



(86) Date de dépôt PCT/PCT Filing Date: 1996/03/06
(87) Date publication PCT/PCT Publication Date: 1996/09/19
(45) Date de délivrance/Issue Date: 2001/10/30
(85) Entrée phase nationale/National Entry: 1997/08/29
(86) N° demande PCT/PCT Application No.: US 96/03006
(87) N° publication PCT/PCT Publication No.: WO 96/28557
(30) Priorité/Priority: 1995/03/09 (401,575) US

(51) Cl.Int.⁶/Int.Cl.⁶ C12N 15/57, A61K 7/28, C12S 11/00,
C12N 9/54, C11D 3/386, C12S 9/00, G02C 13/00
(72) Inventeurs/Inventors:
RUBINGH, Donn Nelson, US;
BARNETT, Bobby Lee, US;
BRODE, Philip Frederick III, US
(73) Propriétaire/Owner:
THE PROCTER & GAMBLE COMPANY, US
(74) Agent: KIRBY EADES GALE BAKER

(54) Titre : VARIANTS DE LA SUBTILISINE DY DOTES D'UN POUVOIR D'ADSORPTION AFFAIBLI ET D'UNE
CAPACITE D'HYDROLYSE ACCRUE

(54) Title: SUBTILISIN DY VARIANTS HAVING DECREASED ADSORPTION AND INCREASED HYDROLYSIS

(57) **Abrégé/Abstract:**

The present invention relates to Subtilisin DY variants having a modified amino acid sequence of wild-type Subtilisin DY amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region, a fifth loop region and a sixth loop region; wherein the modified amino acid sequence comprises different amino acids than that occurring in wild-type Subtilisin DY (i.e., substitution) at specifically identified positions in one or more of the loop regions whereby the Subtilisin DY variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type Subtilisin DY. The present invention also relates to DNA sequences encoding such Subtilisin DY variants. The present invention also relates to compositions comprising such Subtilisin DY variants for cleaning a variety of surfaces.

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/57, 9/56, C11D 3/386	A3	(11) International Publication Number: WO 96/28557 (43) International Publication Date: 19 September 1996 (19.09.96)
(21) International Application Number: PCT/US96/03006 (22) International Filing Date: 6 March 1996 (06.03.96) (30) Priority Data: 08/401,575 9 March 1995 (09.03.95) US (71) Applicant: THE PROCTER & GAMBLE COMPANY [US/US]; One Procter & Gamble Plaza, Cincinnati, OH 45202 (US). (72) Inventors: BRODE, Philip, Frederick, III; 5780 Squirrelsneest Lane, Cincinnati, OH 45252 (US). BARNETT, Bobby, Lee; 12175 Elkwood Drive, Cincinnati, OH 45240 (US). RUBINGH, Donn, Nelton; 8224 Sheed Road, Cincinnati, OH 45247 (US). (74) Agents: REED, T., David et al.; The Procter & Gamble Company, 5299 Spring Grove Avenue, Cincinnati, OH 45217 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 7 August 1997 (07.08.97)
(54) Title: SUBTILISIN DY VARIANTS HAVING DECREASED ADSORPTION AND INCREASED HYDROLYSIS (57) Abstract The present invention relates to Subtilisin DY variants having a modified amino acid sequence of wild-type Subtilisin DY amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region, a fifth loop region and a sixth loop region; wherein the modified amino acid sequence comprises different amino acids than that occurring in wild-type Subtilisin DY (i.e., substitution) at specifically identified positions in one or more of the loop regions whereby the Subtilisin DY variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type Subtilisin DY. The present invention also relates to DNA sequences encoding such Subtilisin DY variants. The present invention also relates to compositions comprising such Subtilisin DY variants for cleaning a variety of surfaces.		

SUBTILISIN DY VARIANTS HAVING DECREASED ADSORPTION AND INCREASED HYDROLYSIS

TECHNICAL FIELD

The present invention relates to novel enzyme variants useful in a variety of cleaning compositions, and DNA sequences encoding such enzyme variants.

BACKGROUND

Enzymes make up the largest class of naturally occurring proteins. Each class of enzyme generally catalyzes (accelerates a reaction without being consumed) a different kind of chemical reaction. One class of enzymes known as proteases, are known for their ability to hydrolyze (break down a compound into two or more simpler compounds with the uptake of the H and OH parts of a water molecule on either side of the chemical bond cleaved) other proteins. This ability to hydrolyze proteins has been taken advantage of by incorporating naturally occurring and protein engineered proteases as an additive to laundry detergent preparations. Many stains on clothes are proteinaceous and wide-specificity proteases can substantially improve removal of such stains.

Unfortunately, the efficacy level of these proteins in their natural, bacterial environment, frequently does not translate into the relatively unnatural wash environment. Specifically, protease characteristics such as thermal stability, pH stability, oxidative stability and substrate specificity are not necessarily optimized for utilization outside the natural environment of the enzyme.

The amino acid sequence of the protease determines the characteristics of the protease. A change of the amino acid sequence of the protease may alter the properties of the enzyme to varying degrees, or may even inactivate the enzyme, depending upon the location, nature and/or magnitude of the change in the amino acid sequence. Several approaches have been taken to alter the wild-type amino acid sequence of proteases in an attempt to improve their properties, with the goal of increasing the efficacy of the protease in the wash environment. These approaches include altering the amino acid sequence to enhance thermal stability and to

improve oxidation stability under quite diverse conditions.

Despite the variety of approaches described in the art, there is a continuing need for new effective variants of proteases useful for cleaning a variety of surfaces.

Objects of the Present Invention

It is an object of the present invention to provide Subtilisin DY enzyme variants having improved hydrolysis versus the wild-type of the enzyme.

It is also an object of the present invention to provide cleaning compositions comprising these subtilisin enzyme variants.

SUMMARY

The present invention relates to Subtilisin DY variants having a modified amino acid sequence of wild-type Subtilisin DY amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region, a fifth loop region and a sixth loop region; wherein the modified amino acid sequence comprises different amino acids than that occurring in wild-type Subtilisin DY (i.e., substitution) at specifically identified positions in one or more of the loop regions whereby the Subtilisin DY variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type Subtilisin DY. The present invention also relates to DNA sequences encoding such Subtilisin DY variants. The present invention also relates to compositions comprising such Subtilisin DY variants for cleaning a variety of surfaces.

DESCRIPTION

I. Subtilisin DY Variants

This invention pertains to subtilisin enzymes, in particular Subtilisin DY, that have been modified by mutating the various nucleotide sequences that code for the enzyme, thereby modifying the amino acid sequence of the enzyme. The modified subtilisin enzymes (hereinafter, "Subtilisin DY variants") of the present invention have decreased adsorption to and increased hydrolysis of an insoluble substrate as compared to the wild-type subtilisin. The present invention also pertains to DNA sequences encoding for such Subtilisin DY variants.

The subtilisin enzymes of this invention belong to a class of enzymes known as proteases. A protease is a catalyst for the cleavage of peptide bonds. One type of protease is a serine protease. A serine protease is

distinguished by the fact that there is an essential serine residue at the active site.

The observation that an enzyme's rate of hydrolysis of soluble substrates increases with enzyme concentration is well documented. It would therefore seem plausible that for surface bound substrates, such as is encountered in many cleaning applications, the rate of hydrolysis would increase with increasing surface concentration. This has been shown to be the case. (Brode, P.F. III and D. S. Rauch, *LANGMUIR*, "Subtilisin BPN': Activity on an Immobilized Substrate", Vol. 8, pp. 1325-1329 (1992)). In fact, a linear dependence of rate upon surface concentration was found for insoluble substrates when the surface concentration of the enzyme was varied. (Rubingh, D. N. and M. D. Bauer, "Catalysis of Hydrolysis by Proteases at the Protein-Solution Interface," in *POLYMER SOLUTIONS, BLENDS AND INTERFACES*, Ed. by I. Noda and D. N. Rubingh, Elsevier, p. 464 (1992)). Surprisingly, when seeking to apply this principle in the search for variant proteases which give better cleaning performance, we did not find that enzymes which adsorb more give better performance. In fact, we surprisingly determined the opposite to be the case: decreased adsorption by an enzyme to a substrate resulted in increased hydrolysis of the substrate (i.e., better cleaning performance).

While not wishing to be bound by theory, it is believed that improved performance, when comparing one variant to another, is a result of the fact that enzymes which adsorb less are also less tightly bound and therefore more highly mobile on the surface from which the insoluble protein substrate is to be removed. At comparable enzyme solution concentrations, this increased mobility is sufficient to outweigh any advantage that is conferred by delivering a higher concentration of enzyme to the surface.

The mutations described herein are designed to change (i.e., decrease) the adsorption of the enzyme to surface-bound soils. In Subtilisin DY, certain amino acids form exterior loops on the enzyme molecule. For purposes of discussion, these loops shall be referred to as first, second, third, fourth and fifth loop regions. Specifically, positions 58-65 form the first loop region; positions 94-106 form the second loop region; positions 125-132 form the third loop region; positions 153-166 form the fourth loop region; positions 186-190 form the fifth loop region; and positions 199-219 form the sixth loop region (position numbering analagous to positions in the amino acid sequence for wild-type subtilisin Subtilisin DY (SEQ ID NO:1)).

It is believed that these loop regions play a significant role in the adsorption of the enzyme molecule to a surface-bound peptide, and specific mutations in one or more of these loop regions will have a significant effect on this adsorption. While not wishing to be bound by theory, it is believed that the loop regions are important to the adsorption of the Subtilisin DY molecule for at least two reasons. First, the amino acids which comprise the loop regions can make close contacts with any surfaces to which the molecule is exposed. Second, the proximity of the loop regions to the active-site and binding pocket of the Subtilisin DY molecule gives them a role in the catalytically productive adsorption of the enzyme to surface-bound substrates (peptides/protein soils).

As used herein, "variant" means an enzyme having an amino acid sequence which differs from that of wild-type.

As used herein, "mutant Subtilisin DY DNA" means a DNA sequence coding for a Subtilisin DY variant.

As used herein, "wild-type Subtilisin DY" refers to an enzyme represented by SEQ ID NO:1. The amino acid sequence for Subtilisin DY is further described by Nedkov, P., Oberthur, W. and Braunitzer, G., *BioL. CHEM.*, Vol. 366, pp. 421-430 (1985).

As used herein, the term "Subtilisin DY wild-type amino acid sequence" encompasses SEQ ID NO:1 as well as SEQ ID NO:1 having modifications to the amino acid sequence other than at any of positions 58, 59, 60, 61, 62, 64, 65, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 125, 126, 127, 128, 129, 130, 131, 132, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 186, 187, 188, 189, 190, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218 or 219.

As used herein, "more hydrophilic amino acid" refers to any other amino acid having greater hydrophilicity than a subject amino acid with reference to the hydrophilicity table below. The following hydrophilicity table (Table 1) lists amino acids in descending order of increasing hydrophilicity (see Hopp, T.P., and Woods, K.R., "Prediction of Protein Antigenic Determinants from Amino Acid Sequences", *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE USA*, Vol. 78, pp. 3824-3828, 1981).

TABLE 1

Amino Acid	Hydrophilicity Value
Trp	-3.4
Phe	-2.5
Tyr	-2.3
Leu, Ile	-1.8
Val	-1.5
Met	-1.3
Cys	-1.0
Ala, His	-0.5
Thr	-0.4
Pro, Gly	-0.0
Gln, Asn	0.2
Ser	0.3
Arg ⁺ , Lys ⁺ , Glu ⁻ , Asp ⁻	3.0

Table 1 also indicates which amino acids carry a charge (this characteristic being based on a pH of from about 8-9). The positively charged amino acids are Arg and Lys, the negatively charged amino acids are Glu and Asp, and the remaining amino acids are neutral. In a preferred embodiment of the present invention, the substituting amino acid is either neutral or negatively charged, more preferably negatively charged (i.e., Glu or Asp).

Therefore, for example, the statement "substitute Gln with an equally or more hydrophilic amino acid which is neutral or has a negative charge" means Gln would be substituted with Asn (which is equally hydrophilic to Gln), or Ser, Glu or Asp (which are more hydrophilic than Gln); each of which are neutral or have a negative charge, and have a greater hydrophilicity value as compared to Gln. Likewise, the statement "substitute Pro with a more hydrophilic amino acid which is neutral or has a negative charge" means Pro would be substituted with Gln, Asn, Ser, Glu or Asp.

In one embodiment of the present invention, the Subtilisin DY variant has a modified amino acid sequence of Subtilisin DY wild-type amino acid sequence, wherein the wild-type amino acid sequence comprises a substitution at one or more positions in one or more of the first loop region, the second loop region, the third loop region, the fourth loop region, the fifth loop region or the sixth loop region; whereby the Subtilisin DY variant has

decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to the wild-type Subtilisin DY.

