTGAACAAATGGTGAARAGGTTGAGTTTACAGGAATCACACCTGCACAGGCAGATGTTGAGTGCATTCTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG
F_SN2_1 AAATTGAACAAATGGTGAARAGGTTGAGTTTACAGGAATCACACCTGCACAGGCAGATGTTGAGTGCATTCTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG
F_SN2_3 AAATTGAACAAATGGTGAARAGGTTGAGTTTACAGGAATCACACCTGCACAGGCAGATGTTGAGTGCATTCTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG
F_SN2_2 AAATTGAACAAATGGTGAARAGGTTGAGTTTACAGGAATCACACCTGCACAGGCAGATGTTGAGTGCATTCTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG
Consensus AAATTGAACAAATGGTGAARAGGTTGAGTTTACAGGAATCACACCTGCACAGGCAGATGTTGAGTGCATTCTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
F_SN2 CACAGATGTTTGTGGACTCAAGGGTGCAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACA
F_SN2_1 CACAGATGTTTGTGGACTCAAGGGTGCAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACA
F_SN2_3 CACAGATGTTTGTGGACTCAAGGGTGCAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACA
F_SN2_2 CACAGATGTTTGTGGACTCAAGGGTGCAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACA
Consensus CACAGATGTTTGTGGACTCAAGGGTGCAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACA

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
F_SN2 AAATTACAGGTTCTCTTGTGTTCTGACGCTAAAGGTCACCTCTTAAAGTTCAATGAACAAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTGGCAGACAGAATTTGTCCTCAGAGC
F_SN2_1 AAATTACAGGTTCTCTTGTGTTCTGACGCTAAAGGTCACCTCTTAAAGTTCAATGAACAAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTGGCAGACAGAATTTGTCCTCAGAGC
F_SN2_3 AAATTACAGGTTCTCTTGTGTTCTGACGCTAAAGGTCACCTCTTAAAGTTCAATGAACAAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTGGCAGACAGAATTTGTCCTCAGAGC
F_SN2_2 AAATTACAGGTTCTCTTGTGTTCTGACGCTAAAGGTCACCTCTTAAAGTTCAATGAACAAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTGGCAGACAGAATTTGTCCTCAGAGC
Consensus AAATTACAGGTTCTCTTGTGTTCTGACGCTAAAGGTCACCTCTTAAAGTTCAATGAACAAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTGGCAGACAGAATTTGTCCTCAGAGC

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
F_SN2 TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCACATTGGCGATTAAATGAGAGGACATTTATTTGCGTTCAACACGGAACTGAGCCAA
F_SN2_1 TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCACATTGGCGATTAAATGAGAGGACATTTATTTGCGTTCAACACGGAACTGAGCCAA
F_SN2_3 TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCACATTGGCGATTAAATGAGAGGACATTTATTTGCGTTCAACACGGAACTGAGCCAA
F_SN2_2 TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCACATTGGCGATTAAATGAGAGGACATTTATTTGCGTTCAACACGGAACTGAGCCAA
Consensus TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCACATTGGCGATTAAATGAGAGGACATTTATTTGCGTTCAACACGGAACTGAGCCAA

1691 1700 1710 1720 1730 1740 1746
F_SN2 TCACAAATGGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA
F_SN2_1 TCACAAATGGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA
F_SN2_3 TCACAAATGGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA
F_SN2_2 TCACAAATGGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA
Consensus TCACAAATGGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
F_SN3 ATGGATTATTCATCTAATTCTCGTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCACTTGCARTCTCAAATGCTG
F_SN3_1 ATGGATTATTCATCTAATTCTCGTAGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCACTTGCARTCTCAAATGCTG
F_SN3_2 ATGGATTATTCATCTAATTCTCGTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCACTTGCARTCTCAAATGCTG
F_SN3_3 ATGGATTATTCATCTAATTCTCGTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCACTTGCARTCTCAAATGCTG
Consensus ATGGATTATTCATCTAATTCTCGTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCACTTGCARTCTCAAATGCTG

131 140 150 160 170 180 190 200 210 220 230 240 250 260
F_SN3 TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTTTACCATCTATTCTACCAATACACCCAAACGGGTC
F_SN3_1 TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTTTACCATCTATTCTACCAATACACCCAAACGGGTC
F_SN3_2 TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTTTACCATCTATTCTACCAATACACCCAAACGGGTC
F_SN3_3 TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTTTACCATCTATTCTACCAATACACCCAAACGGGTC
Consensus TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTTTACCATCTATTCTACCAATACACCCAAACGGGTC

261 270 280 290 300 310 320 330 340 350 360 370 380 390
F_SN3 AGTATGGGGCAATATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCAGCAATTTACCCATCTAAACCATTGATCAATTCGGTACGTGGTCTGGGCTGCAACTATT
F_SN3_1 AGTATGGGGCAATATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCAGCAATTTACCCATCTAAACCATTGATCAATTCGGTACGTGGTCTGGGCTGCAACTATT
F_SN3_2 AGTATGGGGCAATATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCAGCAATTTACCCATCTAAACCATTGATCAATTCGGTACGTGGTCTGGGCTGCAACTATT
F_SN3_3 AGTATGGGGCAATATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCAGCAATTTACCCATCTAAACCATTGATCAATTCGGTACGTGGTCTGGGCTGCAACTATT
Consensus AGTATGGGGCAATATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCAGCAATTTACCCATCTAAACCATTGATCAATTCGGTACGTGGTCTGGGCTGCAACTATT

391 400 410 420 430 440 450 460 470 480 490 500 510 520
F_SN3 CTACCTGGTAAACAGCCTGTTATCTTGTAACCTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAACACGCGATTCCAGCTAACGATCTGATCCTTATCTCCGTGAATGGATCAGCCTGATAATA
F_SN3_1 CTACCTGGTAAACAGCCTGTTATCTTGTAACCTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAACACGCGATTCCAGCTAACGATCTGATCCTTATCTCCGTGAATGGATCAGCCTGATAATA
F_SN3_2 CTACCTGGTAAACAGCCTGTTATCTTGTAACCTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAACACGCGATTCCAGCTAACGATCTGATCCTTATCTCCGTGAATGGATCAGCCTGATAATA
F_SN3_3 CTACCTGGTAAACAGCCTGTTATCTTGTAACCTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAACACGCGATTCCAGCTAACGATCTGATCCTTATCTCCGTGAATGGATCAGCCTGATAATA
Consensus CTACCTGGTAAACAGCCTGTTATCTTGTAACCTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAACACGCGATTCCAGCTAACGATCTGATCCTTATCTCCGTGAATGGATCAGCCTGATAATA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
F_SN3 ATCCATTGATTGTAGCTGATGATAGTATCAACAGACCAAAATTCGTGATCCAACTGCATGGATGGGTAAGATGGACATTGGAGAATTGTGATGGGAAGTTGAGAAAACATAGTAGGGGTTAGC
F_SN3_1 ATCCATTGATTGTAGCTGATGATAGTATCAACAGACCAAAATTCGTGATCCAACTGCATGGATGGGTAAGATGGACATTGGAGAATTGTGATGGGAAGTTGAGAAAACATAGTAGGGGTTAGC
F_SN3_2 ATCCATTGATTGTAGCTGATGATAGTATCAACAGACCAAAATTCGTGATCCAACTGCATGGATGGGTAAGATGGACATTGGAGAATTGTGATGGGAAGTTGAGAAAACATAGTAGGGGTTAGC
F_SN3_3 ATCCATTGATTGTAGCTGATGATAGTATCAACAGACCAAAATTCGTGATCCAACTGCATGGATGGGTAAGATGGACATTGGAGAATTGTGATGGGAAGTTGAGAAAACATAGTAGGGGTTAGC
Consensus ATCCATTGATTGTAGCTGATGATAGTATCAACAGACCAAAATTCGTGATCCAACTGCATGGATGGGTAAGATGGACATTGGAGAATTGTGATGGGAAGTTGAGAAAACATAGTAGGGGTTAGC

651 660 670 680 690 700 710 720 730 740 750 760 770 780
F_SN3 AATAATGTATGAAAGCAAGATTTATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAATTTGGGATGTCTGATTTTTTCTGTTGCATTGAARAGGAACATAGGGGATA
F_SN3_1 AATAATGTATGAAAGCAAGATTTATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAATTTGGGATGTCTGATTTTTTCTGTTGCATTGAARAGGAACATAGGGGATA
F_SN3_2 AATAATGTATGAAAGCAAGATTTATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAATTTGGGATGTCTGATTTTTTCTGTTGCATTGAARAGGAACATAGGGGATA
F_SN3_3 AATAATGTATGAAAGCAAGATTTATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAATTTGGGATGTCTGATTTTTTCTGTTGCATTGAARAGGAACATAGGGGATA
Consensus AATAATGTATGAAAGCAAGATTTATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAATTTGGGATGTCTGATTTTTTCTGTTGCATTGAARAGGAACATAGGGGATA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
F_SN3 GATCAATATGGTGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTCCATTGATA
F_SN3_1 GATCAATATGGTGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTCCATTGATA
F_SN3_2 GATCAATATGGTGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTCCATTGATA
F_SN3_3 GATCAATATGGTGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTCCATTGATA
Consensus GATCAATATGGTGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTCCATTGATA

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
F_SN3 GTTGGAAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATCTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAAGATGATAATGC
F_SN3_1 GTTGGAAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATCTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAAGATGATAATGC
F_SN3_2 GTTGGAAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATCTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAAGATGATAATGC
F_SN3_3 GTTGGAAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATCTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAAGATGATAATGC
Consensus GTTGGAAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATCTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAAGATGATAATGC

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
F_SN3 GAARAGGATGGGCTGGAATTCATTGATCCACGTAAGTATGGCTTGACCCAGTGGTAACCAATTGATTCATGGCCTGTTGAGAATTAGAACCCTAAGAACCCAAAGGTTCAATTGAGTAAACAA
F_SN3_1 GAARAGGATGGGCTGGAATTCATTGATCCACGTAAGTATGGCTTGACCCAGTGGTAACCAATTGATTCATGGCCTGTTGAGAATTAGAACCCTAAGAACCCAAAGGTTCAATTGAGTAAACAA
F_SN3_2 GAARAGGATGGGCTGGAATTCATTGATCCACGTAAGTATGGCTTGACCCAGTGGTAACCAATTGATTCATGGCCTGTTGAGAATTAGAACCCTAAGAACCCAAAGGTTCAATTGAGTAAACAA
F_SN3_3 GAARAGGATGGGCTGGAATTCATTGATCCACGTAAGTATGGCTTGACCCAGTGGTAACCAATTGATTCATGGCCTGTTGAGAATTAGAACCCTAAGAACCCAAAGGTTCAATTGAGTAAACAA
Consensus GAARAGGATGGGCTGGAATTCATTGATCCACGTAAGTATGGCTTGACCCAGTGGTAACCAATTGATTCATGGCCTGTTGAGAATTAGAACCCTAAGAACCCAAAGGTTCAATTGAGTAAACAA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
F_SN3 AAATTGAACAAATGGTGAARAGGTTGAAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG
F_SN3_1 AAATTGAACAAATGGTGAARAGGTTGAAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG
F_SN3_2 AAATTGAACAAATGGTGAARAGGTTGAAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG
F_SN3_3 AAATTGAACAAATGGTGAARAGGTTGAAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG
Consensus AAATTGAACAAATGGTGAARAGGTTGAAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
F_SN3 CACAGATGTTTGTGGACTCAAGGGTGCAGATGTACAGGTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACAC
F_SN3_1 CACAGATGTTTGTGGACTCAAGGGTGCAGATGTACAGGTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACAC
F_SN3_2 CACAGATGTTTGTGGACTCAAGGGTGCAGATGTACAGGTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACAC
F_SN3_3 CACAGATGTTTGTGGACTCAAGGGTGCAGATGTACAGGTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACAC
Consensus CACAGATGTTTGTGGACTCAAGGGTGCAGATGTACAGGTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACAC

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
F_SN3 AAATTACAGGTTCTCTTGTGTTCTGACGCTAAAGGTCACACTCTTAGTTCAATGAACAAATGTACAAGTTTCATTTGCTGGATTGTGGATGTTGATTGGCAGACAGAATTGTCACCTCAGAGC
F_SN3_1 AAATTACAGGTTCTCTTGTGTTCTGACGCTAAAGGTCACACTCTTAGTTCAATGAACAAATGTACAAGTTTCATTTGCTGGATTGTGGATGTTGATTGGCAGACAGAATTGTCACCTCAGAGC
F_SN3_2 AAATTACAGGTTCTCTTGTGTTCTGACGCTAAAGGTCACACTCTTAGTTCAATGAACAAATGTACAAGTTTCATTTGCTGGATTGTGGATGTTGATTGGCAGACAGAATTGTCACCTCAGAGC
F_SN3_3 AAATTACAGGTTCTCTTGTGTTCTGACGCTAAAGGTCACACTCTTAGTTCAATGAACAAATGTACAAGTTTCATTTGCTGGATTGTGGATGTTGATTGGCAGACAGAATTGTCACCTCAGAGC
Consensus AAATTACAGGTTCTCTTGTGTTCTGACGCTAAAGGTCACACTCTTAGTTCAATGAACAAATGTACAAGTTTCATTTGCTGGATTGTGGATGTTGATTGGCAGACAGAATTGTCACCTCAGAGC

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
F_SN3 TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTATGAGAGGCACATTATTTGCGTTCAACACGGAACTGAGCCAA
F_SN3_1 TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTATGAGAGGCACATTATTTGCGTTCAACACGGAACTGAGCCAA
F_SN3_2 TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTATGAGAGGCACATTATTTGCGTTCAACACGGAACTGAGCCAA
F_SN3_3 TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTATGAGAGGCACATTATTTGCGTTCAACACGGAACTGAGCCAA
Consensus TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTATGAGAGGCACATTATTTGCGTTCAACACGGAACTGAGCCAA

1691 1700 1710 1720 1730 1740 1746
F_SN3 TCACAAATGGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA
F_SN3_1 TCACAAATGGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA
F_SN3_2 TCACAAATGGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA
F_SN3_3 TCACAAATGGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA
Consensus TCACAAATGGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
F_SN4 ATGGATTATTCATCTAATTCCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTGTAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCATAAAGTTTTATTCACTTGCARTCTCAAATGCTG
F_SN4_2 ATGGATTATTCATCTAATTCCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTGTAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCATAAAGTTTTATTCACTTGCARTCTCAAATGCTG
F_SN4_3 ATGGATTATTCATCTAATTCCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTGTAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCATAAAGTTTTATTCACTTGCARTCTCAAATGCTG
F_SN4_1 ATGGATTATTCATCTAATTCCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTGTAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCATAAAGTTTTATTCACTTGCARTCTCAAATGCTG
Consensus ATGGATTATTCATCTAATTCCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTGTAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCATAAAGTTTTATTCACTTGCARTCTCAAATGCTG

131 140 150 160 170 180 190 200 210 220 230 240 250 260
F_SN4 TAATGTTCCACTGTTTCATAGAACTGGTTATCATTTTCAGCCAGAAAACATGGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTATCATCTATTCTACCAATCAACCCAAACGGGTC
F_SN4_2 TAATGTTCCACTGTTTCATAGAACTGGTTATCATTTTCAGCCAGAAAACATGGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTATCATCTATTCTACCAATCAACCCAAACGGGTC
F_SN4_3 TAATGTTCCACTGTTTCATAGAACTGGTTATCATTTTCAGCCAGAAAACATGGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTATCATCTATTCTACCAATCAACCCAAACGGGTC
F_SN4_1 TAATGTTCCACTGTTTCATAGAACTGGTTATCATTTTCAGCCAGAAAACATGGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTATCATCTATTCTACCAATCAACCCAAACGGGTC
Consensus TAATGTTCCACTGTTTCATAGAACTGGTTATCATTTTCAGCCAGAAAACATGGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTATCATCTATTCTACCAATCAACCCAAACGGGTC

261 270 280 290 300 310 320 330 340 350 360 370 380 390
F_SN4 AGTATGGGGCAACATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCAGCAATTTACCCATCTAACCATTGATCAATTTGGTACATGGTCTGGATCAGCAACTATT
F_SN4_2 AGTATGGGGCAACATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCAGCAATTTACCCATCTAACCATTGATCAATTTGGTACATGGTCTGGATCAGCAACTATT
F_SN4_3 AGTATGGGGCAACATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCAGCAATTTACCCATCTAACCATTGATCAATTTGGTACATGGTCTGGATCAGCAACTATT
F_SN4_1 AGTATGGGGCAACATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCAGCAATTTACCCATCTAACCATTGATCAATTTGGTACATGGTCTGGATCAGCAACTATT
Consensus AGTATGGGGCAACATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCAGCAATTTACCCATCTAACCATTGATCAATTTGGTACATGGTCTGGATCAGCAACTATT

391 400 410 420 430 440 450 460 470 480 490 500 510 520
F_SN4 CTACCTGGTAAACAACTGTTATCTTGTACACTGGAAATAGTGGATGCTAACCAACTCAAGTTCAAACACGCGGTTCCAGTAAACATATCTGATCCTTATCTACGTGAATGGATCAGGCTGATAACA
F_SN4_2 CTACCTGGTAAACAACTGTTATCTTGTACACTGGAAATAGTGGATGCTAACCAACTCAAGTTCAAACACGCGGTTCCAGTAAACATATCTGATCCTTATCTACGTGAATGGATCAGGCTGATAACA
F_SN4_3 CTACCTGGTAAACAACTGTTATCTTGTACACTGGAAATAGTGGATGCTAACCAACTCAAGTTCAAACACGCGGTTCCAGTAAACATATCTGATCCTTATCTACGTGAATGGATCAGGCTGATAACA
F_SN4_1 CTACCTGGTAAACAACTGTTATCTTGTACACTGGAAATAGTGGATGCTAACCAACTCAAGTTCAAACACGCGGTTCCAGTAAACATATCTGATCCTTATCTACGTGAATGGATCAGGCTGATAACA
Consensus CTACCTGGTAAACAACTGTTATCTTGTACACTGGAAATAGTGGATGCTAACCAACTCAAGTTCAAACACGCGGTTCCAGTAAACATATCTGATCCTTATCTACGTGAATGGATCAGGCTGATAACA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
F_SN4 ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAATTTCTGATCCACACCCGCTTGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGAGAAACATAGTAGGGGTTAGC
F_SN4_2 ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAATTTCTGATCCACACCCGCTTGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGAGAAACATAGTAGGGGTTAGC
F_SN4_3 ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAATTTCTGATCCACACCCGCTTGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGAGAAACATAGTAGGGGTTAGC
F_SN4_1 ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAATTTCTGATCCACACCCGCTTGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGAGAAACATAGTAGGGGTTAGC
Consensus ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAATTTCTGATCCACACCCGCTTGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGAGAAACATAGTAGGGGTTAGC

651 660 670 680 690 700 710 720 730 740 750 760 770 780
F_SN4 TATAATGTATGAGCAAGACTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAACGGTACTGGAATTTGGGATGTCCTGATTTTTCCCGTTGCATTGAARAGGAACATAGGGCAA
F_SN4_2 TATAATGTATGAGCAAGACTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAACGGTACTGGAATTTGGGATGTCCTGATTTTTCCCGTTGCATTGAARAGGAACATAGGGCAA
F_SN4_3 TATAATGTATGAGCAAGACTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAACGGTACTGGAATTTGGGATGTCCTGATTTTTCCCGTTGCATTGAARAGGAACATAGGGCAA
F_SN4_1 TATAATGTATGAGCAAGACTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAACGGTACTGGAATTTGGGATGTCCTGATTTTTCCCGTTGCATTGAARAGGAACATAGGGCAA
Consensus TATAATGTATGAGCAAGACTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAACGGTACTGGAATTTGGGATGTCCTGATTTTTCCCGTTGCATTGAARAGGAACATAGGGCAA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
F_SN4 GATCAATATGGTGAAGACACAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTACATACCAGATGTTGGTCTATTGATA
F_SN4_2 GATCAATATGGTGAAGACACAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTACATACCAGATGTTGGTCTATTGATA
F_SN4_3 GATCAATATGGTGAAGACACAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTACATACCAGATGTTGGTCTATTGATA
F_SN4_1 GATCAATATGGTGAAGACACAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTACATACCAGATGTTGGTCTATTGATA
Consensus GATCAATATGGTGAAGACACAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTACATACCAGATGTTGGTCTATTGATA

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
F_SN4 GTTGGARAGGGATTGAGATTGACTATGGTAAATTTCTATGCATCAAGTCTTTCTATGATCCTAGCAAAATCGAAGGGTATTTGGGGTTGGTCAATGAATCAGATATATCCCTGAGGATGATAACGC
F_SN4_2 GTTGGARAGGGATTGAGATTGACTATGGTAAATTTCTATGCATCAAGTCTTTCTATGATCCTAGCAAAATCGAAGGGTATTTGGGGTTGGTCAATGAATCAGATATATCCCTGAGGATGATAACGC
F_SN4_3 GTTGGARAGGGATTGAGATTGACTATGGTAAATTTCTATGCATCAAGTCTTTCTATGATCCTAGCAAAATCGAAGGGTATTTGGGGTTGGTCAATGAATCAGATATATCCCTGAGGATGATAACGC
F_SN4_1 GTTGGARAGGGATTGAGATTGACTATGGTAAATTTCTATGCATCAAGTCTTTCTATGATCCTAGCAAAATCGAAGGGTATTTGGGGTTGGTCAATGAATCAGATATATCCCTGAGGATGATAACGC
Consensus GTTGGARAGGGATTGAGATTGACTATGGTAAATTTCTATGCATCAAGTCTTTCTATGATCCTAGCAAAATCGAAGGGTATTTGGGGTTGGTCAATGAATCAGATATATCCCTGAGGATGATAACGC

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
F_SN4 TAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGATCCAGTGGTAACCAATTGATTCATGGCCTGTTGAGAATTAGAAACCTAAGAACCRAAGGTTCAATTGAGCAACAG
F_SN4_2 TAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGATCCAGTGGTAACCAATTGATTCATGGCCTGTTGAGAATTAGAAACCTAAGAACCRAAGGTTCAATTGAGCAACAG
F_SN4_3 TAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGATCCAGTGGTAACCAATTGATTCATGGCCTGTTGAGAATTAGAAACCTAAGAACCRAAGGTTCAATTGAGCAACAG
F_SN4_1 TAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGATCCAGTGGTAACCAATTGATTCATGGCCTGTTGAGAATTAGAAACCTAAGAACCRAAGGTTCAATTGAGCAACAG
Consensus TAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGATCCAGTGGTAACCAATTGATTCATGGCCTGTTGAGAATTAGAAACCTAAGAACCRAAGGTTCAATTGAGCAACAG

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
F_SN4 AAGTTGAACAAATGGTGAARAGGTTGAAGTTACAGGGATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTGCAAGTTGGATAGGCAGAGTCATTTGATCCTAGTTGGACTGATATGATG
F_SN4_2 AAGTTGAACAAATGGTGAARAGGTTGAAGTTACAGGGATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTGCAAGTTGGATAGGCAGAGTCATTTGATCCTAGTTGGACTGATATGATG
F_SN4_3 AAGTTGAACAAATGGTGAARAGGTTGAAGTTACAGGGATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTGCAAGTTGGATAGGCAGAGTCATTTGATCCTAGTTGGACTGATATGATG
F_SN4_1 AAGTTGAACAAATGGTGAARAGGTTGAAGTTACAGGGATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTGCAAGTTGGATAGGCAGAGTCATTTGATCCTAGTTGGACTGATATGATG
Consensus AAGTTGAACAAATGGTGAARAGGTTGAAGTTACAGGGATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTGCAAGTTGGATAGGCAGAGTCATTTGATCCTAGTTGGACTGATATGATG

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
F_SN4 CACAGATGTTTGTGGACTCAAGGTTGCAGATGTTCAAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACA
F_SN4_2 CACAGATGTTTGTGGACTCAAGGTTGCAGATGTTCAAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACA
F_SN4_3 CACAGATGTTTGTGGACTCAAGGTTGCAGATGTTCAAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACA
F_SN4_1 CACAGATGTTTGTGGACTCAAGGTTGCAGATGTTCAAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACA
Consensus CACAGATGTTTGTGGACTCAAGGTTGCAGATGTTCAAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTAGAAGAAACACACCTGTTTCTCCGAGTTTTCARAGCACACA

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
F_SN4 AAATTACAGGTTCTCTTGTGCTCTGATGCTAAAGGTCACACTTAAAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCAGACAGAATTGTCACCTCAGAGC
F_SN4_2 AAATTACAGGTTCTCTTGTGCTCTGATGCTAAAGGTCACACTTAAAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCAGACAGAATTGTCACCTCAGAGC
F_SN4_3 AAATTACAGGTTCTCTTGTGCTCTGATGCTAAAGGTCACACTTAAAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCAGACAGAATTGTCACCTCAGAGC
F_SN4_1 AAATTACAGGTTCTCTTGTGCTCTGATGCTAAAGGTCACACTTAAAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCAGACAGAATTGTCACCTCAGAGC
Consensus AAATTACAGGTTCTCTTGTGCTCTGATGCTAAAGGTCACACTTAAAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCAGACAGAATTGTCACCTCAGAGC

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
F_SN4 TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTAAATGACAGGGCACATTTATTTGCGTTCAACACGGAACTGAGCCAA
F_SN4_2 TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTAAATGACAGGGCACATTTATTTGCGTTCAACACGGAACTGAGCCAA
F_SN4_3 TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTAAATGACAGGGCACATTTATTTGCGTTCAACACGGAACTGAGCCAA
F_SN4_1 TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTAAATGACAGGGCACATTTATTTGCGTTCAACACGGAACTGAGCCAA
Consensus TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTAAATGACAGGGCACATTTATTTGCGTTCAACACGGAACTGAGCCAA

1691 1700 1710 1720 1730 1740 1746
F_SN4 TCACAATTGAGACTTTGGATGATGGAGTATGGGCAAGCTAAGATACAAATTTGA
F_SN4_2 TCACAATTGAGACTTTGGATGATGGAGTATGGGCAAGCTAAGATACAAATTTGA
F_SN4_3 TCACAATTGAGACTTTGGATGATGGAGTATGGGCAAGCTAAGATACAAATTTGA
F_SN4_1 TCACAATTGAGACTTTGGATGATGGAGTATGGGCAAGCTAAGATACAAATTTGA
Consensus TCACAATTGAGACTTTGGATGATGGAGTATGGGCAAGCTAAGATACAAATTTGA

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
F_DN2	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN2_1	ATGGATTATTCATCTAATTCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCACAAGTTTTATTCACTTGCAATCTCAAATGCTG													
Consensus	ATGGATTATTCATCTAATTCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCACAAGTTTTATTCAcTTGCAATCTCAAATGCTG													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
F_DN2	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN2_1	TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGTATTTCAATGGAGTCTATCATCTATTCTACCAATACAATCCAAACGGGTC													
Consensus	TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGTATTTCAATGGAGTCTAcCATCTATTCTACCAATACAACcCAAACGGGTC													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
F_DN2	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN2_1	AGTATGGGGCAATATTGTGTGGGCTCATTTCAGTTTCAAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAACCCATTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT													
Consensus	AGTATGGGGCAATATTGTgTGGGCTCATTTCAGTTTCAAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAACCCaTTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
F_DN2	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN2_1	CTACCTGGTAAACAGCCTGTTATCTTGTAACCTGGATTGTAGATGCCAACCAAACTCAAGTTCAAACTACGCGATTCCAGCTAACTTATCTGATCCATATCTTCGTGAATGGATCAAGCCTGATAACA													
Consensus	CTACCTGGTAAACAGCCTGTTATCTTGTAACCTGGATTGTAGATGCCAACCAAACTCAAGTTCAAACTACGCGATTCCAGCTAACTTATCTGATCCATATCTTCGTGAATGGATCAAGCCTGATAACA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
F_DN2	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN2_1	ATCCATTGATTGTAGCTGATGCTAGTATCAACAAGACCAAAATTCGTGATCCAAACACCCGATGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGAGGAACATAGTAGGGGTTAGC													
Consensus	ATCCATTGATTGTAGCTGATGCTAGTATCAACAAGACCAAAATTCGTGATCCAAACACCCGATGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGAGGAACATAGTAGGGGTTAGC													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
F_DN2	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN2_1	TATAATGTATAGAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCAACTAATGGTACTGGAATTTGGGATGTCCGATTTTTCCCTGTTGCATTGAAGGAACATAGGGATA													
Consensus	TATAATGTATAGAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCAACTAATGGTACTGGAATTTGGGATGTCCcGATTTTTCCCTGTTGCATTGAAGGAACATAGGGATA													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
F_DN2	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN2_1	GATCAATATGGTGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTCTATTGATA													
Consensus	GATCAATATGGTGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTCTATTGATA													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
F_DN2	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN2_1	GTTGGAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC													
Consensus	GTTGGAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
F_DN2	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN2_1	GAAAGGATGGGCTGGAATTCATTGATCCACGTAAGTATGGCTTGATCCAAAGTGGTAAACCAATTGGTTCAATGGCCTGTTGAGGATTTAGAACCCTAGAACCCAAAGGTTCAATTGAGTACAAA													
Consensus	GAAAGGATGGGCTGGAATTCATTGATCCACGTAAGTATGGCTTGATCCAAAGTGGTAAACCAATTGGTTCAATGGCCTGTTGAGGATTTAGAACCCTAGAACCCAAAGGTTCAATTGAGTACAAA													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
F_DN2	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN2_1	AAGTTGAACAATGGTGAAGAGGTTGAAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTGCAAGTTTGGATAAAGCAGAGTCATTTGATCCTAGTTGGACTGATATGTATG													
Consensus	AAGTTGAACAATGGTGAAGAGGTTGAAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTGCAAGTTTGGATAAAGCAGAGTCATTTGATCCTAGTTGGACTGATATGTATG													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
F_DN2	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN2_1	CACAAAGATGTTTGTGGACTTAAAGGTCAGATGTTCAAGGTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACTTAGAAGAAAACACACCTGTTTTCTCCGAGTTTTCAAGCACAACA													
Consensus	CACAAAGATGTTTGTGGACTTAAAGGTCAGATGTTCAAGGTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACTTAGAAGAAAACACACCTGTTTTCTCCGAGTTTTCAAGCACAACA													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
F_DN2	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN2_1	AAATTACAAGGTTCTCTTGTTCTGACGCTAAAAGGTCAACTCTTAAAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCTGACAGAATTTGTCCTCAGAGC													
Consensus	AAATTACAAGGTTCTCTTGTTcCTGACGCTAAAAGGTCAACTCTTAAAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCTGACAGAATTTGTCCTCAGAGC													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
F_DN2	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_DN2_1	TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATAACATCGAGAGTTTATCCAACTGGCGATTAAATGACAGGCACATTTATTTGCGTTCAACACGGAACTGAGCCAA													
Consensus	TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATAACATCGAGAGTTTATCCAACTGGCGATTAAATGACAGGCACATTTATTTGCGTTCAACACGGAACTGAGCCAA													
	1691	1700	1710	1720	1730	1740	1746							
F_DN2	-----+-----+-----+-----+-----+-----+-----													
F_DN2_1	TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAATATTGA													
Consensus	TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAATATTaA													

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
F_TN1	-----													
F_TN1_1	ATGGATTATTCATCTAATTCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTGTAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCATAAAGTTTTATTCACTTGCAATCTCAAATGCTG													
Consensus	ATGGATTATTCATCTAATTCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTGTAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCATAAAGTTTTATTCACTTGCAATCTCAAATGCTG													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
F_TN1	-----													
F_TN1_1	TAATGTTCACTGTTTCATAGAAGTGGTTATCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGTATTTCAATGGAGTCTATCATCTATTCTACCAATACACCCAAACGGGTC													
Consensus	TAATGTTCACTGTTTCATAGAAGTGGTTATCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGTATTTCAATGGAGTCTATCATCTATTCTACCAATACACCCAAACGGGTC													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
F_TN1	-----													
F_TN1_1	AGTATGGGGCAACATTGTTTGGGCTCATTCAAGTTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCTAAACCATTGGATCAATTTGGTACATGGTCTGGATCAGCAACTATT													
Consensus	AGTATGGGGCAACATTGTTTGGGCTCATTCAAGTTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCTAAACCATTGGATCAATTTGGTACATGGTCTGGATCAGCAACTATT													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
F_TN1	-----													
F_TN1_1	CTACCTGGTAAACAACCTGTTATCTTGTAAGTGGATGCTAACCAACTCAAGTTTCAAACTACGCGGTTCCAGCTAACATATCTGATCCTTATCTACGTGAATGGATCAAGCCTGATAACA													
Consensus	CTACCTGGTAAACAACCTGTTATCTTGTAAGTGGATGCTAACCAACTCAAGTTTCAAACTACGCGGTTCCAGCTAACATATCTGATCCTTATCTACGTGAATGGATCAAGCCTGATAACA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
F_TN1	-----													
F_TN1_1	ATCCATTGATTGTAGCTGATGCTAGCATCAACAAGACCAATTTTCGTGATCCACACCCGCTTGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGAGAAACATAGTAGGGGTTAGC													
Consensus	ATCCATTGATTGTAGCTGATGCTAGCATCAACAAGACCAATTTTCGTGATCCACACCCGCTTGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGAGAAACATAGTAGGGGTTAGC													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
F_TN1	-----													
F_TN1_1	TATAATGTATAGAAGCAAGACTTCATGAATGGTTAAGGCTAACACCCACTTCACTCAACTAACGGTACTGGAATTGGGATGTCTGATTTTTCCCGTTGCATTGAAGGAACTAATGGGCAR													
Consensus	TATAATGTATAGAAGCAAGACTTCATGAATGGTTAAGGCTAACACCCACTTCACTCAACTAACGGTACTGGAATTGGGATGTCTGATTTTTCCCGTTGCATTGAAGGAACTAATGGGCAR													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
F_TN1	-----													
F_TN1_1	GATCAATATGGTGAAGAACACAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTACATACCAGATGTTGGTCTATTGATA													
Consensus	GATCAATATGGTGAAGAACACAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTACATACCAGATGTTGGTCTATTGATA													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
F_TN1	-----													
F_TN1_1	GTTGGAAGGGATTGAGATTCGACTATGGTAATTTCTATGCATCAAGTCTTTCTATGATCCTAGCAAAAATCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAACGC													
Consensus	GTTGGAAGGGATTGAGATTCGACTATGGTAATTTCTATGCATCAAGTCTTTCTATGATCCTAGCAAAAATCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAACGC													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
F_TN1	-----													
F_TN1_1	TAAGGATGGGCTGGAATTCATTGATCCACGTAAGTATGGCTTGATCCAGTGGTAAACAATTGATTCATGGCCTGTTGAGGATTAGAACCCTAGAACCACAAGGTTCAATTGAGCACACAG													
Consensus	TAAGGATGGGCTGGAATTCATTGATCCACGTAAGTATGGCTTGATCCAGTGGTAAACAATTGATTCATGGCCTGTTGAGGATTAGAACCCTAGAACCACAAGGTTCAATTGAGCACACAG													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
F_TN1	-----													
F_TN1_1	AAGTTGAACAATGGTGAAGAGGTTGAGTTACAGGGATCACACCTGCACAGGCAGATGTTGAGTGCATTCTCTTTGCAAGTTTGGATAGGCAGAGTCATTTGATCCTAGTTGGACTGATATGTATG													
Consensus	AAGTTGAACAATGGTGAAGAGGTTGAGTTACAGGGATCACACCTGCACAGGCAGATGTTGAGTGCATTCTCTTTGCAAGTTTGGATAGGCAGAGTCATTTGATCCTAGTTGGACTGATATGTATG													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
F_TN1	-----													
F_TN1_1	CACAGATGTTTGTGGACTCAAGGTGCAGATGTTCAAGGTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACTTAGAAGAAAACACACCTGTTTTCTCCGAGTTTTCAAGCACACACA													
Consensus	CACAGATGTTTGTGGACTCAAGGTGCAGATGTTCAAGGTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACTTAGAAGAAAACACACCTGTTTTCTCCGAGTTTTCAAGCACACACA													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
F_TN1	-----													
F_TN1_1	AAATTACAAGTTCTCTTGTGCTCTGATGCTAAAAGGTCAACTCTTAAAGTTCAATGAACAATGTACAGAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCAGACAGAARTTGTCACTCAGAAGC													
Consensus	AAATTACAAGTTCTCTTGTGCTCTGATGCTAAAAGGTCAACTCTTAAAGTTCAATGAACAATGTACAGAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCAGACAGAARTTGTCACTCAGAAGC													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
F_TN1	-----													
F_TN1_1	TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATAACATCGAGGGTTTATCCACATTGGCGATTAAATGACAGGCACATTTATTTGCGTTCAACACGGAACTGAGCCAA													
Consensus	TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATAACATCGAGGGTTTATCCACATTGGCGATTAAATGACAGGCACATTTATTTGCGTTCAACACGGAACTGAGCCAA													
	1691	1700	1710	1720	1730	1740	1746							
F_TN1	-----													
F_TN1_1	TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAATATTGA													
Consensus	TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAATATTGA													