In a preferred embodiment of the present invention, the substituting amino acid for one or more of the positions in one or more of the loop regions is, with reference to Table 1, neutral or negatively charged and equally or more hydrophylic, preferably more hydrophylic, than the amino acid at the subject position in the wild-type amino acid sequence.

A. Substitutions in the First Loop Region

When a substitution occurs in the first loop region, the substitution occurs at one or more of positions 58, 59, 60, 61, 62, 64 or 65.

When a substitution occurs at position 58, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 59, the substituting amino acid is Glu.

When a substitution occurs at position 60, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 61, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 62, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 64, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 65, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

B. Substitutions in the Second Loop Region

When a substitution occurs in the second loop region, the substitution occurs at one or more of positions 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105 or 106.

When a substitution occurs at position 94, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

When a substitution occurs at position 95, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 96, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 97, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 98, the substituting amino acid

is Asp or Glu.

When a substitution occurs at position 99, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 100, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 101, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 102, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 103, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 104, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 105, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 106, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val.

C. Substitutions in the Third Loop Region

When a substitution occurs in the third loop region, the substitution occurs at one or more of positions 125, 126, 127, 128, 129, 130, 131 or 132.

When a substitution occurs at position 125, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 126, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 127, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 128, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser.

When a substitution occurs at position 129, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 130, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 131, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 132, the substituting amino

acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

D. Substitutions in the Fourth Loop Region

When a substitution occurs in the fourth loop region, the substitution occurs at one or more of positions 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165 or 166.

When a substitution occurs at position 153, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 154, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 155, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 156, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 157, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 158, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 159, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 160, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 161, the substituting amino acid is Asn, Asp, Glu or Ser.

When a substitution occurs at position 162, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 163, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 164, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly His, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 165, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 166, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

E. Substitutions in the Fifth Loop Region

When a substitution occurs in the fifth loop region, the substitution occurs at one or more of positions 186, 187, 188, 189 or 190.

When a substitution occurs at position 186, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 187, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 188, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr, Tyr or Val.

When a substitution occurs at position 189, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 190, the substituting amino acid is Asp or Glu.

F. Substitutions in the Sixth Loop Region

When a substitution occurs in the sixth loop region, the substitution occurs at one or more of positions 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218 or 219.

When a substitution occurs at position 199, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr.

When a substitution occurs at position 200, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser.

When a substitution occurs at position 201, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 202, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr.

When a substitution occurs at position 203, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 204, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser, or Thr.

When a substitution occurs at position 205, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 206, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 207, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 208, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr or Val.

When a substitution occurs at position 209, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser.

When a substitution occurs at position 210, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 211, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 212, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 213, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 214, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

When a substitution occurs at position 215, the substituting amino acid is Asp or Glu.

When a substitution occurs at position 216, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val.

When a substitution occurs at position 217, the substituting amino acid is Asp, Gln, Glu or Ser.

When a substitution occurs at position 218, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser.

When a substitution occurs at position 219, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

G. Preparation of enzyme variants

Example 1

Mutant Subtilisin DY DNA

A phagemid ("DYP") containing the wild type Subtilisin DY gene is constructed. The 2.8 Kbp *Pvu* II restriction enzyme fragment of plasmid pUC119, (Vieira, J. and Messing, J., "Production of Single-Stranded Plasmid DNA", 153 METHODS IN ENZYMOLOGY 3-11 (1989)) is cloned into the *Pvu* II site of plasmid pUB110 (Bacillus Genetic Stock Center, Columbus, OH 1E9). The pUC119-pUB110 hybrid plasmid is named pJMA601. Into the *Bam*HI restriction site of pJMA601 is cloned the polymerase chain reaction - amplified subtilisin DY gene from chromosomal DNA giving phagemid DYP. Phagemid DYP is transformed into *Escherichia coli* Ung⁻ strain CJ236 and a single stranded uracil-containing DNA template is produced using the VCSM13 helper phage (Kunkel, T.A., J.D. Roberts and

R.A. Zakour, "Rapid and efficient site-specific mutagenesis without phenotypic selection", METHODS IN ENZYMOLOGY, Vol. 154, pp. 367-382, (1987); as modified by Yuckenberg, P.D., F. Witney, J. Geisselsoder and J. McClary, "Site-directed in vitro mutagenesis using uracil-containing DNA and phagemid vectors", DIRECTED MUTAGENESIS - A PRACTICAL APPROACH, ed. M.J. McPherson, pp. 27-48, (1991)). A single primer site-directed mutagenesis modification of the method of Zoller and Smith (Zoller, M.J., and M. Smith, "Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any fragment of DNA", NUCLEIC ACIDS RESEARCH, Vol. 10, pp. 6487-6500, (1982)), is used to produce all mutants (basically as presented by Yuckenberg, et al., 1991, above). Oligonucleotides are made using an Applied Biosystem Inc. 380B DNA synthesizer. Mutagenesis reaction products are transformed into *Escherichia coli* strain MM294 (American Type Culture Collection *E. coli* 33625). All mutants are confirmed by DNA sequencing and the isolated DNA is transformed into the *Bacillus subtilis* expression strain BG2036 (Yang, M. Y., E. Ferrari and D. J. Henner, (1984), "Cloning of the Neutral Protease Gene of *Bacillus subtilis* and the Use of the Cloned Gene to Create an In Vitro-derived Deletion Mutation", JOURNAL OF BACTERIOLOGY, Vol. 160, pp. 15-21). For some of the loop mutants a modified DYP with a frameshift-stop codon mutation in the corresponding loop is used to produce the uracil template. Oligonucleotides are designed to restore the proper reading frame and to encode for random substitutions at positions 58, 59, 60, 61, 62, 64, 65, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 125, 126, 127, 128, 129, 130, 131, 132, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 186, 187, 188, 189, 190, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218 or 219 (equimolar and/or variable mixtures of all four nucleotides for all three bases at these codons). Mutations that correct for the frameshift-stop and produce a functional enzyme are identified by their ability to digest casein. The random substitutions are determined by DNA sequencing.

Example 2

Fermentation

The *Bacillus subtilis* cells (BG2036) containing a subtilisin mutant of

interest are grown to mid-log phase in a one liter culture of LB-glucose broth and inoculated into a Biostat ED fermenter (B. Braun Biotech, Inc., Allentown, Pennsylvania) in a total volume of 10 liters. The fermentation media contains Yeast Extract, starch, antifoam, buffers and trace minerals (see FERMENTATION: A PRACTICAL APPROACH, Ed. B. McNeil and L. M. Harvey, 1990). The broth is kept at a constant pH of 7.0 during the fermentation run. Chloramphenicol is added for antibiotic selection of mutagenized plasmid. The cells are grown overnight at 37°C to an A₆₀₀ of about 60 and harvested.

Example 3

Purification

The fermentation broth is taken through the following steps to obtain pure enzyme. The broth is cleared of *Bacillus subtilis* cells by centrifugation, and clarified by removing fine particulates with a 100K cutoff membrane. This is followed by concentration on a 10K cutoff membrane, and flow dialysis to reduce the ionic strength and adjust the pH to 5.5 using 0.025M MES buffer (2-(*N*-morpholino)ethanesulfonic acid). The enzyme is further purified by loading it onto either a cation exchange chromatography column or an affinity adsorption chromatography column and eluting it from the column with a NaCl or a propylene glycol gradient (see Scopes, R. K., PROTEIN PURIFICATION PRINCIPLES AND PRACTICE, Springer-Verlag, New York (1984)).

The pNA assay (DelMar, E.G., C. Largman, J.W. Brodrick and M.C. Geokas, ANAL. BIOCHEM., Vol. 99, pp. 316-320, (1979)) is used to determine the active enzyme concentration for fractions collected during gradient elution. This assay measures the rate at which *p*-nitroaniline is released as the enzyme hydrolyzes the soluble synthetic substrate, succinyl-alanine-alanine-proline-phenylalanine-*p*-nitroanilide (sAAPF-*p*NA). The rate of production of yellow color from the hydrolysis reaction is measured at 410 nm on a spectrophotometer and is proportional to the active enzyme concentration. In addition, absorbance measurements at 280 nm are used to determine the total protein concentration. The active enzyme/total-protein ratio gives the enzyme purity, and is used to identify fractions to be pooled for the stock solution.

To avoid autolysis of the enzyme during storage, an equal weight of propylene glycol is added to the pooled fractions obtained from the chromatography column. Upon completion of the purification procedure the

purity of the stock enzyme solution is checked with SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and the absolute enzyme concentration is determined via an active site titration method using trypsin inhibitor type II-T: turkey egg white purchased from Sigma Chemical Company (St. Louis, Missouri). The measured conversion factors will show which changes made in the enzyme molecule at the various positions result in the enzyme variant having increased activity over the wild-type, against the soluble substrate pNA.

In preparation for use, the enzyme stock solution is eluted through a Sephadex-G25TM (Pharmacia, Piscataway, New Jersey) size exclusion column to remove the propylene glycol and exchange the buffer. The MES buffer in the enzyme stock solution is exchanged for 0.1 M Tris buffer (Tris(hydroxymethyl-aminomethane) containing 0.01M CaCl₂ and pH adjusted to 8.6 with HCl. All experiments are carried out at pH 8.6 in Tris buffer thermostated at 25°C.

H. Characterization of enzyme variants

Example 4

Model Surface Preparation

Aminopropyl controlled pore glass (CPG) purchased from CPG Inc. (Fairfield, New Jersey) is used as a support for covalently attaching the sAAPF-pNA substrate purchased from Bachem, Inc. (Torrence, California). The reaction is carried out in dimethyl sulfoxide and (1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride) (EDC) is used as a coupling agent. Upon completion (monitored by pNA assay), the excess solvent is removed, and the CPG:sAAPF-pNA is rinsed with dimethyl sulfoxide (DMSO) and doubly-distilled water. This is followed by oven drying with a N₂ purge at about 70°C. The reaction scheme and preparation of the immobilized substrate are conducted as described by Brode, P.F. III, and D.S. Rauch, "Subtilisin BPN': Activity on an Immobilized Substrate," LANGMUIR, Vol. 8, p. 1325-1329, (1992).

The CPG surface will have $62,000 \pm 7,000$ pNA molecules/ μm^2 . The surface area will remain unchanged from the value of $50.0\text{m}^2/\text{g}$ reported by CPG Inc. for the CPG as received. This suggests that the procedure used to add sAAPF-pNA to CPG does not damage the porous structure (mean diameter is 486 Å).

Example 5

Surface Hydrolysis Assay

Using CPG:sAAPF-pNA, adsorption of an enzyme variant and hydrolysis of a CPG-bound peptide can be measured in a single experiment. A small volume of enzyme variant stock solution is added to a flask containing Tris buffer and CPG:sAAPF-pNA which has been degassed. The flask is shaken on a wrist-action shaker for a period of 90 minutes during which the shaker is stopped at various time intervals (for example, every 2 minutes during the early stages of adsorption hydrolysis - e.g., the first 20 minutes - and every 10 minutes towards the end of the experiment). The CPG:sAAPF-pNA is allowed to settle and the solution is sampled. Both the experimental procedure and the calculation of the adsorption and hydrolysis are conducted as described by Brode *et al.*, 1992, above.

All enzymes are monitored for stability against autolysis and should show no appreciable autolytic loss over the time course of this experiment. Therefore, enzyme adsorption can be determined by measuring solution depletion. The difference between the initial enzyme variant concentration and the concentration measured at each individual time point gives the amount of enzyme variant adsorbed. The amount of pNA hydrolyzed from the surface is measured by taking an absorbance reading on an aliquot of the sample at 410 nm. The total amount of pNA hydrolyzed is calculated by adding the amount sampled and the amount remaining in the flask. This value is corrected by subtracting the amount of pNA that is hydrolyzed by Tris buffer at pH 8.6 when no enzyme is present. This base-hydrolysis ranges from 7-29% of the total hydrolysis depending on the efficiency of the enzyme.

Example 6

Soluble Substrate Kinetic Analysis

The rates of hydrolysis of the soluble substrate sAAPF-pNA are monitored by measuring the absorbance increase as a function of time at 410 nm on a DU-70 spectrophotometer. The enzyme concentration is held constant and is prepared to be in the range of 6-10 nanomolar while the substrate concentration is varied from 90-700 μ M sAAPF-pNA for each kinetic determination. An absorbance data point is taken each second over a period of 900 seconds and the data are transferred to a LOTUSTM spreadsheet (Lotus Development Corporation, Cambridge, Massachusetts).

Analysis for kinetic parameters is conducted by the standard Lineweaver Burk analysis in which the data in the initial part of the run (generally the first minute) are fit to a linear regression curve to give v_0 . The v_0 and s_0 data are plotted in the standard inverse fashion to give K_M and k_{cat} .

I. Example Subtilisin DY variants

Subtilisin DY variants of the present invention which have decreased adsorption to and increased hydrolysis of surface bound substrates are exemplified in Tables 2-36, below. In describing the specific mutations, the original amino acid occurring in wild-type is given first, the position number second, and the substituted amino acid third.

TABLE 2

Loop 1 - Single Mutation Variants	
	Thr58Asn
	Thr58Asp
	Thr58Gln
	Thr58Glu
	Thr58Gly
	Thr58Pro
	Thr58Ser
	Asp59Glu
	Gly60Asn
	Gly60Asp
	Gly60Gln
	Gly60Glu
	Gly60Pro
	Gly60Ser
	Asn61Asp
	Asn61Gln
	Asn61Glu
	Asn61Ser
	Gly62Asn
	Gly62Asp
	Gly62Gln
	Gly62Glu
	Gly62Pro
	Gly62Ser
	Gly64Asn
	Gly64Asp
	Gly64Gln
	Gly64Glu
	Gly64Pro
	Gly64Ser
	Thr65Asn
	Thr65Asp
	Thr65Gln
	Thr65Glu
	Thr65Gly

16

Thr65Pro

Thr65Ser

TABLE 3

Loop 1 - Double Mutation Variants

Thr58Gln + Asn61Glu
Thr58Asn + Asn61Asp
Asp59Glu + Gly64Pro
Thr58Glu + Gly62Pro
Asp59Glu + Gly60Ser
Gly60Asn + Gly64Asn
Asn61Asp + Thr65Gln
Thr58Asp + Asn61Ser
Thr58Asp + Thr65Pro
Gly64Glu + Thr65Gly
Gly64Asp + Thr65Asn
Thr58Glu + Gly62Gln
Gly60Asn + Asn61Glu
Thr58Asn + Gly64Ser
Thr58Pro + Asp59Glu
Gly64Glu + Thr65Gln
Thr58Pro + Gly62Ser
Thr58Gly + Gly64Asn
Asn61Gln + Gly64Gln
Thr58Gly + Asn61Asp
Gly60Pro + Asn61Asp
Gly62Asn + Gly64Pro
Gly62Asp + Thr65Asn
Thr58Asn + Gly62Asn
Thr58Pro + Gly62Asn
Gly64Glu + Thr65Ser
Gly60Pro + Gly62Glu
Gly60Glu + Thr65Gln
Gly60Pro + Gly62Ser
Thr58Asp + Asn61Gln
Gly60Asn + Asn61Asp
Thr58Ser + Gly62Glu
Gly60Glu + Gly64Ser
Gly60Pro + Asn61Glu
Thr58Gln + Asn61Ser
Gly60Asp + Thr65Pro
Asn61Asp + Thr65Ser
Asn61Ser + Gly64Asp

TABLE 4

Loop 1 - Triple Mutation Variants

Thr58Asn + Gly60Gln + Gly64Pro
Thr58Gly + Asn61Ser + Thr65Ser
Asp59Glu + Gly60Pro + Thr65Ser

Asn61Glu + Gly62Pro + Gly64Asn
 Thr58Asp + Gly60Asn + Thr65Gln
 Thr58Ser + Gly62Ser + Thr65Gly
 Thr58Pro + Asp59Glu + Gly62Pro
 Gly60Glu + Asn61Gln + Thr65Gln
 Gly60Glu + Gly64Asn + Thr65Pro
 Gly60Asn + Gly62Asp + Thr65Ser
 Thr58Asn + Asn61Glu + Thr65Gln
 Thr58Asn + Asp59Glu + Gly62Pro
 Gly60Gln + Gly62Gln + Gly64Glu
 Asn61Gln + Gly62Pro + Thr65Ser
 Asn61Gln + Gly62Asn + Thr65Gln
 Asn61Ser + Gly62Gln + Thr65Gly
 Gly60Ser + Gly64Gln + Thr65Gly
 Thr58Pro + Gly60Pro + Gly64Gln
 Thr58Asn + Gly60Asn + Thr65Pro
 Asp59Glu + Gly62Gln + Thr65Asn
 Thr58Ser + Gly60Gln + Gly62Asp
 Thr58Asn + Gly64Ser + Thr65Asn
 Gly60Pro + Gly62Asp + Thr65Gln
 Asn61Glu + Gly64Ser + Thr65Gly
 Thr58Pro + Asn61Asp + Gly64Gln
 Asp59Glu + Asn61Ser + Gly62Ser
 Thr58Asn + Gly60Asp + Thr65Asn
 Asp59Glu + Asn61Gln + Gly62Asn
 Thr58Asn + Gly60Asp + Asn61Asp
 Thr58Asn + Gly60Glu + Asn61Glu

TABLE 5

 Loop 1 - Quadruple Mutation Variants

Thr58Asn + Gly60Glu + Gly64Pro + Thr65Gln
 Asp59Glu + Gly60Ser + Gly64Pro + Thr65Pro
 Thr58Ser + Gly60Asn + Asn61Gln + Thr65Pro
 Asp59Glu + Gly60Ser + Gly64Ser + Thr65Gly
 Asp59Glu + Gly60Asn + Asn61Ser + Gly62Ser
 Asp59Glu + Gly60Asn + Asn61Ser + Gly64Asn
 Thr58Gln + Asn61Gln + Gly62Glu + Gly64Asn
 Thr58Asn + Asp59Glu + Gly60Gln + Thr65Pro
 Thr58Gly + Gly60Gln + Gly62Pro + Gly64Glu
 Gly60Asp + Asn61Asp + Gly62Pro + Gly64Gln
 Thr58Pro + Gly60Glu + Asn61Asp + Gly64Asn
 Thr58Asp + Asp59Glu + Gly62Asn + Thr65Gln
 Thr58Asp + Asp59Glu + Gly60Ser + Gly64Asn
 Thr58Glu + Asp59Glu + Gly62Asn + Thr65Ser
 Thr58Asp + Asp59Glu + Gly60Pro + Asn61Ser
 Thr58Asn + Gly60Gln + Asn61Glu + Gly62Asp
 Asp59Glu + Gly60Glu + Asn61Glu + Gly62Ser
 Thr58Gln + Asp59Glu + Gly60Glu + Asn61Glu
 Thr58Pro + Asp59Glu + Gly60Glu + Asn61Glu
 Thr58Gln + Asp59Glu + Gly60Asp + Asn61Asp
 Thr58Pro + Gly60Glu + Asn61Asp + Gly62Glu

18

Gly60Glu + Asn61Glu + Gly62Glu + Thr65Gly
 Gly60Asp + Asn61Asp + Gly62Asp + Thr65Gln
 Thr58Asp + Asp59Glu + Gly60Glu + Thr65Ser
 Asp59Glu + Asn61Asp + Gly62Asp + Thr65Gln
 Asp59Glu + Asn61Asp + Gly62Glu + Thr65Gly
 Asp59Glu + Gly60Asp + Gly62Asp + Thr65Gln
 Thr58Ser + Asp59Glu + Gly60Asp + Gly62Asp

TABLE 6

Loop 2 - Single Mutation Variants

Val 94Ala
 Val 94Asn
 Val 94Asp
 Val 94Cys
 Val 94Gln
 Val 94Glu
 Val 94Gly
 Val 94His
 Val 94Met
 Val 94Pro
 Val 94Ser
 Val 94Thr
 Leu 95Ala
 Leu 95Asn
 Leu 95Asp
 Leu 95Cys
 Leu 95Gln
 Leu 95Glu
 Leu 95Gly
 Leu 95His
 Leu 95Ile
 Leu 95Met
 Leu 95Pro
 Leu 95Ser
 Leu 95Thr
 Leu 95Val
 Asn 96Asp
 Asn 96Gln
 Asn 96Glu
 Asn 96Ser
 Ser 97Asp
 Ser 97Glu
 Ser 98Asp
 Ser 98Glu
 Gly 99Asn
 Gly 99Asp
 Gly 99Gln
 Gly 99Glu
 Gly 99Pro
 Gly 99Ser
 Ser100Asp

19

Ser100Glu
Gly101Asn
Gly101Asp
Gly101Gln
Gly101Glu
Gly101Pro
Gly101Ser
Thr102Asn
Thr102Asp
Thr102Gln
Thr102Glu
Thr102Gly
Thr102Pro
Thr102Ser
Tyr103Ala
Tyr103Asn
Tyr103Asp
Tyr103Cys
Tyr103Gln
Tyr103Glu
Tyr103Gly
Tyr103His
Tyr103Ile
Tyr103Leu
Tyr103Met
Tyr103Pro
Tyr103Ser
Tyr103Thr
Tyr103Val
Ser104Asp
Ser104Glu
Ala105Asn
Ala105Asp
Ala105Gln
Ala105Glu
Ala105Gly
Ala105His
Ala105Pro
Ala105Ser
Ala105Thr
Ile106Ala
Ile106Asn
Ile106Asp
Ile106Cys
Ile106Gln
Ile106Glu
Ile106Gly
Ile106His
Ile106Leu
Ile106Met
Ile106Pro
Ile106Ser
Ile106Thr

Ile106Val

TABLE 7

Loop 2 - Double Mutation Variants

Val 94Gln + Ser100Glu
Asn 96Ser + Gly 99Gln
Val 94Asp + Thr102Gln
Val 94His + Ala105Pro
Val 94Pro + Ser 97Glu
Asn 96Ser + Ile106Asp
Ser 97Glu + Gly101Ser
Leu 95Gly + Thr102Glu
Ser100Glu + Ile106Thr
Asn 96Ser + Gly 99Ser
Ala105Ser + Ile106Pro
Asn 96Ser + Gly101Ser
Thr102Asn + Ile106Gln
Val 94His + Asn 96Gln
Leu 95Cys + Ala105Asp
Leu 95Gly + Asn 96Asp
Leu 95Cys + Asn 96Asp
Thr102Ser + Ile106Cys
Asn 96Gln + Thr102Gln
Gly101Ser + Tyr103Gly
Asn 96Ser + Ile106Thr
Ser 98Asp + Gly 99Gln
Leu 95Ser + Ser100Glu
Leu 95Met + Thr102Gly
Leu 95His + Thr102Glu
Asn 96Asp + Tyr103His
Tyr103Gln + Ile106Asp
Ser104Asp + Ile106Gln
Thr102Asn + Tyr103Asp
Val 94Asn + Ser 97Glu
Val 94Gly + Gly101Ser
Leu 95Gln + Ile106Asp
Asn 96Ser + Ser104Asp
Tyr103Glu + Ile106His
Thr102Ser + Tyr103Glu
Val 94Cys + Ser104Asp
Leu 95Gln + Thr102Asp
Asn 96Glu + Gly101Ser
Leu 95Glu + Ala105Ser
Val 94Met + Leu 95Pro
Val 94Thr + Gly101Gln
Tyr103Gly + Ile106His
Thr102Asn + Ile106Gly
Ser104Asp + Ile106Thr
Gly 99Ser + Ser100Asp
Val 94Glu + Gly 99Asn
Gly 99Gln + Gly101Ser

21

Val 94Thr + Asn 96Asp
 Ser 97Glu + Ile106Leu
 Thr102Asp + Tyr103Met
 Ser 97Asp + Ala105His
 Asn 96Gln + Ser100Asp
 Gly101Asn + Ile106Gln
 Asn 96Glu + Tyr103Ser
 Tyr103Thr + Ile106Pro
 Ser100Asp + Tyr103Ile
 Gly101Glu + Tyr103Leu
 Ser100Asp + Thr102Asn
 Val 94His + Leu 95Thr
 Ser104Asp + Ala105Asn