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
F_TN2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_TN2_1	ATGGATTATTCATCTAATTCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCACAAGTTTTATTCACTTGCATCTCAAATGCTG													
Consensus	ATGGATTATTCATCTAATTCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCACAAGTTTTATTCACTTGCATCTCAAATGCTG													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
F_TN2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_TN2_1	TAAATGTTCAAACGTTTCATAGAAGTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCATGCACCAATGTATTTCAATGGAGTCTATCATCTATTCTACCAATACATCCAACGGGTC													
Consensus	TAAATGTTCAAACGTTTCATAGAAGTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCATGCACCAATGTATTTCAATGGAGTCTATCATCTATTCTACCAATACATCCAACGGGTC													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
F_TN2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_TN2_1	AGTATGGGGCAATATTGTGTGGGCTCATTCAAGTTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAACCATTGGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT													
Consensus	AGTATGGGGCAATATTGTGTGGGCTCATTCAAGTTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAACCATTGGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
F_TN2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_TN2_1	CTACCTGGTAAACAGCCTGTTATCTTGTAAGTGGATAGTAGATGCCAACCAACTCAAGTTTCAAACTACGCGGTTCCAGCTAACTTATCTGATCCATCTTCGTTGGAATGGATCAAGCCTGATAATA													
Consensus	CTACCTGGTAAACAGCCTGTTATCTTGTAAGTGGATAGTAGATGCCAACCAACTCAAGTTTCAAACTACGCGGTTCCAGCTAACTTATCTGATCCATCTTCGTTGGAATGGATCAAGCCTGATAATA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
F_TN2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_TN2_1	ATCCATTGATTGTAGCTGATGCTAGTATCAACAAGACCAATTTTCGTGATCCAAACACCGCATGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGAGAAACATAGTAGGGGTTAGC													
Consensus	ATCCATTGATTGTAGCTGATGCTAGTATCAACAAGACCAATTTTCGTGATCCAAACACCGCATGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGAGAAACATAGTAGGGGTTAGC													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
F_TN2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_TN2_1	TATAATGTATAGGAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCAACTAACGGTACTGGAATTTGGGATGTCTTGATTTTTCCCTGTTGCATTGAAGGAACTAATGGGATA													
Consensus	TATAATGTATAGGAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCAACTAACGGTACTGGAATTTGGGATGTCTTGATTTTTCCCTGTTGCATTGAAGGAACTAATGGGATA													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
F_TN2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_TN2_1	GATCAATATGGTGAAGATATAAAATGTGCTTAAAGATGGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTACGTACCAGATGTTGGTCTATTGATA													
Consensus	GATCAATATGGTGAAGATATAAAaATGTGCTTAAAGATaGGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTACGTACCAGATGTTGGTCTATTGATA													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
F_TN2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_TN2_1	GTTGGAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC													
Consensus	GTTGGAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
F_TN2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_TN2_1	GAAAGGGTGGGCTGGAATTCATTGATCCACGTAAGTATGGCTTGACCCAGTGGTAAACCAATTGATTCATGGCCTGTTGAGGATTAGAACCCTAGAACCRAAGGTTCAATTGAGTACACAG													
Consensus	GAAAGGatGGGCTGGAATTCATTGATCCACGTAAGTATGGCTTGACCCAGTGGTAAACCAATTGATTCATGGCCTGTTGAGGATTAGAACCCTAGAACCRAAGGTTCAATTGAGTACACAG													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
F_TN2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_TN2_1	AAGTTGAACAATGGTGAAGGTTGAAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTTGCAGTTTGGATAAAGCAGAGTATTTGATTCTAGTTGGACTGATATGTATG													
Consensus	AAGTTGAACAATGGTGAAGGTTGAAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTTGCAGTTTGGATAAAGCAGAGTATTTGATTCTAGTTGGACTGATATGTATG													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
F_TN2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_TN2_1	CACAGATGTTTGTGGACTCAGGGTGCAGATGTACAAGGTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACTTAGAAGAAACACACCTGTTTTCTCCGAGTTTTCAAGCACAACA													
Consensus	CACAGATGTTTGTGGACTCAGGGTGCAGATGTACAAGGTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACTTAGAAGAAACACACCTGTTTTCTCCGAGTTTTCAAGCACAACA													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
F_TN2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_TN2_1	AAATTACAAGTTCTCTTGTGCTCTGACGCTAAAAGGTCACCTCTTAAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCAGACAGAARTTGTCACTCAGAAGC													
Consensus	AAATTACAAGTTCTCTTGTGCTCTGACGCTAAAAGGTCACCTCTTAAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCAGACAGAARTTGTCACTCAGAAGC													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
F_TN2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_TN2_1	TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATAACATCGAGGGTTTATCCACATTAGCGATTAATGAGAGGGCACATTTATTTGCGTTCAACACGGAACTGAGCCAA													
Consensus	TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATAACATCGAGGGTTTATCCACATTAGCGATTAATGAGAGGGCACATTTATTTGCGTTCAACACGGAACTGAGCCAA													
	1691	1700	1710	1720	1730	1740	1746							
F_TN2	----- ----- ----- ----- ----- -----													
F_TN2_1	TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAATATTAA													
Consensus	TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAATCACTAGacacaAG.....													

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_P18N	MDYSSNSRWALPVILVCFEIVLLSNVYVFASHKVF IHLQSQNAVNVQTVHRTGYHFQPEKHWINDPNAPHYFNGVYHLFYQYNPNGSVHGNIYVAHSVSKDLINWINLEPAIYPSKPFDDQFGTWSGSATI													
F_P18N_1	MDYSSNSRWALPVILVCFEIVLLSNVYVFASHKVF IHLQSQNAVNVQTVHRTGYHFQPEKHWINDPNAPHYFNGVYHLFYQYNPNGSVHGNIYVAHSVSKDLINWINLEPAIYPSKPFDDQFGTWSGSATI													
Consensus	MDYSSNSRWALPVILVCFEIVLLSNVYVFASHKVF IHLQSQNAVNVQTVHRTGYHFQPEKHWINDPNAPHYFNGVYHLFYQYNPNGSVHGNIYVAHSVSKDLINWINLEPAIYPSKPFDDQFGTWSGSATI													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_P18N	LPGNKPVILYTGIVDANQTQVQNYAIPANLSDPYLREIHKPDNPLIVADASINKTKFRDPTTAMHGKDGHWRIYVHGSLRKHSRGLAIHYRSKDFHKWYKAKHPLHSTNGTGNWECPDFFPVALKGTNGI													
F_P18N_1	LPGNKPVILYTGIVDANQTQVQNYAIPANLSDPYLREIHKPDNPLIVADASINKTKFRDPTTAMHGKDGHWRIYVHGSLRKHSRGLAIHYRSKDFHKWYKAKHPLHSTNGTGNWECPDFFPVALKGTNGI													
Consensus	LPGNKPVILYTGIVDANQTQVQNYAIPANLSDPYLREIHKPDNPLIVADASINKTKFRDPTTAMHGKDGHWRIYVHGSLRKHSRGLAIHYRSKDFHKWYKAKHPLHSTNGTGNWECPDFFPVALKGTNGI													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_P18N	DQYGEEYKYVLKNSHDLTRFEYITLGKYDTKKDRYVDPVGSIDSWKGLRFDYGNFYASKTFYDTSKNRRYVHGWSNESDIFPEDDNAKGWAGIQLIPRKVHLDPGKQLVQWPVEELETLRQKVQLSNK													
F_P18N_1	DQYGEEYKYVLKNSHDLTRFEYITLGKYDTKKDRYVDPVGSIDSWKGLRFDYGNFYASKTFYDTSKNRRYVHGWSNESDIFPEDDNAKGWAGIQLIPRKVHLDPGKQLVQWPVEELETLRQKVQLSNK													
Consensus	DQYGEEYKYVLKNSHDLTRFEYITLGKYDTKKDRYVDPVGSIDSWKGLRFDYGNFYASKTFYDTSKNRRYVHGWSNESDIFPEDDNAKGWAGIQLIPRKVHLDPGKQLVQWPVEELETLRQKVQLSNK													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_P18N	KLNNGEKVEYTGITPAQADVEYTFASFADKAESFSSWTDHYAQDYCGLKGADYQGGGLGPFGLATLATENLEENTPVFFRYVFAQQNYKYVLLCSDAKRSTLKFNETHYKVSFAGFYDVLADKKLSLRS													
F_P18N_1	KLNNGEKVEYTGITPAQADVEYTFASFADKAESFSSWTDHYAQDYCGLKGADYQGGGLGPFGLATLATENLEENTPVFFRYVFAQQNYKYVLLCSDAKRSTLKFNETHYKVSFAGFYDVLADKKLSLRS													
Consensus	KLNNGEKVEYTGITPAQADVEYTFASFADKAESFSSWTDHYAQDYCGLKGADYQGGGLGPFGLATLATENLEENTPVFFRYVFAQQNYKYVLLCSDAKRSTLKFNETHYKVSFAGFYDVLADKKLSLRS													
	521	530	540	550	560	570	581							
	-----+-----+-----+-----+-----+-----+-----													
F_P18N	LIDNSVIESFGAGGKTCITSRVYPTLAINAKAHLFAFNNGTEPITTIETLDAWSHGKAKIQY													
F_P18N_1	LIDNSVIESFGAGGKTCITSRVYPTLAINAKAHLFAFNNGTEPITTIETLDAWSHGKAKIQY													
Consensus	LIDNSVIESFGAGGKTCITSRVYPTLAINAKAHLFAFNNGTEPITTIETLDAWSHGKAKIQY													

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
F_P18N	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_P18N_1	ATGGATTATTCATCTAATTCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCACAAAGTTTTATTTCATTTGCAATCTCAAATGCTG													
Consensus	ATGGATTATTCATCTAATTCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCACAAAGTTTTATTTCATTTGCAATCTCAAATGCTG													
F_P18N	131	140	150	160	170	180	190	200	210	220	230	240	250	260
F_P18N_1	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_P18N_1	TAAATGTTCAAACCTGTTTCATAGAAGTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCATGCACCAATGTATTTCAATGGAGTCTACCATCTATTCTACCAATACACCCAATGGGTC													
Consensus	TAAATGTTCAAACCTGTTTCATAGAAGTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCATGCACCAATGTATTTCAATGGAGTCTACCATCTATTCTACCAATACACCCAATGGGTC													
F_P18N	261	270	280	290	300	310	320	330	340	350	360	370	380	390
F_P18N_1	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_P18N_1	AGTATGGGGCAATATTGTTTGGGCTCATTTCAGTTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAACCCTTTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT													
Consensus	AGTATGGGGCAATATTGTTTGGGCTCATTTCAGTTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAACCCTTTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT													
F_P18N	391	400	410	420	430	440	450	460	470	480	490	500	510	520
F_P18N_1	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_P18N_1	CTACCTGGTAAACAGCCTGTTATCTTGACACTGGAATAGTAGATGCCAACCAACTCAAGTTCCAAACTACGCGATTCCAGCTAATCTGATCCATATCTCCGTGAATGGATCAAGCCTGATAACA													
Consensus	CTACCTGGTAAACAGCCTGTTATCTTGACACTGGAATAGTAGATGCCAACCAACTCAAGTTCCAAACTACGCGATTCCAGCTAATCTGATCCATATCTCCGTGAATGGATCAAGCCTGATAACA													
F_P18N	521	530	540	550	560	570	580	590	600	610	620	630	640	650
F_P18N_1	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_P18N_1	ATCCATTGATTGTAGCTGATGCTAGCATCAACAAGACCAATTTTCGTGATCCAAACACCCGATGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGAGGAACATAGTAGGGGTTAGC													
Consensus	ATCCATTGATTGTAGCTGATGCTAGCATCAACAAGACCAATTTTCGTGATCCAAACACCCGATGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGAGGAACATAGTAGGGGTTAGC													
F_P18N	651	660	670	680	690	700	710	720	730	740	750	760	770	780
F_P18N_1	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_P18N_1	TATAATGTATAGAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCAACTAATGGTACTGGAACCTGGGATGTCTTGATTTTTCCCTGTTGCATTGAAGGAACATAGGGATA													
Consensus	TATAATGTATAGAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCAACTAATGGTACTGGAACCTGGGATGTCTTGATTTTTCCCTGTTGCATTGAAGGAACATAGGGATA													
F_P18N	781	790	800	810	820	830	840	850	860	870	880	890	900	910
F_P18N_1	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_P18N_1	GATCAATATGGTGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTCTATTGATA													
Consensus	GATCAATATGGTGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAAAGTACGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTCTATTGATA													
F_P18N	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
F_P18N_1	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_P18N_1	GTTGGAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC													
Consensus	GTTGGAAGGGATTGAGATTCGATTATGGTAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC													
F_P18N	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
F_P18N_1	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_P18N_1	GAAAGGATGGGCTGGAATTCATTGATCCACGTAAGTATGGCTTGATCCAAAGTGGTAAACCAATTGGTTCAATGGCCTGTTGAGGATTAGAACCCTAGAACCACAAAGGTTCAATTGAGTACAAA													
Consensus	GAAAGGATGGGCTGGAATTCATTGATCCACGTAAGTATGGCTTGATCCAAAGTGGTAAACCAATTGGTTCAATGGCCTGTTGAGGATTAGAACCCTAGAACCACAAAGGTTCAATTGAGTACAAA													
F_P18N	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
F_P18N_1	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_P18N_1	AAGTTGAACAATGGTGAAGGTTGAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAGTGCATTTTCTTTTGCAGTTTGGATAAGGCAGAGTCATTTGATTCTAGTTGGACTGATATGTATG													
Consensus	AAGTTGAACAATGGTGAAGGTTGAGTTACAGGAATCACACCTGCACAGGCAGATGTTGAGTGCATTTTCTTTTGCAGTTTGGATAAGGCAGAGTCATTTGATTCTAGTTGGACTGATATGTATG													
F_P18N	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
F_P18N_1	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_P18N_1	CACAAAGATGTTTGTGGACTCAAGGGTGCAGATGTACAAGGTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACTTGGAGAAACACACCTGTTTTCTCCGAGTTTTCAAGCACAACA													
Consensus	CACAAAGATGTTTGTGGACTCAAGGGTGCAGATGTACAAGGTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACTTGGAGAAACACACCTGTTTTCTCCGAGTTTTCAAGCACAACA													
F_P18N	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
F_P18N_1	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_P18N_1	AAATTACAAGGTTCTTTTGTGCTCTGACGCTAAAAGGTCACCTCTTAAAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCTGACAGAATTTGTCACCTCAGAAGC													
Consensus	AAATTACAAGGTTCTTTTGTGCTCTGACGCTAAAAGGTCACCTCTTAAAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCTGACAGAATTTGTCACCTCAGAAGC													
F_P18N	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
F_P18N_1	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
F_P18N_1	TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATAACATCGAGGGTTTATCCACATTGGCGATTAAATGAGAGGGCACATTTATTTGCGTTCAACCAATGGAACCTGAGCCAA													
Consensus	TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATAACATCGAGGGTTTATCCACATTGGCGATTAAATGAGAGGGCACATTTATTTGCGTTCAACCAATGGAACCTGAGCCAA													
F_P18N	1691	1700	1710	1720	1730	1740	1746							
F_P18N_1	----- ----- ----- ----- ----- ----- -----													
F_P18N_1	TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAATATTGA													
Consensus	TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAATATTGA													

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
F_P40N2	-----													
F_P40N2_2	ATGGATTATTCATCTAATTCCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCCTTTTATAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCACAAAGTTTTATTCACTTGCATCTCAAAATGCTG													
F_P40N2_1	ATGGATTATTCATCTAATTCCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCCTTTTATAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCACAAAGTTTTATTCACTTGCATCTCAAAATGCTG													
Consensus	ATGGATTATTCATCTAATTCCTCGTTGGGCTTTGCCAGTTATCTTAGTTTGCCTTTTATAGTTTTATTATCCAATAATGTTGTTTTGCTTCTCACAAAGTTTTATTCACTTGCATCTCAAAATGCTG													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
F_P40N2	-----													
F_P40N2_2	TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGTATTTCAATGGAATTTACCATCTATTCTACCAATACAACCCAAACGGGTC													
F_P40N2_1	TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGTATTTCAATGGAATTTACCATCTATTCTACCAATACAACCCAAACGGGTC													
Consensus	TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGTATTTCAATGGAATTTACCATCTATTCTACCAATACAACCCAAACGGGTC													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
F_P40N2	-----													
F_P40N2_2	AGTATGGGGCAATATTGTTTGGGCTCATTTCAGTTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAARCCATTGATCAATTTGGTACGTGGTCTGGATCTGCAACTATT													
F_P40N2_1	AGTATGGGGCAATATTGTTTGGGCTCATTTCAGTTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAARCCATTGATCAATTTGGTACGTGGTCTGGATCTGCAACTATT													
Consensus	AGTATGGGGCAATATTGTTTGGGCTCATTTCAGTTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAARCCATTGATCAATTTGGTACGTGGTCTGGATCTGCAACTATT													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
F_P40N2	-----													
F_P40N2_2	CTACCTGGTAAACAGCCTGTTATCTTGACACTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAACCTACGCGATTCCAGCTAATTATCTGATCCATATCTCCGTGAATGGATCAAGCCTGATAACA													
F_P40N2_1	CTACCTGGTAAACAGCCTGTTATCTTGACACTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAACCTACGCGATTCCAGCTAATTATCTGATCCATATCTCCGTGAATGGATCAAGCCTGATAACA													
Consensus	CTACCTGGTAAACAGCCTGTTATCTTGACACTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAACCTACGCGATTCCAGCTAATTATCTGATCCATATCTCCGTGAATGGATCAAGCCTGATAACA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
F_P40N2	-----													
F_P40N2_2	ATCCATTGATTGTAGCTGATGATAGTATCAACAAGACCAATTTTCGTGATCCAACTGCATGGATGGGTAAGATGGACATTGGAGAATTGTGATGGGAAGTTTGAGAAACATAGTAGGGGTTAGC													
F_P40N2_1	ATCCATTGATTGTAGCTGATGATAGTATCAACAAGACCAATTTTCGTGATCCAACTGCATGGATGGGTAAGATGGACATTGGAGAATTGTGATGGGAAGTTTGAGAAACATAGTAGGGGTTAGC													
Consensus	ATCCATTGATTGTAGCTGATGATAGTATCAACAAGACCAATTTTCGTGATCCAACTGCATGGATGGGTAAGATGGACATTGGAGAATTGTGATGGGAAGTTTGAGAAACATAGTAGGGGTTAGC													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
F_P40N2	-----													
F_P40N2_2	AATATGTATAGAGCAAGATTTTCATGAATGGGTTAAGGCTAACACCCACTTCACTCAACTAACGGTACTGGAATTTGGGAATGTCTGATTTTTTCTGTTGCATTGAAGGAACTAATGGGATA													
F_P40N2_1	AATATGTATAGAGCAAGATTTTCATGAATGGGTTAAGGCTAACACCCACTTCACTCAACTAACGGTACTGGAATTTGGGAATGTCTGATTTTTTCTGTTGCATTGAAGGAACTAATGGGATA													
Consensus	AATATGTATAGAGCAAGATTTTCATGAATGGGTTAAGGCTAACACCCACTTCACTCAACTAACGGTACTGGAATTTGGGAATGTCTGATTTTTTCTGTTGCATTGAAGGAACTAATGGGATA													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
F_P40N2	-----													
F_P40N2_2	GATCAATATGATGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAGTACGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTTCTATTGATA													
F_P40N2_1	GATCAATATGATGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAGTACGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTTCTATTGATA													
Consensus	GATCAATATGATGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAGTACGATACAAAAAAGATAGGTATGTTCCAGATGTTGGTTCTATTGATA													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
F_P40N2	-----													
F_P40N2_2	GTTGGAAGGGATTGAGATTCGATTATGGTAACTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC													
F_P40N2_1	GTTGGAAGGGATTGAGATTCGATTATGGTAACTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC													
Consensus	GTTGGAAGGGATTGAGATTCGATTATGGTAACTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
F_P40N2	-----													
F_P40N2_2	GAAAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGACGCAAGTGGTAACAATTTGGTTCAATGGCCTGTTGAGAATTAGAACCCTAAGAACCCAAAGGTTCAATTGAGTAAACAA													
F_P40N2_1	GAAAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGACGCAAGTGGTAACAATTTGGTTCAATGGCCTGTTGAGAATTAGAACCCTAAGAACCCAAAGGTTCAATTGAGTAAACAA													
Consensus	GAAAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGACGCAAGTGGTAACAATTTGGTTCAATGGCCTGTTGAGAATTAGAACCCTAAGAACCCAAAGGTTCAATTGAGTAAACAA													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
F_P40N2	-----													
F_P40N2_2	AAGTTGAACAATGGTGAAGGTTGAGTTACAGGGATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTGCAAGTTTGGATAAAGCAGAGTCATTTGATTCAGTTGGACTGATATGTATG													
F_P40N2_1	AAGTTGAACAATGGTGAAGGTTGAGTTACAGGGATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTGCAAGTTTGGATAAAGCAGAGTCATTTGATTCAGTTGGACTGATATGTATG													
Consensus	AAGTTGAACAATGGTGAAGGTTGAGTTACAGGGATCACACCTGCACAGGCAGATGTTGAAGTGACATTCTCTTTGCAAGTTTGGATAAAGCAGAGTCATTTGATTCAGTTGGACTGATATGTATG													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
F_P40N2	-----													
F_P40N2_2	CACAGATGTTTGTGGACTCAAGGGTGCAGATGTACAGGTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACTTAGAAGAAACACACCTGTTTTCTCCGAGTTTTCAAGCACAAACA													
F_P40N2_1	CACAGATGTTTGTGGACTCAAGGGTGCAGATGTACAGGTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACTTAGAAGAAACACACCTGTTTTCTCCGAGTTTTCAAGCACAAACA													
Consensus	CACAGATGTTTGTGGACTCAAGGGTGCAGATGTACAGGTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACTTAGAAGAAACACACCTGTTTTCTCCGAGTTTTCAAGCACAAACA													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
F_P40N2	-----													
F_P40N2_2	AAATTACAAGGTTCTCTTGTGTTCTGACGCTAAAAGGTCACCTTAAAGTTCAATGAACAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGACAGACAGAAATTGTCCTCAGAGC													
F_P40N2_1	AAATTACAAGGTTCTCTTGTGTTCTGACGCTAAAAGGTCACCTTAAAGTTCAATGAACAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGACAGACAGAAATTGTCCTCAGAGC													
Consensus	AAATTACAAGGTTCTCTTGTGTTCTGACGCTAAAAGGTCACCTTAAAGTTCAATGAACAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGACAGACAGAAATTGTCCTCAGAGC													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
F_P40N2	-----													
F_P40N2_2	TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTAAAGAGAGGCACATTTATTTGCGTTCAACACGGAACCTGAGCCAA													
F_P40N2_1	TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTAAAGAGAGGCACATTTATTTGCGTTCAACACGGAACCTGAGCCAA													
Consensus	TTGATTGATAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTAAAGAGAGGCACATTTATTTGCGTTCAACACGGAACCTGAGCCAA													
	1691	1700	1710	1720	1730	1740	1746	-----						
F_P40N2	TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAATATTGA													
F_P40N2_2	TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAATATTGA													
F_P40N2_1	TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAATATTGA													
Consensus	TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAATATTGA													

1 10 20 30 40 50 60 70 80 90 100 110 120 130
F_P54N2 ATGGATTATTCATCTAATTCTCGTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCATTTGCAATCTCAAATGCTG
F_P54N2_3 ATGGATTATTCATCTAATTCTCGTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCATTTGCAATCTCAAATGCTG
F_P54N2_1 ATGGATTATTCATCTAATTCTCGTAGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCATTTGCAATCTCAAATGCTG
F_P54N2_2 ATGGATTATTCATCTAATTCTCGTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCATTTGCAATCTCAAATGCTG
Consensus ATGGATTATTCATCTAATTCTCGTGGGCTTTGCCAGTTATCTTAGTTTGCTTTTTATAGTTTATTATCCAATATGTTGTTTTGCTTCTCACAAGTTTTTATTCATTTGCAATCTCAAATGCTG

131 140 150 160 170 180 190 200 210 220 230 240 250 260
F_P54N2 TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTACCATCTATTCTACCAATCAACCCAAATGGGTC
F_P54N2_3 TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTACCATCTATTCTACCAATCAACCCAAATGGGTC
F_P54N2_1 TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTACCATCTATTCTACCAATCAACCCAAATGGGTC
F_P54N2_2 TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTACCATCTATTCTACCAATCAACCCAAATGGGTC
Consensus TAATGTTCAAACTGTTTCATAGAACTGGTTACCATTTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTACCATCTATTCTACCAATCAACCCAAATGGGTC

261 270 280 290 300 310 320 330 340 350 360 370 380 390
F_P54N2 AGTATGGGCAATATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAACCCCTTTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT
F_P54N2_3 AGTATGGGCAATATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAACCCCTTTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT
F_P54N2_1 AGTATGGGCAATATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAACCCCTTTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT
F_P54N2_2 AGTATGGGCAATATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAACCCCTTTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT
Consensus AGTATGGGCAATATTGTTGGGCTCATTGTTCAAGGACTTAATCAATTGGATCAATTTAGAACCCGCAATTTACCCATCCAACCCCTTTGATCAATTCGGTACGTGGTCTGGATCTGCAACTATT

391 400 410 420 430 440 450 460 470 480 490 500 510 520
F_P54N2 CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAACACGCGATTCCAGTAACTTATCTGATCCATATCTCCGTGAATGGATCAGCCTGATAACA
F_P54N2_3 CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAACACGCGATTCCAGTAACTTATCTGATCCATATCTCCGTGAATGGATCAGCCTGATAACA
F_P54N2_1 CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAACACGCGATTCCAGTAACTTATCTGATCCATATCTCCGTGAATGGATCAGCCTGATAACA
F_P54N2_2 CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAACACGCGATTCCAGTAACTTATCTGATCCATATCTCCGTGAATGGATCAGCCTGATAACA
Consensus CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAAACTCAAGTTCAAACACGCGATTCCAGTAACTTATCTGATCCATATCTCCGTGAATGGATCAGCCTGATAACA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
F_P54N2 ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAAAATTTCTGATCCACACCCGATGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGGGAAACATAGTAGGGGTTTACG
F_P54N2_3 ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAAAATTTCTGATCCACACCCGATGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGGGAAACATAGTAGGGGTTTACG
F_P54N2_1 ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAAAATTTCTGATCCACACCCGATGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGGGAAACATAGTAGGGGTTTACG
F_P54N2_2 ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAAAATTTCTGATCCACACCCGATGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGGGAAACATAGTAGGGGTTTACG
Consensus ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAAAATTTCTGATCCACACCCGATGGATGGGTAAGATGGACATTGGAGATTGTGATGGGAGTTTGGGAAACATAGTAGGGGTTTACG

651 660 670 680 690 700 710 720 730 740 750 760 770 780
F_P54N2 TATAATGTATAGAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAACCTGGGATGTCTGATTTTTCCCTGTTGCATTGAARAGGAATATGGGATA
F_P54N2_3 TATAATGTATAGAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAACCTGGGATGTCTGATTTTTCCCTGTTGCATTGAARAGGAATATGGGATA
F_P54N2_1 TATAATGTATAGAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAACCTGGGATGTCTGATTTTTCCCTGTTGCATTGAARAGGAATATGGGATA
F_P54N2_2 TATAATGTATAGAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAACCTGGGATGTCTGATTTTTCCCTGTTGCATTGAARAGGAATATGGGATA
Consensus TATAATGTATAGAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCACTAATGGTACTGGAACCTGGGATGTCTGATTTTTCCCTGTTGCATTGAARAGGAATATGGGATA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
F_P54N2 GATCAATATGGTGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAACTAGACATAAAAAAGATAGGTATGTTCCAGATGTTGGTCTATTGATA
F_P54N2_3 GATCAATATGGTGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAACTAGACATAAAAAAGATAGGTATGTTCCAGATGTTGGTCTATTGATA
F_P54N2_1 GATCAATATGGTGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAACTAGACATAAAAAAGATAGGTATGTTCCAGATGTTGGTCTATTGATA
F_P54N2_2 GATCAATATGGTGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAACTAGACATAAAAAAGATAGGTATGTTCCAGATGTTGGTCTATTGATA
Consensus GATCAATATGGTGAAGATATAAATATGTGCTTAAAGATAGTATGGATCTTACTCGATTTGAGTACTATACTCTTGGTAACTAGACATAAAAAAGATAGGTATGTTCCAGATGTTGGTCTATTGATA

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
F_P54N2 GTTGGAGGGGATTGAGATTCGATTATGGTAAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC
F_P54N2_3 GTTGGAGGGGATTGAGATTCGATTATGGTAAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC
F_P54N2_1 GTTGGAGGGGATTGAGATTCGATTATGGTAAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC
F_P54N2_2 GTTGGAGGGGATTGAGATTCGATTATGGTAAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC
Consensus GTTGGAGGGGATTGAGATTCGATTATGGTAAATTTCTATGCATCAAGACTTTCTATGATACTAGCAAAAACCGAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
F_P54N2 GAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGTCCAGTGGTAACCAATTGGTTCAATGGCCTGTTGAGAATTAGAACCTTAGAACCCAAAGGTTCAATTTGAGTAAACAA
F_P54N2_3 GAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGTCCAGTGGTAACCAATTGGTTCAATGGCCTGTTGAGAATTAGAACCTTAGAACCCAAAGGTTCAATTTGAGTAAACAA
F_P54N2_1 GAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGTCCAGTGGTAACCAATTGGTTCAATGGCCTGTTGAGAATTAGAACCTTAGAACCCAAAGGTTCAATTTGAGTAAACAA
F_P54N2_2 GAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGTCCAGTGGTAACCAATTGGTTCAATGGCCTGTTGAGAATTAGAACCTTAGAACCCAAAGGTTCAATTTGAGTAAACAA
Consensus GAARAGGATGGGCTGGAATTCATTTGATCCACGTAAGTATGGCTTGTCCAGTGGTAACCAATTGGTTCAATGGCCTGTTGAGAATTAGAACCTTAGAACCCAAAGGTTCAATTTGAGTAAACAA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
F_P54N2 AAGTTGAACAAATGGTGAARAGGTTGAAGTTACAGGAATCACACCTGCACAGGCGGATGTTGAAGTATATTTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG
F_P54N2_3 AAGTTGAACAAATGGTGAARAGGTTGAAGTTACAGGAATCACACCTGCACAGGCGGATGTTGAAGTATATTTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG
F_P54N2_1 AAGTTGAACAAATGGTGAARAGGTTGAAGTTACAGGAATCACACCTGCACAGGCGGATGTTGAAGTATATTTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG
F_P54N2_2 AAGTTGAACAAATGGTGAARAGGTTGAAGTTACAGGAATCACACCTGCACAGGCGGATGTTGAAGTATATTTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG
Consensus AAGTTGAACAAATGGTGAARAGGTTGAAGTTACAGGAATCACACCTGCACAGGCGGATGTTGAAGTATATTTCTTTTGCAGTTTGGATAAAGCAGAGTCATTTGATTCTAGTTGGACTGATATGATG

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
F_P54N2 CACAGATGTTTGTGGACTTAAAGGTTGACAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTTAGAAGAARACACACCTGTTTCTCCGAGTTTTCARAGCACACA
F_P54N2_3 CACAGATGTTTGTGGACTTAAAGGTTGACAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTTAGAAGAARACACACCTGTTTCTCCGAGTTTTCARAGCACACA
F_P54N2_1 CACAGATGTTTGTGGACTTAAAGGTTGACAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTTAGAAGAARACACACCTGTTTCTCCGAGTTTTCARAGCACACA
F_P54N2_2 CACAGATGTTTGTGGACTTAAAGGTTGACAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTTAGAAGAARACACACCTGTTTCTCCGAGTTTTCARAGCACACA
Consensus CACAGATGTTTGTGGACTTAAAGGTTGACAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGTACATTGGCTACTGAAACCTTAGAAGAARACACACCTGTTTCTCCGAGTTTTCARAGCACACA

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
F_P54N2 AAATTACAAGGTTCTCTTGTGCTCTGACGCTAAAGGTCACACTCTTAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCTGACAGAARATGTCACCTCAGAGC
F_P54N2_3 AAATTACAAGGTTCTCTTGTGCTCTGACGCTAAAGGTCACACTCTTAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCTGACAGAARATGTCACCTCAGAGC
F_P54N2_1 AAATTACAAGGTTCTCTTGTGCTCTGACGCTAAAGGTCACACTCTTAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCTGACAGAARATGTCACCTCAGAGC
F_P54N2_2 AAATTACAAGGTTCTCTTGTGCTCTGACGCTAAAGGTCACACTCTTAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCTGACAGAARATGTCACCTCAGAGC
Consensus AAATTACAAGGTTCTCTTGTGCTCTGACGCTAAAGGTCACACTCTTAGTTCAATGAACCAATGTACAAGTTTCATTTGCTGGATTTGTGGATGTTGATTTGGCTGACAGAARATGTCACCTCAGAGC

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
F_P54N2 TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTATGAGAGGACACATTTATTTGCATTCAACACGGAACTGAGCCAA
F_P54N2_3 TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTATGAGAGGACACATTTATTTGCATTCAACACGGAACTGAGCCAA
F_P54N2_1 TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTATGAGAGGACACATTTATTTGCATTCAACACGGAACTGAGCCAA
F_P54N2_2 TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTATGAGAGGACACATTTATTTGCATTCAACACGGAACTGAGCCAA
Consensus TTGATTGATAATTCAGTTATAGAAGTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTATCCAACATTGGCGATTATGAGAGGACACATTTATTTGCATTCAACACGGAACTGAGCCAA