TABLE 8

 Loop 2 - Triple Mutation Variants

Val 94Gln + Leu 95Thr + Ser100Glu
 Ser 98Asp + Ala105Ser + Ile106Asn
 Val 94Gly + Gly 99Pro + Ile106Val
 Val 94Ser + Leu 95Gln + Ser104Glu
 Val 94His + Tyr103Glu + Ile106Gly
 Val 94Pro + Asn 96Gln + Ile106Cys
 Val 94Thr + Ser 98Asp + Ile106Cys
 Val 94Met + Ser100Asp + Gly101Ser
 Val 94Ala + Ser 97Asp + Gly 99Gln
 Leu 95Gly + Gly 99Pro + Ala105Gly
 Val 94Cys + Ser100Asp + Tyr103Ala
 Gly101Asn + Thr102Asp + Ile106Ala
 Gly 99Ser + Ser104Glu + Ile106Val
 Asn 96Gln + Ser100Glu + Tyr103Thr
 Leu 95Met + Thr102Pro + Ile106Leu
 Asn 96Glu + Thr102Ser + Ala105His
 Ser100Glu + Thr102Asn + Tyr103Val
 Ser 98Glu + Gly 99Gln + Ile106Met
 Asn 96Asp + Gly 99Ser + Ala105Thr
 Gly101Asp + Thr102Asn + Tyr103Thr
 Val 94Ala + Gly 99Gln + Tyr103His
 Thr102Gly + Tyr103Glu + Ala105Gly
 Ser 97Glu + Gly101Ser + Thr102Gln
 Val 94Pro + Gly 99Ser + Gly101Asp
 Ser 97Asp + Gly 99Ser + Ile106Gly
 Val 94Cys + Leu 95Val + Gly101Gln
 Ser100Asp + Gly101Asn + Tyr103Gln
 Thr102Asp + Tyr103Leu + Ile106Val
 Val 94Cys + Ser104Glu + Ala105Asn
 Val 94Ser + Thr102Asn + Ala105Thr
 Ser 98Glu + Gly 99Asn + Tyr103Val
 Leu 95Val + Gly101Gln + Ala105Asp
 Ser 97Glu + Gly 99Pro + Thr102Ser
 Val 94Ser + Gly101Glu + Ile106Ala
 Gly 99Asn + Gly101Asp + Thr102Glu

Gly 99Asp + Ser100Glu + Thr102Asn
 Val 94Thr + Gly 99Glu + Ser100Asp
 Gly 99Asp + Ser100Asp + Tyr103Pro
 Gly 99Glu + Ser100Glu + Gly101Gln
 Asn 96Glu + Ser 97Glu + Ala105Asn
 Asn 96Glu + Ser 97Asp + Gly101Pro
 Leu 95Met + Asn 96Asp + Ser 97Glu
 Gly 99Ser + Ser104Asp + Ala105Glu
 Ser 97Asp + Ser 98Glu + Gly101Ser
 Asn 96Asp + Ser 98Asp + Gly 99Glu
 Asn 96Glu + Ser 97Glu + Gly 99Glu
 Leu 95Asp + Ser100Glu + Ile106Leu
 Asn 96Asp + Ser100Glu + Gly101Glu
 Ser104Asp + Ala105Thr + Ile106Glu
 Asn 96Glu + Ser 98Asp + Tyr103Ala
 Leu 95Asn + Ser 98Asp + Ser100Glu
 Leu 95Gly + Ser 98Glu + Ser100Glu
 Ser 98Asp + Ser100Glu + Ala105Ser
 Ser 98Glu + Ser100Asp + Thr102Asn
 Val 94Asn + Ser 97Glu + Gly 99Glu
 Asn 96Glu + Ser100Glu + Thr102Pro
 Val 94Asp + Gly101Asp + Thr102Pro
 Gly101Glu + Thr102Asp + Ser104Asp
 Ser 98Glu + Ser100Asp + Gly101Asp
 Leu 95Glu + Gly101Asp + Ile106Glu

TABLE 9

 Loop 2 - Quadruple Mutation Variants

Gly 99Asn + Gly101Gln + Thr102Glu + Tyr103Val
 Val 94Asn + Gly101Asp + Tyr103Ile + Ala105Thr
 Val 94Asn + Gly 99Ser + Ser100Glu + Thr102Ser
 Leu 95Cys + Ser 98Asp + Gly101Pro + Ile106Ala
 Leu 95His + Gly101Ser + Tyr103Ile + Ala105Ser
 Val 94Ser + Leu 95Val + Gly 99Pro + Ser100Glu
 Gly 99Ser + Ser100Glu + Tyr103Ile + Ala105Ser
 Val 94Ala + Ser 97Glu + Thr102Ser + Ala105Pro
 Val 94Thr + Tyr103Gly + Ser104Asp + Ala105Gln
 Val 94Ala + Leu 95Pro + Ser100Glu + Ile106Ser
 Leu 95Asn + Ser 98Asp + Tyr103Gly + Ala105Pro
 Asn 96Ser + Gly101Glu + Tyr103Leu + Ala105Pro
 Leu 95Met + Ser 98Glu + Gly 99Asn + Tyr103Thr
 Val 94Ala + Leu 95Ala + Ser 97Asp + Thr102Asn
 Gly 99Gln + Ser100Glu + Thr102Ser + Ile106Leu
 Asn 96Glu + Gly 99Gln + Ala105Pro + Ile106Thr
 Val 94Gly + Gly101Glu + Tyr103Gln + Ile106His
 Val 94Thr + Ser 97Asp + Gly101Gln + Ala105Thr
 Val 94Gly + Leu 95Val + Asn 96Gln + Ser104Glu
 Leu 95Gln + Gly 99Glu + Ser100Glu + Thr102Pro
 Leu 95Met + Ser100Asp + Gly101Asp + Tyr103Ser
 Leu 95Ile + Thr102Pro + Ser104Asp + Ala105Asp
 Ser 97Asp + Ser 98Asp + Tyr103Met + Ala105Pro

23

Leu 95Ser + Ser 97Asp + Ser 98Asp + Thr102Ser
 Ser 97Glu + Ser 98Glu + Ala105Pro + Ile106Asn
 Ser 97Glu + Ser 98Glu + Gly101Ser + Ile106Cys
 Ser 97Asp + Ser 98Asp + Gly101Pro + Thr102Pro
 Leu 95Glu + Ser100Glu + Gly101Asp + Thr102Ser
 Val 94Met + Leu 95Glu + Asn 96Glu + Gly101Asp
 Leu 95His + Gly 99Glu + Ser100Glu + Gly101Glu
 Asn 96Glu + Gly 99Glu + Gly101Ser + Ile106Asn
 Leu 95Val + Asn 96Asp + Ser 98Glu + Tyr103Gln
 Asn 96Asp + Ser 98Glu + Gly 99Ser + Thr102Asn
 Asn 96Glu + Ser 98Glu + Gly101Ser + Thr102Pro
 Leu 95Val + Asn 96Glu + Ser 98Asp + Ile106Ala
 Val 94Gly + Asn 96Glu + Ser 98Asp + Tyr103Thr
 Ser 98Glu + Gly 99Gln + Ser100Asp + Gly101Pro
 Ser 98Glu + Gly 99Gln + Ser100Asp + Thr102Gln
 Asn 96Glu + Ser100Glu + Tyr103Gln + Ile106Pro
 Asn 96Asp + Gly 99Asn + Ser100Glu + Tyr103Ile
 Leu 95Glu + Asn 96Gln + Ser 98Glu + Gly 99Glu
 Ser 97Asp + Ser 98Glu + Ser100Asp + Ile106Val
 Ser 97Asp + Ser 98Asp + Ser100Glu + Thr102Ser
 Ser 97Asp + Ser 98Asp + Ser100Glu + Gly101Gln
 Leu 95Thr + Ser 97Asp + Ser 98Glu + Ser100Asp
 Val 94Met + Asn 96Glu + Gly 99Asp + Gly101Asp
 Ser 98Asp + Ser100Glu + Gly101Glu + Ala105Ser
 Val 94Cys + Ser 98Asp + Ser100Asp + Gly101Asp
 Val 94Glu + Ser100Glu + Gly101Glu + Tyr103Ile
 Leu 95Glu + Ser 97Asp + Ser 98Asp + Ala105Ser
 Leu 95Ala + Asn 96Asp + Gly101Asp + Thr102Asp
 Val 94Cys + Gly 99Asp + Gly101Asp + Ile106Pro
 Leu 95Ser + Ser100Glu + Gly101Gln + Thr102Glu
 Leu 95Met + Ser100Asp + Thr102Glu + Ile106Gln
 Asn 96Ser + Gly101Asp + Tyr103Asn + Ile106Asp
 Leu 95Cys + Gly101Glu + Ala105Pro + Ile106Glu
 Val 94Asp + Gly 99Glu + Ser100Asp + Ala105Ser
 Ser 97Glu + Ser 98Glu + Gly101Asp + Tyr103Ser
 Ser 97Asp + Ser100Glu + Thr102Gln + Tyr103Thr
 Ser 97Glu + Ser100Asp + Tyr103Ser + Ala105Gly

TABLE 10

Loop 3 - Single Mutation Variants

Leu125Ala
 Leu125Asn
 Leu125Asp
 Leu125Cys
 Leu125Gln
 Leu125Glu
 Leu125Gly
 Leu125His
 Leu125Ile
 Leu125Met
 Leu125Pro

24

Leu125Ser
 Leu125Thr
 Leu125Val
 Gly126Asn
 Gly126Asp
 Gly126Gln
 Gly126Glu
 Gly126Pro
 Gly126Ser
 Gly127Asn
 Gly127Asp
 Gly127Gln
 Gly127Glu
 Gly127Pro
 Gly127Ser
 Pro128Asn
 Pro128Asp
 Pro128Gln
 Pro128Glu
 Pro128Gly
 Pro128Ser
 Ser129Asp
 Ser129Glu
 Gly130Asn
 Gly130Asp
 Gly130Gln
 Gly130Glu
 Gly130Pro
 Gly130Ser
 Ser131Asp
 Ser131Glu
 Thr132Asn
 Thr132Asp
 Thr132Gln
 Thr132Glu
 Thr132Gly
 Thr132Pro
 Thr132Ser

TABLE 11

 Loop 3 - Double Mutation Variants

Leu125Gln + Ser129Glu
 Leu125Pro + Gly126Ser
 Gly127Gln + Ser131Asp
 Gly130Ser + Thr132Gly
 Leu125Met + Gly130Asp
 Leu125Pro + Pro128Asp
 Ser129Asp + Gly130Asn
 Leu125Asp + Pro128Asn
 Gly127Ser + Ser131Glu
 Leu125Ile + Gly130Ser

25

Leu125Thr + Thr132Asp
Leu125Thr + Thr132Asn
Leu125Pro + Gly127Glu
Leu125Glu + Thr132Asn
Pro128Asn + Thr132Pro
Leu125Thr + Gly130Asp
Gly127Pro + Thr132Ser
Gly127Asn + Thr132Asn
Gly126Gln + Ser131Asp
Leu125Val + Thr132Gly
Gly126Gln + Gly130Ser
Ser129Asp + Thr132Gly
Gly126Asp + Gly130Pro
Leu125Cys + Pro128Gln
Gly127Gln + Thr132Gly
Leu125His + Gly126Asp
Leu125Val + Ser129Glu
Gly126Ser + Pro128Asp
Leu125Val + Gly126Glu
Gly127Glu + Pro128Ser
Leu125Asn + Thr132Pro
Leu125Met + Gly126Asp
Leu125Ser + Ser129Glu
Leu125Ser + Gly126Asn
Leu125Val + Thr132Gln
Leu125Val + Gly130Asp
Ser129Glu + Thr132Ser
Pro128Gly + Ser129Asp
Gly126Pro + Thr132Asp
Gly126Asp + Thr132Ser
Pro128Asp + Thr132Gln
Gly126Pro + Ser129Asp
Gly130Ser + Thr132Glu
Gly127Glu + Pro128Gln
Gly126Asn + Gly130Pro
Pro128Asn + Ser129Glu
Gly130Asp + Thr132Gly
Gly126Gln + Ser131Glu
Gly127Glu + Gly130Ser
Gly126Asn + Ser129Glu
Pro128Asp + Gly130Asn
Pro128Asp + Thr132Gly
Gly126Gln + Pro128Asp
Ser131Glu + Thr132Gly
Gly130Glu + Thr132Asn
Leu125Val + Pro128Gly
Leu125Ile + Gly130Pro
Pro128Asn + Ser131Asp
Gly130Asn + Thr132Asp
Gly127Ser + Pro128Glu

TABLE 12

Loop 3 - Triple Mutation Variants

Gly127Asp	+	Gly130Gln	+	Thr132Ser
Pro128Asp	+	Gly130Pro	+	Thr132Ser
Gly127Asn	+	Ser131Asp	+	Thr132Pro
Leu125Ser	+	Ser131Asp	+	Thr132Pro
Leu125Met	+	Pro128Glu	+	Thr132Pro
Leu125Asp	+	Gly126Ser	+	Thr132Gln
Gly127Asn	+	Pro128Glu	+	Gly130Asn
Gly127Ser	+	Pro128Gln	+	Ser129Glu
Gly126Pro	+	Gly127Pro	+	Ser131Glu
Leu125Ile	+	Gly127Asp	+	Thr132Gln
Gly126Asn	+	Gly130Ser	+	Thr132Pro
Gly126Pro	+	Gly127Gln	+	Thr132Asp
Gly126Asp	+	Gly130Asn	+	Thr132Gln
Leu125Ser	+	Pro128Gln	+	Thr132Asn
Gly126Pro	+	Gly127Pro	+	Pro128Glu
Leu125Met	+	Gly126Glu	+	Gly130Gln
Leu125Met	+	Ser129Asp	+	Thr132Gln
Leu125His	+	Ser129Asp	+	Gly130Ser
Gly127Pro	+	Ser129Glu	+	Gly130Ser
Pro128Gln	+	Gly130Asn	+	Thr132Gln
Gly126Asn	+	Gly127Pro	+	Gly130Gln
Leu125Pro	+	Gly126Ser	+	Gly127Gln
Leu125His	+	Gly127Ser	+	Ser131Glu
Leu125His	+	Gly127Asn	+	Thr132Glu
Gly126Glu	+	Gly130Pro	+	Thr132Gln
Leu125His	+	Gly127Gln	+	Gly130Glu
Gly127Pro	+	Pro128Gly	+	Thr132Asn
Leu125Gly	+	Pro128Gly	+	Gly130Asp
Gly126Asn	+	Pro128Asn	+	Ser129Glu
Gly126Pro	+	Gly130Ser	+	Thr132Gly
Leu125Ser	+	Gly126Pro	+	Ser131Glu
Leu125Val	+	Gly126Glu	+	Thr132Gly
Pro128Asn	+	Ser131Asp	+	Thr132Pro
Leu125Gly	+	Pro128Asn	+	Thr132Pro
Gly127Glu	+	Pro128Ser	+	Thr132Pro
Gly127Gln	+	Ser129Glu	+	Thr132Gln
Leu125Ile	+	Gly127Pro	+	Gly130Asp
Gly126Ser	+	Gly127Asp	+	Gly130Asn
Pro128Glu	+	Gly130Pro	+	Thr132Gln
Leu125Val	+	Gly126Asp	+	Gly127Asp
Pro128Asn	+	Gly130Asp	+	Ser131Asp
Gly130Glu	+	Ser131Asp	+	Thr132Gln
Gly126Pro	+	Gly130Glu	+	Ser131Asp
Gly127Glu	+	Pro128Glu	+	Thr132Ser
Gly130Gln	+	Ser131Glu	+	Thr132Asp
Leu125Gln	+	Ser131Asp	+	Thr132Asp
Gly130Ser	+	Ser131Glu	+	Thr132Glu
Gly130Pro	+	Ser131Asp	+	Thr132Asp

27

Gly127Pro + Ser131Glu + Thr132Asp
 Leu125Asn + Ser131Asp + Thr132Asp
 Pro128Gln + Ser131Asp + Thr132Glu
 Pro128Gly + Ser131Asp + Thr132Asp
 Gly126Gln + Ser129Asp + Gly130Asp
 Leu125Ile + Ser129Asp + Gly130Glu
 Gly127Glu + Pro128Asp + Ser129Glu
 Ser129Asp + Gly130Asp + Ser131Asp
 Ser129Glu + Gly130Asp + Ser131Glu
 Ser129Glu + Gly130Glu + Ser131Asp
 Gly126Glu + Gly127Glu + Pro128Glu
 Gly130Glu + Ser131Asp + Thr132Asp

TABLE 13

Loop 3 - Quadruple Mutation Variants

Gly126Ser	+	Pro128Glu	+	Gly130Ser	+	Thr132Ser
Leu125Cys	+	Gly127Gln	+	Ser129Glu	+	Thr132Pro
Leu125Gln	+	Gly126Pro	+	Pro128Ser	+	Ser129Glu
Leu125Thr	+	Ser129Glu	+	Gly130Ser	+	Thr132Pro
Gly127Gln	+	Pro128Gln	+	Ser129Glu	+	Gly130Pro
Gly127Asn	+	Pro128Asn	+	Ser131Glu	+	Thr132Gly
Leu125Met	+	Gly127Glu	+	Gly130Asn	+	Thr132Gly
Leu125Met	+	Gly127Asn	+	Pro128Asn	+	Ser129Glu
Leu125Met	+	Gly126Ser	+	Gly127Gln	+	Ser131Asp
Leu125Asn	+	Gly126Asp	+	Gly127Gln	+	Thr132Ser
Leu125Gln	+	Gly127Gln	+	Gly130Glu	+	Thr132Pro
Leu125Ala	+	Gly130Asn	+	Ser131Asp	+	Thr132Pro
Leu125Met	+	Gly126Pro	+	Gly127Ser	+	Ser131Asp
Leu125Cys	+	Gly126Pro	+	Ser129Glu	+	Thr132Asn
Leu125Gly	+	Pro128Gly	+	Ser131Asp	+	Thr132Gln
Leu125Met	+	Gly130Asn	+	Ser131Asp	+	Thr132Gln
Leu125Val	+	Gly126Pro	+	Gly130Gln	+	Thr132Asp
Gly126Asn	+	Gly127Pro	+	Pro128Gln	+	Ser129Glu
Leu125Gln	+	Gly126Glu	+	Gly127Glu	+	Gly130Asn
Leu125Val	+	Gly126Asp	+	Gly127Glu	+	Gly130Pro
Leu125Asp	+	Gly126Glu	+	Gly127Pro	+	Thr132Pro
Leu125Glu	+	Gly126Glu	+	Gly127Asn	+	Thr132Ser
Leu125Gln	+	Gly127Pro	+	Gly130Glu	+	Ser131Asp
Leu125Cys	+	Gly130Glu	+	Ser131Asp	+	Thr132Gln
Gly126Gln	+	Gly127Glu	+	Pro128Glu	+	Gly130Asn
Gly126Ser	+	Gly127Asp	+	Pro128Asp	+	Gly130Ser
Leu125Thr	+	Gly127Ser	+	Ser131Glu	+	Thr132Asp
Pro128Asn	+	Gly130Asn	+	Ser131Asp	+	Thr132Glu
Leu125Met	+	Gly126Ser	+	Ser129Asp	+	Gly130Asp
Gly126Gln	+	Pro128Gln	+	Ser129Glu	+	Gly130Glu
Gly127Asn	+	Pro128Asn	+	Ser129Glu	+	Gly130Glu
Gly127Ser	+	Ser129Glu	+	Gly130Asp	+	Thr132Gln
Gly127Asp	+	Pro128Glu	+	Ser129Asp	+	Gly130Asn
Gly127Asp	+	Pro128Glu	+	Ser129Asp	+	Thr132Ser
Leu125Gln	+	Ser129Glu	+	Gly130Asp	+	Ser131Asp
Leu125Cys	+	Ser129Glu	+	Gly130Asp	+	Ser131Glu

28

Gly127Gln + Pro128Glu + Ser129Glu + Gly130Asp
 Leu125Met + Gly126Asp + Gly127Glu + Pro128Glu
 Gly127Glu + Pro128Gln + Ser129Glu + Thr132Ser
 Leu125Pro + Gly127Asp + Pro128Ser + Ser129Asp
 Gly126Pro + Gly127Glu + Pro128Gly + Ser129Asp
 Leu125Pro + Gly127Asp + Ser129Glu + Gly130Glu
 Gly127Asp + Pro128Ser + Ser129Glu + Gly130Asp
 Gly127Asp + Ser129Glu + Gly130Glu + Thr132Pro
 Leu125Ile + Gly127Glu + Ser129Asp + Gly130Asp
 Gly126Glu + Gly127Asp + Ser129Glu + Gly130Pro
 Gly126Asp + Gly127Glu + Ser129Asp + Thr132Pro
 Leu125Met + Gly126Asp + Gly127Asp + Ser129Asp
 Gly126Ser + Ser129Asp + Ser131Glu + Thr132Gln
 Gly127Gln + Pro128Asn + Ser129Asp + Ser131Asp
 Pro128Gln + Ser129Glu + Ser131Asp + Thr132Ser
 Gly126Pro + Ser129Asp + Ser131Glu + Thr132Ser
 Leu125Ala + Ser129Glu + Gly130Pro + Ser131Asp
 Gly127Gln + Ser129Glu + Gly130Ser + Ser131Asp
 Gly127Ser + Ser129Glu + Ser131Glu + Thr132Gln
 Gly127Gln + Pro128Gln + Ser129Glu + Ser131Asp
 Leu125Ala + Gly126Ser + Ser129Asp + Ser131Glu
 Gly126Pro + Pro128Gln + Ser129Asp + Ser131Asp
 Gly126Ser + Pro128Ser + Ser129Asp + Ser131Glu
 Gly126Glu + Pro128Asp + Ser129Asp + Thr132Asn

TABLE 14

Loop 4 - Single Mutation Variants

Gly153Asn
 Gly153Asp
 Gly153Gln
 Gly153Glu
 Gly153Pro
 Gly153Ser
 Asn154Asp
 Asn154Gln
 Asn154Glu
 Asn154Ser
 Ser155Asp
 Ser155Glu
 Gly156Asn
 Gly156Asp
 Gly156Gln
 Gly156Glu
 Gly156Pro
 Gly156Ser
 Ser157Asp
 Ser157Glu
 Ser158Asp
 Ser158Glu
 Gly159Asn
 Gly159Asp

29

Gly159Gln
Gly159Glu
Gly159Pro
Gly159Ser
Ser160Asp
Ser160Glu
Gln161Asn
Gln161Asp
Gln161Glu
Gln161Ser
Asn162Asp
Asn162Gln
Asn162Glu
Asn162Ser
Thr163Asn
Thr163Asp
Thr163Gln
Thr163Glu
Thr163Gly
Thr163Pro
Thr163Ser
Ile164Ala
Ile164Asn
Ile164Asp
Ile164Cys
Ile164Gln
Ile164Glu
Ile164Gly
Ile164His
Ile164Leu
Ile164Met
Ile164Pro
Ile164Ser
Ile164Thr
Ile164Val
Gly165Asn
Gly165Asp
Gly165Gln
Gly165Glu
Gly165Pro
Gly165Ser
Tyr166Ala
Tyr166Asn
Tyr166Asp
Tyr166Cys
Tyr166Gln
Tyr166Glu
Tyr166Gly
Tyr166His
Tyr166Ile
Tyr166Leu
Tyr166Met
Tyr166Pro

30

Tyr166Ser
 Tyr166Thr
 Tyr166Val

TABLE 15

Loop 4 - Double Mutation Variants

Asn154Ser	+	Ser155Glu
Gly165Glu	+	Tyr166Cys
Ser158Asp	+	Gly165Pro
Ser157Asp	+	Thr163Asn
Gly156Pro	+	Thr163Ser
Asn154Glu	+	Ile164Cys
Gly153Asn	+	Thr163Asn
Thr163Glu	+	Ile164Gly
Gly156Gln	+	Ile164Thr
Ser157Asp	+	Ile164His
Ser158Glu	+	Tyr166His
Gly153Pro	+	Tyr166Asp
Gly153Pro	+	Gly165Gln
Ser157Glu	+	Gly159Asn
Asn154Ser	+	Gly159Pro
Gly156Glu	+	Asn162Gln
Gln161Asp	+	Ile164Met
Ser157Asp	+	Thr163Ser
Ser157Glu	+	Tyr166Ile
Gln161Glu	+	Ile164His
Ser157Glu	+	Asn162Gln
Gly159Pro	+	Tyr166Asp
Asn162Asp	+	Ile164Cys
Gln161Ser	+	Asn162Asp
Asn154Asp	+	Asn162Gln
Ser158Glu	+	Gly165Asn
Ile164Met	+	Tyr166Val
Gln161Asn	+	Gly165Pro
Ser155Asp	+	Gly159Pro
Ser160Asp	+	Asn162Ser
Gly156Glu	+	Ile164Cys
Thr163Asp	+	Gly165Pro
Gly153Asn	+	Ser155Glu
Ser158Asp	+	Thr163Gly
Ser160Asp	+	Ile164Met
Gly159Asp	+	Thr163Gln
Ser155Glu	+	Tyr166Cys
Gln161Ser	+	Tyr166Gly
Ser160Asp	+	Ile164Ala
Ser157Asp	+	Gln161Ser
Ser160Glu	+	Asn162Gln
Gly153Asn	+	Gly156Gln
Ser157Asp	+	Ile164Val
Asn162Gln	+	Thr163Glu
Ser155Asp	+	Gln161Ser

Gly159Glu + Gly165Gln
 Ser160Glu + Ile164His
 Gly153Ser + Ser160Asp
 Gly159Gln + Gly165Glu
 Gly153Glu + Gly159Gln
 Gly156Glu + Thr163Gly
 Gly156Ser + Tyr166Asn
 Gly153Asp + Thr163Asn
 Asn154Ser + Ser157Glu
 Thr163Ser + Ile164Pro
 Ser155Glu + Asn162Ser
 Gly156Pro + Ile164Gly
 Thr163Glu + Gly165Asn
 Gly153Gln + Ser155Glu
 Ile164Ser + Tyr166Cys