1691 1700 1710 1720 1730 1740 1746
F_P54N2 TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA
F_P54N2_3 TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA
F_P54N2_1 TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA
F_P54N2_2 TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA
Consensus TCACAATTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACAAATTTGA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
F_P18N ATGGATTATTCATCTAATTCCTGTTGGGCTTTGCCAGTTATCTTAGTGTGCTTTTTATAGTTTTATTATCCAAATGTTGTTTTGCTTCTCACAAAGTTTTATTTCATTTGCAATCTCAAATGCTG
F_P54N1 ATGGATTATTCATCTAATTCCTGTTGGGCTTTGCCAGTTATCTTAGTGTGCTTTTTATAGTTTTATTATCCAAATGTTGTTTTGCTTCTCACAAAGTTTTATTTCATTTGCAATCTCAAATGCTG
F_P40N1 ATGGATTATTCATCTAATTCCTGTTGGGCTTTGCCAGTTATCTTAGTGTGCTTTTTATAGTTTTATTATCCAAATGTTGTTTTGCTTCTCACAAAGTTTTATTTCATTTGCAATCTCAAATGCTG
F_P54N2 ATGGATTATTCATCTAATTCCTGTTGGGCTTTGCCAGTTATCTTAGTGTGCTTTTTATAGTTTTATTATCCAAATGTTGTTTTGCTTCTCACAAAGTTTTATTTCATTTGCAATCTCAAATGCTG
F_P40N2 ATGGATTATTCATCTAATTCCTGTTGGGCTTTGCCAGTTATCTTAGTGTGCTTTTTATAGTTTTATTATCCAAATGTTGTTTTGCTTCTCACAAAGTTTTATTTCATTTGCAATCTCAAATGCTG
Consensus ATGGATTATTCATCTAATTCCTGTTGGGCTTTGCCAGTTATCTTAGTGTGCTTTTTATAGTTTTATTATCCAAATGTTGTTTTGCTTCTCACAAAGTTTTATTTCATTTGCAATCTCAAATGCTG

131 140 150 160 170 180 190 200 210 220 230 240 250 260
F_P18N TAARTGTTCAAACTGTTTCATAGAAGCTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTACCATCTATTCTACCAATACACCCAAATGGGTC
F_P54N1 TAARTGTTCAAACTGTTTCATAGAAGCTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTACCATCTATTCTACCAATACACCCAAATGGGTC
F_P40N1 TAARTGTTCAAACTGTTTCATAGAAGCTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTACCATCTATTCTACCAATACACCCAAATGGGTC
F_P54N2 TAARTGTTCAAACTGTTTCATAGAAGCTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTACCATCTATTCTACCAATACACCCAAATGGGTC
F_P40N2 TAARTGTTCAAACTGTTTCATAGAAGCTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTACCATCTATTCTACCAATACACCCAAATGGGTC
Consensus TAARTGTTCAAACTGTTTCATAGAAGCTGGTTACCATTTCAGCCAGAAAACATTGGATCAATGATCCCAATGCACCAATGATTTCAATGGAGTCTACCATCTATTCTACCAATACACCCAAATGGGTC

261 270 280 290 300 310 320 330 340 350 360 370 380 390
F_P18N AGTATGGGGCAATATTGTTTGGGCTCATTCAAGTTCARAGGACTTAATCAATTGGATCAATTTAGAACCCGCATTTACCATCCAAACCCTTTGATCAATTGGTACGTGGTCTGGATCTGCACCTATT
F_P54N1 AGTATGGGGCAATATTGTTTGGGCTCATTCAAGTTCARAGGACTTAATCAATTGGATCAATTTAGAACCCGCATTTACCATCCAAACCCTTTGATCAATTGGTACGTGGTCTGGATCTGCACCTATT
F_P40N1 AGTATGGGGCAATATTGTTTGGGCTCATTCAAGTTCARAGGACTTAATCAATTGGATCAATTTAGAACCCGCATTTACCATCCAAACCCTTTGATCAATTGGTACGTGGTCTGGATCTGCACCTATT
F_P54N2 AGTATGGGGCAATATTGTTTGGGCTCATTCAAGTTCARAGGACTTAATCAATTGGATCAATTTAGAACCCGCATTTACCATCCAAACCCTTTGATCAATTGGTACGTGGTCTGGATCTGCACCTATT
F_P40N2 AGTATGGGGCAATATTGTTTGGGCTCATTCAAGTTCARAGGACTTAATCAATTGGATCAATTTAGAACCCGCATTTACCATCCAAACCCTTTGATCAATTGGTACGTGGTCTGGATCTGCACCTATT
Consensus AGTATGGGGCAATATTGTTTGGGCTCATTCAAGTTCARAGGACTTAATCAATTGGATCAATTTAGAACCCGCATTTACCATCCAAACCCTTTGATCAATTGGTACGTGGTCTGGATCTGCACCTATT

391 400 410 420 430 440 450 460 470 480 490 500 510 520
F_P18N CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAACTCAAGTTCAAAACACGCGATTCCAGTCACTTATCTGATCCATATCTCCGTGAATGGATCAGCCTGATAACA
F_P54N1 CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAACTCAAGTTCAAAACACGCGATTCCAGTCACTTATCTGATCCATATCTCCGTGAATGGATCAGCCTGATAACA
F_P40N1 CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAACTCAAGTTCAAAACACGCGATTCCAGTCACTTATCTGATCCATATCTCCGTGAATGGATCAGCCTGATAACA
F_P54N2 CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAACTCAAGTTCAAAACACGCGATTCCAGTCACTTATCTGATCCATATCTCCGTGAATGGATCAGCCTGATAACA
F_P40N2 CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAACTCAAGTTCAAAACACGCGATTCCAGTCACTTATCTGATCCATATCTCCGTGAATGGATCAGCCTGATAACA
Consensus CTACCTGGTAAACAGCCTGTTATCTTGTACACTGGAAATAGTAGATGCCAACCAACTCAAGTTCAAAACACGCGATTCCAGTCACTTATCTGATCCATATCTCCGTGAATGGATCAGCCTGATAACA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
F_P18N ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAAAATTCGTGATCCAAACACCGCATGGATGGGTAAAGATGGACATTGGAGATTGTGATGGGAGTTTGGAGAACATAGTAGGGGTTTAGC
F_P54N1 ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAAAATTCGTGATCCAAACACCGCATGGATGGGTAAAGATGGACATTGGAGATTGTGATGGGAGTTTGGAGAACATAGTAGGGGTTTAGC
F_P40N1 ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAAAATTCGTGATCCAAACACCGCATGGATGGGTAAAGATGGACATTGGAGATTGTGATGGGAGTTTGGAGAACATAGTAGGGGTTTAGC
F_P54N2 ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAAAATTCGTGATCCAAACACCGCATGGATGGGTAAAGATGGACATTGGAGATTGTGATGGGAGTTTGGAGAACATAGTAGGGGTTTAGC
F_P40N2 ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAAAATTCGTGATCCAAACACCGCATGGATGGGTAAAGATGGACATTGGAGATTGTGATGGGAGTTTGGAGAACATAGTAGGGGTTTAGC
Consensus ATCCATTGATTGTAGCTGATGCTAGCATCAACAGACCAAAATTCGTGATCCAAACACCGCATGGATGGGTAAAGATGGACATTGGAGATTGTGATGGGAGTTTGGAGAACATAGTAGGGGTTTAGC

651 660 670 680 690 700 710 720 730 740 750 760 770 780
F_P18N TATAATGTATAGAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCAACTAATGGTACTGGAAACCTGGGAATGTCTGATTTTTTCCCTGTTGCATTGAAAGGAACTAATGGGATA
F_P54N1 TATAATGTATAGAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCAACTAATGGTACTGGAAACCTGGGAATGTCTGATTTTTTCCCTGTTGCATTGAAAGGAACTAATGGGATA
F_P40N1 TATAATGTATAGAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCAACTAATGGTACTGGAAACCTGGGAATGTCTGATTTTTTCCCTGTTGCATTGAAAGGAACTAATGGGATA
F_P54N2 TATAATGTATAGAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCAACTAATGGTACTGGAAACCTGGGAATGTCTGATTTTTTCCCTGTTGCATTGAAAGGAACTAATGGGATA
F_P40N2 TATAATGTATAGAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCAACTAATGGTACTGGAAACCTGGGAATGTCTGATTTTTTCCCTGTTGCATTGAAAGGAACTAATGGGATA
Consensus TATAATGTATAGAAGCAAGATTTTCATGAATGGGTTAAGGCTAAACACCCACTTCACTCAACTAATGGTACTGGAAACCTGGGAATGTCTGATTTTTTCCCTGTTGCATTGAAAGGAACTAATGGGATA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
F_P18N GATCAATATGTTGAGGAAATATAAATATGTGCTTAAGAAATAGTAGGATCTTACTCGATTGAGTACTATACTCTTGGTAAAGTACGATACAAAAGGATAGGTATGTTCCAGATGTTGGTCTATTGATA
F_P54N1 GATCAATATGTTGAGGAAATATAAATATGTGCTTAAGAAATAGTAGGATCTTACTCGATTGAGTACTATACTCTTGGTAAAGTACGATACAAAAGGATAGGTATGTTCCAGATGTTGGTCTATTGATA
F_P40N1 GATCAATATGTTGAGGAAATATAAATATGTGCTTAAGAAATAGTAGGATCTTACTCGATTGAGTACTATACTCTTGGTAAAGTACGATACAAAAGGATAGGTATGTTCCAGATGTTGGTCTATTGATA
F_P54N2 GATCAATATGTTGAGGAAATATAAATATGTGCTTAAGAAATAGTAGGATCTTACTCGATTGAGTACTATACTCTTGGTAAAGTACGATACAAAAGGATAGGTATGTTCCAGATGTTGGTCTATTGATA
F_P40N2 GATCAATATGTTGAGGAAATATAAATATGTGCTTAAGAAATAGTAGGATCTTACTCGATTGAGTACTATACTCTTGGTAAAGTACGATACAAAAGGATAGGTATGTTCCAGATGTTGGTCTATTGATA
Consensus GATCAATATGTTGAGGAAATATAAATATGTGCTTAAGAAATAGTAGGATCTTACTCGATTGAGTACTATACTCTTGGTAAAGTACGATACAAAAGGATAGGTATGTTCCAGATGTTGGTCTATTGATA

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
F_P18N GTTGGAGGGGATTGAGATTCGATTATGGTAAATTTCTATGCATCAAGACCTTCTATGATAC TAGCAAAAACCGAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC
F_P54N1 GTTGGAGGGGATTGAGATTCGATTATGGTAAATTTCTATGCATCAAGACCTTCTATGATAC TAGCAAAAACCGAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC
F_P40N1 GTTGGAGGGGATTGAGATTCGATTATGGTAAATTTCTATGCATCAAGACCTTCTATGATAC TAGCAAAAACCGAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC
F_P54N2 GTTGGAGGGGATTGAGATTCGATTATGGTAAATTTCTATGCATCAAGACCTTCTATGATAC TAGCAAAAACCGAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC
F_P40N2 GTTGGAGGGGATTGAGATTCGATTATGGTAAATTTCTATGCATCAAGACCTTCTATGATAC TAGCAAAAACCGAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC
Consensus GTTGGAGGGGATTGAGATTCGATTATGGTAAATTTCTATGCATCAAGACCTTCTATGATAC TAGCAAAAACCGAGGGTTATTTGGGGTTGGTCTAATGAATCAGATATATCCCTGAGGATGATAATGC

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
F_P18N GAARGGATGGGCTGGAAATTCATTTGATCCACGTAAGTATGGCTTGTCCAAAGTGGTAAACAATGGTTCARTGGCCTGTTGAGGAAATAGAAACCTAGAACCCAAAGGTTCAATTTGAGTAAACAA
F_P54N1 GAARGGATGGGCTGGAAATTCATTTGATCCACGTAAGTATGGCTTGTCCAAAGTGGTAAACAATGGTTCARTGGCCTGTTGAGGAAATAGAAACCTAGAACCCAAAGGTTCAATTTGAGTAAACAA
F_P40N1 GAARGGATGGGCTGGAAATTCATTTGATCCACGTAAGTATGGCTTGTCCAAAGTGGTAAACAATGGTTCARTGGCCTGTTGAGGAAATAGAAACCTAGAACCCAAAGGTTCAATTTGAGTAAACAA
F_P54N2 GAARGGATGGGCTGGAAATTCATTTGATCCACGTAAGTATGGCTTGTCCAAAGTGGTAAACAATGGTTCARTGGCCTGTTGAGGAAATAGAAACCTAGAACCCAAAGGTTCAATTTGAGTAAACAA
F_P40N2 GAARGGATGGGCTGGAAATTCATTTGATCCACGTAAGTATGGCTTGTCCAAAGTGGTAAACAATGGTTCARTGGCCTGTTGAGGAAATAGAAACCTAGAACCCAAAGGTTCAATTTGAGTAAACAA
Consensus GAARGGATGGGCTGGAAATTCATTTGATCCACGTAAGTATGGCTTGTCCAAAGTGGTAAACAATGGTTCARTGGCCTGTTGAGGAAATAGAAACCTAGAACCCAAAGGTTCAATTTGAGTAAACAA

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
F_P18N AAGTTGACCAATGGTGAARAGGTTGAGGTTACAGGAAATCACACCTGCACAGGCAAGATGTTGAGGTGACATTTCCTTTTGCAGTTTGGATAAGGCAGAGTCAATTTGATTCTAGTTGGACTGATATGTATG
F_P54N1 AAGTTGACCAATGGTGAARAGGTTGAGGTTACAGGAAATCACACCTGCACAGGCAAGATGTTGAGGTGACATTTCCTTTTGCAGTTTGGATAAGGCAGAGTCAATTTGATTCTAGTTGGACTGATATGTATG
F_P40N1 AAGTTGACCAATGGTGAARAGGTTGAGGTTACAGGAAATCACACCTGCACAGGCAAGATGTTGAGGTGACATTTCCTTTTGCAGTTTGGATAAGGCAGAGTCAATTTGATTCTAGTTGGACTGATATGTATG
F_P54N2 AAGTTGACCAATGGTGAARAGGTTGAGGTTACAGGAAATCACACCTGCACAGGCAAGATGTTGAGGTGACATTTCCTTTTGCAGTTTGGATAAGGCAGAGTCAATTTGATTCTAGTTGGACTGATATGTATG
F_P40N2 AAGTTGACCAATGGTGAARAGGTTGAGGTTACAGGAAATCACACCTGCACAGGCAAGATGTTGAGGTGACATTTCCTTTTGCAGTTTGGATAAGGCAGAGTCAATTTGATTCTAGTTGGACTGATATGTATG
Consensus AAGTTGACCAATGGTGAARAGGTTGAGGTTACAGGAAATCACACCTGCACAGGCAAGATGTTGAGGTGACATTTCCTTTTGCAGTTTGGATAAGGCAGAGTCAATTTGATTCTAGTTGGACTGATATGTATG

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
F_P18N CACAGATGTTTGTGGACTCAGGGGTGCAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACCTTGGAGAAACACACCTGTTTTCTTCCGAGTTTTCAAGCACACCA
F_P54N1 CACAGATGTTTGTGGACTCAGGGGTGCAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACCTTGGAGAAACACACCTGTTTTCTTCCGAGTTTTCAAGCACACCA
F_P40N1 CACAGATGTTTGTGGACTCAGGGGTGCAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACCTTGGAGAAACACACCTGTTTTCTTCCGAGTTTTCAAGCACACCA
F_P54N2 CACAGATGTTTGTGGACTCAGGGGTGCAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACCTTGGAGAAACACACCTGTTTTCTTCCGAGTTTTCAAGCACACCA
F_P40N2 CACAGATGTTTGTGGACTCAGGGGTGCAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACCTTGGAGAAACACACCTGTTTTCTTCCGAGTTTTCAAGCACACCA
Consensus CACAGATGTTTGTGGACTCAGGGGTGCAGATGTACAGGTTGGGCTTGGGCCATTTGGTCTTGCTACATTGGCTACTGAAACCTTGGAGAAACACACCTGTTTTCTTCCGAGTTTTCAAGCACACCA

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
F_P18N AAATTACAGGTTCTTTTGTGCTCTGACGCTAAAGGTCACCTTAAAGTTCATGAACCAATGTACAAAGTTTCATTTGCTGGATTGTGGATGTTGATTTGGCTGACAGAAATGTCACCTCAGAGC
F_P54N1 AAATTACAGGTTCTTTTGTGCTCTGACGCTAAAGGTCACCTTAAAGTTCATGAACCAATGTACAAAGTTTCATTTGCTGGATTGTGGATGTTGATTTGGCTGACAGAAATGTCACCTCAGAGC
F_P40N1 AAATTACAGGTTCTTTTGTGCTCTGACGCTAAAGGTCACCTTAAAGTTCATGAACCAATGTACAAAGTTTCATTTGCTGGATTGTGGATGTTGATTTGGCTGACAGAAATGTCACCTCAGAGC
F_P54N2 AAATTACAGGTTCTTTTGTGCTCTGACGCTAAAGGTCACCTTAAAGTTCATGAACCAATGTACAAAGTTTCATTTGCTGGATTGTGGATGTTGATTTGGCTGACAGAAATGTCACCTCAGAGC
F_P40N2 AAATTACAGGTTCTTTTGTGCTCTGACGCTAAAGGTCACCTTAAAGTTCATGAACCAATGTACAAAGTTTCATTTGCTGGATTGTGGATGTTGATTTGGCTGACAGAAATGTCACCTCAGAGC
Consensus AAATTACAGGTTCTTTTGTGCTCTGACGCTAAAGGTCACCTTAAAGTTCATGAACCAATGTACAAAGTTTCATTTGCTGGATTGTGGATGTTGATTTGGCTGACAGAAATGTCACCTCAGAGC

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
F_P18N TTGATTGATTAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTTATCCACATTTGGCGATTAAATGAGAGGCCACATTTATTTGGCTTCACCAATGGAACTGAGCCAA
F_P54N1 TTGATTGATTAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTTATCCACATTTGGCGATTAAATGAGAGGCCACATTTATTTGGCTTCACCAATGGAACTGAGCCAA
F_P40N1 TTGATTGATTAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTTATCCACATTTGGCGATTAAATGAGAGGCCACATTTATTTGGCTTCACCAATGGAACTGAGCCAA
F_P54N2 TTGATTGATTAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTTATCCACATTTGGCGATTAAATGAGAGGCCACATTTATTTGGCTTCACCAATGGAACTGAGCCAA
F_P40N2 TTGATTGATTAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTTATCCACATTTGGCGATTAAATGAGAGGCCACATTTATTTGGCTTCACCAATGGAACTGAGCCAA
Consensus TTGATTGATTAATTCAGTTATAGAAGTTTTGGTGTGGTGGAAAGACATGTATACATCGAGGGTTTATCCACATTTGGCGATTAAATGAGAGGCCACATTTATTTGGCTTCACCAATGGAACTGAGCCAA

1691 1700 1710 1720 1730 1740 1746
F_P18N TCACAATTTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACATATTGA
F_P54N1 TCACAATTTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACATATTGA
F_P40N1 TCACAATTTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACATATTGA
F_P54N2 TCACAATTTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACATATTGA
F_P40N2 TCACAATTTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACATATTGA
Consensus TCACAATTTGAGACTTTGGATGCATGGAGTATGGGCAAGCTAAGATACATATTGA

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
E_SA	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_SN4	MELFMKSSSLWGLEIYLF CFFIVLSNINKVFGSHNVFLDLQSSSAISVKNVHRTGFHFQPPKHWINDPNAPHMYNGVYHLFYQYNPKGSVWGMNIYWAHSVSKDLINWIHLEPAIYPSKFDKYGAWSGSA													
Consensus	M#.%.kSnSIWaleiilfCFFiVlIninkVfaSHkVfidLQsqnAinVhnVHRTG%HFQPeKHWINDPNAPHY%NGVYHLFYQYNPkGSVWGMNIYWAHSVSKDLINWIhLEPAIYPSKkFDk%GaWSGSA													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
E_SA	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_SN4	TILPNMKPVILYTGIVDANQTQVQNYAVPANI SDPYLREWIKPDNNPLIYADASINKTKFRDPTTAWMGKDGHWRIYVIGSLRKH-RGMALLYRSRDFIKHWYKAKHPLHSTNGTGNWECDFFPVALKGTN													
Consensus	TILPgNKPVILYTGIVDah#TQVQNYAiPANI SDP%LRWiKP#NPNLIiaDaSINKTKFRDPTTAWMGkDghWRIYiGS\$RKH.RG\$Ai\$YRSkDFiKHWYKakHPLHSngTGNWECDFFPVaLkgTN													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
E_SA	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_SN4	GLDASYRGKNVYKYLKNSLDVNRFDYITIGMYDTRKDRYIPDNN SIDGCKGLRLDYGNFYASKSFYDPTKNRRIYVWGMTNESDVL PDEIKKGWAGIQAIPRKVWLDHSGKQLIQWPIEELETLRKQKIQ													
Consensus	GldA.Y.Ge#hKYVLKNS\$DlnRF#YyTiGkYDTkKDRYIPDngSIDgcKGLRfDYGNFYASKSFYDp\$KNRRiVWGMTNESDifP#D#iAKGWAGIQAIPRKVWLDhSGKQLIQWpiEELETLRkQkiQ													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
E_SA	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
F_SN4	LNNKKLSKGEFVKGISASQSDIEVSFSFSSLNKAEQFDPNWAADLYAQDVC AIKGSTIQGGLGPFGLATLASKNLEEYTPVFFRVFKAQKNYKVLCSDAKRSTLRQNEAMYKPSFAGFYDYDLAD-KK													
Consensus	LnNKKLnkGEkfEvkGI\$aaQaDiEv\$FSFaSL#KAEqFDPnWaD\$YAQDVCaiKGadiQGGLGPFGLATLaseNLEENTPVFFRVFKAQkNYKVL\$CDAkrST\$kfNEaMYkpsFAG%YDYDLad.KK													
	521	530	540	550	560	570	580	586						
E_SA	-----+-----+-----+-----+-----+-----+-----+-----													
F_SN4	LSLRSLIDNSVIESFGAGGKTCITSRYPTLAIHDKAHLFAFNNGTEPITIETLDAMSHGKAKIQY													
Consensus	LSLRSLIDNSViESFGAGGKTCITSRYPTLAIhdkAHLfAFNNGsEpITIETL#AMSHdkaKih.													

Appendix 3.3

A sequence was defined as an allele when it was found twice in two independent PCRs. Additionally, the consensus sequence of all alleles found in one genotype was used for allele definition when variable sequence polymorphisms occurred.

In the following Table, *pCD111* and *pCD141* alleles are listed, which do not correspond to this definition because only one full-length sequence was obtained. Nevertheless, for some of the listed alleles independent PCR amplifications gave rise to sequences that after partial sequencing indicated the existence of the alleles as mentioned above, clearly implying their existence. These additional sequences exhibited frame shifts or other modifications (e.g. missing or modified start and stop codons) and, therefore, were not completely sequenced. Cloning of *pCD111* and *pCD141* alleles was carried out in addition to *Pain-1*, *invGE*, and *invGF* allele cloning, which were in the focus of the present study.

Table A3.3.1: Overview of fully and partially sequenced *pCD111* and *pCD141* alleles.

Gene	Full-length allele	Additional partial sequences
<i>pCD111</i>	<i>pCD111_S3</i>	No additional sequences
	<i>pCD111_D1</i>	No additional sequences
	<i>pCD111_T1</i>	No additional sequences
	<i>pCD111_P40_1</i>	2
	<i>pCD111_P54_2</i>	No additional sequences
<i>pCD141</i>	<i>pCD141_S1</i>	2
	<i>pCD141_D2</i>	No additional sequences
	<i>pCD141_T3</i>	No additional sequences

1 10 20 30 40 50 60 70 80 90 100 110 120 130
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD111_S1 MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPPK**N**WINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSVSTDLINWIPLEPGIYPSEVFDKYGTWSGSAT
CD111_S1_1 MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPPK**N**WINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSVSTDLINWIPLEPGIYPSEVFDKYGTWSGSAT
CD111_S3 MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPPK**N**WINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSVSTDLINWIPLEPGIYPSEVFDKYGTWSGSAT
CD111_S2 MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPP**N**WINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSVSTDLINWIPLEPGIYPSEVFDKYGTWSGSAT
CD111_S2_1 MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPP**N**WINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSVSTDLINWIPLEPGIYPSEVFDKYGTWSGSAT
Consensus MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPP**k**NWINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSVSTDLINWIPLEPGIYPSEVFDKYGTWSGSAT

131 140 150 160 170 180 190 200 210 220 230 240 250 260
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD111_S1 ILPNNKPIILYTGIVDAKNTQVQNYAIPANISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSY**H**GKQGLAILYK**S**K**N**FMKWT**K**IQ**H**PLHSVDGTGNWECPDFFPVLLHGTNGL
CD111_S1_1 ILPNNKPIILYTGIVDAKNTQVQNYAIPANISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSY**H**GKQGLAILYK**S**K**N**FMKWT**K**IQ**H**PLHSVDGTGNWECPDFFPVLLHGTNGL
CD111_S3 ILPNNKPIILYTGIVDAKNTQVQNYAIPADISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSY**H**GKQGLAILYK**S**K**N**FMKWT**K**IQ**H**PLHSVDGTGNWECPDFFPVLLHGTNGL
CD111_S2 ILPNNKPIILYTGIVDAKNTQVQNYAIPADISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSY**H**EKKGLAILYK**S**R**D**FMKWT**K**V**D**PLHSVDGTGNWECPDFFPVLLHGTNGL
CD111_S2_1 ILPNNKPIILYTGIVDAKNTQVQNYAIPADISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSY**H**EKKGLAILYK**S**R**D**FMKWT**K**V**D**PLHSVDGTGNWECPDFFPVLLHGTNGL
Consensus ILPNNKPIILYTGIVDAKNTQVQNYAIP**A**#ISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSY**H**g**k**qGLAILYK**s****k**#**F**MKWT**K**!**q**HPLHSVDGTGNWECPDFFPVLLHGTNGL

261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD111_S1 DASYNKKNIK**H**ALKVS LDVTRFEYTYVGIYDTKKDRYIPDKTSIDG**W**KGLRLDYGNYYASKSFYD**P**SKNRRIMHG**W**ANESDTYNDDYKKG**W**AGIQTIPRKL**W**LDPSGKQLVQWPVEELET**L**REQVQLSN
CD111_S1_1 DASYNKKNIK**H**ALKVS LDVTRFEYTYVGIYDTKKDRYIPDKTSIDG**W**KGLRLDYGNYYASKSFYD**P**SKNRRIMHG**W**ANESDTYNDDYKKG**W**AGIQTIPRKL**W**LDPSGKQLVQWPVEELET**L**REQVQLSN
CD111_S3 DASYNKKNIK**H**V**L**KVS LDVTRFEYTYVGIYDTKKDRYIPDKTSIDG**W**KGLRLDYGNYYASKSFYD**P**SKNRRIMHG**W**ANESDTYNDDI**K**KG**W**AGIQTIPRKL**W**LDPSGKQLVQWPVEELET**F**REQVQLSN
CD111_S2 DASYNKKNIK**H**ALKVS LDVTRFEYTYV**G**KYDTKKDRYIPDKTSIDG**L**NGLRLDYGNYYASKSFYD**L**RKNRRIMHG**W**ANESDTYNDDYKKG**W**AGIQTIPRKL**W**LDPSGKQLVQWPVEELET**L**REQVQLSN
CD111_S2_1 DASYNKKNIK**H**ALKVS LDVTRFEYTYV**G**KYDTKKDRYIPDKTSIDG**L**NGLRLDYGNYYASKSFYD**L**RKNRRIMHG**W**ANESDTYNDDYKKG**W**AGIQTIPRKL**W**LDPSGKQLVQWPVEELET**L**REQVQLSN
Consensus DASYNKKNIK**H**a**L**KVS LDVTRFEYTYV**g**iYDTKKDRYIPDKTSIDG**w**kGLRLDYGNYYASKSFYD**p**sKNRRIMHG**W**ANESDTYNDD!**k**KG**W**AGIQTIPRKL**W**LDPSGKQLVQWPVEELET**l**REQVQLSN

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD111_S1 RKLKKGDKIEVKGITPAQADVEYTFSSLDKAEFPD**N**W**D**NLYAQDYCAIKGSTVQGG**L**GPFG**L**TLASQ**N**LEEYTPVFFRV**F**K**A**QDKYK**V**L**H**CS**D**ASR**S**T**L**K**N**D**K**THY**K**PS**F**AGY**V**D**V**D**L**IN**K**T**L**SLR
CD111_S1_1 RKLKKGDKIEVKGITPAQADVEYTFSSLDKAEFPD**N**W**D**NLYAQDYCAIKGSTVQGG**L**GPFG**L**TLASQ**N**LEEYTPVFFRV**F**K**A**QDKYK**V**L**H**CS**D**ASR**S**T**L**K**N**D**K**THY**K**PS**F**AGY**V**D**V**D**L**IN**K**T**L**SLR
CD111_S3 RKL**N**KGDKIEVKGITPAQADVEYTFSSLDKAEFPD**S**W**A**NLYAQDYCAIKGSTVQGG**L**GPFG**L**TLASQ**N**LEEYTPVFFRV**F**K**T**QDKYK**V**L**H**CS**D**ASR**S**T**L**K**N**D**K**THY**K**PS**F**AGY**V**D**V**D**L**T**N**K**T**L**S**L**R**
CD111_S2 RKL**N**KGDKIEVKGITPAQADVEYTFSSLDKAEFPD**N**W**A**NLYAQDYCAIKGSTVQGG**L**GPFG**L**TLASQ**N**LEEYTPVFFRV**F**K**A**Q**N**KYK**V**L**H**CS**D**ASR**S**T**L**K**N**D**K**THY**K**PS**F**AGY**V**D**V**D**L**T**N**K**T**L**S**L**R**
CD111_S2_1 RKL**N**KGDKIEVKGITPAQADVEYTFSSLDKAEFPD**N**W**A**NLYAQDYCAIKGSTVQGG**L**GPFG**L**TLASQ**N**LEEYTPVFFRV**F**K**A**Q**N**KYK**V**L**H**CS**D**ASR**S**T**L**K**N**D**K**THY**K**PS**F**AGY**V**D**V**D**L**T**N**K**T**L**S**L**R**
Consensus RKL**n**KGDKIEVKGITPAQADVEYTFSSLDKAEFPD**n**W**a**nLYAQDYCAIKGSTVQGG**L**GPFG**L**TLASQ**N**LEEYTPVFFRV**F**K**a**q**#**KYK**V**L**H**CS**D**ASR**S**T**L**K**N**D**K**THY**K**PS**F**AGY**V**D**V**D**l**T**n**K**T**L**S**L**R**

521 530 540 550 560 570 580 589
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD111_S1 SLIDHSV**V**ES**F**GGAG**G**K**T**C**I**S**R**Y**P**T**L**A**I**Y**D**NA**H**L**F**V**F**N**G**T**E**T**I**K**I**K**S**L**N**A**W**T**H**G**K**P**K**M**N**S**F**G**H**S**S****Y**
CD111_S1_1 SLIDHSV**V**ES**F**GGAG**G**K**T**C**I**S**R**Y**P**T**L**A**I**Y**D**NA**H**L**F**V**F**N**G**T**E**T**I**K**I**K**S**L**N**A**W**T**H**G**K**P**K**M**N**S**F**G**H**S**N****H**
CD111_S3 SLIDHSV**V**ES**F**GGAG**G**K**T**C**I**S**R**Y**P**T**L**A**I**Y**D**NA**H**L**F**V**F**N**G**T**E**T**I**K**I**K**S**L**N**A**W**T**H**G**K**P**K**M**N**S**F**G**H**S**S****Y**
CD111_S2 SLIDHSV**V**ES**F**GGAG**G**K**T**C**I**S**R**Y**P**T**L**A**I**Y**D**NA**H**L**F**V**F**N**G**T**E**T**I**K**I**K**S**L**N**A**W**T**H**G**K**P**K**M**N**S**F**G**H**S**S****Y**
CD111_S2_1 SLIDHSV**V**ES**F**GGAG**G**K**T**C**I**S**R**Y**P**T**L**A**I**Y**D**NA**H**L**F**V**F**N**G**T**E**T**I**K**I**K**S**L**N**A**W**T**H**G**K**P**K**M**N**S**F**G**H**S**S****Y**
Consensus SLIDHSV**V**ES**F**GGAG**G**K**T**C**I**S**R**Y**P**T**L**A**I**Y**D**NA**H**L**F**V**F**N**G**T**E**T**I**K**I**K**S**L**N**A**W**T**H**G**K**P**K**M**N**S**F**G**H**S**sy**

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
CD111_T1	MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPPQNWINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSYSTDLINWIPLLEPGIYPSEVFDKYGTWSGSAT													
CD111_T2	MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPPNMWINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSYSTDLINWIPLLEPGIYPSEVFDKYGTWSGSAT													
CD111_T2_2	MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPPNMWINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSYSTDLINWIPLLEPGIYPSEVFDKYGTWSGSAT													
CD111_T2_1	MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPPNMWINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSYSTDLINWIPLLEPGIYPSEVFDKYGTWSGSAT													
Consensus	MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPP#NWINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSYSTDLINWIPLLEPGIYPSEVFDKYGTWSGSAT													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
CD111_T1	ILPNKPIILYTGIVDAKNTQVQNYAIPADISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSYWgkqglailyksknlhkwtkiqhplhsydggtgnwecpddfpyllhgtngl													
CD111_T2	ILPNKPIILYTGIVDAKNTQVQNYAIPADISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSYWekkglaailyksrdfmkwtkyqdpplhsydggtgnwecpddfpyllhgtngl													
CD111_T2_2	ILPNKPIILYTGIVDAKNTQVQNYAIPADISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSYWekkglaailyksrdfmkwtkyqdpplhsydggtgnwecpddfpyllhgtngl													
CD111_T2_1	ILPNKPIILYTGIVDAKNTQVQNYAIPADISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSYWekkglaailyksrdfmkwtkyqdpplhsydggtgnwecpddfpyllhgtngl													
Consensus	ILPNKPIILYTGIVDAKNTQVQNYAIPADISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSYWeKkGLAILYKSr#fMKWTk!QdPLHSYDGTGNWECPDFFPVLLHGTNGL													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
CD111_T1	DASYNKKNIKHVlKVSldvtrfeyytvgqydtkkdryipdktsidgwnkglrldygnyyasksfydlrknrrimhgwanesdtynddykkgwagiqtiprklwldpsgkqlvqmpveeletlreqkvqlsn													
CD111_T2	DASYNKKNIKHAlKVSldvtrfeyytvgkydtkkdryipdktsidgwnGLRLDYGNYYASKSFYDLRknrrimhgwanesdtynddykkgwagiqtiprklwldpsgkqlvqmpveeletlreqkvqlsn													
CD111_T2_2	DASYNKKNIKHAlKVSldvtrfeyytvgkydtkkdryipdktsidgwnGLRLDYGNYYASKSFYDLRknrrimhgwanesdtynddykkgwagiqtiprklwldpsgkqlvqmpveeletlreqkvqlsn													
CD111_T2_1	DASYNKKNIKHAlKVSldvtrfeyytvgkydtkkdryipdktsidgwnGLRLDYGNYYASKSFYDLRknrrimhgwanesdtynddykkgwagiqtiprklwldpsgkqlvqmpveeletlreqkvqlsn													
Consensus	DASYNKKNIKHAlKVSldvtrfeyytvgkydtkkdryipdktsidgwnGLRLDYGNYYASKSFYDLrknrrimhgwanesdtynddykkgwagiqtiprklwldpsgkqlvqmpveeletlreqkvqlsn													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
CD111_T1	RKLNKGDKIEVKGITPAQADYEYTFsFTSLDKAETFDPNWAnLYAQDYCAIKGStVQGGGLGPFGLLTLASQNLEEYTPVFFRYFKAQNKYKVLHCSDASRSTLKNdkThyKPSFAGYyDvDLtnKtLSLr													
CD111_T2	RKLNKGDKIEVKGITPAQADYEYTFsSSLDKAEPFDPNWAnLYAQDYCAIKGStVQGGGLGPFGLLTLASQNLEEYTPVFFRYFKAQNKYKVLHCSDASRSTLKNdkThyKPSFAGYyDvDLtnKtLSLr													
CD111_T2_2	RKLNKGDKIEVKGITPAQADYEYTFsSSLDKAEPFDPNWAnLYAQDYCAIKGStVQGGGLGPFGLLTLASQNLEEYTPVFFRYFKAQNKYKVLHCSDASRSTLKNdkThyKPSFAGYyDvDLtnKtLSLr													
CD111_T2_1	RKLNKGDKIEVKGITPAQADYEYTFsSSLDKAEPFDPNWAnLYAQDYCAIKGStVQGGGLGPFGLLTLASQNLEEYTPVFFRYFKAQNKYKVLHCSDASRSTLKNdkThyKPSFAGYyDvDLtnKtLSLr													
Consensus	RKLNKGDKIEVKGITPAQADYEYTFsSSLDKAEPFDPNWAnLYAQDYCAIKGStVQGGGLGPFGLLTLASQNLEEYTPVFFRYFKAQ#KYKVLHCSDASRSTLKNdkThyKPSFAGYyDvDLtnKtLSLr													
	521	530	540	550	560	570	580	589						
	-----+-----+-----+-----+-----+-----+-----+-----													
CD111_T1	SLIDHSVYESFGAGGKTCITSRVYPTLAIYDNaHlFvFNNGTETIKIKSLNawThGKPKMnWSFGHSSY													
CD111_T2	SLIDHSVYESFGAGGKTCITSRVYPTLAIYDNaHlFvFNNGTETIKIKSLNawThGKPKMnWSFGHSSY													
CD111_T2_2	SLIDHSVYESFGAGGKTCITSRVYPTLAIYDNaHlFvFNNGTETIKIKSLNawThGKPKMnWSFGHSSY													
CD111_T2_1	SLIDHSVYESFGAGGKTRITSRVYPTLAIYDNaHlFvFNNGTETIKIKSLNawThGKPKMnWSFGHSSY													
Consensus	SLIDHSVYESFGAGGKTCITSRVYPTLAIYDNaHlFvFNNGTETIKIKSLNawThGKPKMnWSFGHSSY													