TABLE 16

Loop 4 - Triple Mutation Variants

Gly153Gln + Asn154Ser + Ser155Glu
 Ser155Asp + Thr163Pro + Tyr166Gln
 Gly156Gln + Thr163Glu + Ile164Gly
 Gly159Gln + Gln161Ser + Gly165Ser
 Asn154Gln + Thr163Asp + Tyr166Gln
 Asn154Gln + Gly159Pro + Ile164Thr
 Asn162Gln + Ile164Gln + Gly165Glu
 Gly153Asp + Gln161Ser + Thr163Pro
 Gly153Gln + Asn154Gln + Tyr166Ile
 Asn154Gln + Gln161Asn + Tyr166Pro
 Gly153Gln + Ser158Glu + Tyr166Ser
 Ser157Asp + Ile164Val + Tyr166Ala
 Thr163Gly + Gly165Asp + Tyr166Pro
 Gln161Glu + Asn162Gln + Tyr166Thr
 Gly156Pro + Gln161Glu + Thr163Ser
 Ser158Glu + Gly159Pro + Gln161Asn
 Ser158Glu + Gln161Asn + Asn162Ser
 Asn154Glu + Gln161Ser + Gly165Asn
 Gly153Asn + Asn154Gln + Ser158Asp
 Ser157Glu + Ile164Val + Tyr166Val
 Ser155Asp + Gln161Asn + Tyr166Ile
 Gly153Glu + Gln161Asn + Gly165Ser
 Gly156Pro + Asn162Asp + Gly165Pro
 Gly156Asn + Asn162Asp + Ile164Leu
 Asn154Gln + Thr163Asn + Gly165Ser
 Ser158Asp + Gly159Asn + Gln161Asn
 Ser155Glu + Gln161Asn + Tyr166Leu
 Ser158Glu + Asn162Ser + Tyr166His
 Asn154Asp + Thr163Gln + Ile164Met
 Gly156Asp + Ser157Asp + Gly159Ser
 Gly159Asp + Ser160Glu + Thr163Gln
 Ser158Asp + Gly159Asp + Tyr166Cys
 Asn154Glu + Ser155Glu + Gly156Ser

Ser155Asp + Gly156Asp + Asn162Ser
 Ser157Asp + Ser158Glu + Gly159Pro
 Ser160Asp + Gln161Asp + Ile164Ala
 Gly159Gln + Ser160Asp + Gln161Asp
 Gly156Asp + Ser157Asp + Ser158Asp
 Gly159Asp + Gln161Glu + Gly165Gln
 Gly153Pro + Gly159Glu + Gln161Glu
 Ser157Glu + Ser158Asp + Gln161Glu
 Ser157Glu + Gly159Glu + Gly165Ser
 Ser157Glu + Gly159Glu + Ser160Glu
 Ser158Asp + Ser160Asp + Ile164Thr
 Ser158Asp + Ser160Glu + Tyr166Val
 Asn154Gln + Ser158Asp + Ser160Asp
 Ser158Glu + Ser160Asp + Tyr166His
 Gly153Asp + Asn154Glu + Gly165Glu
 Ser155Asp + Ser157Asp + Tyr166Ile
 Ser155Glu + Ser157Asp + Gly159Pro
 Gly153Asn + Ser157Glu + Thr163Glu
 Ser155Asp + Ser157Asp + Ser158Glu
 Ser157Asp + Gly159Asp + Gln161Glu
 Ser155Asp + Gly156Glu + Ser158Asp
 Asn154Gln + Ser160Glu + Asn162Asp
 Ser158Glu + Ser160Glu + Asn162Glu
 Ser157Glu + Gln161Asp + Thr163Asp
 Asn154Glu + Ser155Glu + Ser157Glu
 Ser157Asp + Ser160Glu + Tyr166Gly
 Gly153Asn + Ser157Glu + Ser160Asp

TABLE 17

Loop 4 - Quadruple Mutation Variants

Asn154Ser + Gly159Asp + Ile164Met + Gly165Pro
 Ser155Glu + Gly156Ser + Asn162Gln + Thr163Asn
 Gly156Gln + Ser157Asp + Gly159Ser + Asn162Ser
 Ser155Asp + Gly159Pro + Gln161Ser + Ile164Cys
 Asn154Gln + Gly156Asp + Thr163Gly + Tyr166Met
 Asn154Gln + Ser155Asp + Thr163Asn + Gly165Ser
 Gln161Ser + Asn162Gln + Thr163Gly + Tyr166Gly
 Ser155Asp + Gly159Pro + Gly165Pro + Tyr166Gln
 Asn154Ser + Ser155Asp + Thr163Gly + Ile164Val
 Gly159Asp + Gln161Ser + Thr163Gln + Ile164Thr
 Gly153Pro + Ser158Asp + Asn162Gln + Ile164Cys
 Gly153Pro + Gly156Asp + Ile164Ser + Tyr166Leu
 Gly153Glu + Gly156Pro + Gln161Ser + Tyr166Met
 Ser155Glu + Thr163Ser + Gly165Pro + Tyr166Leu
 Ser157Asp + Thr163Gly + Ile164Gln + Tyr166Gly
 Gly153Ser + Ser155Asp + Thr163Pro + Ile164Pro
 Asn154Ser + Gly159Gln + Gln161Glu + Tyr166Met
 Asn154Ser + Ser160Asp + Ile164Gln + Gly165Ser
 Gly159Asn + Asn162Glu + Gly165Gln + Tyr166Cys
 Gly156Pro + Gly159Pro + Ile164Gln + Gly165Gln
 Gly153Ser + Gly156Asn + Asn162Gln + Gly165Asp

Ser158Asp + Gly159Glu + Gln161Ser + Ile164Thr
 Gly153Gln + Asn154Glu + Ser155Glu + Tyr166Gly
 Gly153Ser + Ser155Asp + Gly156Asp + Ile164Gln
 Asn154Ser + Ser155Glu + Gly156Asp + Tyr166Val
 Ser155Glu + Gly156Glu + Thr163Asn + Ile164Leu
 Ser157Glu + Ser158Asp + Gln161Asn + Ile164Cys
 Ser160Glu + Gln161Asp + Thr163Gln + Gly165Pro
 Ser157Asp + Ser158Glu + Gly159Asp + Ile164Cys
 Asn154Gln + Ser160Glu + Gln161Glu + Asn162Asp
 Gly153Glu + Gly159Gln + Gln161Asn + Gly165Glu
 Asn154Ser + Ser158Asp + Gly159Asn + Gln161Glu
 Asn154Glu + Gly156Asp + Thr163Ser + Tyr166Asn
 Ser158Glu + Ser160Asp + Gln161Asn + Thr163Pro
 Asn154Glu + Ser155Asp + Gly156Gln + Thr163Asp
 Ser158Glu + Gln161Glu + Thr163Asp + Ile164His
 Ser155Glu + Ser157Asp + Asn162Ser + Gly165Pro
 Ser155Asp + Ser157Asp + Gly159Pro + Ile164Cys
 Gly159Glu + Ser160Asp + Asn162Glu + Ile164Asn
 Ser157Glu + Gln161Asp + Thr163Asp + Gly165Gln
 Asn154Glu + Ser155Glu + Ser157Asp + Gly159Pro
 Asn154Gln + Ser160Asp + Gln161Asp + Thr163Asp
 Ser160Asp + Gln161Asp + Asn162Gln + Thr163Asp
 Ser155Asp + Thr163Asp + Ile164Gly + Gly165Asp
 Gly156Ser + Ser158Asp + Thr163Asp + Ile164Leu
 Ser155Glu + Ser158Glu + Thr163Glu + Ile164Met
 Gly153Glu + Gly156Pro + Thr163Asp + Tyr166His
 Gly156Ser + Ser157Asp + Ser160Glu + Asn162Asp
 Gly153Asn + Ser157Glu + Asn162Asp + Ile164Leu
 Gly153Glu + Ser155Glu + Ser157Asp + Ile164Met
 Gly156Pro + Ser157Asp + Gly159Gln + Ser160Glu
 Gly153Pro + Ser155Glu + Gly156Glu + Gly165Asp
 Ser155Asp + Ser158Glu + Gln161Asn + Gly165Pro
 Ser155Asp + Ser158Glu + Gly159Gln + Ile164Gly
 Gly153Asp + Ser157Glu + Gly159Pro + Thr163Glu
 Ser155Asp + Gly156Pro + Gln161Ser + Gly165Asp
 Gly156Glu + Ser157Glu + Ser160Asp + Ile164Ser
 Asn154Ser + Gly156Asp + Ser158Asp + Ser160Asp
 Gly153Gln + Ser155Asp + Ser158Asp + Gly159Glu
 Asn154Asp + Gly156Glu + Ser158Glu + Ile164Ala

TABLE 18

Loop 5 - Single Mutation Variants

Ala186Asn
 Ala186Asp
 Ala186Gln
 Ala186Glu
 Ala186Gly
 Ala186His
 Ala186Pro
 Ala186Ser
 Ala186Thr

34

Ser187Asp
 Ser187Glu
 Phe188Ala
 Phe188Asn
 Phe188Asp
 Phe188Cys
 Phe188Gln
 Phe188Glu
 Phe188Gly
 Phe188His
 Phe188Ile
 Phe188Leu
 Phe188Met
 Phe188Pro
 Phe188Ser
 Phe188Thr
 Phe188Tyr
 Phe188Val
 Ser189Asp
 Ser189Glu
 Ser190Asp
 Ser190Glu

TABLE 19

 Loop 5 - Double Mutation Variants

Phe188Met + Ser190Glu
 Ala186Ser + Ser187Glu
 Phe188Ser + Ser190Asp
 Ala186His + Ser187Glu
 Ser187Asp + Phe188Cys
 Ala186Ser + Ser190Glu
 Ala186Pro + Ser190Asp
 Phe188Ser + Ser190Glu
 Ala186Gln + Ser187Glu
 Phe188Asn + Ser190Asp
 Ala186Glu + Phe188Met
 Ala186Ser + Phe188Ala
 Ser187Glu + Phe188Thr
 Ala186Thr + Phe188Asp
 Ser187Asp + Phe188Leu
 Ala186Gly + Phe188Cys
 Phe188Val + Ser190Glu
 Ala186Asp + Phe188Pro
 Ala186Asn + Ser187Asp
 Phe188Leu + Ser190Glu
 Ser187Asp + Phe188Asn
 Ala186Ser + Ser187Asp
 Phe188Ala + Ser190Glu
 Ala186Gly + Ser187Glu
 Ala186Glu + Phe188His
 Ala186Gly + Phe188Glu

35

Ser187Glu + Phe188Gly
 Ala186Glu + Phe188Asn
 Ser187Glu + Phe188His
 Ala186Ser + Phe188Asp
 Ala186Gly + Ser187Asp
 Phe188Ile + Ser190Glu
 Ala186Thr + Ser190Glu
 Ala186Gly + Ser190Glu
 Phe188His + Ser190Asp
 Phe188Leu + Ser190Asp
 Ala186Ser + Ser190Asp
 Ser187Glu + Phe188Ile
 Ala186Thr + Phe188His
 Phe188Gln + Ser190Glu
 Ala186His + Ser187Asp
 Phe188Tyr + Ser190Glu
 Ala186Pro + Phe188Thr
 Ala186Gln + Ser187Asp
 Ser187Glu + Phe188Ala
 Ala186Asp + Phe188Ile
 Ala186Ser + Phe188Pro
 Ala186Asn + Ser187Glu
 Phe188His + Ser190Glu
 Ala186Glu + Phe188Ser
 Ala186Ser + Phe188Leu
 Phe188Pro + Ser190Glu
 Ser187Asp + Phe188Thr
 Ala186His + Phe188Ala
 Ala186Asn + Phe188Asp
 Phe188Ile + Ser190Asp
 Ala186Thr + Phe188Met
 Ala186Asp + Phe188His
 Ser187Asp + Phe188Ala
 Ala186Pro + Ser190Glu

TABLE 20

Loop 5 - Triple Mutation Variants

Ala186His + Phe188Ala + Ser190Asp
 Ala186Asn + Ser187Asp + Phe188Ala
 Ala186Gly + Phe188Asn + Ser190Asp
 Ala186Pro + Phe188Met + Ser190Asp
 Ala186Gly + Phe188Leu + Ser190Glu
 Ala186Gln + Ser187Asp + Phe188Ile
 Ala186Thr + Ser187Glu + Phe188Cys
 Ala186Ser + Phe188Ala + Ser190Glu
 Ala186Gln + Ser187Asp + Phe188Ser
 Ala186Gln + Ser187Glu + Phe188Ile
 Ala186Ser + Phe188Gly + Ser190Asp
 Ala186Ser + Ser187Asp + Phe188Met
 Ala186Gly + Ser187Glu + Phe188Gly
 Ala186Gln + Phe188Pro + Ser190Asp

Ala186Asn + Phe188Ala + Ser190Glu
 Ala186Ser + Phe188Pro + Ser190Glu
 Ala186Asn + Ser187Glu + Phe188Pro
 Ala186Gln + Phe188Gly + Ser190Glu
 Ala186Asn + Ser187Glu + Phe188Ile
 Ala186His + Ser187Glu + Phe188Gly
 Ala186His + Phe188Cys + Ser190Asp
 Ala186Thr + Ser187Glu + Phe188Val
 Ala186Gln + Ser187Glu + Phe188Met
 Ala186His + Phe188Ala + Ser190Glu
 Ala186Gly + Phe188Met + Ser190Glu
 Ala186Ser + Ser187Glu + Phe188His
 Ala186Asn + Phe188Gly + Ser190Glu
 Ala186Asn + Phe188Pro + Ser190Asp
 Ala186Asn + Phe188Tyr + Ser190Asp
 Ala186Gly + Phe188Thr + Ser190Glu
 Ala186His + Ser187Asp + Phe188Leu
 Ala186Ser + Phe188Pro + Ser190Asp
 Ala186Thr + Ser187Glu + Phe188Pro
 Ala186Pro + Phe188Pro + Ser190Glu
 Ala186Thr + Phe188Asn + Ser190Asp
 Ala186Pro + Ser187Glu + Phe188His
 Ala186Asp + Ser187Glu + Phe188Met
 Ala186Asp + Ser187Glu + Phe188Gln
 Ala186Asp + Ser187Glu + Phe188Cys
 Ala186Glu + Ser187Asp + Phe188Pro
 Ala186Asp + Ser187Glu + Phe188Tyr
 Ala186Glu + Ser187Asp + Phe188Thr
 Ala186Asp + Ser187Asp + Phe188Val
 Ala186Glu + Ser187Glu + Phe188Asn
 Ala186Asp + Ser187Asp + Phe188His
 Ala186Asp + Ser187Glu + Phe188Ala
 Ala186His + Ser187Asp + Phe188Asp
 Ala186Gly + Ser187Asp + Phe188Asp
 Ala186Gln + Ser187Glu + Phe188Glu
 Ala186Asn + Ser187Asp + Phe188Glu
 Ala186Pro + Ser187Glu + Phe188Asp
 Ala186Asp + Ser187Glu + Phe188Asp
 Ala186Glu + Ser187Glu + Phe188Glu
 Ala186Asp + Ser187Asp + Phe188Glu
 Ala186Thr + Phe188Asp + Ser190Glu
 Ala186Ser + Phe188Glu + Ser190Glu
 Ala186Thr + Phe188Glu + Ser190Asp
 Ala186Pro + Phe188Glu + Ser190Glu
 Ser187Glu + Phe188Glu + Ser190Glu
 Ser187Asp + Phe188Asp + Ser190Glu

TABLE 21

 Loop 5 - Quadruple Mutation Variants

Ala186Gly + Ser187Glu + Phe188Asp + Ser190Glu
 Ala186Gly + Ser187Asp + Phe188Asp + Ser190Asp

Ala186Gln + Ser187Asp + Phe188Asp + Ser190Asp
Ala186Gln + Ser187Glu + Phe188Asp + Ser190Glu
Ala186Ser + Ser187Asp + Phe188Glu + Ser190Glu
Ala186Pro + Ser187Glu + Phe188Asp + Ser190Asp
Ala186Thr + Ser187Glu + Phe188Asp + Ser190Asp
Ala186Thr + Ser187Glu + Phe188Asp + Ser190Glu
Ala186His + Ser187Asp + Phe188Glu + Ser190Asp
Ala186Asn + Ser187Glu + Phe188Asp + Ser190Asp
Ala186Gly + Ser187Glu + Phe188Glu + Ser190Asp
Ala186Gln + Ser187Asp + Phe188Glu + Ser190Glu
Ala186Gln + Ser187Asp + Phe188Glu + Ser190Asp
Ala186Gly + Ser187Glu + Phe188Asp + Ser190Asp
Ala186Asp + Ser187Glu + Phe188Gly + Ser190Asp
Ala186Asp + Ser187Asp + Phe188Gln + Ser190Asp
Ala186Glu + Ser187Glu + Phe188Val + Ser190Asp
Ala186Asp + Ser187Glu + Phe188Pro + Ser190Glu
Ala186Glu + Ser187Asp + Phe188Met + Ser190Glu
Ala186Glu + Ser187Asp + Phe188Ala + Ser190Glu
Ala186Asp + Ser187Asp + Phe188His + Ser190Asp
Ala186Glu + Ser187Glu + Phe188Met + Ser190Glu
Ala186Asp + Ser187Asp + Phe188Ala + Ser190Glu
Ala186Glu + Ser187Glu + Phe188Cys + Ser190Asp
Ala186Glu + Ser187Glu + Phe188Thr + Ser190Glu
Ala186Asp + Ser187Asp + Phe188His + Ser190Glu
Ala186Asp + Ser187Asp + Phe188Gln + Ser190Glu
Ala186Asp + Ser187Asp + Phe188Tyr + Ser190Asp
Ala186Glu + Ser187Asp + Phe188Ser + Ser190Asp
Ala186Glu + Ser187Asp + Phe188Gly + Ser190Asp
Ala186Asp + Ser187Asp + Phe188Asn + Ser190Asp
Ala186Glu + Ser187Glu + Phe188Cys + Ser190Glu
Ala186Glu + Ser187Asp + Phe188Ile + Ser190Glu
Ala186Glu + Ser187Glu + Phe188Val + Ser190Glu
Ala186Asp + Ser187Asp + Phe188Cys + Ser190Asp
Ala186Asp + Ser187Glu + Phe188Val + Ser190Glu
Ala186Asp + Ser187Glu + Phe188Ser + Ser190Asp
Ala186Asp + Ser187Asp + Phe188Ala + Ser190Asp
Ala186Asp + Ser187Asp + Phe188Gly + Ser190Glu
Ala186Asp + Ser187Asp + Phe188Thr + Ser190Glu
Ala186Glu + Ser187Glu + Phe188His + Ser190Glu
Ala186Glu + Ser187Asp + Phe188Pro + Ser190Glu
Ala186Glu + Ser187Glu + Phe188Ile + Ser190Glu
Ala186Glu + Ser187Glu + Phe188Pro + Ser190Glu
Ala186Glu + Ser187Glu + Phe188Gly + Ser190Asp
Ala186Glu + Ser187Glu + Phe188Pro + Ser190Asp
Ala186Glu + Ser187Asp + Phe188Ser + Ser190Glu
Ala186Asp + Ser187Asp + Phe188Tyr + Ser190Glu
Ala186Asp + Ser187Glu + Phe188His + Ser190Glu
Ala186Ser + Ser187Glu + Phe188Ser + Ser190Glu
Ala186His + Ser187Glu + Phe188Ser + Ser190Glu
Ala186Ser + Ser187Asp + Phe188Cys + Ser190Glu
Ala186Ser + Ser187Glu + Phe188Asn + Ser190Glu
Ala186Gln + Ser187Glu + Phe188Ala + Ser190Asp
Ala186Gln + Ser187Asp + Phe188Met + Ser190Glu

38

Ala186Asn + Ser187Glu + Phe188His + Ser190Asp
 Ala186Thr + Ser187Glu + Phe188Ala + Ser190Glu
 Ala186Pro + Ser187Asp + Phe188Cys + Ser190Glu
 Ala186Pro + Ser187Asp + Phe188Leu + Ser190Asp
 Ala186Asn + Ser187Asp + Phe188Gln + Ser190Glu

TABLE 22

Loop 6 - Single Mutation Variants

Ala199Asn
 Ala199Asp
 Ala199Gln
 Ala199Glu
 Ala199Gly
 Ala199His
 Ala199Pro
 Ala199Ser
 Ala199Thr
 Pro200Asn
 Pro200Asp
 Pro200Gln
 Pro200Glu
 Pro200Gly
 Pro200Ser
 Gly201Asn
 Gly201Asp
 Gly201Gln
 Gly201Glu
 Gly201Pro
 Gly201Ser
 Val202Ala
 Val202Asn
 Val202Asp
 Val202Cys
 Val202Gln
 Val202Glu
 Val202Gly
 Val202His
 Val202Met
 Val202Pro
 Val202Ser
 Val202Thr
 Ser203Asp
 Ser203Glu
 Val204Ala
 Val204Asn
 Val204Asp
 Val204Cys
 Val204Gln
 Val204Glu
 Val204Gly
 Val204His

39

Val204Met
Val204Pro
Val204Ser
Val204Thr
Tyr205Ala
Tyr205Asn
Tyr205Asp
Tyr205Cys
Tyr205Gln
Tyr205Glu
Tyr205Gly
Tyr205His
Tyr205Ile
Tyr205Leu
Tyr205Met
Tyr205Pro
Tyr205Ser
Tyr205Thr
Tyr205Val
Ser206Asp
Ser206Glu
Thr207Asn
Thr207Asp
Thr207Gln
Thr207Glu
Thr207Gly
Thr207Pro
Thr207Ser
Tyr208Ala
Tyr208Asn
Tyr208Asp
Tyr208Cys
Tyr208Gln
Tyr208Glu
Tyr208Gly
Tyr208His
Tyr208Ile
Tyr208Leu
Tyr208Met
Tyr208Pro
Tyr208Ser
Tyr208Thr
Tyr208Val
Pro209Asn
Pro209Asp
Pro209Gln
Pro209Glu
Pro209Gly
Pro209Ser
Ser210Asp
Ser210Glu
Asn211Asp
Asn211Gln

40

Asn211Glu
Asn211Ser
Thr212Asn
Thr212Asp
Thr212Gln
Thr212Glu
Thr212Gly
Thr212Pro
Thr212Ser
Tyr213Ala
Tyr213Asn
Tyr213Asp
Tyr213Cys
Tyr213Gln
Tyr213Glu
Tyr213Gly
Tyr213His
Tyr213Ile
Tyr213Leu
Tyr213Met
Tyr213Pro
Tyr213Ser
Tyr213Thr
Tyr213Val
Thr214Asn
Thr214Asp
Thr214Gln
Thr214Glu
Thr214Gly
Thr214Pro
Thr214Ser
Ser215Asp
Ser215Glu
Leu216Ala
Leu216Asn
Leu216Asp
Leu216Cys
Leu216Gln
Leu216Glu
Leu216Gly
Leu216His
Leu216Ile
Leu216Met
Leu216Pro
Leu216Ser
Leu216Thr
Leu216Val
Asn217Asp
Asn217Gln
Asn217Glu
Asn217Ser
Gly218Asn
Gly218Asp

41

Gly218Gln
 Gly218Glu
 Gly218Pro
 Gly218Ser
 Thr219Asn
 Thr219Asp
 Thr219Gln
 Thr219Glu
 Thr219Gly
 Thr219Pro
 Thr219Ser

TABLE 23

 Loop 6 - Double Mutation Variants

Val204Ser + Ser210Glu
 Pro200Gly + Thr214Gly
 Thr212Asn + Thr219Gly
 Thr207Ser + Asn211Glu
 Val204Asn + Tyr208Thr
 Ser215Asp + Asn217Gln
 Pro200Gly + Ser215Glu
 Tyr208Gln + Tyr213Glu
 Pro200Gln + Leu216Glu
 Thr207Gln + Ser215Asp
 Tyr205Gly + Thr219Gly
 Tyr208Gly + Asn211Glu
 Val202Cys + Gly218Asn
 Tyr205Ser + Tyr213Leu
 Ala199Asn + Gly218Pro
 Thr207Gln + Thr214Glu
 Asn211Gln + Ser215Asp
 Ala199Gly + Gly218Glu
 Pro200Asn + Thr214Glu
 Val204Thr + Pro209Asp
 Pro209Gly + Tyr213Cys
 Thr214Gln + Asn217Glu
 Pro200Gln + Pro209Glu
 Asn211Asp + Thr219Gly
 Ser203Asp + Val204Gln
 Tyr205Val + Gly218Glu
 Tyr205Gln + Thr219Glu
 Ser203Asp + Leu216Ala
 Ser210Asp + Gly218Pro
 Val202Ala + Val204Ser
 Ala199Pro + Val204Ala
 Tyr205Gln + Tyr213Gln
 Tyr205Leu + Pro209Asp
 Val204Ser + Tyr208Met
 Thr212Asp + Tyr213Met
 Val202Ser + Asn211Asp
 Val202Asp + Tyr213Ser