1 10 20 30 40 50 60 70 80 90 100 110 120 130
|-----|
CD111_S1 ATGGATTGTTAAAAAGTCTTCTCTTTTTCTTTGCCAATTTTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTAAATGCTTCACACAAGTTTTTCCAGGGTTGCAATCTACAAGCA
CD111_S1_1 ATGGATTGTTAAAAAGTCTTCTCTTTTTCTTTGCCAATTTTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTAAATGCTTCACACAAGTTTTTCCAGGGTTGCAATCTACAAGCA
Consensus ATGGATTGTTAAAAAGTCTTCTCTTTTTCTTTGCCAATTTTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTAAATGCTTCACACAAGTTTTTCCAGGGTTGCAATCTACAAGCA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
|-----|
CD111_S1 CGGTTGATGTGAAAATGTTTCATAGAACTGGTTATCATTTCACCTCTAAAACCTGGATCAATGATCCCAATGCCCAATGTAATAATGGTGTCTATCATCTATTCTACCAATACATCCATATGG
CD111_S1_1 CGGTTGATGTGAAAATGTTTCATAGAACTGGTTATCATTTCACCTCTAAAACCTGGATCAATGATCCCAATGCCCAATGTAATAATGGTGTCTATCATCTATTCTACCAATACATCCATATGG
Consensus CGGTTGATGTGAAAATGTTTCATAGAACTGGTTATCATTTCACCTCTAAAACCTGGATCAATGATCCCAATGCCCAATGTAATAATGGTGTCTATCATCTATTCTACCAATACATCCATATGG

261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----|
CD111_S1 ATCAGTTTGGGGAATATTGTTGGGCCATTGATTTCAACCGACTTGATTAATTGGATCCCACTTGAGCCCGGAATCTATCCATCCGAGTATTGACAATATGGTACATGGTCCGGGTCAGCCACA
CD111_S1_1 ATCAGTTTGGGGAATATTGTTGGGCCATTGATTTCAACCGACTTGATTAATTGGATCCCACTTGAGCCCGGAATCTATCCATCCGAGTATTGACAATATGGTACATGGTCCGGGTCAGCCACA
Consensus ATCAGTTTGGGGAATATTGTTGGGCCATTGATTTCAACCGACTTGATTAATTGGATCCCACTTGAGCCCGGAATCTATCCATCCGAGTATTGACAATATGGTACATGGTCCGGGTCAGCCACA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----|
CD111_S1 ATCTTACCAACAACAAGCCATTATCCTCTACACCGGAATCGTCGATGCAAAAAATACCCAAGTCCAAAACCTATGCAATCCCAGCCAACATATCCGATCCATTTCTTCGTAATGGATCAAGCCCGATA
CD111_S1_1 ATCTTACCAACAACAAGCCATTATCCTCTACACCGGAATCGTCGATGCAAAAAATACCCAAGTCCAAAACCTATGCAATCCCAGCCAACATATCCGATCCATTTCTTCGTAATGGATCAAGCCCGATA
Consensus ATCTTACCAACAACAAGCCATTATCCTCTACACCGGAATCGTCGATGCAAAAAATACCCAAGTCCAAAACCTATGCAATCCCAGCCAACATATCCGATCCATTTCTTCGTAATGGATCAAGCCCGATA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
|-----|
CD111_S1 ACAACCCATTAAATTGTCGCTGACGTTAGCATTAAACAAGACCCAAATCCGTGACCCAAACCACATGTTGGTTGGGTGAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAACAGGATTGGC
CD111_S1_1 ACAACCCATTAAATTGTCGCTGACGTTAGCATTAAACAAGACCCAAATCCGTGACCCAAACCACATGTTGGTTGGGTGAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAACAGGATTGGC
Consensus ACAACCCATTAAATTGTCGCTGACGTTAGCATTAAACAAGACCCAAATCCGTGACCCAAACCACATGTTGGTTGGGTGAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAACAGGATTGGC

651 660 670 680 690 700 710 720 730 740 750 760 770 780
|-----|
CD111_S1 AATATTGTATAAAGTAAAAATTTTCATGAATGGACCAAGATTCAACATCCACTTCATTCAGTTGACGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTATTGCATGGTACAARTGGATTG
CD111_S1_1 AATATTGTATAAAGTAAAAATTTTCATGAATGGACCAAGATTCAACATCCACTTCATTCAGTTGACGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTATTGCATGGTACAARTGGATTG
Consensus AATATTGTATAAAGTAAAAATTTTCATGAATGGACCAAGATTCAACATCCACTTCATTCAGTTGACGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTATTGCATGGTACAARTGGATTG

781 790 800 810 820 830 840 850 860 870 880 890 900 910
|-----|
CD111_S1 GATGCCTCATACAACAAGAAAATATTAAACATGCTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATACAGTTGGTATATATGATACAAAAAAGATAGGTATATTCCAGATAAGACTTCTA
CD111_S1_1 GATGCCTCATACAACAAGAAAATATTAAACATGCTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATACAGTTGGTATATATGATACAAAAAAGATAGGTATATTCCAGATAAGACTTCTA
Consensus GATGCCTCATACAACAAGAAAATATTAAACATGCTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATACAGTTGGTATATATGATACAAAAAAGATAGGTATATTCCAGATAAGACTTCTA

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
|-----|
CD111_S1 TCGATGGTTGGAAGGGATTGAGGCTTGACTATGGTAATTATTATGCATCTAAATCATTCTATGACCCTAGCAAAAATCGAAGAATTATGTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATGT
CD111_S1_1 TCGATGGTTGGAAGGGATTGAGGCTTGACTATGGTAATTATTATGCATCTAAATCATTCTATGACCCTAGCAAAAATCGAAGAATTATGTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATGT
Consensus TCGATGGTTGGAAGGGATTGAGGCTTGACTATGGTAATTATTATGCATCTAAATCATTCTATGACCCTAGCAAAAATCGAAGAATTATGTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATGT

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
|-----|
CD111_S1 CAAGAAAGGATGGGCAGGAATTCAAACTATTCCCCGCAATTTATGGCTTGATCCTAGTGGAAGCAATTTGGTCCATGGCCTGTCGAGAATTAGAARTCTAAGAGAGCAAAAGGTGCATTAAGTAAT
CD111_S1_1 CAAGAAAGGATGGGCAGGAATTCAAACTATTCCCCGCAATTTATGGCTTGATCCTAGTGGAAGCAATTTGGTCCATGGCCTGTCGAGAATTAGAARTCTAAGAGAGCAAAAGGTGCATTAAGTAAT
Consensus CAAGAAAGGATGGGCAGGAATTCAAACTATTCCCCGCAATTTATGGCTTGATCCTAGTGGAAGCAATTTGGTCCATGGCCTGTCGAGAATTAGAARTCTAAGAGAGCAAAAGGTGCATTAAGTAAT

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
|-----|
CD111_S1 CGCAGTTAAGAAAGGAGATAAATTGAAGTTAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTTTCATTTTCAGTTTGGATAAGGCAGAGCCATTTGATCCCAATTGGGATAACCTTT
CD111_S1_1 CGCAGTTAAGAAAGGAGATAAATTGAAGTTAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTTTCATTTTCAGTTTGGATAAGGCAGAGCCATTTGATCCCAATTGGGATAACCTTT
Consensus CGCAGTTAAGAAAGGAGATAAATTGAAGTTAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTTTCATTTTCAGTTTGGATAAGGCAGAGCCATTTGATCCCAATTGGGATAACCTTT

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
|-----|
CD111_S1 ATGCTCAAGATGTATGCGCCATTAAAGGCTCAACGGTGCAGGGTGGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAATTTGGAGAAATATACGCCGGTATTTTTTCAAGATTTTTAAGGCCCA
CD111_S1_1 ATGCTCAAGATGTATGCGCCATTAAAGGCTCAACGGTGCAGGGTGGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAATTTGGAGAAATATACGCCGGTATTTTTTCAAGATTTTTAAGGCCCA
Consensus ATGCTCAAGATGTATGCGCCATTAAAGGCTCAACGGTGCAGGGTGGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAATTTGGAGAAATATACGCCGGTATTTTTTCAAGATTTTTAAGGCCCA

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
|-----|
CD111_S1 AGATAAATATAAGGTTCTCATGTGCTCTGATGCATCAAGATCAACCTCAAGAAATGATAGACATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTTAATAACAAGACATTGTCTTTAAGG
CD111_S1_1 AGATAAATATAAGGTTCTCATGTGCTCTGATGCATCAAGATCAACCTCAAGAAATGATAGACATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTTAATAACAAGACATTGTCTTTAAGG
Consensus AGATAAATATAAGGTTCTCATGTGCTCTGATGCATCAAGATCAACCTCAAGAAATGATAGACATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTTAATAACAAGACATTGTCTTTAAGG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
|-----|
CD111_S1 AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAACATGTATCACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA
CD111_S1_1 AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAACATGTATCACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA
Consensus AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAACATGTATCACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA

1691 1700 1710 1720 1730 1740 1750 1760 1770
|-----|
CD111_S1 CTATCAAATTAAGTCATTGAATGCATGGACCATGGGTAACCTAAGATGAATTGGTCATTTGGTCACTCTTCTTATTGA
CD111_S1_1 CTATCAAATTAAGTCATTGAATGCATGGACCATGGGTAACCTAAGATGAATTGGTCATTTGGTCACTCTAATCACTAG
Consensus CTATCAAATTAAGTCATTGAATGCATGGACCATGGGTAACCTAAGATGAATTGGTCATTTGGTCACTCTaaTcAcTaa

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
CD111_S2	-----													
CD111_S2_1	ATGGATTGTTAAAAAGTCTTCTCTTTTTCTTTGCCAATTTTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTAAATGCTTCACACAAGTTTTTCCAGGGTTGCAATCTACAAGCA													
Consensus	ATGGATTGTTAAAAAGTCTTCTCTTTTTCTTTGCCAATTTTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTAAATGCTTCACACAAGTTTTTCCAGGGTTGCAATCTACAAGCA													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
CD111_S2	-----													
CD111_S2_1	CGGTTGATGTGAAAATGTTTCATAGAACTGGTTATCATTTCACCTCCTAATAACTGGATCAATGATCCCAATGCCCAATGTAATAATGGTGTCTATCATCTATTCTACCAATACATCCATATGG													
Consensus	CGGTTGATGTGAAAATGTTTCATAGAACTGGTTATCATTTCACCTCCTAATAACTGGATCAATGATCCCAATGCCCAATGTAATAATGGTGTCTATCATCTATTCTACCAATACATCCATATGG													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
CD111_S2	-----													
CD111_S2_1	ATCAGTTTGGGGAATATTGTTGGGCCATTGAGTTTCAACCGACTTGATTAAATGGATCCCACTTGAGCCCGGAATCTATCCATCCGAGTATTCGACAATATGGTACATGGTCCGGGTCAGCCACA													
Consensus	ATCAGTTTGGGGAATATTGTTGGGCCATTGAGTTTCAACCGACTTGATTAAATGGATCCCACTTGAGCCCGGAATCTATCCATCCGAGTATTCGACAATATGGTACATGGTCCGGGTCAGCCACA													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
CD111_S2	-----													
CD111_S2_1	ATCTTACCAACAACAAGCCATTATCCTCTACACCGGAATCGTCGATGCAAAAAATACCCAAGTCCAAAATATGCAATCCCAGCCGACATATCCGATCCATTTCTTCGTAATGGATCAAGCCCGATA													
Consensus	ATCTTACCAACAACAAGCCATTATCCTCTACACCGGAATCGTCGATGCAAAAAATACCCAAGTCCAAAATATGCAATCCCAGCCGACATATCCGATCCATTTCTTCGTAATGGATCAAGCCCGATA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
CD111_S2	-----													
CD111_S2_1	ACAACCCATTAAATTGTCGCTGACGTTAGCATTAAACAAGACCCAAATCCGTGACCCAAACCACATGTTGGTTGGGTGAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAAAAAGGATTGGC													
Consensus	ACAACCCATTAAATTGTCGCTGACGTTAGCATTAAACAAGACCCAAATCCGTGACCCAAACCACATGTTGGTTGGGTGAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAAAAAGGATTGGC													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
CD111_S2	-----													
CD111_S2_1	AATATTGTATAAAGTAGAGATTTTCATGAATGGACCAAGGTCCAAGATCCACTTCATTCAGTTGACGGTACTGGAATTTGGGATGTCTTGATTTTTCCAGTGTATTGCATGGTACAARTGGATTA													
Consensus	AATATTGTATAAAGTAGAGATTTTCATGAATGGACCAAGGTCCAAGATCCACTTCATTCAGTTGACGGTACTGGAATTTGGGATGTCTTGATTTTTCCAGTGTATTGCATGGTACAARTGGATTA													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
CD111_S2	-----													
CD111_S2_1	GATGCCTCATACAACAAGAAAAATATTAACATGCTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATAACCGTTGGTAATATGATACAAAAAGGATAGGTATATTTCCCGATAAGACTTCTA													
Consensus	GATGCCTCATACAACAAGAAAAATATTAACATGCTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATAACCGTTGGTAATATGATACAAAAAGGATAGGTATATTTCCCGATAAGACTTCTA													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
CD111_S2	-----													
CD111_S2_1	TCGATGGTTTGAACGGATTGAGACTTGACTATGGTAATTATTATGCATCTAATCATTCTATGACCTTAGGAAAAATCGAAGAATTATGTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATGT													
Consensus	TCGATGGTTTGAACGGATTGAGACTTGACTATGGTAATTATTATGCATCTAATCATTCTATGACCTTAGGAAAAATCGAAGAATTATGTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATGT													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
CD111_S2	-----													
CD111_S2_1	CAAGAAAGGATGGGCGGGAATTCAAACTATTCCCCGCAACTATGGCTTGATCCTAGTGGAAGCAATTGGTCCATGGCCTGTCGAGAATTAGAARTCTAAGAGAGCAAAAGGTGCATTAAGTAAT													
Consensus	CAAGAAAGGATGGGCGGGAATTCAAACTATTCCCCGCAACTATGGCTTGATCCTAGTGGAAGCAATTGGTCCATGGCCTGTCGAGAATTAGAARTCTAAGAGAGCAAAAGGTGCATTAAGTAAT													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
CD111_S2	-----													
CD111_S2_1	CGCAGTTAAACAAGGAGATAAATTGAAGTTAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTCTCATTTCAGTTTGGATAAGGCAGAGCCATTTGATCCCAATTGGGCTAACCTTT													
Consensus	CGCAGTTAAACAAGGAGATAAATTGAAGTTAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTCTCATTTCAGTTTGGATAAGGCAGAGCCATTTGATCCCAATTGGGCTAACCTTT													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
CD111_S2	-----													
CD111_S2_1	ATGCTCAAGATGTATGCGCCATTAAAGGCTCAACGGTGCAGGGTGGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAATTTGGAGAGATATACACCGGTATTTTTCAGAGTTTTAAGGCCCA													
Consensus	ATGCTCAAGATGTATGCGCCATTAAAGGCTCAACGGTGCAGGGTGGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAATTTGGAGAGATATACACCGGTATTTTTCAGAGTTTTAAGGCCCA													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
CD111_S2	-----													
CD111_S2_1	AAATAAATATAAGGTTCTCATGTGCTCTGATGCATCAAGATCAACCTCAAGAAATGATAGACATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTAAACAACAAGACATTGTCTTTAAGG													
Consensus	AAATAAATATAAGGTTCTCATGTGCTCTGATGCATCAAGATCAACCTCAAGAAATGATAGACATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTAAACAACAAGACATTGTCTTTAAGG													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
CD111_S2	-----													
CD111_S2_1	AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAAACATGTATCACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA													
Consensus	AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAAACATGTATCACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA													
	1691	1700	1710	1720	1730	1740	1750	1760	1770					
CD111_S2	-----													
CD111_S2_1	CTATCAAATTAAGTCATTGAATGCATGGACCATGGGTAACCTAAGATGAATTGGTCATTTGGTCACTCTTCTTATTGA													
Consensus	CTATCAAATTAAGTCATTGAATGCATGGACCATGGGTAACCTAAGATGAATTGGTCATTTGGTCACTCTTCTTATTGA													

1 10 20 30 40 50 60 70 80 90 100 110 120 130
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_T2 ATGGATTGTTAAAAAGTCCTCTCTTTTTCTTTGCCAATTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTTAATGCTTCACACAAGTTTTCCAGGGTTGCATCTACAGCA
CD111_T2_1 ATGGATTGTTAAAAAGTCCTCTCTTTTTCTTTGCCAATTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTTAATGCTTCACACAAGTTTTCCAGGGTTGCATCTACAGCA
CD111_T2_2 ATGGATTGTTAAAAAGTCCTCTCTTTTTCTTTGCCAATTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTTAATGCTTCACACAAGTTTTCCAGGGTTGCATCTACAGCA
Consensus ATGGATTGTTAAAAAGTCCTCTCTTTTTCTTTGCCAATTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTTAATGCTTCACACAAGTTTTCCAGGGTTGCATCTACAGCA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_T2 CGTTGATGTGAAAAATGTTTCATAGAACTGGTTATCATTTCACCTCCTAATAACTGGATCAATGATCCCAATGCCCAATGTACTATAATGGTGTCTATCATCTATTCTACCAATACAATCCATATGG
CD111_T2_1 CGTTGATGTGAAAAATGTTTCATAGAACTGGTTATCATTTCACCTCCTAATAACTGGATCAATGATCCCAATGCCCAATGTACTATAATGGTGTCTATCATCTATTCTACCAATACAATCCATATGG
CD111_T2_2 CGTTGATGTGAAAAATGTTTCATAGAACTGGTTATCATTTCACCTCCTAATAACTGGATCAATGATCCCAATGCCCAATGTACTATAATGGTGTCTATCATCTATTCTACCAATACAATCCATATGG
Consensus CGTTGATGTGAAAAATGTTTCATAGAACTGGTTATCATTTCACCTCCTAATAACTGGATCAATGATCCCAATGCCCAATGTACTATAATGGTGTCTATCATCTATTCTACCAATACAATCCATATGG

261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_T2 ATCAGTTTGGGGAAATATTGTTTGGGCCCATTCAGTTTCACCCGACTTGATTAAATTGGATCCCACTTGAAGCCCGAATCTATCCATCCGAGGATTCGACAAATATGGTACATGGTCCGGGTCAGCCACA
CD111_T2_1 ATCAGTTTGGGGAAATATTGTTTGGGCCCATTCAGTTTCACCCGACTTGATTAAATTGGATCCCACTTGAAGCCCGAATCTATCCATCCGAGGATTCGACAAATATGGTACATGGTCCGGGTCAGCCACA
CD111_T2_2 ATCAGTTTGGGGAAATATTGTTTGGGCCCATTCAGTTTCACCCGACTTGATTAAATTGGATCCCACTTGAAGCCCGAATCTATCCATCCGAGGATTCGACAAATATGGTACATGGTCCGGGTCAGCCACA
Consensus ATCAGTTTGGGGAAATATTGTTTGGGCCCATTCAGTTTCACCCGACTTGATTAAATTGGATCCCACTTGAAGCCCGAATCTATCCATCCGAGGATTCGACAAATATGGTACATGGTCCGGGTCAGCCACA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_T2 ATCTTACCCACACACAGCCATTATCCTCTACACCCGGAACTCGTCGATGCAAAAATACCCAGTCCAAACTATGCATCCAGCCGACATATCCGATCCATTTCTTCGTAATGGATCAGCCCGATA
CD111_T2_1 ATCTTACCCACACACAGCCATTATCCTCTACACCCGGAACTCGTCGATGCAAAAATACCCAGTCCAAACTATGCATCCAGCCGACATATCCGATCCATTTCTTCGTAATGGATCAGCCCGATA
CD111_T2_2 ATCTTACCCACACACAGCCATTATCCTCTACACCCGGAACTCGTCGATGCAAAAATACCCAGTCCAAACTATGCATCCAGCCGACATATCCGATCCATTTCTTCGTAATGGATCAGCCCGATA
Consensus ATCTTACCCACACACAGCCATTATCCTCTACACCCGGAACTCGTCGATGCAAAAATACCCAGTCCAAACTATGCATCCAGCCGACATATCCGATCCATTTCTTCGTAATGGATCAGCCCGATA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_T2 ACAACCCATTAAATTGTCGCTGACGTTAGCATTAAACAGACCCAACTCCGTGACCCACCCACATGTTGGTTGGGTCAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAAAAAGGATTGGC
CD111_T2_1 ACAACCCATTAAATTGTCGCTGACGTTAGCATTAAACAGACCCAACTCCGTGACCCACCCACATGTTGGTTGGGTCAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAAAAAGGATTGGC
CD111_T2_2 ACAACCCATTAAATTGTCGCTGACGTTAGCATTAAACAGACCCAACTCCGTGACCCACCCACATGTTGGTTGGGTCAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAAAAAGGATTGGC
Consensus ACAACCCATTAAATTGTCGCTGACGTTAGCATTAAACAGACCCAACTCCGTGACCCACCCACATGTTGGTTGGGTCAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAAAAAGGATTGGC

651 660 670 680 690 700 710 720 730 740 750 760 770 780
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_T2 AATATTGTATAAAGTAGAGATTTTCATGAATGGACCAAGGTCAGATCCACTTCATTTCAGTTGACGGTACTGGAATTTGGGAAATGTCCTGATTTTTCCAGTGTATTGCATGGTACAATGGATTA
CD111_T2_1 AATATTGTATAAAGTAGAGATTTTCATGAATGGACCAAGGTCAGATCCACTTCATTTCAGTTGACGGTACTGGAATTTGGGAAATGTCCTGATTTTTCCAGTGTATTGCATGGTACAATGGATTA
CD111_T2_2 AATATTGTATAAAGTAGAGATTTTCATGAATGGACCAAGGTCAGATCCACTTCATTTCAGTTGACGGTACTGGAATTTGGGAAATGTCCTGATTTTTCCAGTGTATTGCATGGTACAATGGATTA
Consensus AATATTGTATAAAGTAGAGATTTTCATGAATGGACCAAGGTCAGATCCACTTCATTTCAGTTGACGGTACTGGAATTTGGGAAATGTCCTGATTTTTCCAGTGTATTGCATGGTACAATGGATTA

781 790 800 810 820 830 840 850 860 870 880 890 900 910
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_T2 GATGCTCATAACACAGAAATATTAAACATGCTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATACCGTTGGTAAATATGATACAAAAAAGATAGGTATATCCCGATAGACTTCTA
CD111_T2_1 GATGCTCATAACACAGAAATATTAAACATGCTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATACCGTTGGTAAATATGATACAAAAAAGATAGGTATATCCCGATAGACTTCTA
CD111_T2_2 GATGCTCATAACACAGAAATATTAAACATGCTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATACCGTTGGTAAATATGATACAAAAAAGATAGGTATATCCCGATAGACTTCTA
Consensus GATGCTCATAACACAGAAATATTAAACATGCTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATACCGTTGGTAAATATGATACAAAAAAGATAGGTATATCCCGATAGACTTCTA

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_T2 TCGATGGTTGGACCGGATTGAGACTTGACTATGGTAATTTATGATCTAATCATTCTATGACCTTAGGAAGAAATCGAAGAATATGTTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATGT
CD111_T2_1 TCGATGGTTGGACCGGATTGAGACTTGACTATGGTAATTTATGATCTAATCATTCTATGACCTTAGGAAGAAATCGAAGAATATGTTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATGT
CD111_T2_2 TCGATGGTTGGACCGGATTGAGACTTGACTATGGTAATTTATGATCTAATCATTCTATGACCTTAGGAAGAAATCGAAGAATATGTTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATGT
Consensus TCGATGGTTGGACCGGATTGAGACTTGACTATGGTAATTTATGATCTAATCATTCTATGACCTTAGGAAGAAATCGAAGAATATGTTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATGT

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_T2 CAAGAAAGGATGGGCGGAATTCAACTATTCCCCGCAACTATGGCTTGATCCAGTGGAAAGCAATTTGGTCCATGGCTGTGCAAGAAATAGAACTCTAAGAGAGCAAAAGGTGCAATTAAGTAAT
CD111_T2_1 CAAGAAAGGATGGGCGGAATTCAACTATTCCCCGCAACTATGGCTTGATCCAGTGGAAAGCAATTTGGTCCATGGCTGTGCAAGAAATAGAACTCTAAGAGAGCAAAAGGTGCAATTAAGTAAT
CD111_T2_2 CAAGAAAGGATGGGCGGAATTCAACTATTCCCCGCAACTATGGCTTGATCCAGTGGAAAGCAATTTGGTCCATGGCTGTGCAAGAAATAGAACTCTAAGAGAGCAAAAGGTGCAATTAAGTAAT
Consensus CAAGAAAGGATGGGCGGAATTCAACTATTCCCCGCAACTATGGCTTGATCCAGTGGAAAGCAATTTGGTCCATGGCTGTGCAAGAAATAGAACTCTAAGAGAGCAAAAGGTGCAATTAAGTAAT

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_T2 CGCAAGTTAAACAAGGAGATAAATTGAAGTTAAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTCTCATTTTCAAGTTTGGATAGGCGAGGCCATTTGATCCCAATTGGGCTAACCTTT
CD111_T2_1 CGCAAGTTAAACAAGGAGATAAATTGAAGTTAAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTCTCATTTTCAAGTTTGGATAGGCGAGGCCATTTGATCCCAATTGGGCTAACCTTT
CD111_T2_2 CGCAAGTTAAACAAGGAGATAAATTGAAGTTAAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTCTCATTTTCAAGTTTGGATAGGCGAGGCCATTTGATCCCAATTGGGCTAACCTTT
Consensus CGCAAGTTAAACAAGGAGATAAATTGAAGTTAAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTCTCATTTTCAAGTTTGGATAGGCGAGGCCATTTGATCCCAATTGGGCTAACCTTT

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_T2 ATGCTCAGATGTATGCGCCATTAAAGGCTCAACGGTGCAGGTGGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAATTTGGAAGAAATACACCGGATTTTTTCAGAGTTTTAAGGCCCA
CD111_T2_1 ATGCTCAGATGTATGCGCCATTAAAGGCTCAACGGTGCAGGTGGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAATTTGGAAGAAATACACCGGATTTTTTCAGAGTTTTAAGGCCCA
CD111_T2_2 ATGCTCAGATGTATGCGCCATTAAAGGCTCAACGGTGCAGGTGGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAATTTGGAAGAAATACACCGGATTTTTTCAGAGTTTTAAGGCCCA
Consensus ATGCTCAGATGTATGCGCCATTAAAGGCTCAACGGTGCAGGTGGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAATTTGGAAGAAATACACCGGATTTTTTCAGAGTTTTAAGGCCCA

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_T2 AAATAAATATAAGGTTCTCATGTGCTCTGATGCATCAAGATCAACCTCAAGAAATGATAAGACAATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTTAACAAACAGACATTGTCTTTAAGG
CD111_T2_1 AAATAAATATAAGGTTCTCATGTGCTCTGATGCATCAAGATCAACCTCAAGAAATGATAAGACAATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTTAACAAACAGACATTGTCTTTAAGG
CD111_T2_2 AAATAAATATAAGGTTCTCATGTGCTCTGATGCATCAAGATCAACCTCAAGAAATGATAAGACAATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTTAACAAACAGACATTGTCTTTAAGG
Consensus AAATAAATATAAGGTTCTCATGTGCTCTGATGCATCAAGATCAACCTCAAGAAATGATAAGACAATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTTAACAAACAGACATTGTCTTTAAGG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_T2 AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAAACATGTATACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA
CD111_T2_1 AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAAACATGTATACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA
CD111_T2_2 AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAAACATGTATACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA
Consensus AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAAACATGTATACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA

1691 1700 1710 1720 1730 1740 1750 1760 1770 1782
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_T2 CTATCAAATTAAGTCATTGAATGCATGGACCATGGTAAACCTAAGATGAATTGGTCATTTGGTCACTCTTCTTATTGA
CD111_T2_1 CTATCAAATTAAGTCATTGAATGCATGGACCATGGTAAACCTAAGATGAATTGGTCATTTGGTCACTCTTCTTATTGA
CD111_T2_2 CTATCAAATTAAGTCATTGAATGCATGGACCATGGTAAACCTAAGATGAATTGGTCATTTGGTCACTCTTCTTATTGA
Consensus CTATCAAATTAAGTCATTGAATGCATGGACCATGGTAAACCTAAGATGAATTGGTCATTTGGTCACTCTTCTTATTGA.....

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
CD111_P541	MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPPNMWINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSYSTDLINWIPLEPGIYPSEVFDKYGTWSGSAT													
CD111_P541_1	MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPPNMWINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSYSTDLINWIPLEPGIYPSEVFDKYGTWSGSAT													
CD111_P542	MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPPNMWINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSYSTDLINWIPLEPGIYPSEVFDKYGTWSGSAT													
Consensus	MDCLKKSSLFSLPIFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDYKNVHRTGYHFQPPNMWINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSYSTDLINWIPLEPGIYPSEVFDKYGTWSGSAT													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
CD111_P541	ILPNNKPIILYTGIVDAKNTQVQNYAIPADISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSYEKKGLAILYKSRDFHKWTKVQDPLHSVDGTGNWECPDFFPVLLHGTNGL													
CD111_P541_1	ILPNNKPIILYTGIVDAKNTQVQNYAIPADISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSYEKKGLAILYKSRDFHKWTKVQDPLHSVDGTGNWECPDFFPVLLHGTNGL													
CD111_P542	ILLNNTPIILHTGIYDAKNTQVQNYAIPADISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSYCEKKGLAILYKSRDFHKWTKVQDPLHSVDGTGNWECPDFFPVLLHGTNGL													
Consensus	ILpNNkPIILyTGIVDAKNTQVQNYAIPADISDPFLRKWIKPDNNPLIYADVSINKTQFRDPTTCWLGQDGYWRTLIGSYeEKKGLAILYKSRDFHKWTKVQDPLHSVDGTGNWECPDFFPVLLHGTNGL													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
CD111_P541	DASYNKKNIKHALKVS LDYTRFEYTYGKYDTKKDRYIPDKTSIDGMNGLRLDYGNYYASKSFYDLRKNRRIMHGWANESDYNDDYKKGWAGIQTIPRKLWLDPSGKQLYQWPVEELETREQKVQLSN													
CD111_P541_1	DASYNKKNIKHALKVS LDYTRFEYTYGKYDTKKDRYIPDKTSIDGMNGLRLDYGNYYASKSFYDLRKNRRIMHGWANESDYNDDYKKGWAGIQTIPRKLWLDPSGKQLYQWPVEELETREQKVQLSN													
CD111_P542	DASYNKKNIKHV LKVS LDYTRFEYTYGKYDTKKDRYIPDKTSIDGMKGLRLDYGNYYASKSFYDLRKNRRIMHGWANESDYNDDYKKGWAGIQTIPRKLWLDPSGKQLYQWPVEELETREQKVQLSN													
Consensus	DASYNKKNIKHaLKVS LDYTRFEYTYGKYDTKKDRYIPDKTSIDGMnGLRLDYGNYYASKSFYDLRKNRRIMHGWANESDYNDDYKKGWAGIQTIPRKLWLDPSGKQLYQWPVEELETREQKVQLSN													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
CD111_P541	RKLNKGDKIEVKGITPAQADYEYTFSSLDKAEPDPNHWANLYAQDYCAIKGSTVQGG LGPFGLLTLASQNL E EYTPVFFRVFKAQNKYKYLHCSDASRSTLKN DKTHYKPSFAGYVDYDLTNKTL SLR													
CD111_P541_1	RKLNKGDKIEVKGITPAQADYEYTFSSLDKAEPDPNHWANLYAQDYCAIKGSTVQGG LGPFGLLTLASQNL E EYTPVFFRVFKAQNKYKYLHCSDASRSTLKN DKTHYKPSFAGYVDYDLTNKTL SLR													
CD111_P542	RKLNKGDKIEVKGITPAQADYEYTFSSLDKAEPDPNHWANLYAQDYCAIKGSTVQGG LGPFGLLTLASRN L E EYTPVFFRVFKAQDKYKYLHCSDASRSTLKN DKTHYKPSFAGYVDYDLTNKTL SLR													
Consensus	RKLNKGDKIEVKGITPAQADYEYTFSSLDKAEPDPNHWANLYAQDYCAIKGSTVQGG LGPFGLLTLASqNL E EYTPVFFRVFKAQ#KYKYLHCSDASRSTLKN DKTHYKPSFAGYVDYDLTNKTL SLR													
	521	530	540	550	560	570	580	589						
	-----+-----+-----+-----+-----+-----+-----+-----													
CD111_P541	SLIDHSVYESFGAGGKTCITSRVYPTLAIYDNAHLVFNNGTETIKIKSLNAWTHGKPKMNSFGHSSY													
CD111_P541_1	SLIDHSVYESFGAGGKTCITSRVYPTLAIYDNAHLVFNNGTETIKIKSLNAWTHGKPKMNSFGHSSI													
CD111_P542	SLIDHSVYESFGAGGKTCITSRVYPTLAIYDNAHLVFNNGTETIKIKSLNAWTHGKPKMNSFGHSSY													
Consensus	SLIDHSVYESFGAGGKTCITSRVYPTLAIYDNAHLVFNNGTETIKIKSLNAWTHGKPKMNSFGHSSy													

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
CD111_P541	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD111_P541_1	ATGGATTGTTAAAAAGTCTTCTCTTTTTCTTTGCCAATTTTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTAAATGCTTCACACAAGTTTTTCCAGGGTTGCAATCTACAAGCA													
Consensus	ATGGATTGTTAAAAAGTCTTCTCTTTTTCTTTGCCAATTTTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTAAATGCTTCACACAAGTTTTTCCAGGGTTGCAATCTACAAGCA													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
CD111_P541	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD111_P541_1	CGGTTGATGTGAAAATGTTTCATAGAACTGGTTATCATTTCACCTCCTAATAACTGGATCAATGATCCCAATGCCCAATGTAATAATGGTGTCTATCATCTATTCTACCAATACATCCATATGG													
Consensus	CGGTTGATGTGAAAATGTTTCATAGAACTGGTTATCATTTCACCTCCTAATAACTGGATCAATGATCCCAATGCCCAATGTAATAATGGTGTCTATCATCTATTCTACCAATACATCCATATGG													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
CD111_P541	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD111_P541_1	ATCAGTTTGGGGAATATTGTTGGGCCATTGAGTTTCAACCGACTTGATTAAATGGATCCCACTTGAGCCCGGAATCTATCCATCCGAGTATTCGACAATATGGTACATGGTCCGGGTCAGCCACA													
Consensus	ATCAGTTTGGGGAATATTGTTGGGCCATTGAGTTTCAACCGACTTGATTAAATGGATCCCACTTGAGCCCGGAATCTATCCATCCGAGTATTCGACAATATGGTACATGGTCCGGGTCAGCCACA													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
CD111_P541	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD111_P541_1	ATCTTACCAACAACAAGCCATTATCCTCTACACCGGAATCGTCGATGCAAAAAATACCCAAGTCCAAAATATGCAATCCCAGCCGACATATCCGATCCATTTCTTCGTAATGGATCAAGCCCGATA													
Consensus	ATCTTACCAACAACAAGCCATTATCCTCTACACCGGAATCGTCGATGCAAAAAATACCCAAGTCCAAAATATGCAATCCCAGCCGACATATCCGATCCATTTCTTCGTAATGGATCAAGCCCGATA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
CD111_P541	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD111_P541_1	ACAACCCATTAAATTGTCGCTGACGTTAGCATTAAACAAGACCCAAATCCGTGACCCAAACCACATGTTGGTTGGGTGAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAAAAAGGATTGGC													
Consensus	ACAACCCATTAAATTGTCGCTGACGTTAGCATTAAACAAGACCCAAATCCGTGACCCAAACCACATGTTGGTTGGGTGAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAAAAAGGATTGGC													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
CD111_P541	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD111_P541_1	AATATTGTATAAAGTAGAGATTTTCATGAAGTGGACCAAGGTCCAAGATCCACTTCATTCAGTTGACGGTACTGGAATTTGGGATGTCTTGATTTTTCCAGTGTATTGCATGGTACAARTGGATTA													
Consensus	AATATTGTATAAAGTAGAGATTTTCATGAAGTGGACCAAGGTCCAAGATCCACTTCATTCAGTTGACGGTACTGGAATTTGGGATGTCTTGATTTTTCCAGTGTATTGCATGGTACAARTGGATTA													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
CD111_P541	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD111_P541_1	GATGCCTCATACAACAAGAAAAATATTAAACATGCTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATAACCGTTGGTAATATGATACAAAAAAGATAGGTATATTCCCGATAAGACTTCTA													
Consensus	GATGCCTCATACAACAAGAAAAATATTAAACATGCTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATAACCGTTGGTAATATGATACAAAAAAGATAGGTATATTCCCGATAAGACTTCTA													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
CD111_P541	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD111_P541_1	TCGATGGTTGGAACGGATTGAGACTTGACTATGGTAATTATTATGCATCTAAATCATTCTATGACCTTAGGAAAAATCGAAGAATTATGTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATGT													
Consensus	TCGATGGTTGGAACGGATTGAGACTTGACTATGGTAATTATTATGCATCTAAATCATTCTATGACCTTAGGAAAAATCGAAGAATTATGTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATGT													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
CD111_P541	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD111_P541_1	CAAGAAAGGATGGGCGGGAATTCAAACTATTCCCCGCAACTATGGCTTGATCCTAGTGGAAGCAATTGGTCCAATGGCCTGTCGAGAATTAGAARTCTAAGAGAGCAAAAGGTGCATTAAGTAAT													
Consensus	CAAGAAAGGATGGGCGGGAATTCAAACTATTCCCCGCAACTATGGCTTGATCCTAGTGGAAGCAATTGGTCCAATGGCCTGTCGAGAATTAGAARTCTAAGAGAGCAAAAGGTGCATTAAGTAAT													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
CD111_P541	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD111_P541_1	CGCAGTTAAACAAGGAGATAAATTGAAGTTAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTCTCATTTTTCAAGTTTGGATAAGGCAGAGCCATTTGATCCCAATTGGGCTAACCTTT													
Consensus	CGCAGTTAAACAAGGAGATAAATTGAAGTTAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTCTCATTTTTCAAGTTTGGATAAGGCAGAGCCATTTGATCCCAATTGGGCTAACCTTT													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
CD111_P541	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD111_P541_1	ATGCTCAAGATGTATGCGCCATTAAAGGCTCAACGGTGCACAGGTTGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAATTTGGAGAAATATACACCGGATTTTTTTCAGAGTTTTTAAAGGCCCA													
Consensus	ATGCTCAAGATGTATGCGCCATTAAAGGCTCAACGGTGCACAGGTTGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAATTTGGAGAAATATACACCGGATTTTTTTCAGAGTTTTTAAAGGCCCA													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
CD111_P541	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD111_P541_1	AAATAAATATAAGGTTCTCATGTGCTCTGATGCATCAAGATCAACCTCAAGAAATGATAGACATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTAAACAACAAGACATTGTCTTTAAGG													
Consensus	AAATAAATATAAGGTTCTCATGTGCTCTGATGCATCAAGATCAACCTCAAGAAATGATAGACATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTAAACAACAAGACATTGTCTTTAAGG													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
CD111_P541	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD111_P541_1	AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAAACATGTATCACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA													
Consensus	AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAAACATGTATCACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA													
	1691	1700	1710	1720	1730	1740	1750	1760	1770					
CD111_P541	----- ----- ----- ----- ----- ----- ----- -----													
CD111_P541_1	CTATCAAATTAAGTCATTGAATGCATGGACCATGGGTAACCTAAGATGAATTGGTCATTTGGTCACTCTTCTTATTGA													
Consensus	CTATCAAATTAAGTCATTGAATGCATGGACCATGGGTAACCTAAGATGAATTGGTCATTTGGTCACTCTTCTTATTGA													

1 10 20 30 40 50 60 70 80 90 100 110 120 130
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_P40_1 ATGGATTGTTAAAAAGTCTTCTTTTTCTTTGCCAATTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTTAATGCTTCACACAAGTTTTCCAGGGTTGCAATCTACAGCA
CD111_P54_1 ATGGATTGTTAAAAAGTCTTCTTTTTCTTTGCCAATTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTTAATGCTTCACACAAGTTTTCCAGGGTTGCAATCTACAGCA
CD111_P54_2 ATGGATTGTTAAAAAGTCTTCTTTTTCTTTGCCAATTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTTAATGCTTCACACAAGTTTTCCAGGGTTGCAATCTACAGCA
Consensus ATGGATTGTTAAAAAGTCTTCTTTTTCTTTGCCAATTTTTGTTATATTTCTCCATAATTTTATCTTTCAACAATGGCGTTAATGCTTCACACAAGTTTTCCAGGGTTGCAATCTACAGCA

131 140 150 160 170 180 190 200 210 220 230 240 250 260
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_P40_1 CGTTGATGTGAAAAATGTTTCATAGAACTGGTTATCATTTCACCTCCTAAACTGGATCAATGATCCCAATGCTCCAATGTACTATAATGGTGTCTATCATCTATTCTACCAATACAATCCATATGG
CD111_P54_1 CGTTGATGTGAAAAATGTTTCATAGAACTGGTTATCATTTCACCTCCTAAACTGGATCAATGATCCCAATGCTCCAATGTACTATAATGGTGTCTATCATCTATTCTACCAATACAATCCATATGG
CD111_P54_2 CGTTGATGTGAAAAATGTTTCATAGAACTGGTTATCATTTCACCTCCTAAACTGGATCAATGATCCCAATGCTCCAATGTACTATAATGGTGTCTATCATCTATTCTACCAATACAATCCATATGG
Consensus CGTTGATGTGAAAAATGTTTCATAGAACTGGTTATCATTTCACCTCCTAAACTGGATCAATGATCCCAATGCTCCAATGTACTATAATGGTGTCTATCATCTATTCTACCAATACAATCCATATGG

261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_P40_1 ATCAGTATGGGGAATATTGTTTGGGCCATTGATTTCACCGGACTTGGATCCACTTGGAGCCCGAATCTATCCATCCGAGTATTGACCAATATGGTACATGGTCCGGGTCAGCCACA
CD111_P54_1 ATCAGTATGGGGAATATTGTTTGGGCCATTGATTTCACCGGACTTGGATCCACTTGGAGCCCGAATCTATCCATCCGAGTATTGACCAATATGGTACATGGTCCGGGTCAGCCACA
CD111_P54_2 ATCAGTATGGGGAATATTGTTTGGGCCATTGATTTCACCGGACTTGGATCCACTTGGAGCCCGAATCTATCCATCCGAGTATTGACCAATATGGTACATGGTCCGGGTCAGCCACA
Consensus ATCAGTATGGGGAATATTGTTTGGGCCATTGATTTCACCGGACTTGGATCCACTTGGAGCCCGAATCTATCCATCCGAGTATTGACCAATATGGTACATGGTCCGGGTCAGCCACA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_P40_1 ATCTTACCACACACAGGCCATTATCCTCTACACCGGAATCGTCGATGCAAAAATACCCAAGTCCAAACTATGCATCCAGCCGACATATCCGATCCATTTCTTCGTAATGGATCAAGCCGATA
CD111_P54_1 ATCTTACCACACACAGGCCATTATCCTCTACACCGGAATCGTCGATGCAAAAATACCCAAGTCCAAACTATGCATCCAGCCGACATATCCGATCCATTTCTTCGTAATGGATCAAGCCGATA
CD111_P54_2 ATCTTACCACACACAGGCCATTATCCTCTACACCGGAATCGTCGATGCAAAAATACCCAAGTCCAAACTATGCATCCAGCCGACATATCCGATCCATTTCTTCGTAATGGATCAAGCCGATA
Consensus ATCTTACCACACACAGGCCATTATCCTCTACACCGGAATCGTCGATGCAAAAATACCCAAGTCCAAACTATGCATCCAGCCGACATATCCGATCCATTTCTTCGTAATGGATCAAGCCGATA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_P40_1 ACAACCCATTAAATGTCGCTGACGTTAGCATTAAACAGACCAATTCGTCGACCAACCCACATGTTGGTTGGGTCAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAAACAGGATTGGC
CD111_P54_1 ACAACCCATTAAATGTCGCTGACGTTAGCATTAAACAGACCAATTCGTCGACCAACCCACATGTTGGTTGGGTCAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAAACAGGATTGGC
CD111_P54_2 ACAACCCATTAAATGTCGCTGACGTTAGCATTAAACAGACCAATTCGTCGACCAACCCACATGTTGGTTGGGTCAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAAACAGGATTGGC
Consensus ACAACCCATTAAATGTCGCTGACGTTAGCATTAAACAGACCAATTCGTCGACCAACCCACATGTTGGTTGGGTCAGGATGGTTATTGGAGAACCTTGATAGGGAGTGTGTGGGAAAACAGGATTGGC

651 660 670 680 690 700 710 720 730 740 750 760 770 780
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_P40_1 AATATTGTATAAAGTAAATTTTCATGAATGGACCAAGTTCACATCCACTTCATTTCAGTTGACGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTATTGCATGGTACAATGGATTG
CD111_P54_1 AATATTGTATAAAGTAAATTTTCATGAATGGACCAAGTTCACATCCACTTCATTTCAGTTGACGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTATTGCATGGTACAATGGATTG
CD111_P54_2 AATATTGTATAAAGTAAATTTTCATGAATGGACCAAGTTCACATCCACTTCATTTCAGTTGACGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTATTGCATGGTACAATGGATTG
Consensus AATATTGTATAAAGTAAATTTTCATGAATGGACCAAGTTCACATCCACTTCATTTCAGTTGACGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTATTGCATGGTACAATGGATTG

781 790 800 810 820 830 840 850 860 870 880 890 900 910
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_P40_1 GATGCCTCATAACACAGAAAATATTAACATGTTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATACGTTGGTATATATGATACAAAAAAGATAGGTATATCCAGATAGACTTCTA
CD111_P54_1 GATGCCTCATAACACAGAAAATATTAACATGTTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATACGTTGGTAAATATGATACAAAAAAGATAGGTATATCCAGATAGACTTCTA
CD111_P54_2 GATGCCTCATAACACAGAAAATATTAACATGTTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATACGTTGGTAAATATGATACAAAAAAGATAGGTATATCCAGATAGACTTCTA
Consensus GATGCCTCATAACACAGAAAATATTAACATGTTCTTAAGTTAGTCTTGATGTTACGAGGTTTGAATACTATACGTTGGTAAATATGATACAAAAAAGATAGGTATATCCAGATAGACTTCTA

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_P40_1 TCGATGGTTGGAAAGGATTGAGACTTGATTTATGGTAATTTATGATCTAATCATTCTATGACCTTAGCAAAAATCGAAGAATATGTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATAT
CD111_P54_1 TCGATGGTTGGAAAGGATTGAGACTTGATTTATGGTAATTTATGATCTAATCATTCTATGACCTTAGCAAAAATCGAAGAATATGTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATAT
CD111_P54_2 TCGATGGTTGGAAAGGATTGAGACTTGATTTATGGTAATTTATGATCTAATCATTCTATGACCTTAGCAAAAATCGAAGAATATGTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATAT
Consensus TCGATGGTTGGAAAGGATTGAGACTTGATTTATGGTAATTTATGATCTAATCATTCTATGACCTTAGCAAAAATCGAAGAATATGTGGGGTTGGGCTAATGAATCAGATACTGTCAATGACGATAT

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_P40_1 CAAGAAAGGATGGGCGGAATTCAACTATTCCCCGCAATTTATGGCTTGATCCAGTGGAAACCAATTAGTCCAATGGCTGTGCAAGAATTAGAACTTTAGAGAGCAAAAGGTGCAATTAAGTAAT
CD111_P54_1 CAAGAAAGGATGGGCGGAATTCAACTATTCCCCGCAACTATGGCTTGATCCAGTGGAAACCAATTAGTCCAATGGCTGTGCAAGAATTAGAACTTTAGAGAGCAAAAGGTGCAATTAAGTAAT
CD111_P54_2 CAAGAAAGGATGGGCGGAATTCAACTATTCCCCGCAACTATGGCTTGATCCAGTGGAAACCAATTAGTCCAATGGCTGTGCAAGAATTAGAACTTTAGAGAGCAAAAGGTGCAATTAAGTAAT
Consensus CAAGAAAGGATGGGCGGAATTCAACTATTCCCCGCAACTATGGCTTGATCCAGTGGAAACCAATTAGTCCAATGGCTGTGCAAGAATTAGAACTTTAGAGAGCAAAAGGTGCAATTAAGTAAT

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_P40_1 CGCAAGTTAAACAAGGAGATAAATTTGAAGTTAAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTCTCATTTTCAAGTTTGGATAGGCGAGGCCATTTGATCCCAATTTGGGCTAACCTTT
CD111_P54_1 CGCAAGTTAAACAAGGAGATAAATTTGAAGTTAAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTCTCATTTTCAAGTTTGGATAGGCGAGGCCATTTGATCCCAATTTGGGCTAACCTTT
CD111_P54_2 CGCAAGTTAAACAAGGAGATAAATTTGAAGTTAAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTCTCATTTTCAAGTTTGGATAGGCGAGGCCATTTGATCCCAATTTGGGCTAACCTTT
Consensus CGCAAGTTAAACAAGGAGATAAATTTGAAGTTAAAGGAATCACACCTGCACAGGCTGATGTTGAAGTGACATTCTCATTTTCAAGTTTGGATAGGCGAGGCCATTTGATCCCAATTTGGGCTAACCTTT

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_P40_1 ATGCTCAGATGTATGCGCCATTAAAGGCTCAACGGTGCAGGTTGGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAAATTTGGAAGAATATACCGGGTATTTTTCAGAGTTTTTAAAGCCCA
CD111_P54_1 ATGCTCAGATGTATGCGCCATTAAAGGCTCAACGGTGCAGGTTGGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAAATTTGGAAGAATATACCGGGTATTTTTCAGAGTTTTTAAAGCCCA
CD111_P54_2 ATGCTCAGATGTATGCGCCATTAAAGGCTCAACGGTGCAGGTTGGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAAATTTGGAAGAATATACCGGGTATTTTTCAGAGTTTTTAAAGCCCA
Consensus ATGCTCAGATGTATGCGCCATTAAAGGCTCAACGGTGCAGGTTGGACTTGGGCCATTTGGGCTCTTAACTTTGGCTTCTCAAAATTTGGAAGAATATACCGGGTATTTTTCAGAGTTTTTAAAGCCCA

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_P40_1 AGATAAATATAAGGTTCTCATGTGCTCTGATGCTCAAGATCAACCTCAAGATGATAAGACAATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTTAACAAACAGACATTGTCTTTAAGG
CD111_P54_1 AGATAAATATAAGGTTCTCATGTGCTCTGATGCTCAAGATCAACCTCAAGATGATAAGACAATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTTAACAAACAGACATTGTCTTTAAGG
CD111_P54_2 AGATAAATATAAGGTTCTCATGTGCTCTGATGCTCAAGATCAACCTCAAGATGATAAGACAATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTTAACAAACAGACATTGTCTTTAAGG
Consensus AGATAAATATAAGGTTCTCATGTGCTCTGATGCTCAAGATCAACCTCAAGATGATAAGACAATGTACAGCCATCATTGCTGGATATGTAGATGTAGATTTAACAAACAGACATTGTCTTTAAGG

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_P40_1 AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAACATGTATCACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA
CD111_P54_1 AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAACATGTATCACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA
CD111_P54_2 AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAACATGTATCACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA
Consensus AGTTTGATTGATCACTCAGTAGTAGAAGCTTTGGTGCCGGAGGAAAACATGTATCACATCAAGGGTTTATCCGACATTAGCAATTTATGATAATGCACATCTATTTGTCTTTAATAATGGGACAGAGA

1691 1700 1710 1720 1730 1740 1750 1760 1770
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD111_P40_1 CTATCAAATTAAGTCATTGAATGCATGGACCATGGGTAACCTAAGATGAATTGGTCATTTGGTCTCTTCTTATTGA
CD111_P54_1 CTATCAAATTAAGTCATTGAATGCATGGACCATGGGTAACCTAAGATGAATTGGTCATTTGGTCTCTTCTTATTGA
CD111_P54_2 CTATCAAATTAAGTCATTGAATGCATGGACCATGGGTAACCTAAGATGAATTGGTCATTTGGTCTCTTCTTATTGA
Consensus CTATCAAATTAAGTCATTGAATGCATGGACCATGGGTAACCTAAGATGAATTGGTCATTTGGTCTCTTCTTATTGA

1 10 20 30 40 50 60 70 80 90 100 110 120 130
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD141_T1 MEILRRSSSLWVLPILLLCFFINNGVFDASHKYYMHLQSTTSHVDVSKYHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIMGNIYVAHSVSKDLINWIPLPAIYPSKYFDKYGTWSGSAT
CD141_T1_2 MEILRRSSSLWVLPILLLCFFINNGVFDASHKYYMHLQSTTSHVDVSKYHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIMGNIYVAHSVSKDLINWIPLPAIYPSKYFDKYGTWSGSAT
CD141_T2 MEILRRSSSLWVLPILLLCFFINNGVFDASHKYYMHLQSTTSHVDASKYHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIMGNIYVAHSVSKDLINWIPLPAIYPSKYFDKYGTWSGSAT
CD141_T2_1 MEILRRSSSLWVLPILLLCFFINNGVFDASHKYYMHLQSTTSHVDASKYHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIMGNIYVAHSVSKDLINWIPLPAIYPSKYFDKYGTWSGSAT
CD141_T3 MEILRRSSSLWVLPILLLCFFINNGVFDASHKYYMHLQSTTSHVDASKYHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIMGNIYVAHSVSKDLINWIPLPAIYPSKYFDKYGTWSGSAT
CD141_T1_1 MEILRRSSSLWVLPILLLCFFINNGVFDASHKYYMHLQSTTSHVDVSKYHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIMGNIYVAHSVSKDLINWIPLPAIYPSKYFDKYGTWSGSAT
Consensus MEILRRSSSLWVLPILLLCFFINNGVFDASHKYYMHLQSTTSHVDVSKYHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIMGNIYVAHSVSKDLINWIPLPAIYPSKYFDKYGTWSGSAT

131 140 150 160 170 180 190 200 210 220 230 240 250 260
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD141_T1 ILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYLRKWKIPDNPLIYADKTINKSQFRDPTTAMHGRDGNWRILVGSYRNHRGKVIHYKSNKNFMKTKAKHPLHSAPGTGNWECPDFFPVSLKNKDG
CD141_T1_2 ILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYLRKWKIPDNPLIYADKTINKSQFRDPTTAMHGRDGNWRILVGSYRNHRGKVIHYKSNKNFMKTKAKHPLHSAPGTGNWECPDFFPVSLKNKDG
CD141_T2 ILPGNKPVILYTGIVDGNKTQVQNYAIPANMSDPYLRKWKIPDNPLIYADKTINKSQFRDPTTAMHGRDGNWRILVGSYRNHRGKVIHYKSNKNFMKTKAKHPLHSAPGTGNWECPDFFPVSLKNKDG
CD141_T2_1 ILPGNKPVILYTGIVDGNKTQVQNYAIPANMSDPYLRKWKIPDNPLIYADKTINKSQFRDPTTAMHGRDGNWRILVGSYRNHRGKVIHYKSNKNFMKTKAKHPLHSAPGTGNWECPDFFPVSLKNKDG
CD141_T3 ILPGNKPVILYTGIVDGNKTQVQNYAIPANMSDPYLRKWKIPDNPLIYADKTINKSQFRDPTTAMHGRDGNWRILVGSYRNHRGKVIHYKSNKNFMKTKAKHPLHSAPGTGNWECPDFFPVSLKNKDG
CD141_T1_1 ILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYLRKWKIPDNPLIYADKTINKSQFRDPTTAMHGRDGNWRILVGSYRNHRGKVIHYKSNKNFMKTKAKHPLHSAPGTGNWECPDFFPVSLKNKDG
Consensus ILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYLRKWKIPDNPLIYADKTINKSQFRDPTTAMHGRDGNWRILVGSYRNHRGKVIHYKSNKNFMKTKAKHPLHSAPGTGNWECPDFFPVSLKNKDG

261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD141_T1 LDTSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYYASKTFFDSGKNRRILLGHANESDVTVDNDYRKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKVQLN
CD141_T1_2 LDTSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYYASKTFFDSGKNRRILLGHANESDVTVDNDYRKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKVQLN
CD141_T2 LDTSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYYASKTFFDSGKNRRILLGHANESDVTVDNDYRKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKVQLN
CD141_T2_1 LDTSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYYASKTFFDSGKNRRILLGHANESDVTVDNDYRKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKVQLN
CD141_T3 LDTSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYYASKTFFDSGKNRRILLGHANESDVTVDNDYRKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKVQLN
CD141_T1_1 LDTSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYYASKTFFDSGKNRRILLGHANESDVTVDNDYRKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKVQLN
Consensus LDTSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYYASKTFFDSGKNRRILLGHANESDVTVDNDYRKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKVQLN

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD141_T1 NKKLNKGEKVEIKGITVAQADVEYIFSFSLDKAEPFDPADWLYAQDYCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDVLDADKKLSL
CD141_T1_2 NKKLNKGEKVEIKGITVAQADVEYIFSFSLDKAEPFDPADWLYAQDYCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDVLDADKKLSL
CD141_T2 NKKLNKGEKVEIKGITVAQADVEYIFSFSLDKAEPFDPADWLYAQDYCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDVLDADKKLSL
CD141_T2_1 NKKLNKGEKVEIKGITVAQADVEYIFSFSLDKAEPFDPADWLYAQDYCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDVLDADKKLSL
CD141_T3 NKKLNKGEKVEIKGITVAQADVEYIFSFSLDKAEPFDPADWLYAQDYCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDVLDADKKLSL
CD141_T1_1 NKKLNKGEKVEIKGITVAQADVEYIFSFSLDKAEPFDPADWLYAQDYCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDVLDADKKLSL
Consensus NKKLNKGEKVEIKGITVAQADVEYIFSFSLDKAEPFDPADWLYAQDYCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDVLDADKKLSL

521 530 540 550 560 570 580 582
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD141_T1 RSLIDHSIVESFGAGGKTCITSRYPTLAIFDKAHLFAFNNGAERITITLNAWSMANAKLH
CD141_T1_2 RSLIDHSIVESFGAGGKTCITSRYPTLAIFDKAHLFAFNNGAERITITLNAWSMANAKLH
CD141_T2 RSLIDHSIVESFGAGGKTCITSRYPTLAIFDKAHLFAFNNGAERITITLNAWSMANAKLH
CD141_T2_1 RSLIDHSIVESFGAGGKTCITSRYPTLAIFDKAHLFAFNNGAERITITLNAWSMANAKLH
CD141_T3 RSLIDHSIVESFGAGGKTCITSRYPTLAIFDKAHLFAFNNGAERITITLNAWSMANAKLH
CD141_T1_1 RSLIDHSIVESFGAGGKTCITSRYPTLAIFDKAHLFAFNNGAERITITLNAWSMANAKLH
Consensus RSLIDHSIVESFGAGGKTCITSRYPTLAIFDKAHLFAFNNGAERITITLNAWSMANAKLH

1 10 20 30 40 50 60 70 80 90 100 110 120 130
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_S2 ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTGCCAAATCTTTTGTGTGTTCTTTATCAACAATGGAGTATTTGTTGATGCTTCTCATAAAGTTTATATGCACTTGCATCTACTACTAGCC
CD141_S2_1 ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTGCCAAATCTTTTGTGTGTTCTTTATCAACAATGGAGTATTTGTTGATGCTTCTCAAAAGTTTATATGCACTTGCATCTACTACTAGCC
CD141_S2_2 ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTGCCAAATCTTTTGTGTGTTCTTTATCAACAATGGAGTATTTGTTGATGCTTCTCAAAAGTTTATATGCACTTGCATCTACTACTAGCC
Consensus ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTGCCAAATCTTTTGTGTGTTCTTTATCAACAATGGAGTATTTGTTGATGCTTCTCAAAAGTTTATATGCACTTGCATCTACTACTAGCC

131 140 150 160 170 180 190 200 210 220 230 240 250 260
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_S2 ATGTTGATGTTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCCTAAAATTGGATCAACGATCCAATGGTCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACAACCCAAAGGG
CD141_S2_1 ATGTTGATGTTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCCTAAAATTGGATCAACGATCCAATGGTCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACAACCCAAAGGG
CD141_S2_2 ATGTTGATGTTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCCTAAAATTGGATCAACGATCCAATGGTCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACAACCCAAAGGG
Consensus ATGTTGATGTTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCCTAAAATTGGATCAACGATCCAATGGTCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACAACCCAAAGGG

261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_S2 AGCGATATGGGGCAATATTGTTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACCTTGAAACCCGCTATCTACCCGTCCAAAGTATTTGACAAAGTATGGTACATGGTCCGGGTCAGCCACA
CD141_S2_1 AGCGATATGGGGCAATATTGTTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACCTTGAAACCCGCTATCTACCCGTCCAAAGTATTTGACAAAGTATGGTACATGGTCCGGGTCAGCCACA
CD141_S2_2 AGCGATATGGGGCAATATTGTTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACCTTGAAACCCGCTATCTACCCGTCCAAAGTATTTGACAAAGTATGGTACATGGTCCGGGTCAGCCACA
Consensus AGCGATATGGGGCAATATTGTTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACCTTGAAACCCGCTATCTACCCGTCCAAAGTATTTGACAAAGTATGGTACATGGTCCGGGTCAGCCACA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_S2 ATCTTGCCAGGCACAAGCCTGTTATTCTTACACTGGAAATGTGGATGCTAACAGACACAAGTCCAAACTATGCATCCAGCTAACATATCTGATCCATATCTTCGTAAGTGGATCAAGCCGATA
CD141_S2_1 ATCTTGCCAGGCACAAGCCTGTTATTCTTACACTGGAAATGTGGATGCTAACAGACACAAGTCCAAACTATGCATCCAGCTAACATATCTGATCCATATCTTCGTAAGTGGATCAAGCCGATA
CD141_S2_2 ATCTTGCCAGGCACAAGCCTGTTATTCTTACACTGGAAATGTGGATGCTAACAGACACAAGTCCAAACTATGCATCCAGCTAACATATCTGATCCATATCTTCGTAAGTGGATCAAGCCGATA
Consensus ATCTTGCCAGGCACAAGCCTGTTATTCTTACACTGGAAATGTGGATGCTAACAGACACAAGTCCAAACTATGCATCCAGCTAACATATCTGATCCATATCTTCGTAAGTGGATCAAGCCGATA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_S2 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCAAACACAGCATGGATGGGCCGAGATGGAAATGGAGATCTTGGTTGGGAGTGTGAGGAATCATAGGGGAAGGT
CD141_S2_1 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCAAACACAGCATGGATGGGCCGAGATGGAAATGGAGATCTTGGTTGGGAGTGTGAGGAATCATAGGGGAAGGT
CD141_S2_2 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCAAACACAGCATGGATGGGCCGAGATGGAAATGGAGATCTTGGTTGGGAGTGTGAGGAATCATAGGGGAAGGT
Consensus ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCAAACACAGCATGGATGGGCCGAGATGGAAATGGAGATCTTGGTTGGGAGTGTGAGGAATCATAGGGGAAGGT

651 660 670 680 690 700 710 720 730 740 750 760 770 780
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_S2 TATAATGTACAAAGTGATAAGGACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCCGCCCGGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTGATTGAAAATAAAATGGT
CD141_S2_1 TATAATGTACAAAGTGATAAGGACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCCGCCCGGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTGATTGAAAATAAAATGGT
CD141_S2_2 TATAATGTACAAAGTGATAAGGACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCCGCCCGGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTGATTGAAAATAAAATGGT
Consensus TATAATGTACAAAGTGATAAGGACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCCGCCCGGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTGATTGAAAATAAAATGGT

781 790 800 810 820 830 840 850 860 870 880 890 900 910
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_S2 TTGGACACGTCATACATGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACTAGGTTTGATCATTACACAGTTGGTACATATGACACCAAAAGGATAAATACTTTCCGGATACACTT
CD141_S2_1 TTGGACACGTCATACATGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACTAGGTTTGATCATTACACAGTTGGTACATATGACACCAAAAGGATAAATACTTTCCGGATACACTT
CD141_S2_2 TTGGACACGTCATACATGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACTAGGTTTGATCATTACACAGTTGGTACATATGACACCAAAAGGATAAATACTTTCCGGATACACTT
Consensus TTGGACACGTCATACATGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACTAGGTTTGATCATTACACAGTTGGTACATATGACACCAAAAGGATAAATACTTTCCGGATACACTT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_S2 CTATTGATGGATGGAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAGACATTCTTTGATAATGGCAAGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA
CD141_S2_1 CTATTGATGGATGGAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAGACATTCTTTGATAATGGCAAGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA
CD141_S2_2 CTATTGATGGATGGAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAGACATTCTTTGATAATGGCAAGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA
Consensus CTATTGATGGATGGAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAGACATTCTTTGATAATGGCAAGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_S2 TGTGAGGAAGGATGGGCTGGAGTTCACCCATTCTCCTCGTAAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTCAAGAAATAGAACTCTAAGAAAGAAAAGGTCCAAATTAAT
CD141_S2_1 TGTGAGGAAGGATGGGCTGGAGTTCACCCATTCTCCTCGTAAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTCAAGAAATAGAACTCTAAGAAAGAAAAGGTCCAAATTAAT
CD141_S2_2 TGTGAGGAAGGATGGGCTGGAGTTCACCCATTCTCCTCGTAAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTCAAGAAATAGAACTCTAAGAAAGAAAAGGTCCAAATTAAT
Consensus TGTGAGGAAGGATGGGCTGGAGTTCACCCATTCTCCTCGTAAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTCAAGAAATAGAACTCTAAGAAAGAAAAGGTCCAAATTAAT

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_S2 AACAAAAGTTGAACAAAGGAGAAAAGGTTGAATCAAGGAATCACAGTTGCTCAGGCTGATGTTGAAGTGATTTTCTCATTACAAAGTTTGGATAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC
CD141_S2_1 AACAAAAGTTGAACAAAGGAGAAAAGGTTGAATCAAGGAATCACAGTTGCTCAGGCTGATGTTGAAGTGATTTTCTCATTACAAAGTTTGGATAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC
CD141_S2_2 AACAAAAGTTGAACAAAGGAGAAAAGGTTGAATCAAGGAATCACAGTTGCTCAGGCTGATGTTGAAGTGATTTTCTCATTACAAAGTTTGGATAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC
Consensus AACAAAAGTTGAACAAAGGAGAAAAGGTTGAATCAAGGAATCACAGTTGCTCAGGCTGATGTTGAAGTGATTTTCTCATTACAAAGTTTGGATAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_S2 TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTGGACTTGGGCTTTTGGGCTCCTAACTTTGGCTTCTAAAACCTTAGAAGATACACACCCGTTTTCTTTAGAAATTTCAAGGC
CD141_S2_1 TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTGGACTTGGGCTTTTGGGCTCCTAACTTTGGCTTCTAAAACCTTAGAAGATACACACCCGTTTTCTTTAGAAATTTCAAGGC
CD141_S2_2 TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTGGACTTGGGCTTTTGGGCTCCTAACTTTGGCTTCTAAAACCTTAGAAGATACACACCCGTTTTCTTTAGAAATTTCAAGGC
Consensus TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTGGACTTGGGCTTTTGGGCTCCTAACTTTGGCTTCTAAAACCTTAGAAGATACACACCCGTTTTCTTTAGAAATTTCAAGGC