42

Ser203Asp + Tyr205Ser
Tyr213His + Thr219Glu
Val202Pro + Asn217Glu
Val202Ser + Ser210Asp
Val202Thr + Ser215Asp
Tyr205Ser + Tyr208Pro
Val204Gly + Ser210Glu
Thr212Glu + Thr219Gln
Tyr208Ala + Gly218Glu
Pro200Gly + Tyr213Asp
Thr207Pro + Asn211Asp
Pro209Gly + Tyr213Ala
Val204Met + Ser210Asp
Thr214Pro + Asn217Ser
Val204Gln + Gly218Pro
Tyr205Pro + Thr214Glu
Ala199Gln + Ser215Asp
Ala199Pro + Ser203Asp
Gly201Pro + Thr212Ser
Tyr205Pro + Tyr213His
Val204Gln + Thr207Ser
Thr207Gly + Thr219Asn
Pro200Gln + Thr214Pro
Val202Cys + Ser215Asp
Gly201Pro + Thr214Ser
Val204Ser + Pro209Glu
Ser203Asp + Asn211Gln
Val202Met + Thr212Pro
Tyr205Gln + Thr207Asn
Ala199Gln + Tyr213Met
Gly201Ser + Ser210Asp
Gly201Ser + Thr212Asn
Tyr208His + Pro209Gly
Pro209Gln + Ser215Glu
Tyr208Pro + Leu216Thr
Tyr213Asp + Thr219Gln
Pro209Gln + Thr212Gly
Tyr208Met + Pro209Gly
Pro209Gly + Asn211Asp
Asn211Asp + Tyr213Ser
Val202Gln + Ser210Glu
Ser203Glu + Thr207Gln
Pro200Gln + Val204Thr
Asn211Asp + Thr214Ser
Ser210Asp + Thr212Gln
Val204Gln + Thr219Glu
Val202Gln + Tyr205His
Thr214Glu + Gly218Pro
Pro209Asn + Asn211Ser
Ala199Gln + Asn217Glu
Pro200Gly + Asn211Ser
Gly201Pro + Tyr208Val
Val202Asn + Tyr208Cys

43

Val202Gln + Ser215Asp
Ala199Pro + Thr219Ser
Gly218Pro + Thr219Glu
Ala199Gln + Pro200Ser
Thr207Pro + Asn217Asp
Val204Ser + Asn217Gln
Ser203Asp + Val204Met
Ser203Asp + Thr219Pro
Thr212Pro + Thr219Pro
Tyr205Ser + Ser210Glu
Ala199His + Gly201Gln
Pro209Gly + Ser210Glu
Tyr208Gln + Leu216Asn
Gly201Ser + Tyr205Leu
Val202Met + Thr214Glu
Tyr205Ser + Pro209Asp
Pro209Ser + Thr212Glu
Tyr205Gly + Asn217Asp
Pro200Ser + Leu216Ser
Ala199His + Tyr208His
Asn211Glu + Thr219Asn
Ala199Asn + Ser210Glu
Tyr205Val + Tyr208His
Thr207Ser + Asn211Asp
Gly201Ser + Thr207Ser
Thr207Asn + Tyr208Val
Tyr205Glu + Asn217Gln
Tyr208Pro + Thr212Glu
Val204Thr + Asn211Glu
Tyr205Cys + Asn217Asp
Leu216Glu + Thr219Ser
Tyr208Ala + Thr212Pro
Ala199Ser + Ser203Glu
Val204His + Thr212Ser
Val204Gln + Gly218Ser
Ala199Asn + Ser203Glu
Pro200Gln + Val202Met
Ala199Asn + Ser203Asp
Tyr205Pro + Leu216Asp
Tyr205Gln + Ser210Asp
Ser210Asp + Asn211Gln
Ala199Pro + Asn217Asp
Ala199Gly + Asn211Asp
Tyr205Ile + Leu216Met
Tyr205Gly + Thr214Asn
Pro209Glu + Thr219Ser
Pro200Gln + Ser210Glu
Ala199Gly + Pro200Asn
Tyr205Asp + Tyr213Val
Ser203Asp + Asn217Gln
Val204Met + Thr219Ser
Val202Thr + Gly218Ser
Pro200Gly + Tyr205Cys

44

Thr207Gly + Thr219Asp
 Ser215Glu + Gly218Pro
 Gly201Pro + Tyr205Met
 Tyr205Ser + Asn217Asp
 Val202Ser + Pro209Gln
 Pro200Gly + Gly218Asn
 Tyr208Ser + Pro209Asp
 Pro209Gln + Ser215Asp
 Ala199Thr + Asn217Glu
 Thr214Asn + Leu216Asp
 Ser210Asp + Tyr213Val
 Thr207Asn + Gly218Asp
 Thr212Pro + Thr219Glu
 Tyr213Asn + Leu216Glu
 Gly201Pro + Thr212Asp
 Gly201Gln + Asn211Ser
 Val202Met + Leu216Ala
 Ser215Asp + Leu216Gly
 Gly201Gln + Leu216Cys
 Leu216Gly + Thr219Ser
 Val204Gln + Thr214Gln
 Thr207Ser + Ser215Glu
 Thr207Gly + Asn217Glu
 Pro200Asn + Tyr213Ala
 Thr212Asp + Thr219Pro
 Tyr205Glu + Leu216Ile
 Val202Asp + Tyr208Gly
 Tyr208Cys + Pro209Asp
 Thr207Asn + Tyr208Ile
 Pro200Gly + Ser210Asp
 Asn211Glu + Thr212Pro
 Ala199His + Leu216Ser
 Val204Thr + Asn211Asp
 Gly218Ser + Thr219Gly
 Ser203Glu + Val204Ala
 Val202Thr + Thr219Gly
 Thr212Asp + Asn217Gln

TABLE 24

Loop 6 - Triple Mutation Variants

Thr207Ser + Thr212Asn + Thr219Gly
 Ala199Asn + Thr207Ser + Asn211Glu
 Pro200Gln + Leu216Glu + Gly218Asn
 Tyr205Gly + Thr207Gln + Ser215Asp
 Tyr208Gly + Asn211Glu + Thr219Gly
 Val204Thr + Pro209Asp + Tyr213Cys
 Val204Asn + Thr212Gly + Asn217Glu
 Pro200Ser + Tyr208Ser + Thr214Gly
 Ala199Ser + Gly201Ser + Thr214Ser
 Ala199Gly + Val202Met + Ser210Glu
 Gly201Asn + Tyr205His + Thr214Gln

45

Ala199Thr + Asn211Asp + Gly218Ser
Thr207Gln + Tyr213Gln + Leu216Met
Ala199Gly + Val202Cys + Ser210Asp
Val204Gln + Tyr213Ala + Thr214Pro
Tyr213Leu + Asn217Ser + Thr219Glu
Pro200Gly + Thr214Pro + Asn217Asp
Pro200Gly + Leu216Val + Thr219Gln
Pro200Gly + Gly201Ser + Ser210Asp
Gly201Gln + Val202His + Thr212Glu
Tyr208His + Asn211Ser + Asn217Asp
Thr207Ser + Ser210Asp + Gly218Ser
Thr212Asn + Leu216Asn + Thr219Glu
Val202Pro + Val204Met + Pro209Asp
Val202Asn + Thr212Pro + Thr214Glu
Tyr208Ile + Thr212Asp + Tyr213His
Ser210Asp + Thr212Ser + Leu216Ala
Pro200Gln + Thr214Pro + Leu216His
Tyr208Met + Pro209Asp + Leu216Ile
Val202Met + Leu216Gly + Thr219Gly
Pro200Ser + Tyr205Val + Leu216Cys
Val204Thr + Tyr205Glu + Asn217Gln
Thr212Glu + Leu216Ile + Asn217Ser
Val204Thr + Tyr208Pro + Asn211Glu
Thr207Gly + Leu216Glu + Thr219Ser
Ser203Glu + Tyr208Ala + Thr212Pro
Ala199Ser + Val204His + Thr212Ser
Ala199Asn + Val204Gln + Gly218Ser
Pro200Gln + Thr214Pro + Gly218Asp
Ala199Asn + Val202Met + Ser203Asp
Pro200Gln + Ser210Asp + Asn211Gln
Tyr205Ile + Thr214Asn + Leu216Met
Pro200Gln + Pro209Glu + Thr219Ser
Ala199Gly + Pro200Asn + Ser210Glu
Val204Met + Asn217Gln + Thr219Ser
Val202Thr + Thr214Asp + Gly218Ser
Gly201Pro + Tyr205Met + Gly218Pro
Val202Ser + Tyr205Ser + Asn217Asp
Pro200Gly + Pro209Gln + Gly218Asn
Ala199Thr + Thr214Asn + Asn217Glu
Thr207Asn + Thr212Pro + Gly218Asp
Gly201Pro + Asn211Ser + Thr212Asp
Gly201Gln + Ser215Asp + Leu216Gly
Val204Gln + Leu216Cys + Thr219Ser
Thr207Ser + Thr214Gln + Ser215Glu
Pro200Asn + Thr207Gly + Asn217Glu
Thr212Asp + Tyr213Ala + Thr219Pro
Thr207Asn + Tyr208Ile + Pro209Glu
Ala199His + Asn211Glu + Thr212Pro
Val204Thr + Asn211Asp + Leu216Ser
Ser203Glu + Val204Ala + Thr219Gly
Thr207Gly + Thr212Gly + Leu216His
Gly201Asn + Thr207Gly + Tyr208Gly
Gly201Asn + Val202Glu + Thr207Ser

Thr207Gln + Leu216Glu + Gly218Ser
Tyr205His + Ser210Asp + Thr219Pro
Pro200Gly + Tyr208Val + Ser210Asp
Ala199Ser + Ser203Asp + Tyr208Gln
Gly201Ser + Asn211Glu + Tyr213Pro
Ala199Gln + Tyr205His + Thr219Glu
Pro200Ser + Thr207Pro + Thr214Ser
Pro200Gln + Tyr205Met + Thr212Pro
Ala199Gly + Val204His + Asn211Glu
Thr207Asn + Thr212Ser + Thr219Asn
Tyr205Pro + Thr207Gln + Ser215Glu
Val204His + Gly218Glu + Thr219Gly
Tyr205Pro + Tyr208Ile + Ser215Glu
Ala199Asn + Pro200Ser + Ser203Glu
Tyr208Gln + Tyr213Met + Thr219Glu
Val202Glu + Val204Thr + Asn217Ser
Val204Gln + Pro209Gln + Thr214Gly
Thr214Gln + Ser215Asp + Leu216Gly
Val204Thr + Tyr213Ile + Ser215Glu
Val204Gly + Asn211Gln + Ser215Glu
Pro200Ser + Val204Gly + Tyr213Ile
Pro200Gly + Thr212Asp + Leu216His
Asn211Glu + Tyr213Thr + Leu216Pro
Leu216Asn + Asn217Asp + Gly218Ser
Gly201Gln + Pro209Asn + Ser215Glu
Gly201Asn + Ser203Glu + Pro209Asn
Gly201Asn + Val202Ser + Leu216Gly
Asn211Asp + Tyr213His + Thr219Gln
Thr207Gln + Asn217Asp + Gly218Ser
Tyr208Gly + Ser210Glu + Thr214Gly
Thr207Gln + Tyr208Gln + Tyr213Asp
Ala199Asn + Tyr205Ile + Gly218Pro
Pro200Asn + Val202Gln + Val204Ser
Val204Asn + Ser215Glu + Leu216Pro
Pro200Ser + Gly201Ser + Tyr208Ala
Tyr208Thr + Tyr213Val + Leu216Asp
Thr207Gly + Thr212Ser + Tyr213Ala
Gly201Asn + Gly218Asp + Thr219Asn
Ala199Gly + Tyr205Cys + Tyr208Thr
Gly201Gln + Tyr213Cys + Thr214Gly
Ala199Gln + Pro200Ser + Thr214Asp
Ala199Ser + Gly201Asn + Thr219Pro
Val204Gly + Leu216Val + Thr219Gln
Gly201Asn + Ser203Glu + Thr212Gly
Gly201Ser + Ser203Glu + Asn217Gln
Pro200Gly + Thr207Gln + Asn217Asp
Ala199Thr + Tyr208Gln + Thr219Pro
Thr207Ser + Thr212Ser + Gly218Asp
Pro200Ser + Tyr208Ile + Asn217Ser
Val204Pro + Tyr205Asp + Thr214Pro
Gly201Ser + Tyr205Glu + Thr207Asn
Pro200Ser + Thr207Gln + Asn217Gln
Gly201Asn + Val202Glu + Leu216Cys

Ala199Ser + Pro209Ser + Gly218Asp
Gly201Pro + Tyr205Met + Asn211Asp
Gly201Ser + Thr214Pro + Ser215Asp
Val204