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_S2 TCAAGATAAATCAAAAGTCTTATGTGTTCCGATGCTTCAGGTCAGCCTAAGGAATGAACAACATATGTACAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAAAGAAATGTCTCTT
CD141_S2_1 TCAAGATAAATCAAAAGTCTTATGTGTTCCGATGCTTCAGGTCAGCCTAAGGAATGAACAACATATGTACAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAAAGAAATGTCTCTT
CD141_S2_2 TCAAGATAAATCAAAAGTCTTATGTGTTCCGATGCTTCAGGTCAGCCTAAGGAATGAACAACATATGTACAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAAAGAAATGTCTCTT
Consensus TCAAGATAAATCAAAAGTCTTATGTGTTCCGATGCTTCAGGTCAGCCTAAGGAATGAACAACATATGTACAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAAAGAAATGTCTCTT

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_S2 AGAAGTTTGATTGATCATTGCGTAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAAGGGTTATCCACGTTGGCGATATTTGACAAAGCACATTTATTTGCATTCAACACGGCGCGG
CD141_S2_1 AGAAGTTTGATTGATCATTGCGTAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAAGGGTTATCCACGTTGGCGATATTTGACAAAGCACATTTATTTGCATTCAACACGGCGCGG
CD141_S2_2 AGAAGTTTGATTGATCATTGCGTAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAAGGGTTATCCACGTTGGCGATATTTGACAAAGCACATTTATTTGCATTCAACACGGCGCGG
Consensus AGAAGTTTGATTGATCATTGCGTAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAAGGGTTATCCACGTTGGCGATATTTGACAAAGCACATTTATTTGCATTCAACACGGCGCGG

1691 1700 1710 1720 1730 1740 1749
|-----|-----|-----|-----|-----|-----|-----|
CD141_S2 AGAGAATCACAAATGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
CD141_S2_1 AGAGAATCACAAATGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
CD141_S2_2 AGAGAATCACAAATGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
Consensus AGAGAATCACAAATGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
CD141_S3	-----													
CD141_S3_1	ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTTGCCAATCTTTTGTGTGCTTCTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCATCTACTACTAGCC													
Consensus	ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTTGCCAATCTTTTGTGTGCTTCTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCATCTACTACTAGCC													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
CD141_S3	-----													
CD141_S3_1	ATGTTGATGCTAGCAAGGTCATAGAAGTGGTTATCATTTCACCTTCTAAAACTGGATCAACGATCCAATGGTCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACACCCAAGGG													
Consensus	ATGTTGATGCTAGCAAGGTCATAGAAGTGGTTATCATTTCACCTTCTAAAACTGGATCAACGATCCAATGGTCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACACCCAAGGG													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
CD141_S3	-----													
CD141_S3_1	AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCCTTGAACCCGCTATCTACCCGTCGAAGTATTTGACAGTATGGTACATGGTCCGGGTCAGCCACA													
Consensus	AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCCTTGAACCCGCTATCTACCCGTCGAAGTATTTGACAGTATGGTACATGGTCCGGGTCAGCCACA													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
CD141_S3	-----													
CD141_S3_1	ATCTTGCCAGGCAACAAGCCTGTTATTCTTACACTGGAAATGTTGGATGCTAACAGACACAGTCCAAATTTATGCAATCCCAGGTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCCGATA													
Consensus	ATCTTGCCAGGCAACAAGCCTGTTATTCTTACACTGGAAATGTTGGATGCTAACAGACACAGTCCAAATTTATGCAATCCCAGGTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCCGATA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
CD141_S3	-----													
CD141_S3_1	ACAATCCATTGATTGTTGCTGACAAAACCATCAACAAAAGCCAAATTCGTGATCCACACACAGCATGGATGGGCCGAGATAGAATTGGAGAACTTGGTTGGGAGTGTGAGGAATCATAGGGGAAGGT													
Consensus	ACAATCCATTGATTGTTGCTGACAAAACCATCAACAAAAGCCAAATTCGTGATCCACACACAGCATGGATGGGCCGAGATAGAATTGGAGAACTTGGTTGGGAGTGTGAGGAATCATAGGGGAAGGT													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
CD141_S3	-----													
CD141_S3_1	TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCCGTACTGGAAATTTGGGAATGTCTGATTTTTTTCCAGTGTCAATAAAAATAAAGATGGT													
Consensus	TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCCGTACTGGAAATTTGGGAATGTCTGATTTTTTTCCAGTGTCAATAAAAATAAAGATGGT													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
CD141_S3	-----													
CD141_S3_1	TTGGACACGTATACAAATGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACTAGGTTTGGATCATTACACAATTGGTACATATGACACCAAAAAGGATAAATACTTTCCGGATAACACTT													
Consensus	TTGGACACGTATACAAATGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACTAGGTTTGGATCATTACACAATTGGTACATATGACACCAAAAAGGATAAATACTTTCCGGATAACACTT													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
CD141_S3	-----													
CD141_S3_1	CTATTGATGGATGGAAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAAGACATTCTTTGATAGTGGCAAGAATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACGGTTGATAACGA													
Consensus	CTATTGATGGATGGAAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAAGACATTCTTTGATAGTGGCAAGAATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACGGTTGATAACGA													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
CD141_S3	-----													
CD141_S3_1	TGTGAGGAAGGATGGGCCGGAGTTCACCCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACCAATTGGTTCAATGGCCTGTTCAAGAACTAGAARCTTAGAAGAAAAGGTCCAATTAAT													
Consensus	TGTGAGGAAGGATGGGCCGGAGTTCACCCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACCAATTGGTTCAATGGCCTGTTCAAGAACTAGAARCTTAGAAGAAAAGGTCCAATTAAT													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
CD141_S3	-----													
CD141_S3_1	AACAAAAGTTGAACAAGGGAGAAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTCATTACAAAGTTGGATAAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC													
Consensus	AACAAAAGTTGAACAAGGGAGAAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTCATTACAAAGTTGGATAAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
CD141_S3	-----													
CD141_S3_1	TTTACGCGCAAGATGTATGCGCCATTAAAGGTTCAACGGTCCAAGGTGGACTTGGGCCTTTTGGGCTCCTAACTTTGGCTTCTAAAACTTAGAAGATAACACACCCGTTTTCTTTAGAATTTCAAGGC													
Consensus	TTTACGCGCAAGATGTATGCGCCATTAAAGGTTCAACGGTCCAAGGTGGACTTGGGCCTTTTGGGCTCCTAACTTTGGCTTCTAAAACTTAGAAGATAACACACCCGTTTTCTTTAGAATTTCAAGGC													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
CD141_S3	-----													
CD141_S3_1	TCAGATAGATACAAGTTCTTATGTGTTCCGATGCTTCAAGGTCAGCCTAAGAAATGAACAACTATGTACAACCCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAGAARTTGTCTCTT													
Consensus	TCAGATAaATACAAGTTCTTATGTGTTCCGATGCTTCAAGGTCAGCCTAAGAAATGAACAACTATGTACAACCCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAGAARTTGTCTCTT													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
CD141_S3	-----													
CD141_S3_1	AGAAGTTTGATTGATCATTCCGTAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAAGGGTTATCCACGTTGGCGATATTTGACAAGGCACATTTATTTGCATTCAATACGGCGCGG													
Consensus	AGAAGTTTGATTGATCATTCCGTAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAAGGGTTATCCACGTTGGCGATATTTGACAAGGCACATTTATTTGCATTCAATACGGCGCGG													
	1691	1700	1710	1720	1730	1740	1749							
CD141_S3	-----													
CD141_S3_1	AGAGAATCACAAATTGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG													
Consensus	AGAGAATCACAAATTGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG													

1 10 20 30 40 50 60 70 80 90 100 110 120 130
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_D1 ATGGAGATTTAAGAAGATCTTCTCTCTTTGGGTTTGGCAATCTTTTGTGTGCTTCTTATCAACAAATGGAGTATTGTTGATGCTTCTCACAAGTTTATATGCACCTGCAATCTACTACTAGCC
CD141_D1_2 ATGGAGATTTAAGAAGATCTTCTCTCTTTGGGTTTGGCAATCTTTTGTGTGCTTCTTATCAACAAATGGAGTATTGTTGATGCTTCTCACAAGTTTATATGCACCTGCAATCTACTACTAGCC
CD141_D1_3 ATGGAGATTTAAGAAGATCTTCTCTCTTTGGGTTTGGCAATCTTTTGTGTGCTTCTTATCAACAAATGGAGTATTGTTGATGCTTCTCACAAGTTTATATGCACCTGCAATCTACTACTAGCC
CD141_D1_1 ATGGAGATTTAAGAAGATCTTCTCTCTTTGGGTTTGGCAATCTTTTGTGTGCTTCTTATCAACAAATGGAGTATTGTTGATGCTTCTCACAAGTTTATATGCACCTGCAATCTACTACTAGCC
Consensus ATGGAGATTTAAGAAGATCTTCTCTCTTTGGGTTTGGCAATCTTTTGTGTGCTTCTTATCAACAAATGGAGTATTGTTGATGCTTCTCACAAGTTTATATGCACCTGCAATCTACTACTAGCC

131 140 150 160 170 180 190 200 210 220 230 240 250 260
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_D1 ATGTTGATGCTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCTAAAACTGGATCAACGATCCAAATGGCCCAATGTATTACAAATGGAGTGTATCATTATTCTACCAGTACACCCAAAGGG
CD141_D1_2 ATGTTGATGCTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCTAAAACTGGATCAACGATCCAAATGGCCCAATGTATTACAAATGGAGTGTATCATTATTCTACCAGTACACCCAAAGGG
CD141_D1_3 ATGTTGATGCTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCTAAAACTGGATCAACGATCCAAATGGCCCAATGTATTACAAATGGAGTGTATCATTATTCTACCAGTACACCCAAAGGG
CD141_D1_1 ATGTTGATGCTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCTAAAACTGGATCAACGATCCAAATGGCCCAATGTATTACAAATGGAGTGTATCATTATTCTACCAGTACACCCAAAGGG
Consensus ATGTTGATGCTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCTAAAACTGGATCAACGATCCAAATGGCCCAATGTATTACAAATGGAGTGTATCATTATTCTACCAGTACACCCAAAGGG

261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_D1 AGCGATATGGGGCAATATTGTTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACTTGACCCGCTATCTACCCGTCCAAGTATTGACAAAGTATGGTACATGGTCTGGGTGAGCCACA
CD141_D1_2 AGCGATATGGGGCAATATTGTTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACTTGACCCGCTATCTACCCGTCCAAGTATTGACAAAGTATGGTACATGGTCTGGGTGAGCCACA
CD141_D1_3 AGCGATATGGGGCAATATTGTTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACTTGACCCGCTATCTACCCGTCCAAGTATTGACAAAGTATGGTACATGGTCTGGGTGAGCCACA
CD141_D1_1 AGCGATATGGGGCAATATTGTTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACTTGACCCGCTATCTACCCGTCCAAGTATTGACAAAGTATGGTACATGGTCTGGGTGAGCCACA
Consensus AGCGATATGGGGCAATATTGTTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACTTGACCCGCTATCTACCCGTCCAAGTATTGACAAAGTATGGTACATGGTCTGGGTGAGCCACA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_D1 ATCTTGCCAGGCAACAAGCCTGTTATTCTCTACACTGGAAATGTGGATGGTAATAGACACAGTCCAAACTATGCATCCCGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAGCCCGATA
CD141_D1_2 ATCTTGCCAGGCAACAAGCCTGTTATTCTCTACACTGGAAATGTGGATGGTAATAGACACAGTCCAAACTATGCATCCCGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAGCCCGATA
CD141_D1_3 ATCTTGCCAGGCAACAAGCCTGTTATTCTCTACACTGGAAATGTGGATGGTAATAGACACAGTCCAAACTATGCATCCCGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAGCCCGATA
CD141_D1_1 ATCTTGCCAGGCAACAAGCCTGTTATTCTCTACACTGGAAATGTGGATGGTAATAGACACAGTCCAAACTATGCATCCCGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAGCCCGATA
Consensus ATCTTGCCAGGCAACAAGCCTGTTATTCTCTACACTGGAAATGTGGATGGTAATAGACACAGTCCAAACTATGCATCCCGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAGCCCGATA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_D1 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTTGATCCAAACACCCGATGGATGGGCCGAGATGGAATTTGGAGATCTGGTAGGCAGTGTGAGGAAATCATAGGGGAAGGT
CD141_D1_2 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTTGATCCAAACACCCGATGGATGGGCCGAGATGGAATTTGGAGATCTGGTAGGCAGTGTGAGGAAATCATAGGGGAAGGT
CD141_D1_3 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTTGATCCAAACACCCGATGGATGGGCCGAGATGGAATTTGGAGATCTGGTAGGCAGTGTGAGGAAATCATAGGGGAAGGT
CD141_D1_1 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTTGATCCAAACACCCGATGGATGGGCCGAGATGGAATTTGGAGATCTGGTAGGCAGTGTGAGGAAATCATAGGGGAAGGT
Consensus ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTTGATCCAAACACCCGATGGATGGGCCGAGATGGAATTTGGAGATCTGGTAGGCAGTGTGAGGAAATCATAGGGGAAGGT

651 660 670 680 690 700 710 720 730 740 750 760 770 780
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_D1 TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCGGTACTGGAAATTTGGAAATGTCTGATTTTTTCCAGTGTCTTAAAAAATAAGATGGT
CD141_D1_2 TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCGGTACTGGAAATTTGGAAATGTCTGATTTTTTCCAGTGTCTTAAAAAATAAGATGGT
CD141_D1_3 TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCGGTACTGGAAATTTGGAAATGTCTGATTTTTTCCAGTGTCTTAAAAAATAAGATGGT
CD141_D1_1 TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCGGTACTGGAAATTTGGAAATGTCTGATTTTTTCCAGTGTCTTAAAAAATAAGATGGT
Consensus TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCGGTACTGGAAATTTGGAAATGTCTGATTTTTTCCAGTGTCTTAAAAAATAAGATGGT

781 790 800 810 820 830 840 850 860 870 880 890 900 910
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_D1 TTGGACACGTATACACACGGCAAGACATTAAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGATCATTACACAATTGGTACATATGACACCAAAAGGATCAGTACTTTCCGGATAGCACTT
CD141_D1_2 TTGGACACGTATACACACGGCAAGACATTAAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGATCATTACACAATTGGTACATATGACACCAAAAGGATCAGTACTTTCCGGATAGCACTT
CD141_D1_3 TTGGACACGTATACACACGGCAAGACATTAAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGATCATTACACAATTGGTACATATGACACCAAAAGGATCAGTACTTTCCGGATAGCACTT
CD141_D1_1 TTGGACACGTATACACACGGCAAGACATTAAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGATCATTACACAATTGGTACATATGACACCAAAAGGATCAGTACTTTCCGGATAGCACTT
Consensus TTGGACACGTATACACACGGCAAGACATTAAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGATCATTACACAATTGGTACATATGACACCAAAAGGATCAGTACTTTCCGGATAGCACTT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_D1 CTATTGATGGATGGAAGGATTGAGACTCGACTATGGTAACTATTACGCGTCCAGACATTCCTTTGATAGTGGCAAGATCGTAGGATTTGTTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA
CD141_D1_2 CTATTGATGGATGGAAGGATTGAGACTCGACTATGGTAACTATTACGCGTCCAGACATTCCTTTGATAGTGGCAAGATCGTAGGATTTGTTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA
CD141_D1_3 CTATTGATGGATGGAAGGATTGAGACTCGACTATGGTAACTATTACGCGTCCAGACATTCCTTTGATAGTGGCAAGATCGTAGGATTTGTTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA
CD141_D1_1 CTATTGATGGATGGAAGGATTGAGACTCGACTATGGTAACTATTACGCGTCCAGACATTCCTTTGATAGTGGCAAGATCGTAGGATTTGTTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA
Consensus CTATTGATGGATGGAAGGATTGAGACTCGACTATGGTAACTATTACGCGTCCAGACATTCCTTTGATAGTGGCAAGATCGTAGGATTTGTTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_D1 TGTGAGGAAGGATGGGCCGGAGTTCACCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTTCAAGATTAGAACTCTAGAAAGAAAAGGTCCAAATTAAT
CD141_D1_2 TGTGAGGAAGGATGGGCCGGAGTTCACCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTTCAAGATTAGAACTCTAGAAAGAAAAGGTCCAAATTAAT
CD141_D1_3 TGTGAGGAAGGATGGGCCGGAGTTCACCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTTCAAGATTAGAACTCTAGAAAGAAAAGGTCCAAATTAAT
CD141_D1_1 TGTGAGGAAGGATGGGCCGGAGTTCACCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTTCAAGATTAGAACTCTAGAAAGAAAAGGTCCAAATTAAT
Consensus TGTGAGGAAGGATGGGCCGGAGTTCACCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTTCAAGATTAGAACTCTAGAAAGAAAAGGTCCAAATTAAT

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_D1 AACAAAAGTTGAACAGGGAGAAAAGGTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTATTCAACAGTTTGGACAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC
CD141_D1_2 AACAAAAGTTGAACAGGGAGAAAAGGTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTATTCAACAGTTTGGACAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC
CD141_D1_3 AACAAAAGTTGAACAGGGAGAAAAGGTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTATTCAACAGTTTGGACAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC
CD141_D1_1 AACAAAAGTTGAACAGGGAGAAAAGGTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTATTCAACAGTTTGGACAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC
Consensus AACAAAAGTTGAACAGGGAGAAAAGGTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTATTCAACAGTTTGGACAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_D1 TTTACGCGCAGATGATGCGCCATTAAGGTTCAACGGTCCAGGTTGGACTTGGGCTTTTGGGCTACTAATTTGGCTTCTAAAACCTTAGAAGAAACACACCCGTTTCTTCAGAAATTTCAAGGC
CD141_D1_2 TTTACGCGCAGATGATGCGCCATTAAGGTTCAACGGTCCAGGTTGGACTTGGGCTTTTGGGCTACTAATTTGGCTTCTAAAACCTTAGAAGAAACACACCCGTTTCTTCAGAAATTTCAAGGC
CD141_D1_3 TTTACGCGCAGATGATGCGCCATTAAGGTTCAACGGTCCAGGTTGGACTTGGGCTTTTGGGCTACTAATTTGGCTTCTAAAACCTTAGAAGAAACACACCCGTTTCTTCAGAAATTTCAAGGC
CD141_D1_1 TTTACGCGCAGATGATGCGCCATTAAGGTTCAACGGTCCAGGTTGGACTTGGGCTTTTGGGCTACTAATTTGGCTTCTAAAACCTTAGAAGAAACACACCCGTTTCTTCAGAAATTTCAAGGC
Consensus TTTACGCGCAGATGATGCGCCATTAAGGTTCAACGGTCCAGGTTGGACTTGGGCTTTTGGGCTACTAATTTGGCTTCTAAAACCTTAGAAGAAACACACCCGTTTCTTCAGAAATTTCAAGGC

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_D1 TCATGATAAATACAAGTTCTTATGTGTTCCGATGCCTCAGGTCAGCCTAAGAAATGAACAACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTAGCGGACAGAAATGTCTCTC
CD141_D1_2 TCATGATAAATACAAGTTCTTATGTGTTCCGATGCCTCAGGTCAGCCTAAGAAATGAACAACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTAGCGGACAGAAATGTCTCTC
CD141_D1_3 TCATGATAAATACAAGTTCTTATGTGTTCCGATGCCTCAGGTCAGCCTAAGAAATGAACAACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTAGCGGACAGAAATGTCTCTC
CD141_D1_1 TCATGATAAATACAAGTTCTTATGTGTTCCGATGCCTCAGGTCAGCCTAAGAAATGAACAACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTAGCGGACAGAAATGTCTCTC
Consensus TCATGATAAATACAAGTTCTTATGTGTTCCGATGCCTCAGGTCAGCCTAAGAAATGAACAACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTAGCGGACAGAAATGTCTCTC

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_D1 AGAAGTTGATTGATCATTGATAGTGGAAAGTTTGGTCTGGAGGAAAACATGCATTACATCAAGAGTTTATCCACGTTGGCGATATTGACAAGGCACATTTATTGCAATCAACACGGCGCGG
CD141_D1_2 AGAAGTTGATTGATCATTGATAGTGGAAAGTTTGGTCTGGAGGAAAACATGCATTACATCAAGAGTTTATCCACGTTGGCGATATTGACAAGGCACATTTATTGCAATCAACACGGCGCGG
CD141_D1_3 AGAAGTTGATTGATCATTGATAGTGGAAAGTTTGGTCTGGAGGAAAACATGCATTACATCAAGAGTTTATCCACGTTGGCGATATTGACAAGGCACATTTATTGCAATCAACACGGCGCGG
CD141_D1_1 AGAAGTTGATTGATCATTGATAGTGGAAAGTTTGGTCTGGAGGAAAACATGCATTACATCAAGAGTTTATCCACGTTGGCGATATTGACAAGGCACATTTATTGCAATCAACACGGCGCGG
Consensus AGAAGTTGATTGATCATTGATAGTGGAAAGTTTGGTCTGGAGGAAAACATGCATTACATCAAGAGTTTATCCACGTTGGCGATATTGACAAGGCACATTTATTGCAATCAACACGGCGCGG

1691 1700 1710 1720 1730 1740 1749
|-----|-----|-----|-----|-----|-----|-----|
CD141_D1 AGAGAATCACAAATGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
CD141_D1_2 AGAGAATCACAAATGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
CD141_D1_3 AGAGAATCACAAATGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
CD141_D1_1 AGAGAATCACAAATGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
Consensus AGAGAATCACAAATGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG

1 10 20 30 40 50 60 70 80 90 100 110 120 130
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_T1 ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTGCCAAATCTTTTGTGTGTTCTTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCAATCTACTACTAGCC
CD141_T1_2 ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTGCCAAATCTTTTGTGTGTTCTTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCAATCTACTACTAGCC
CD141_T1_1 ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTGCCAAATCTTTTGTGTGTTCTTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCAATCTACTACTAGCC
Consensus ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTGCCAAATCTTTTGTGTGTTCTTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCAATCTACTACTAGCC

131 140 150 160 170 180 190 200 210 220 230 240 250 260
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_T1 ATGTTGATGTTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCCTAAAATTGGATCAACGATCCAATGGTCCAATGTATTACATGGAGTGTATCATTATTTCTACCAGTACAACCCAAAGGG
CD141_T1_2 ATGTTGATGTTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCCTAAAATTGGATCAACGATCCAATGGTCCAATGTATTACATGGAGTGTATCATTATTTCTACCAGTACAACCCAAAGGG
CD141_T1_1 ATGTTGATGTTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCCTAAAATTGGATCAACGATCCAATGGTCCAATGTATTACATGGAGTGTATCATTATTTCTACCAGTACAACCCAAAGGG
Consensus ATGTTGATGTTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCCTAAAATTGGATCAACGATCCAATGGTCCAATGTATTACATGGAGTGTATCATTATTTCTACCAGTACAACCCAAAGGG

261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_T1 AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACCTGAAACCCGCTATCTACCCGTCCAAAGTATTTGACAAAGTATGGTACATGGTCCGGGTCAGCCACA
CD141_T1_2 AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACCTGAAACCCGCTATCTACCCGTCCAAAGTATTTGACAAAGTATGGTACATGGTCCGGGTCAGCCACA
CD141_T1_1 AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACCTGAAACCCGCTATCTACCCGTCCAAAGTATTTGACAAAGTATGGTACATGGTCCGGGTCAGCCACA
Consensus AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACCTGAAACCCGCTATCTACCCGTCCAAAGTATTTGACAAAGTATGGTACATGGTCCGGGTCAGCCACA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_T1 ATCTTGCCAGGCACAAGCCTGTTATTCTTACACCCGGATTGTGGATGCTAATAGACACAAGTCCAAACTATGCAATCCCGCTAACATGCTGATCCATATCTTCGTAAGTGGATCAAGCCGATA
CD141_T1_2 ATCTTGCCAGGCACAAGCCTGTTATTCTTACACCCGGATTGTGGATGCTAATAGACACAAGTCCAAACTATGCAATCCCGCTAACATGCTGATCCATATCTTCGTAAGTGGATCAAGCCGATA
CD141_T1_1 ATCTTGCCAGGCACAAGCCTGTTATTCTTACACCCGGATTGTGGATGCTAATAGACACAAGTCCAAACTATGCAATCCCGCTAACATGCTGATCCATATCTTCGTAAGTGGATCAAGCCGATA
Consensus ATCTTGCCAGGCACAAGCCTGTTATTCTTACACCCGGATTGTGGATGCTAATAGACACAAGTCCAAACTATGCAATCCCGCTAACATGCTGATCCATATCTTCGTAAGTGGATCAAGCCGATA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_T1 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCAAACAGCATGGATGGGCCGAGATGGAAATGGAGATCTTGGTAGGCAGTGTGAGGAATCATAGGGGAAGGT
CD141_T1_2 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCAAACAGCATGGATGGGCCGAGATGGAAATGGAGATCTTGGTAGGCAGTGTGAGGAATCATAGGGGAAGGT
CD141_T1_1 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCAAACAGCATGGATGGGCCGAGATGGAAATGGAGATCTTGGTAGGCAGTGTGAGGAATCATAGGGGAAGGT
Consensus ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCAAACAGCATGGATGGGCCGAGATGGAAATGGAGATCTTGGTAGGCAGTGTGAGGAATCATAGGGGAAGGT

651 660 670 680 690 700 710 720 730 740 750 760 770 780
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_T1 TATAATGTACAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTCAATAAAAATAAGATGGT
CD141_T1_2 TATAATGTACAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTCAATAAAAATAAGATGGT
CD141_T1_1 TATAATGTACAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTCAATAAAAATAAGATGGT
Consensus TATAATGTACAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCGGTACTGGAATTTGGGATGTCTGATTTTTTCCAGTGTCAATAAAAATAAGATGGT

781 790 800 810 820 830 840 850 860 870 880 890 900 910
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_T1 TTGGACACGTCATACACGGCAAGACATTAAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGATCATTACACAATTGGTACATATGACACCAAAAGGATCAGTACTTTCCGGATAGCACTT
CD141_T1_2 TTGGACACGTCATACACGGCAAGACATTAAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGATCATTACACAATTGGTACATATGACACCAAAAGGATCAGTACTTTCCGGATAGCACTT
CD141_T1_1 TTGGACACGTCATACACGGCAAGACATTAAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGATCATTACACAATTGGTACATATGACACCAAAAGGATCAGTACTTTCCGGATAGCACTT
Consensus TTGGACACGTCATACACGGCAAGACATTAAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGATCATTACACAATTGGTACATATGACACCAAAAGGATCAGTACTTTCCGGATAGCACTT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_T1 CTATTGATGGATGGAAGGATTGAGACTCGACTATGGTAACTATTACGCGTCCAGACATTCTTTGATAGTGGCAAGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATACGA
CD141_T1_2 CTATTGATGGATGGAAGGATTGAGACTCGACTATGGTAACTATTACGCGTCCAGACATTCTTTGATAGTGGCAAGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATACGA
CD141_T1_1 CTATTGATGGATGGAAGGATTGAGACTCGACTATGGTAACTATTACGCGTCCAGACATTCTTTGATAGTGGCAAGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATACGA
Consensus CTATTGATGGATGGAAGGATTGAGACTCGACTATGGTAACTATTACGCGTCCAGACATTCTTTGATAGTGGCAAGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATACGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_T1 TGTGAGGAAGGATGGGCCGGAGTTCACCCATTCTCCTGTAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTCAAGAAATAGAACTCTAAGAAGAAAAGGTCCAAATTAAT
CD141_T1_2 TGTGAGGAAGGATGGGCCGGAGTTCACCCATTCTCCTGTAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTCAAGAAATAGAACTCTAAGAAGAAAAGGTCCAAATTAAT
CD141_T1_1 TGTGAGGAAGGATGGGCCGGAGTTCACCCATTCTCCTGTAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTCAAGAAATAGAACTCTAAGAAGAAAAGGTCCAAATTAAT
Consensus TGTGAGGAAGGATGGGCCGGAGTTCACCCATTCTCCTGTAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTCAAGAAATAGAACTCTAAGAAGAAAAGGTCCAAATTAAT

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_T1 AACAAAAGTTGAACAGGGGAGAAAAGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGAATTTCTCATTACAAAGTTTGGACAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC
CD141_T1_2 AACAAAAGTTGAACAGGGGAGAAAAGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGAATTTCTCATTACAAAGTTTGGACAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC
CD141_T1_1 AACAAAAGTTGAACAGGGGAGAAAAGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGAATTTCTCATTACAAAGTTTGGACAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC
Consensus AACAAAAGTTGAACAGGGGAGAAAAGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGAATTTCTCATTACAAAGTTTGGACAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_T1 TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTGGACTTGGGCTTTTGGGCTACTAATTTGGCTTCTAAAACCTTAGAAGATACACACCCGTTTTCTTCAGAAATTTCAAGGC
CD141_T1_2 TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTGGACTTGGGCTTTTGGGCTACTAATTTGGCTTCTAAAACCTTAGAAGATACACACCCGTTTTCTTCAGAAATTTCAAGGC
CD141_T1_1 TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTGGACTTGGGCTTTTGGGCTACTAATTTGGCTTCTAAAACCTTAGAAGATACACACCCGTTTTCTTCAGAAATTTCAAGGC
Consensus TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTGGACTTGGGCTTTTGGGCTACTAATTTGGCTTCTAAAACCTTAGAAGATACACACCCGTTTTCTTCAGAAATTTCAAGGC

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_T1 TCATGATAAATACAAGTTCCTATGTGTTCCGATGCCTCAAGGTCAAGCCTAAGAATGAACAACACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAGAATTTGTCTCTC
CD141_T1_2 TCATGATAAATACAAGTTCCTATGTGTTCCGATGCCTCAAGGTCAAGCCTAAGAATGAACAACACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAGAATTTGTCTCTC
CD141_T1_1 TCATGATAAATACAAGTTCCTATGTGTTCCGATGCCTCAAGGTCAAGCCTAAGAATGAACAACACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAGAATTTGTCTCTC
Consensus TCATGATAAATACAAGTTCCTATGTGTTCCGATGCCTCAAGGTCAAGCCTAAGAATGAACAACACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAGAATTTGTCTCTC

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_T1 AGAAGTTTGGATTGATCATTGATAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAGAGTTTATCCACGTTGGCGATATTTGACAAAGCACATTTATTTGCATTCAACACGGCGCGG
CD141_T1_2 AGAAGTTTGGATTGATCATTGATAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAGAGTTTATCCACGTTGGCGATATTTGACAAAGCACATTTATTTGCATTCAACACGGCGCGG
CD141_T1_1 AGAAGTTTGGATTGATCATTGATAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAGAGTTTATCCACGTTGGCGATATTTGACAAAGCACATTTATTTGCATTCAACACGGCGCGG
Consensus AGAAGTTTGGATTGATCATTGATAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAGAGTTTATCCACGTTGGCGATATTTGACAAAGCACATTTATTTGCATTCAACACGGCGCGG

1691 1700 1710 1720 1730 1740 1749
|-----|-----|-----|-----|-----|-----|-----|
CD141_T1 AGAGAATCACAAATGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
CD141_T1_2 AGAGAATCACAAATGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
CD141_T1_1 AGAGAATCACAAATGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
Consensus AGAGAATCACAAATGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
CD141_T2	-----													
CD141_T2_1	ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTTGCCAATCTTTTGTGTGCTTCTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCATCTACTACTAGCC													
Consensus	ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTTGCCAATCTTTTGTGTGCTTCTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCATCTACTACTAGCC													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
CD141_T2	-----													
CD141_T2_1	ATGTTGATGCTAGCAAGGTCATAGAAGTGGTTATCATTTCACCTCCTAAAACTGGATCAACGATCCAATGGCCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACACCCAAGGG													
Consensus	ATGTTGATGCTAGCAAGGTCATAGAAGTGGTTATCATTTCACCTCCTAAAACTGGATCAACGATCCAATGGCCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACACCCAAGGG													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
CD141_T2	-----													
CD141_T2_1	AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCCTTGAACCCGCTATCTACCCGTCCAAGTATTTGACAGTATGGTACATGGTCTGGGTACGCCACA													
Consensus	AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCCTTGAACCCGCTATCTACCCGTCCAAGTATTTGACAGTATGGTACATGGTCTGGGTACGCCACA													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
CD141_T2	-----													
CD141_T2_1	ATCTTGCCAGGCAACAAGCCTGTTATTCTTACACTGGAAATGTTGGATGGTAATAGACACAAAGTCCAAAATATGCAATCCCGGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCCGATA													
Consensus	ATCTTGCCAGGCAACAAGCCTGTTATTCTTACACTGGAAATGTTGGATGGTAATAGACACAAAGTCCAAAATATGCAATCCCGGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCCGATA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
CD141_T2	-----													
CD141_T2_1	ACAATCCATTGATTGTTGCTGACAAAACCATCAACAAAAGCCAAATTCGTGATCCACACCCGCATGGATGGGCCGAGATGGAAATTTGGAGAACTTGGTAGGCAGTGTGAGGAATCATAGGGGAAGGT													
Consensus	ACAATCCATTGATTGTTGCTGACAAAACCATCAACAAAAGCCAAATTCGTGATCCACACCCGCATGGATGGGCCGAGATGGAAATTTGGAGAACTTGGTAGGCAGTGTGAGGAATCATAGGGGAAGGT													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
CD141_T2	-----													
CD141_T2_1	TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCCGTACTGGAAATTTGGGAATGTCTGATTTTTTTCCAGTGTCAATAAAAATAAAGATGGT													
Consensus	TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCCGTACTGGAAATTTGGGAATGTCTGATTTTTTTCCAGTGTCAATAAAAATAAAGATGGT													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
CD141_T2	-----													
CD141_T2_1	TTGGACACGTATACACACGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACAAAGTTTGGTACATATGACACCAAAAAGGATCAGTACTTTCCGGATAGCACTT													
Consensus	TTGGACACGTATACACACGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACAAAGTTTGGTACATATGACACCAAAAAGGATCAGTACTTTCCGGATAGCACTT													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
CD141_T2	-----													
CD141_T2_1	CTATTGATGGATGGAAAGGATTGAGACTCGACTATGGTAACTATTACGCGTCCAAGACATTCTTTGATAGTGGCAAGAAATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA													
Consensus	CTATTGATGGATGGAAAGGATTGAGACTCGACTATGGTAACTATTACGCGTCCAAGACATTCTTTGATAGTGGCAAGAAATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
CD141_T2	-----													
CD141_T2_1	TGTGAGGAAGGATGGGCCGGAGTTCACCCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACCAATTGGTTCAATGGCCTGTTCAAGAAATTAAGAACTCTAAGAAGAAAAGGTCCAATTAAT													
Consensus	TGTGAGGAAGGATGGGCCGGAGTTCACCCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACCAATTGGTTCAATGGCCTGTTCAAGAAATTAAGAACTCTAAGAAGAAAAGGTCCAATTAAT													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
CD141_T2	-----													
CD141_T2_1	AACAAAAGTTGAACAAGGGAGAAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTCATTACAAAGTTTGGACAGGGCAGAGCCATTTGATCCTAGTTGGGCTGATC													
Consensus	AACAAAAGTTGAACAAGGGAGAAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTCATTACAAAGTTTGGACAGGGCAGAGCCATTTGATCCTAGTTGGGCTGATC													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
CD141_T2	-----													
CD141_T2_1	TTTACGCGCAAGATGTATGCGCCATTAAAGGTTCAACGGTCCAAGGTGGACTTGGGCCTTTTGGGCTACTRACTTTGGCTTCTAAAACTTAGAAGAATACACACCCGTTTTCTTCAGAATTTTCAGGC													
Consensus	TTTACGCGCAAGATGTATGCGCCATTAAAGGTTCAACGGTCCAAGGTGGACTTGGGCCTTTTGGGCTACTRACTTTGGCTTCTAAAACTTAGAAGAATACACACCCGTTTTCTTCAGAATTTTCAGGC													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
CD141_T2	-----													
CD141_T2_1	TCATGATAAATACAAGTTCTTATGTGTTCCGATGCCTCAAGGTCAGGCTAAGGAATGAACAACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAGAARTTGTCTCTC													
Consensus	TCATGATAAATACAAGTTCTTATGTGTTCCGATGCCTCAAGGTCAGGCTAAGGAATGAACAACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAGAARTTGTCTCTC													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
CD141_T2	-----													
CD141_T2_1	AGAAGTTTGATTGATCATTTCGATAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAAGAGTTTATCCACGTTGGCGATATTTGACAAGGCACATTTATTTGCATTCAACACGGCGCGG													
Consensus	AGAAGTTTGATTGATCATTTCGATAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAAGAGTTTATCCACGTTGGCGATATTTGACAAGGCACATTTATTTGCATTCAACACGGCGCGG													
	1691	1700	1710	1720	1730	1740	1749							
CD141_T2	-----													
CD141_T2_1	AGAGAATCACAAATTGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG													
Consensus	AGAGAATCACAAATTGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG													

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
CD141_P401	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
CD141_P401_1	MEILRRSSSLWVLPILLLCFFINNGVFDASHKYYMHLQSTTSHVDASKVHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIMGNIYVAHSVSKDLINWIPLPAIYPSKYFDKYGTWSGSAT													
Consensus	MEILRRSSSLWVLPILLLCFFINNGVFDASHKYYMHLQSTTSHVDASKVHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIMGNIYVAHSVSKDLINWIPLPAIYPSKYFDKYGTWSGSAT													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
CD141_P401	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
CD141_P401_1	ILPGNKPVILYTGIVDGNKTQVQNYAIPANMSDPYLRKWKIPDNPLIYADKTINKSQFRDPTTAWMGRDGNWRILVGSYRNHRGKVIHYKSNKNFMKATKAKHPLHSAPGTGNWECPDFFPVSLKNKDG													
Consensus	ILPGNKPVILYTGIVDGNKTQVQNYAIPANMSDPYLRKWKIPDNPLIYADKTINKSQFRDPTTAWMGRDGNWRILVGSYRNHRGKVIHYKSNKNFMKATKAKHPLHSAPGTGNWECPDFFPVSLKNKDG													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
CD141_P401	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
CD141_P401_1	LDSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYYASKTFFDSGKNRRILLGHANESDTVDNDVRKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKYQLN													
Consensus	LDSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYYASKTFFDSGKNRRILLGHANESDTVDNDVRKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKYQLN													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
CD141_P401	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
CD141_P401_1	NKKLNKGEKVEIKGITVAQADVEYIFSFSLDKAEPFDPADLVAQDYCAIKGSTVQGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDVLDADKLSL													
Consensus	NKKLNKGEKVEIKGITVAQADVEYIFSFSLDKAEPFDPADLVAQDYCAIKGSTVQGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDVLDADKLSL													
	521	530	540	550	560	570	58882							
CD141_P401	-----+-----+-----+-----+-----+-----+-----													
CD141_P401_1	RSLIDHSIYESFGAGGKTCITSRVYPTLAIFDKAHLFAFNNGAERITTIETLNAWSMANAKLH													
Consensus	RSLIDHSIYESFGAGGKTCITSRVYPTLAIFDKAHLFAFNNGAERITTIETLNAWSMANAKLH													

1 10 20 30 40 50 60 70 80 90 100 110 120 130
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD141_P541 MEILRRSSSLWVLPILLLCFFINNGVFDASHK**V**YMHQLQSTTSHVDASKVHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIWGNIYVAHSVSKDLINWIPLPAIYPSKYFDKYGTWSGSAT
CD141_P541_1 MEILRRSSSLWVLPILLLCFFINNGVFDASHK**V**YMHQLQSTTSHVDASKVHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIWGNIYVAHSVSKDLINWIPLPAIYPSKYFDKYGTWSGSAT
CD141_P542 MEILRRSSSLWVLPILLLCFFINNGVFDASHK**A**YMHQLQSTTSHVDASKVHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIWGNIYVAHSVSKDLINWIPLPAIYPSKYFDKYGTWSGSAT
CD141_P542_1 MEILRRSSSLWVLPILLLCFFINNGVFDASHK**A**YMHQLQSTTSHVDASKVHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIWGNIYVAHSVSKDLINWIPLPAIYPSKYFDKYGTWSGSAT
Consensus MEILRRSSSLWVLPILLLCFFINNGVFDASHK**v**YMHQLQSTTSHVDASKVHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIWGNIYVAHSVSKDLINWIPLPAIYPSKYFDKYGTWSGSAT

131 140 150 160 170 180 190 200 210 220 230 240 250 260
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD141_P541 ILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYLRKWIKPDNNPLIYADKTINKSQFRDPTTAWMGRDGNWRILVGSYRNHRGKVIHYKSNKNFMKWTAKAHPHLSAPGTGNWECPDFFPV**S**LKNKNG
CD141_P541_1 ILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYLRKWIKPDNNPLIYADKTINKSQFRDPTTAWMGRDGNWRILVGSYRNHRGKVIHYKSNKNFMKWTAKAHPHLSAPGTGNWECPDFFPV**S**LKNKNG
CD141_P542 ILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYLRKWIKPDNNPLIYADKTINKSQFRDPTTAWMGRDGNWRILVGSYRNHRGKVIHYKSNKNFMKWTAKAHPHLSAPGTGNWECPDFFPV**L**LKNKNG
CD141_P542_1 ILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYLRKWIKPDNNPLIYADKTINKSQFRDPTTAWMGRDGNWRILVGSYRNHRGKVIHYKSNKNFMKWTAKAHPHLSAPGTGNWECPDFFPV**L**LKNKNG
Consensus ILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYLRKWIKPDNNPLIYADKTINKSQFRDPTTAWMGRDGNWRILVGSYRNHRGKVIHYKSNKNFMKWTAKAHPHLSAPGTGNWECPDFFPV**s**LKNKNG

261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD141_P541 LDTSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDKYFPDNTSIDGWKGLRLDYGNYYASKTFFDSGKNRRILLGWANESDTVDND**V**RKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKVQLN
CD141_P541_1 LDTSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDKYFPDNTSIDGWKGLRLDYGNYYASKTFFDSGKNRRILLGWANESDTVDND**V**RKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKVQLN
CD141_P542 LDTSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDKYFPDNTSYDGWKGLRLDYGNYYASKTFFDSGKNRRILLGWANESDTVDND**A**RKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKVQLN
CD141_P542_1 LDTSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDKYFPDNTSYDGWKGLRLDYGNYYASKTFFDSGKNRRILLGWANESDTVDND**A**RKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKVQLN
Consensus LDTSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDKYFPDNTS**!**DGWKGLRLDYGNYYASKTFFDSGKNRRILLGWANESDTVDND**v**RKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKVQLN

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD141_P541 NKKLNGKKKVEIKGITVAQADVEYIFSFSTSLDKAEPFDPADWADLYAQDYCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPV**F**FRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDVLDADKKLSL
CD141_P541_1 NKKLNGKKKVEIKGITVAQADVEYIFSFSTSLDKAEPFDPADWADLYAQDYCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPV**F**FRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDVLDADKKLSL
CD141_P542 NKKLNGKKKVEIKGITVAQADVEYIFSFSTSLDKAEPFDPADWADLYAQDYCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPV**S**FRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDVLDADKKLSL
CD141_P542_1 NKKLNGKKKVEIKGITVAQADVEYIFSFSTSLDKAEPFDPADWADLYAQDYCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPV**S**FRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDVLDADKKLSL
Consensus NKKLNGKKKVEIKGITVAQADVEYIFSFSTSLDKAEPFDPADWADLYAQDYCAIKGSTVQGGGLGPFGLLTLASKNLEEYTPV**f**FRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDVLDADKKLSL

521 530 540 550 560 570 580 582
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
CD141_P541 RSLIDHSVYESFGAGGK**T**CITSRVYPTLAIFDKAHL**F**VFNNGAERITIIETLNAWSMANAKLH
CD141_P541_1 RSLIDHSVYESFGAGGK**T**CITSRVYPTLAIFDKAHL**F**VFNNGAERITIIETLNAWSMANAKLH
CD141_P542 RSLIDHSVYESFGAGGK**I**CITSRVYPTLAIFDKAHL**F**AFNNGAERITIIETLNAWSMANAKLH
CD141_P542_1 RSLIDHSVYESFGAGGK**I**CITSRVYPTLAIFDKAHL**F**AFNNGAERITIIETLNAWSMANAKLH
Consensus RSLIDHSVYESFGAGGK**t**CITSRVYPTLAIFDKAHL**f**VFNNGAERITIIETLNAWSMANAKLH

1 10 20 30 40 50 60 70 80 90 100 110 120 130
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_P181 ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTGGCAATTCTTTGTTGTGTTCTTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCAATCTACTACTAGCC
CD141_P181_2 ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTGGCAATTCTTTGTTGTGTTCTTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCAATCTACTACTAGCC
CD141_P181_1 ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTGGCAATTCTTTGTTGTGTTCTTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCAATCTACTACTAGCC
Consensus ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTGGCAATTCTTTGTTGTGTTCTTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCAATCTACTACTAGCC

131 140 150 160 170 180 190 200 210 220 230 240 250 260
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_P181 ATGTTGATGTTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCCTAAAATTGGATCAACGATCCAATGGCCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACAACCCAAAGGG
CD141_P181_2 ATGTTGATGTTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCCTAAAATTGGATCAACGATCCAATGGCCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACAACCCAAAGGG
CD141_P181_1 ATGTTGATGTTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCCTAAAATTGGATCAACGATCCAATGGCCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACAACCCAAAGGG
Consensus ATGTTGATGTTAGCAAGGTCATAGAACTGGTTATCATTTCACCTCCTAAAATTGGATCAACGATCCAATGGCCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACAACCCAAAGGG

261 270 280 290 300 310 320 330 340 350 360 370 380 390
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_P181 AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACCTTGAAACCCGCTATCTACCCGTCCAAGTATTTGACAAAGTATGGTACATGGTCCGGGTCAGCCACA
CD141_P181_2 AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACCTTGAAACCCGCTATCTACCCGTCCAAGTATTTGACAAAGTATGGTACATGGTCCGGGTCAGCCACA
CD141_P181_1 AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACCTTGAAACCCGCTATCTACCCGTCCAAGTATTTGACAAAGTATGGTACATGGTCCGGGTCAGCCACA
Consensus AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCACCTTGAAACCCGCTATCTACCCGTCCAAGTATTTGACAAAGTATGGTACATGGTCCGGGTCAGCCACA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_P181 ATCTTGCCAGGCACAAGCCTGTTATTCTTACACTGGAAATGTGGATGCTAACAGACACAAGTCCAAATATGCAATCCCGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCGACA
CD141_P181_2 ATCTTGCCAGGCACAAGCCTGTTATTCTTACACTGGAAATGTGGATGCTAACAGACACAAGTCCAAATATGCAATCCCGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCGACA
CD141_P181_1 ATCTTGCCAGGCACAAGCCTGTTATTCTTACACTGGAAATGTGGATGCTAACAGACACAAGTCCAAATATGCAATCCCGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCGACA
Consensus ATCTTGCCAGGCACAAGCCTGTTATTCTTACACTGGAAATGTGGATGCTAACAGACACAAGTCCAAATATGCAATCCCGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCGACA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_P181 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCAAACACAGCATGGATGGGCCGAGATGGAAATGGAGATCTTGGTTGGGAGTGTGAGGAATCATAGGGGAAGGT
CD141_P181_2 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCAAACACAGCATGGATGGGCCGAGATGGAAATGGAGATCTTGGTTGGGAGTGTGAGGAATCATAGGGGAAGGT
CD141_P181_1 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCAAACACAGCATGGATGGGCCGAGATGGAAATGGAGATCTTGGTTGGGAGTGTGAGGAATCATAGGGGAAGGT
Consensus ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCAAACACAGCATGGATGGGCCGAGATGGAAATGGAGATCTTGGTTGGGAGTGTGAGGAATCATAGGGGAAGGT

651 660 670 680 690 700 710 720 730 740 750 760 770 780
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_P181 TATAATGTACAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTAGCCCCGGGTACTGGAATTTGGGATGTCTGATTTTTTTCCAGTGTCAATGAAAATAAAATGGT
CD141_P181_2 TATAATGTACAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTAGCCCCGGGTACTGGAATTTGGGATGTCTGATTTTTTTCCAGTGTCAATGAAAATAAAATGGT
CD141_P181_1 TATAATGTACAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTAGCCCCGGGTACTGGAATTTGGGATGTCTGATTTTTTTCCAGTGTCAATGAAAATAAAATGGT
Consensus TATAATGTACAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTAGCCCCGGGTACTGGAATTTGGGATGTCTGATTTTTTTCCAGTGTCAATGAAAATAAAATGGT

781 790 800 810 820 830 840 850 860 870 880 890 900 910
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_P181 TTGGACACGTCATACAAATGGCAAGACGTTAAACATGTTCTTAAGGTTAGCTTTGATGTTACTAGGTTTGATCATTACACAGTTGGTACATATGACCCAAAAGGATAAATACTTTCCGGATACACTT
CD141_P181_2 TTGGACACGTCATACAAATGGCAAGACGTTAAACATGTTCTTAAGGTTAGCTTTGATGTTACTAGGTTTGATCATTACACAGTTGGTACATATGACCCAAAAGGATAAATACTTTCCGGATACACTT
CD141_P181_1 TTGGACACGTCATACAAATGGCAAGACGTTAAACATGTTCTTAAGGTTAGCTTTGATGTTACTAGGTTTGATCATTACACAGTTGGTACATATGACCCAAAAGGATAAATACTTTCCGGATACACTT
Consensus TTGGACACGTCATACAAATGGCAAGACGTTAAACATGTTCTTAAGGTTAGCTTTGATGTTACTAGGTTTGATCATTACACAGTTGGTACATATGACCCAAAAGGATAAATACTTTCCGGATACACTT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_P181 CTATTGATGGATGGAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAGACATTCTTTGATAATGGCAAGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA
CD141_P181_2 CTATTGATGGATGGAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAGACATTCTTTGATAATGGCAAGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA
CD141_P181_1 CTATTGATGGATGGAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAGACATTCTTTGATAATGGCAAGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA
Consensus CTATTGATGGATGGAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAGACATTCTTTGATAATGGCAAGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_P181 TGTGAGGAAGGATGGGCCGGAGTTCACCCATTCTCCTGTAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTCAAGAAATGAAACACTGAGAAAGAAAAGTCCAAATTAAT
CD141_P181_2 TGTGAGGAAGGATGGGCCGGAGTTCACCCATTCTCCTGTAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTCAAGAAATGAAACACTGAGAAAGAAAAGTCCAAATTAAT
CD141_P181_1 TGTGAGGAAGGATGGGCCGGAGTTCACCCATTCTCCTGTAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTCAAGAAATGAAACACTGAGAAAGAAAAGTCCAAATTAAT
Consensus TGTGAGGAAGGATGGGCCGGAGTTCACCCATTCTCCTGTAATTTGGCTTGATCCTAGTGGAAACAAATGGTTCAATGGCTGTTCAAGAAATGAAACACTGAGAAAGAAAAGTCCAAATTAAT

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_P181 AACAAAAGTTGAACAAAGGAGAAAAGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGAATTTCTCATTACAAAGTTTGGATAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC
CD141_P181_2 AACAAAAGTTGAACAAAGGAGAAAAGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGAATTTCTCATTACAAAGTTTGGATAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC
CD141_P181_1 AACAAAAGTTGAACAAAGGAGAAAAGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGAATTTCTCATTACAAAGTTTGGATAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC
Consensus AACAAAAGTTGAACAAAGGAGAAAAGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGAATTTCTCATTACAAAGTTTGGATAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_P181 TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTGGACTTGGGCTTTTGGGCTACTAATTTGGCTTCTAAAACCTTAGAAGATACACACCCGTTTTCTTCAGAAATTTCAAGAC
CD141_P181_2 TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTGGACTTGGGCTTTTGGGCTACTAATTTGGCTTCTAAAACCTTAGAAGATACACACCCGTTTTCTTCAGAAATTTCAAGAC
CD141_P181_1 TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTGGACTTGGGCTTTTGGGCTACTAATTTGGCTTCTAAAACCTTAGAAGATACACACCCGTTTTCTTCAGAAATTTCAAGAC
Consensus TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTGGACTTGGGCTTTTGGGCTACTAATTTGGCTTCTAAAACCTTAGAAGATACACACCCGTTTTCTTCAGAAATTTCAAGAC

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_P181 TCATGATAAATACAGGTTCTTATGTGTTCCGATGCCTCAGGTCAGGCTAAGGAATGAACAACACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCAGACAGAATTTGTCTCTT
CD141_P181_2 TCATGATAAATACAGGTTCTTATGTGTTCCGATGCCTCAGGTCAGGCTAAGGAATGAACAACACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCAGACAGAATTTGTCTCTT
CD141_P181_1 TCATGATAAATACAGGTTCTTATGTGTTCCGATGCCTCAGGTCAGGCTAAGGAATGAACAACACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCAGACAGAATTTGTCTCTT
Consensus TCATGATAAATACAGGTTCTTATGTGTTCCGATGCCTCAGGTCAGGCTAAGGAATGAACAACACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCAGACAGAATTTGTCTCTT

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
CD141_P181 AGAAGTTTGATTGATCATTTCGGTAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAGGGTTTATCCACGTTGGCGATATTTGACAAAGCACATTTATTTGCATTCAACACGGCGCGG
CD141_P181_2 AGAAGTTTGATTGATCATTTCGGTAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAGGGTTTATCCACGTTGGCGATATTTGACAAAGCACATTTATTTGCATTCAACACGGCGCGG
CD141_P181_1 AGAAGTTTGATTGATCATTTCGGTAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAGGGTTTATCCACGTTGGCGATATTTGACAAAGCACATTTATTTGCATTCAACACGGCGCGG
Consensus AGAAGTTTGATTGATCATTTCGGTAGTGGAAAGTTTGGTGTGGAGGAAAACATGCATTACATCAGGGTTTATCCACGTTGGCGATATTTGACAAAGCACATTTATTTGCATTCAACACGGCGCGG

1691 1700 1710 1720 1730 1740 1749
|-----|-----|-----|-----|-----|-----|-----|
CD141_P181 AGAGAATCACAAATGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
CD141_P181_2 AGAGAATCACAAATGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
CD141_P181_1 AGAGAATCACAAATGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
Consensus AGAGAATCACAAATGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
CD141_P182	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P182_1	ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTTGCCAATTCTTTTGTGTGCTTCTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCARTCTACTACTAGCC													
Consensus	ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTTGCCAATTCTTTTGTGTGCTTCTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCARTCTACTACTAGCC													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
CD141_P182	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P182_1	ATGTTGATGCTAGCAAGGTCCATAGAAGTGGTTATCATTTCACCTCCTAAAACTGGATCAACGATCCAATGGTCCAATGTATTACATGGAGTGTACCATTTATTCTACCAGTACRACCCAAGGG													
Consensus	ATGTTGATGCTAGCAAGGTCCACAGAAGTGGTTATCATTTCACCTCCTAAAACTGGATCAACGATCCAATGGCCAATGTATTACATGGAGTGTATCATTTATTCTACCAGTACRACCCAAGGG													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
CD141_P182	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P182_1	AGCGATATGGGGCAACATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCCTTGARCCCGCTATCTACCCGTCGAAGTATTTGACAGTATGGTACATGGTCCGGGTCAGCCACA													
Consensus	AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCCTTGARCCCGCTATCTACCCGTCGAAGTATTTGACAGTATGGTACATGGTCCGGGTCAGCCACA													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
CD141_P182	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P182_1	ATCTTGCCAGGCACRACAGCCTGTTATTCTCTACACTGGAAATTGTGGATGCTAACAGACACRAGTCCAAAATTATGCAATCCCGGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCCGATA													
Consensus	ATCTTGCCAGGCACRACAGCCTGTTATTCTCTACACTGGAAATTGTGGATGCTAACAGACACRAGTCCAAAATTATGCAATCCCGGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCCGATA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
CD141_P182	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P182_1	ACAATCCATTGATTGTTGCTGACAAAACCATCAACAAAAGCCAAATTCGTGATCCACRACCCGCTTGGATGGGCCGAGATGGAAATTGGAGAACTTGGTGGAGGTGTGAGGAATCATAGGGGAAGGT													
Consensus	ACAATCCATTGATTGTTGCTGACAAAACCATCAACAAAAGCCAAATTCGTGATCCACRACCCGCTTGGATGGGCCGAGATGGAAATTGGAGAACTTGGTGGAGGTGTGAGGAATCATAGGGGAAGGT													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
CD141_P182	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P182_1	TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAAATCCCCTCCACTCGCCCCGGTACTGGAAATTGGGAATGTCTGATTTTTTTCCAGTGTCAATAAAAATAAAGATGGT													
Consensus	TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAAATCCCCTCCACTCGCCCCGGTACTGGAAATTGGGAATGTCTGATTTTTTTCCAGTGTCAATAAAAATAAATGGT													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
CD141_P182	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P182_1	TTGGACACGTATACAAATGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACRAGGTTGATCATTACACRATTGGTACATATGACACCAAAAAGGATAAATACATTCCGGATAACACTT													
Consensus	TTGGACACGTATACAAATGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACRAGGTTGATCATTACACRATTGGTACATATGACACCAAAAAGGATAAATACATTCCGGATAACACTT													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
CD141_P182	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P182_1	CTATTGATGGATGGAAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAAGACATTCTTTGATAGTGGCAAGAACTCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA													
Consensus	CTATTGATGGATGGAAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAAGACATTCTTTGATAGTGGCAAGAACTCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
CD141_P182	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P182_1	TGTGAGGAAGGATGGGCCGGAGTTCACCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACRATTGGTTCAATGGCCTGTTCAAGAATTAGAARCTTAGAAGAAAAGGTCCAATTAAT													
Consensus	TGTGAGGAAGGATGGGCCGGAGTTCACCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACRATTGGTTCAATGGCCTGTTCAAGAATTAGAARCTTAGAAGAAAAGGTCCAATTAAT													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
CD141_P182	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P182_1	AACAAAAGTTGAACAAAGGAGAAAAGGTTGAATCAAGGAATCACAGTTGCTCAGGCTGATGTTGAAGTGATTTTCTCATTACRAGTTGGATAGGGCAGAGCCATTTGATCCTAGTTGGGCTGATC													
Consensus	AACAAAAGTTGAACAAAGGAGAAAAGGTTGAATCAAGGAATCACAGTTGCTCAGGCTGATGTTGAAGTGATTTTCTCATTACRAGTTGGATAGGGCAGAGCCATTTGATCCTAGTTGGGCTGATC													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
CD141_P182	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P182_1	TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAAGGTGGACTTGGGCCTTTTGGGCTCCTAACTTTGGCTTCTAAAACTTAGAAGATAACACACCCGTTTTCTTTAGAATTTCAAGGC													
Consensus	TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAAGGTGGACTTGGGCCTTTTGGGCTCCTAACTTTGGCTTCTAAAACTTAGAAGATAACACACCCGTTTTCTTTAGAATTTCAAGGC													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
CD141_P182	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P182_1	TCAGATAAATACAAAGTTCTTATGTGTTCCGATGCTTCRAGGTCRAGCCTAAGAATGAARCACTATGTACRACCATCATTTGCTGGATATGTAGATGTAGATTTAGCGGACRAGAARTTGTCTCTT													
Consensus	TCAGATAAATACAAAGTTCTTATGTGTTCCGATGCTTCRAGGTCRAGCCTAAGAATGAARCACTATGTACRACCATCATTTGCTGGATATGTAGATGTAGATTTAGCGGACRAGAARTTGTCTCTT													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
CD141_P182	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P182_1	AGAAGTTTGATTGATCATTCCGTAGTGGAAAGTTTGGTGCCTGGAGGAAAACATGCATTACATCAAGGGTTATCCRACGTTGGCGATATTTGACRAGCACATTTATTTGCAATTCACRACGGCGCGG													
Consensus	AGAAGTTTGATTGATCATTCCGTAGTGGAAAGTTTGGTGCCTGGAGGAAAACATGCATTACATCAAGGGTTATCCRACGTTGGCGATATTTGACRAGCACATTTATTTGCAATTCACRACGGCGCGG													
	1691	1700	1710	1720	1730	1740	1749							
CD141_P182	----- ----- ----- ----- ----- ----- -----													
CD141_P182_1	AGAGAATCACRATTGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG													
Consensus	AGAGAATCACRATTGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG													

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
CD141_P401	-----													
CD141_P401_1	ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTTGCCAATTCTTTTGTGTGCTTCTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAGTTTATATGCACTTGCATCTACTACTAGCC													
Consensus	ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTTGCCAATTCTTTTGTGTGCTTCTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAGTTTATATGCACTTGCATCTACTACTAGCC													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
CD141_P401	-----													
CD141_P401_1	ATGTTGATGCTAGCAAGGTCATAGAAGTGGTTATCATTTCACCTCCTAAAACTGGATCAACGATCCAATGGCCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACACCCAAGGG													
Consensus	ATGTTGATGCTAGCAAGGTCATAGAAGTGGTTATCATTTCACCTCCTAAAACTGGATCAACGATCCAATGGCCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACACCCAAGGG													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
CD141_P401	-----													
CD141_P401_1	AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCCTTGACCCCGCTATCTACCCGTCCAAGTATTTGACAGTATGGTACATGGTCTGGGTACGCCACA													
Consensus	AGCGATATGGGGCAATATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCCTTGACCCCGCTATCTACCCGTCCAAGTATTTGACAGTATGGTACATGGTCTGGGTACGCCACA													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
CD141_P401	-----													
CD141_P401_1	ATCTTGCCAGGCAACAAGCCTGTTATTCTTACACTGGAAATGTTGGATGGTAATAGACACAGTCCAAAATATGCAATCCCGGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCCGATA													
Consensus	ATCTTGCCAGGCAACAAGCCTGTTATTCTTACACTGGAAATGTTGGATGGTAATAGACACAGTCCAAAATATGCAATCCCGGCTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCCGATA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
CD141_P401	-----													
CD141_P401_1	ACAATCCATTGATTGTTGCTGACAAAACCATCAACAAAAGCCAAATTCGTGATCCACACCCGCATGGATGGGCCGAGATGGAAATGGAGAACTTGGTAGGCAGTGTGAGGAATCATAGGGGAAGGT													
Consensus	ACAATCCATTGATTGTTGCTGACAAAACCATCAACAAAAGCCAAATTCGTGATCCACACCCGCATGGATGGGCCGAGATGGAAATGGAGAACTTGGTAGGCAGTGTGAGGAATCATAGGGGAAGGT													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
CD141_P401	-----													
CD141_P401_1	TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCGGTACTGGAAATGGGAATGTCTGATTTTTTTCCAGTGTCAATAAAAATAAAGATGGT													
Consensus	TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCGGTACTGGAAATGGGAATGTCTGATTTTTTTCCAGTGTCAATAAAAATAAAGATGGT													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
CD141_P401	-----													
CD141_P401_1	TTGGACACGTATACACACGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGTACATATGACACCAAAAAGGATCAGTACTTTCCGGATAGCACTT													
Consensus	TTGGACACGTATACACACGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGTACATATGACACCAAAAAGGATCAGTACTTTCCGGATAGCACTT													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
CD141_P401	-----													
CD141_P401_1	CTATTGATGGATGGAAAGGATTGAGACTCGACTATGGTAATATTACGCGTCCAAGACATTCTTTGATAGTGGCAAGAAATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA													
Consensus	CTATTGATGGATGGAAAGGATTGAGACTCGACTATGGTAATATTACGCGTCCAAGACATTCTTTGATAGTGGCAAGAAATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
CD141_P401	-----													
CD141_P401_1	TGTGAGGAAGGATGGGCCGGAGTTCACCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACCAATTGGTTCAATGGCCTGTTCAAGAATTAGAARTCTAGAAGAAAAGGTCCAATTAAT													
Consensus	TGTGAGGAAGGATGGGCCGGAGTTCACCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACCAATTGGTTCAATGGCCTGTTCAAGAATTAGAARTCTAGAAGAAAAGGTCCAATTAAT													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
CD141_P401	-----													
CD141_P401_1	AACAAAAGTTGAACAAGGGAGAAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTCATTACAGTTTGGACAGGGCAGAGCCATTTGATCCTAGTTGGGCTGATC													
Consensus	AACAAAAGTTGAACAAGGGAGAAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTCATTACAGTTTGGACAGGGCAGAGCCATTTGATCCTAGTTGGGCTGATC													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
CD141_P401	-----													
CD141_P401_1	TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAAGGTGGACTTGGGCCTTTTGGGCTACTRACTTTGGCTTCTAAAACTTAGAAGAATACACACCCGTTTTCTTCAGAATTTTCAGGC													
Consensus	TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAAGGTGGACTTGGGCCTTTTGGGCTACTRACTTTGGCTTCTAAAACTTAGAAGAATACACACCCGTTTTCTTCAGAATTTTCAGGC													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
CD141_P401	-----													
CD141_P401_1	TCATGATAAATACAAGTTCTTATGTGTTCCGATGCCTCAAGGTCAGGCTAAGGAATGAACAACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAGAARTTGTCTCTC													
Consensus	TCATGATAAATACAAGTTCTTATGTGTTCCGATGCCTCAAGGTCAGGCTAAGGAATGAACAACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAGAARTTGTCTCTC													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
CD141_P401	-----													
CD141_P401_1	AGAAGTTTGATTGATCATTTCGATAGTGGAAAGTTTGGTGCcGGAGGAAAACATGCATTACATCAGAGTTTATCCACGTTGGCGATATTTGACAAGGCACATTTATTTGCATTCAACACGGCGCGG													
Consensus	AGAAGTTTGATTGATCATTTCGATAGTGGAAAGTTTGGTGCcGGAGGAAAACATGCATTACATCAGAGTTTATCCACGTTGGCGATATTTGACAAGGCACATTTATTTGCATTCAACACGGCGCGG													
	1691	1700	1710	1720	1730	1740	1749							
CD141_P401	-----													
CD141_P401_1	AGAGAATCACAAATTGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG													
Consensus	AGAGAATCACAAATTGAGACTCTAAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAa													

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
CD141_P541	-----													
CD141_P541_1	ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTTGCCAATCTTTTGTGTGCTTCTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCATCTACTACTAGCC													
Consensus	ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTTGCCAATCTTTTGTGTGCTTCTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAAGTTTATATGCACTTGCATCTACTACTAGCC													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
CD141_P541	-----													
CD141_P541_1	ATGTTGATGCTAGCAAGGTCACAGAAGTGGTTATCATTTCACCTCCTAAAACTGGATCAATGATCCAATGGCCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACACCCAAGGG													
Consensus	ATGTTGATGCTAGCAAGGTCACAGAAGTGGTTATCATTTCACCTCCTAAAACTGGATCAATGATCCAATGGCCCAATGTATTACATGGAGTGTATCATTATTCTACCAGTACACCCAAGGG													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
CD141_P541	-----													
CD141_P541_1	AGCGATATGGGGCAATATTGTTGGGCCATTCCGGTCTCAAGGACTTGATCAATTGGATCCCCTTGACCCCGCTATCTACCCGTCCAAGTATTTGACAGTATGGTACATGGTCCGGGTCAGCCACA													
Consensus	AGCGATATGGGGCAATATTGTTGGGCCATTCCGGTCTCAAGGACTTGATCAATTGGATCCCCTTGACCCCGCTATCTACCCGTCCAAGTATTTGACAGTATGGTACATGGTCCGGGTCAGCCACA													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
CD141_P541	-----													
CD141_P541_1	ATCTTGCCAGGCAACAAGCCTGTTATTCTCTACACTGGAAATGTTGGATGCTAATAAGACACAGTCCAAAATATGCAATCCCAGGTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCCGATA													
Consensus	ATCTTGCCAGGCAACAAGCCTGTTATTCTCTACACTGGAAATGTTGGATGCTAATAAGACACAGTCCAAAATATGCAATCCCAGGTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCCGATA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
CD141_P541	-----													
CD141_P541_1	ACAATCCATTGATTGTTGCTGACAAAACCATCAACAAAAGCCAAATTCGTGATCCACACCCGCTTGGATGGGCCGAGATGGAAATGGAGAACTTGGTTGGGAGTGTGAGGAATCATAGGGGAAGGT													
Consensus	ACAATCCATTGATTGTTGCTGACAAAACCATCAACAAAAGCCAAATTCGTGATCCACACCCGCTTGGATGGGCCGAGATGGAAATGGAGAACTTGGTTGGGAGTGTGAGGAATCATAGGGGAAGGT													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
CD141_P541	-----													
CD141_P541_1	TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCGGGTACTGGAAATGGGAATGTCTGATTTTTTTCCAGTGTCAATTGAAAATAAAAATGGT													
Consensus	TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCCCCGGGTACTGGAAATGGGAATGTCTGATTTTTTTCCAGTGTCAATTGAAAATAAAAATGGT													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
CD141_P541	-----													
CD141_P541_1	TTGGACACGTATACATGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGTACATATGACACCAAAAAGGATAAATACTTTCCGGATAACACTT													
Consensus	TTGGACACGTATACATGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGTACATATGACACCAAAAAGGATAAATACTTTCCGGATAACACTT													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
CD141_P541	-----													
CD141_P541_1	CTATTGATGGATGGAAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAAGACATTCCTTGATAGCGGCAAGAACTCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA													
Consensus	CTATTGATGGATGGAAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAAGACATTCCTTGATAGCGGCAAGAACTCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
CD141_P541	-----													
CD141_P541_1	TGTGAGGAAGGATGGGCCGGAGTTCACCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAARACAAATTGGTTCAATGGCCTGTTCAAGAATTAGAARCTTAGAAGAAAAGGTCCAATTAAT													
Consensus	TGTGAGGAAGGATGGGCCGGAGTTCACCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAARACAAATTGGTTCAATGGCCTGTTCAAGAATTAGAARCTTAGAAGAAAAGGTCCAATTAAT													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
CD141_P541	-----													
CD141_P541_1	AACAAAAGTTGAACAAGGGAAAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTCATTACAGTTTGGATAAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC													
Consensus	AACAAAAGTTGAACAAGGGAAAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTCATTACAGTTTGGATAAGGCAGAGCCATTTGATCCTAGTTGGGCTGATC													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
CD141_P541	-----													
CD141_P541_1	TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAAGGTGGACTTGGGCCTTTTGGGCTCCTAACTTTGGCTTCTAAAACTTAGAAGATAACACACCCGTTTTCTTTAGAATTTCAAGGC													
Consensus	TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAAGGTGGACTTGGGCCTTTTGGGCTCCTAACTTTGGCTTCTAAAACTTAGAAGATAACACACCCGTTTTCTTTAGAATTTCAAGGC													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
CD141_P541	-----													
CD141_P541_1	TCATGATAAATACAAGTTCTTATGTGTTCTGATGCCTCAAGGTCAGGCTAAAGAAATGAARCACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAGAARTTGTCTCTT													
Consensus	TCATGATAAATACAAGTTCTTATGTGTTCTGATGCCTCAAGGTCAGGCTAAAGAAATGAARCACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAGAARTTGTCTCTT													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
CD141_P541	-----													
CD141_P541_1	AGAAGTTTGATTGATCATTCCGTAGTCGAAGTTTTGGTGCCGGAGGAAAACATGCATTACATCAAGGGTTATCCACATTGGCGATATTTGACAAGGCACATTTATTTGTGTTCAACACGGCGCAG													
Consensus	AGAAGTTTGATTGATCATTCCGTAGTCGAAGTTTTGGTGCCGGAGGAAAACATGCATTACATCAAGGGTTATCCACATTGGCGATATTTGACAAGGCACATTTATTTGTGTTCAACACGGCGCAG													
	1691	1700	1710	1720	1730	1740	1749							
CD141_P541	-----													
CD141_P541_1	AGAGAATCACAAATTGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG													
Consensus	AGAGAATCACAAATTGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG													

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
CD141_P542	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P542_1	ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTTGCCAATTCTTTTGTGTGCTTCTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAGCTTATATGCACTTGCARTCTACTACTAGCC													
Consensus	ATGGAGATTTAAGAAGATCTTCTTCTTTGGGTTTTGCCAATTCTTTTGTGTGCTTCTTATCAACAATGGAGTATTTGTTGATGCTTCTCACAAGCTTATATGCACTTGCARTCTACTACTAGCC													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
CD141_P542	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P542_1	ATGTTGATGCAAGCAAGGTCCATAGAAGTGGTTATCATTTCACCTCCTAAAACTGGATCAACGATCCAATGGTCCAATGTATTACATGGAGTGACCATTATTCTACCAGTACRACCCAAGGG													
Consensus	ATGTTGATGCAAGCAAGGTCCATAGAAGTGGTTATCATTTCACCTCCTAAAACTGGATCAACGATCCAATGGTCCAATGTATTACATGGAGTGACCATTATTCTACCAGTACRACCCAAGGG													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
CD141_P542	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P542_1	AGCGATATGGGGCAACATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCCTTGACCCGCTATCTACCCGTCGAAGTATTTGACAGTATGGTACATGGTCCGGGTCAGCCACA													
Consensus	AGCGATATGGGGCAACATTGTTGGGCCATTTCGGTCTCAAGGACTTGATCAATTGGATCCCCTTGACCCGCTATCTACCCGTCGAAGTATTTGACAGTATGGTACATGGTCCGGGTCAGCCACA													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
CD141_P542	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P542_1	ATCTTGCCAGGCAACAAGCTGTTATTCTCTACACTGGAAATGTTGGATGCTAATAAGACACAGTCCAAAATATGCAATCCCAGGTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCCGATA													
Consensus	ATCTTGCCAGGCAACAAGCTGTTATTCTCTACACTGGAAATGTTGGATGCTAATAAGACACAGTCCAAAATATGCAATCCCAGGTAACATGTCTGATCCATATCTTCGTAAGTGGATCAAGCCCGATA													
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
CD141_P542	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P542_1	ACAATCCATTGATTGTTGCTGACAAAACCATCAACAAAAGCCAAATTCGTGATCCACACCCGCTTGGATGGGCCGAGATGGAAATGGGAGATCTTGGTTGGGAGTGAGGAAATCATAGGGGAAGGN													
Consensus	ACAATCCATTGATTGTTGCTGACAAAACCATCAACAAAAGCCAAATTCGTGATCCACACCCGCTTGGATGGGCCGAGATGGAAATGGGAGATCTTGGTTGGGAGTGAGGAAATCATAGGGGAAGGN													
	651	660	670	680	690	700	710	720	730	740	750	760	770	780
CD141_P542	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P542_1	TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCTCCGGGTACTGGAAATGGGAATGTCTGATTTTTTTCCAGTGTcATTGAAAATAAAAATGGT													
Consensus	TATAATGTACAAAAGTAATAAGAACTTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCcCCGGGTACTGGAAATGGGAATGTCTGATTTTTTTCCAGTGTcATTGAAAATAAAAATGGT													
	781	790	800	810	820	830	840	850	860	870	880	890	900	910
CD141_P542	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P542_1	TTGGACACGTATACATGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGTcATTACACAATTGGTACATATGACACCAAAAAGGATAAATACTTTCCGGATAACACTT													
Consensus	TTGGACACGTATACATGGCAAGACATTAACATGTTCTTAAGGTTAGCTTTGATGTTACAGGTTTGGaTCATTACACAATTGGTACATATGACACCAAAAAGGATAAATACTTTCCGGATAACACTT													
	911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
CD141_P542	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P542_1	CTGTTGATGGATGGAAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAAGACATTCTTTGATAGTGGCAGAAATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA													
Consensus	CTGTTGATGGATGGAAAGGATTGAGACTTGACTATGGTAACTATTACGCGTCCAAGACATTCTTTGATAGTGGCAGAAATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACTGTTGATAACGA													
	1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
CD141_P542	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P542_1	TGTGAGGAAGGATGGGCCGGAGTTCACCCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACCAATTGGTTCAATGGCCTGTTCAAGAATTAGAARCTTAGAAGAAAAGGTCCAATTAAT													
Consensus	TGTGAGGAAGGATGGGCCGGAGTTCACCCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACCAATTGGTTCAATGGCCTGTTCAAGAATTAGAARCTTAGAAGAAAAGGTCCAATTAAT													
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
CD141_P542	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P542_1	AACAAAAGTTGAACAAGGGAAAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTCATTACACAGTTTGGATAGGGCAGAGCCATTTGATCCTAGTTGGGCTGATC													
Consensus	AACAAAAGTTGAACAAGGGAAAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTGATTTTCTCATTACACAGTTTGGATAGGGCAGAGCCATTTGATCCTAGTTGGGCTGATC													
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
CD141_P542	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P542_1	TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAAGGTGGACTTGGGCCTTTTGGGCTCCTAACTTTGGCTTCTAAAACTTAGAAGAATACACACCCGTTTTCTCCGAATTTTCAGGC													
Consensus	TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAAGGTGGACTTGGGCCTTTTGGGCTCCTAACTTTGGCTTCTAAAACTTAGAAGAATACACACCCGTTTTCTCCGAATTTTCAGGC													
	1431	1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
CD141_P542	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P542_1	TCATGATAAATACAAGTTCTCATGTGTTCCGATGCCTCAAGGTCAGGCTAAGGAATGAARCACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAGAARTTGTCTCTT													
Consensus	TCATGATAAATACAAGTTCTCATGTGTTCCGATGCCTCAAGGTCAGGCTAAGGAATGAARCACTATGTATAAACCATCATTGCTGGATATGTAGATGTAGATTTAGCGGACAGAARTTGTCTCTT													
	1561	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
CD141_P542	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----													
CD141_P542_1	AGAAGTTTGATTGATCATTCCGTAGTGGAAAGTTTGGTGTGGAGGAAAAATATGCATTACATCAAGGGTTATCCACGTTGGCGATATTTGACAAGCACATTTATTTGCATTCAACATGGCGCGG													
Consensus	AGAAGTTTGATTGATCATTCCGTAGTGGAAAGTTTGGTGTGGAGGAAAAATATGCATTACATCAAGGGTTATCCACGTTGGCGATATTTGACAAGCACATTTATTTGCATTCAACATGGCGCGG													
	1691	1700	1710	1720	1730	1740	1749							
CD141_P542	----- ----- ----- ----- ----- ----- -----													
CD141_P542_1	AGAGAATCACATTGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG													
Consensus	AGAGAATCACATTGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG													

1 10 20 30 40 50 60 70 80 90 100 110 120 130
CD141_P542 ATGGAGATTTAAGAGATCTTCTTCTTTGGGTTTTGCCAATCTTTTGTGTGCTTCTTTATCAACAAATGGAGTATTGTTGATGCTTCTCACAAAGCTTATATGCACCTGCAATCTACTACTAGCC
CD141_P541 ATGGAGATTTAAGAGATCTTCTTCTTTGGGTTTTGCCAATCTTTTGTGTGCTTCTTTATCAACAAATGGAGTATTGTTGATGCTTCTCACAAAGCTTATATGCACCTGCAATCTACTACTAGCC
CD141_P182 ATGGAGATTTAAGAGATCTTCTTCTTTGGGTTTTGCCAATCTTTTGTGTGCTTCTTTATCAACAAATGGAGTATTGTTGATGCTTCTCACAAAGCTTATATGCACCTGCAATCTACTACTAGCC
CD141_P401 ATGGAGATTTAAGAGATCTTCTTCTTTGGGTTTTGCCAATCTTTTGTGTGCTTCTTTATCAACAAATGGAGTATTGTTGATGCTTCTCACAAAGCTTATATGCACCTGCAATCTACTACTAGCC
CD141_P181 ATGGAGATTTAAGAGATCTTCTTCTTTGGGTTTTGCCAATCTTTTGTGTGCTTCTTTATCAACAAATGGAGTATTGTTGATGCTTCTCACAAAGCTTATATGCACCTGCAATCTACTACTAGCC
Consensus ATGGAGATTTAAGAGATCTTCTTCTTTGGGTTTTGCCAATCTTTTGTGTGCTTCTTTATCAACAAATGGAGTATTGTTGATGCTTCTCACAAAGCTTATATGCACCTGCAATCTACTACTAGCC

131 140 150 160 170 180 190 200 210 220 230 240 250 260
CD141_P542 ATGTTGATGCAAGCAGGTCCTAGAACTGGTATCATTTTCACCTCTTAAAACTGGATCAACGATCCAATGGTCCAATGTATTACAAATGGAGTGTACCATTTATTCTACCAGTACACCCAAAGGG
CD141_P541 ATGTTGATGCTAGCAGGTCCTAGAACTGGTATCATTTTCACCTCTTAAAACTGGATCAATGATCCAATGGTCCAATGTATTACAAATGGAGTGTACCATTTATTCTACCAGTACACCCAAAGGG
CD141_P182 ATGTTGATGCTAGCAGGTCCTAGAACTGGTATCATTTTCACCTCTTAAAACTGGATCAACGATCCAATGGTCCAATGTATTACAAATGGAGTGTACCATTTATTCTACCAGTACACCCAAAGGG
CD141_P401 ATGTTGATGCTAGCAGGTCCTAGAACTGGTATCATTTTCACCTCTTAAAACTGGATCAACGATCCAATGGTCCAATGTATTACAAATGGAGTGTACCATTTATTCTACCAGTACACCCAAAGGG
CD141_P181 ATGTTGATGCTAGCAGGTCCTAGAACTGGTATCATTTTCACCTCTTAAAACTGGATCAACGATCCAATGGTCCAATGTATTACAAATGGAGTGTACCATTTATTCTACCAGTACACCCAAAGGG
Consensus ATGTTGATGCTAGCAGGTCCTAGAACTGGTATCATTTTCACCTCTTAAAACTGGATCAACGATCCAATGGTCCAATGTATTACAAATGGAGTGTACCATTTATTCTACCAGTACACCCAAAGGG

261 270 280 290 300 310 320 330 340 350 360 370 380 390
CD141_P542 AGCGATATGGGGCAATATTGTTGGGCCCATTCGGTCTCAAGGACTTGATCAATTGGATCCCACTTGAACCCGCTATCTACCCGTCARAGTATTGACAGTATGGTACATGGTCCGGTCAAGCCACA
CD141_P541 AGCGATATGGGGCAATATTGTTGGGCCCATTCGGTCTCAAGGACTTGATCAATTGGATCCCACTTGAACCCGCTATCTACCCGTCARAGTATTGACAGTATGGTACATGGTCCGGTCAAGCCACA
CD141_P182 AGCGATATGGGGCAATATTGTTGGGCCCATTCGGTCTCAAGGACTTGATCAATTGGATCCCACTTGAACCCGCTATCTACCCGTCARAGTATTGACAGTATGGTACATGGTCCGGTCAAGCCACA
CD141_P401 AGCGATATGGGGCAATATTGTTGGGCCCATTCGGTCTCAAGGACTTGATCAATTGGATCCCACTTGAACCCGCTATCTACCCGTCARAGTATTGACAGTATGGTACATGGTCCGGTCAAGCCACA
CD141_P181 AGCGATATGGGGCAATATTGTTGGGCCCATTCGGTCTCAAGGACTTGATCAATTGGATCCCACTTGAACCCGCTATCTACCCGTCARAGTATTGACAGTATGGTACATGGTCCGGTCAAGCCACA
Consensus AGCGATATGGGGCAATATTGTTGGGCCCATTCGGTCTCAAGGACTTGATCAATTGGATCCCACTTGAACCCGCTATCTACCCGTCARAGTATTGACAGTATGGTACATGGTCCGGTCAAGCCACA

391 400 410 420 430 440 450 460 470 480 490 500 510 520
CD141_P542 ATCTTGGCAGGCACACAGCCTGTTATCTCTACACTGGAAATGTGGATGCTAATAGACACAGTCCAAAATATGCATCCCGCTACATGTCTGATCCATATCTTCGTAGTGGATCAAGCCGATA
CD141_P541 ATCTTGGCAGGCACACAGCCTGTTATCTCTACACTGGAAATGTGGATGCTAATAGACACAGTCCAAAATATGCATCCCGCTACATGTCTGATCCATATCTTCGTAGTGGATCAAGCCGATA
CD141_P182 ATCTTGGCAGGCACACAGCCTGTTATCTCTACACTGGAAATGTGGATGCTAATAGACACAGTCCAAAATATGCATCCCGCTACATGTCTGATCCATATCTTCGTAGTGGATCAAGCCGATA
CD141_P401 ATCTTGGCAGGCACACAGCCTGTTATCTCTACACTGGAAATGTGGATGCTAATAGACACAGTCCAAAATATGCATCCCGCTACATGTCTGATCCATATCTTCGTAGTGGATCAAGCCGATA
CD141_P181 ATCTTGGCAGGCACACAGCCTGTTATCTCTACACTGGAAATGTGGATGCTAATAGACACAGTCCAAAATATGCATCCCGCTACATGTCTGATCCATATCTTCGTAGTGGATCAAGCCGATA
Consensus ATCTTGGCAGGCACACAGCCTGTTATCTCTACACTGGAAATGTGGATGCTAATAGACACAGTCCAAAATATGCATCCCGCTACATGTCTGATCCATATCTTCGTAGTGGATCAAGCCGATA

521 530 540 550 560 570 580 590 600 610 620 630 640 650
CD141_P542 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCACACACCGCTGGATGGCCGAGATGGAATGGAGATCTTGGTGGAGTGTGAGGATCATAGGGAAAGGN
CD141_P541 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCACACACCGCTGGATGGCCGAGATGGAATGGAGATCTTGGTGGAGTGTGAGGATCATAGGGAAAGGN
CD141_P182 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCACACACCGCTGGATGGCCGAGATGGAATGGAGATCTTGGTGGAGTGTGAGGATCATAGGGAAAGGN
CD141_P401 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCACACACCGCTGGATGGCCGAGATGGAATGGAGATCTTGGTGGAGTGTGAGGATCATAGGGAAAGGN
CD141_P181 ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCACACACCGCTGGATGGCCGAGATGGAATGGAGATCTTGGTGGAGTGTGAGGATCATAGGGAAAGGN
Consensus ACAATCCATTGATTGTTGCTGACAAACCATCAACAAAGCCAAATTCGTGATCCACACACCGCTGGATGGCCGAGATGGAATGGAGATCTTGGTGGAGTGTGAGGATCATAGGGAAAGGN

651 660 670 680 690 700 710 720 730 740 750 760 770 780
CD141_P542 TATAATGTACAAAGTAATARGAATTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCTCCGGTACTGGAAATGGGAATGTCCTGATTTTTTCCAGTGTCTTGAARAATAAATGGT
CD141_P541 TATAATGTACAAAGTAATARGAATTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCTCCGGTACTGGAAATGGGAATGTCCTGATTTTTTCCAGTGTCTTGAARAATAAATGGT
CD141_P182 TATAATGTACAAAGTAATARGAATTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCTCCGGTACTGGAAATGGGAATGTCCTGATTTTTTCCAGTGTCTTGAARAATAAATGGT
CD141_P401 TATAATGTACAAAGTAATARGAATTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCTCCGGTACTGGAAATGGGAATGTCCTGATTTTTTCCAGTGTCTTGAARAATAAATGGT
CD141_P181 TATAATGTACAAAGTAATARGAATTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCTCCGGTACTGGAAATGGGAATGTCCTGATTTTTTCCAGTGTCTTGAARAATAAATGGT
Consensus TATAATGTACAAAGTAATARGAATTCATGAATGGACCAAGCCAAACACCCACTCCACTCAGCTCCGGTACTGGAAATGGGAATGTCCTGATTTTTTCCAGTGTCTTGAARAATAAATGGT

781 790 800 810 820 830 840 850 860 870 880 890 900 910
CD141_P542 TTGGACACGTCATACAAATGGCAAGGACATTAACATGTTCTTARAGTTAGCTTTGATGTTACAGGTTTGGTCATTACACAAATGGTACATATGACACCAAAAGGATAAATACTTTCCGGATAACACTT
CD141_P541 TTGGACACGTCATACAAATGGCAAGGACATTAACATGTTCTTARAGTTAGCTTTGATGTTACAGGTTTGGTCATTACACAAATGGTACATATGACACCAAAAGGATAAATACTTTCCGGATAACACTT
CD141_P182 TTGGACACGTCATACAAATGGCAAGGACATTAACATGTTCTTARAGTTAGCTTTGATGTTACAGGTTTGGTCATTACACAAATGGTACATATGACACCAAAAGGATAAATACTTTCCGGATAACACTT
CD141_P401 TTGGACACGTCATACAAATGGCAAGGACATTAACATGTTCTTARAGTTAGCTTTGATGTTACAGGTTTGGTCATTACACAAATGGTACATATGACACCAAAAGGATAAATACTTTCCGGATAACACTT
CD141_P181 TTGGACACGTCATACAAATGGCAAGGACATTAACATGTTCTTARAGTTAGCTTTGATGTTACAGGTTTGGTCATTACACAAATGGTACATATGACACCAAAAGGATAAATACTTTCCGGATAACACTT
Consensus TTGGACACGTCATACAAATGGCAAGGACATTAACATGTTCTTARAGTTAGCTTTGATGTTACAGGTTTGGTCATTACACAAATGGTACATATGACACCAAAAGGATAAATACTTTCCGGATAACACTT

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
CD141_P542 CTATTGATGGATGGAAAGGATTTGAGACTTGACTATGGTAACTATTACGCGTCCAGACATTTCTTGTATGTTGGCAAGGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACGTTGATAACGA
CD141_P541 CTATTGATGGATGGAAAGGATTTGAGACTTGACTATGGTAACTATTACGCGTCCAGACATTTCTTGTATGTTGGCAAGGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACGTTGATAACGA
CD141_P182 CTATTGATGGATGGAAAGGATTTGAGACTTGACTATGGTAACTATTACGCGTCCAGACATTTCTTGTATGTTGGCAAGGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACGTTGATAACGA
CD141_P401 CTATTGATGGATGGAAAGGATTTGAGACTTGACTATGGTAACTATTACGCGTCCAGACATTTCTTGTATGTTGGCAAGGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACGTTGATAACGA
CD141_P181 CTATTGATGGATGGAAAGGATTTGAGACTTGACTATGGTAACTATTACGCGTCCAGACATTTCTTGTATGTTGGCAAGGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACGTTGATAACGA
Consensus CTATTGATGGATGGAAAGGATTTGAGACTTGACTATGGTAACTATTACGCGTCCAGACATTTCTTGTATGTTGGCAAGGATCGTAGGATTTTGTGGGTTGGGCTAATGAATCAGATACGTTGATAACGA

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
CD141_P542 TGTGAGGAAGGATGGCCGGAGTTACCCCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACCAATGGTTCATGGCTGTTCCAGATTAGAARCTTAGAAGAAAGAAAGGTCCTAATTAAT
CD141_P541 TGTGAGGAAGGATGGCCGGAGTTACCCCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACCAATGGTTCATGGCTGTTCCAGATTAGAARCTTAGAAGAAAGAAAGGTCCTAATTAAT
CD141_P182 TGTGAGGAAGGATGGCCGGAGTTACCCCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACCAATGGTTCATGGCTGTTCCAGATTAGAARCTTAGAAGAAAGAAAGGTCCTAATTAAT
CD141_P401 TGTGAGGAAGGATGGCCGGAGTTACCCCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACCAATGGTTCATGGCTGTTCCAGATTAGAARCTTAGAAGAAAGAAAGGTCCTAATTAAT
CD141_P181 TGTGAGGAAGGATGGCCGGAGTTACCCCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACCAATGGTTCATGGCTGTTCCAGATTAGAARCTTAGAAGAAAGAAAGGTCCTAATTAAT
Consensus TGTGAGGAAGGATGGCCGGAGTTACCCCTATTCTCGTAAATTTGGCTTGATCCTAGTGGAAACCAATGGTTCATGGCTGTTCCAGATTAGAARCTTAGAAGAAAGAAAGGTCCTAATTAAT

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
CD141_P542 AACAAAAGTTGAACAAAGGAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTATTCTCATTACACAGTTGGATAGGCAGAGCCATTTGATCCTAGTTGGCTGATC
CD141_P541 AACAAAAGTTGAACAAAGGAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTATTCTCATTACACAGTTGGATAGGCAGAGCCATTTGATCCTAGTTGGCTGATC
CD141_P182 AACAAAAGTTGAACAAAGGAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTATTCTCATTACACAGTTGGATAGGCAGAGCCATTTGATCCTAGTTGGCTGATC
CD141_P401 AACAAAAGTTGAACAAAGGAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTATTCTCATTACACAGTTGGATAGGCAGAGCCATTTGATCCTAGTTGGCTGATC
CD141_P181 AACAAAAGTTGAACAAAGGAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTATTCTCATTACACAGTTGGATAGGCAGAGCCATTTGATCCTAGTTGGCTGATC
Consensus AACAAAAGTTGAACAAAGGAAAGGTTGAATCAAGGAATCACAGTTGCACAGGCTGATGTTGAAGTATTCTCATTACACAGTTGGATAGGCAGAGCCATTTGATCCTAGTTGGCTGATC

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
CD141_P542 TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTTGGACTTGGGCTTTTGGGCTCCTAATTTGGCTTCTAAAACTTAGAAGAAATACACCCGTTTTCTTCAAGTTTCAAGGC
CD141_P541 TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTTGGACTTGGGCTTTTGGGCTCCTAATTTGGCTTCTAAAACTTAGAAGAAATACACCCGTTTTCTTCAAGTTTCAAGGC
CD141_P182 TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTTGGACTTGGGCTTTTGGGCTCCTAATTTGGCTTCTAAAACTTAGAAGAAATACACCCGTTTTCTTCAAGTTTCAAGGC
CD141_P401 TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTTGGACTTGGGCTTTTGGGCTCCTAATTTGGCTTCTAAAACTTAGAAGAAATACACCCGTTTTCTTCAAGTTTCAAGGC
CD141_P181 TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTTGGACTTGGGCTTTTGGGCTCCTAATTTGGCTTCTAAAACTTAGAAGAAATACACCCGTTTTCTTCAAGTTTCAAGGC
Consensus TTTACGCGCAGATGTATGCGCCATTAAAGGTTCAACGGTCCAGGTTGGACTTGGGCTTTTGGGCTCCTAATTTGGCTTCTAAAACTTAGAAGAAATACACCCGTTTTCTTCAAGTTTCAAGGC

1431 1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
CD141_P542 TCATGATAAATACAAAGTTCTATGTGTTCCGATGCTCAAGGTCAGCCTAAGGAATGAACCACTATGTAACACCATCATTTGCTGGATATGTAGATGTAGATTAGCGGACAGAAATTTGTCTCT
CD141_P541 TCATGATAAATACAAAGTTCTATGTGTTCCGATGCTCAAGGTCAGCCTAAGGAATGAACCACTATGTAACACCATCATTTGCTGGATATGTAGATGTAGATTAGCGGACAGAAATTTGTCTCT
CD141_P182 TCATGATAAATACAAAGTTCTATGTGTTCCGATGCTCAAGGTCAGCCTAAGGAATGAACCACTATGTAACACCATCATTTGCTGGATATGTAGATGTAGATTAGCGGACAGAAATTTGTCTCT
CD141_P401 TCATGATAAATACAAAGTTCTATGTGTTCCGATGCTCAAGGTCAGCCTAAGGAATGAACCACTATGTAACACCATCATTTGCTGGATATGTAGATGTAGATTAGCGGACAGAAATTTGTCTCT
CD141_P181 TCATGATAAATACAAAGTTCTATGTGTTCCGATGCTCAAGGTCAGCCTAAGGAATGAACCACTATGTAACACCATCATTTGCTGGATATGTAGATGTAGATTAGCGGACAGAAATTTGTCTCT
Consensus TCATGATAAATACAAAGTTCTATGTGTTCCGATGCTCAAGGTCAGCCTAAGGAATGAACCACTATGTAACACCATCATTTGCTGGATATGTAGATGTAGATTAGCGGACAGAAATTTGTCTCT

1561 1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690
CD141_P542 AGAAGTTTGATTGATCATTCCGTTAGTGGAAAGTTTGGTGTGGAGGAAAAATATGCATTACATCARGGGTTATCCARCGTTGGCGATATTTGACAAAGCACATTTATTTGCAATTCARCAATGGCGCGG
CD141_P541 AGAAGTTTGATTGATCATTCCGTTAGTGGAAAGTTTGGTGTGGAGGAAAAATATGCATTACATCARGGGTTATCCARCGTTGGCGATATTTGACAAAGCACATTTATTTGCAATTCARCAATGGCGCGG
CD141_P182 AGAAGTTTGATTGATCATTCCGTTAGTGGAAAGTTTGGTGTGGAGGAAAAATATGCATTACATCARGGGTTATCCARCGTTGGCGATATTTGACAAAGCACATTTATTTGCAATTCARCAATGGCGCGG
CD141_P401 AGAAGTTTGATTGATCATTCCGTTAGTGGAAAGTTTGGTGTGGAGGAAAAATATGCATTACATCARGGGTTATCCARCGTTGGCGATATTTGACAAAGCACATTTATTTGCAATTCARCAATGGCGCGG
CD141_P181 AGAAGTTTGATTGATCATTCCGTTAGTGGAAAGTTTGGTGTGGAGGAAAAATATGCATTACATCARGGGTTATCCARCGTTGGCGATATTTGACAAAGCACATTTATTTGCAATTCARCAATGGCGCGG
Consensus AGAAGTTTGATTGATCATTCCGTTAGTGGAAAGTTTGGTGTGGAGGAAAAATATGCATTACATCARGGGTTATCCARCGTTGGCGATATTTGACAAAGCACATTTATTTGCAATTCARCAATGGCGCGG

1691 1700 1710 1720 1730 1740 1749
CD141_P542 AGAGAATCAATTTGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
CD141_P541 AGAGAATCAATTTGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
CD141_P182 AGAGAATCAATTTGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
CD141_P401 AGAGAATCAATTTGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
CD141_P181 AGAGAATCAATTTGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG
Consensus AGAGAATCAATTTGAGACTCTAATGCTTGGAGCATGGCTAATGCAAGTTGCACTAG

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
Gene30	MDCLKKSSLFSLPFFLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDVKNVHRTGYHFQPPKNWINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSYSTDLINWIPLEPGIYPSEVFDKYGTWSGSAT													
CD111_P401	MDCLKKSSLFSLP I FLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDVKNVHRTGYHFQPPKNWINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSYSTDLINWIPLEPGIYPSEVFDKYGTWSGSAT													
Consensus	MDCLKKSSLFSLP i FLLYFSIILSFNNGVNAHSHKVFPGLQSTSTVDVKNVHRTGYHFQPPKNWINDPNAPHYYNGVYHLFYQYNPYGSVMGNIYVAHSYSTDLINWIPLEPGIYPSEVFDKYGTWSGSAT													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
Gene30	ILPNNKPIILYTGIVDAKNTQVQNYAIPANISDPFLRKWIKPDNNPLIYADYSINKTQFRDPTTCWLGQDGYWRTLIGSYW E KQGLAILYKSKNFHKWTKYQHPLHS A DGTGNWECPDFFPVLLHGTNGL													
CD111_P401	ILPNNKPIILYTGIVDAKNTQVQNYAIPADISDPFLRKWIKPDNNPLIYADYSINKTQFRDPTTCWLGQDGYWRTLIGSYW G KQGLAILYKSKNFHKWTKIQHPLHS V DGTGNWECPDFFPVLLHGTNGL													
Consensus	ILPNNKPIILYTGIVDAKNTQVQNYAIPA#ISDPFLRKWIKPDNNPLIYADYSINKTQFRDPTTCWLGQDGYWRTLIGSYW e KQGLAILYKSKNFHKWTK! Q HPLHS a DGTGNWECPDFFPVLLHGTNGL													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
Gene30	DASYNKKNIKHVLYKSLDVTRFEYTYVGIYDTKKDRYIPDKTSDIGWKGLRLDYGNYASKSFYDPSKNRRIMHGWANESDTYNDVYKKGWAGIQTSPRKLWLDPSGKQLYQWPVEELET L RENKIQL M													
CD111_P401	DASYNKKNIKHVLYKSLDVTRFEYTYVGI I YDTKKDRYIPDKTSDIGWKGLRLDYGNYASKSFYDPSKNRRIMHGWANESDTYNDVYKKGWAGIQT I PRKLWLDPSGKQLYQWPVEELET F REQKYQL S M													
Consensus	DASYNKKNIKHVLYKSLDVTRFEYTYVGI i YDTKKDRYIPDKTSDIGWKGLRLDYGNYASKSFYDPSKNRRIMHGWANESDTYNDV! K KGWAGIQT i PRKLWLDPSGKQLYQWPVEELET I RE# K ! Q L m													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
Gene30	RKLNKGDKIEYKGITPAQADVEYTFSSLDKAEPDPNHWANLYAQDYCAIKGSTVQGGGLGPFGLLTLASQNLEEYTPVFFRVFK A QDKYKYLHCSDA T RSTLKNDKTHYKPSFAGYVDYDLTNKTL S LR													
CD111_P401	RKLNKGDKIEYKGITPAQADVEYTFSSLDKAEPDPNHWANLYAQDYCAIKGSTVQGGGLGPFGLLTLASQNLEEYTPVFFRVFK T QDKYKYLHCSDA S RSTLKNDKTHYKPSFAGYVDYDLTNKTL S LR													
Consensus	RKLNKGDKIEYKGITPAQADVEYTFSSLDKAEPDPNHWANLYAQDYCAIKGSTVQGGGLGPFGLLTLASQNLEEYTPVFFRVFK a QDKYKYLHCSDA s RSTLKNDKTHYKPSFAGYVDYDLTNKTL S LR													
	521	530	540	550	560	570	580	590						
	-----+-----+-----+-----+-----+-----+-----+-----+-----													
Gene30	SLIDHSVYESFGAGGKTCITSRVYPTLAIYDNAHLVFNNGTETIKIKSLNAWTHGKPKMNWSFGHSSFR													
CD111_P401	SLIDHSVYESFGAGGKTCITSRVYPTLAIYDNAHLVFNNGTETIKIKSLNAWTHGKPKMNWSFG R SSY													
Consensus	SLIDHSVYESFGAGGKTCITSRVYPTLAIYDNAHLVFNNGTETIKIKSLNAWTHGKPKMNWSFG r SS%.													

	1	10	20	30	40	50	60	70	80	90	100	110	120	130
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
Gene40	MEILRKSSSLWALPILLLCFFINNGVFDASHKYYMHLQSTTSHVDASKYHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIMGNIWAHSYSKDLINWIPLPAIYPSKYFDKYGTWSGSAT													
CD141_P401	MEILRRSSSLWVLPILLLCFFINNGVFDASHKYYMHLQSTTSHVDASKYHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIMGNIWAHSYSKDLINWIPLPAIYPSKYFDKYGTWSGSAT													
Consensus	MEILRrSSSLW ^a LPILLLCFFINNGVFDASHKYYMHLQSTTSHVDASKYHRTGYHFQPPKNWINDPNGPHYYNGVYHLFYQYNPKGAIMGNI!WAHSYSKDLINWIPLPAIYPSKYFDKYGTWSGSAT													
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
Gene40	ILPGNKPVILYTGIVDANKTQVQNYAIPANMSDPYLRKWKIKPDNNPLIYADKTINKSQFRDPTTAWMGRDGNWRILVGSYRNHRGKYIHYKSNKNFMKATKAKHPLHSAPGTGNWECPDFFPVSLKNKDG													
CD141_P401	ILPGNKPVILYTGIVDGNKTQVQNYAIPANMSDPYLRKWKIKPDNNPLIYADKTINKSQFRDPTTAWMGRDGNWRILVGSYRNHRGKYIHYKSNKNFMKATKAKHPLHSAPGTGNWECPDFFPVSLKNKDG													
Consensus	ILPGNKPVILYTGIVD ^a NKTQVQNYAIPANMSDPYLRKWKIKPDNNPLIYADKTINKSQFRDPTTAWMGRDGNWRILVGSYRNHRGKYIHYKSNKNFMKATKAKHPLHSAPGTGNWECPDFFPVSLKNKDG													
	261	270	280	290	300	310	320	330	340	350	360	370	380	390
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
Gene40	LDSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDKYFPDNTSIDGWKGLRLDYGNYYASKTFFDSGKNRRILLGWANESDTRDNDVYRKGWAGVHPIPRKIWLDSGKQLVQWPVQELETLRKKKYQLN													
CD141_P401	LDSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKDQYFPDSTSIDGWKGLRLDYGNYYASKTFFDSGKNRRILLGWANESDTRDNDVYRKGWAGVHPIPRKIWLDPGKQLVQWPVQELETLRKKKYQLN													
Consensus	LDSYNGKDIKHVLYKVSFDVTRFDHYTIGTYDTKKD ^q YFPD ⁿ TSIDGWKGLRLDYGNYYASKTFFDSGKNRRILLGWANESDTRDNDVYRKGWAGVHPIPRKIWL ^p SGKQLVQWPVQELETLRKKKYQLN													
	391	400	410	420	430	440	450	460	470	480	490	500	510	520
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----													
Gene40	NKKLNKGEKVEIKGITVAQADVEYIFSFASLEKAELFDPSWADLYAQDYCAIKGSTVQGGLGPFGLLTLASKNLEEYTPVFFRIFKAQDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDYDLADKKLSL													
CD141_P401	NKKLNKGEKVEIKGITVAQADVEYIFSFTSLDKAEPFDPSWADLYAQDYCAIKGSTVQGGLGPFGLLTLASKNLEEYTPVFFRIFKAHDKYKYLHCSDASRSSLKNETTHYKPSFAGYVDYDLADKKLSL													
Consensus	NKKLNKGEKVEIKGITVAQADVEYIFS ^a SL#KAELFDPSWADLYAQDYCAIKGSTVQGGLGPFGLLTLASKNLEEYTPVFFRIFKA ^q DKYKYLHCSDASRSSLKNETTHYKPSFAGYVDYDLADKKLSL													
	521	530	540	550	560	570	58882							
	-----+-----+-----+-----+-----+-----+-----													
Gene40	RSLIDHSYVESFGAGGKTCITSRVYPTLAIFDKAHLFAFNNGAERITTIETLNAWSMANAKLH													
CD141_P401	RSLIDHSIYESFGAGGKTCITSRVYPTLAIFDKAHLFAFNNGAERITTIETLNAWSMANAKLH													
Consensus	RSLIDHS!YESFGAGGKTCITSRVYPTLAIFDKAHLFAFNNGAERITTIETLNAWSMANAKLH													

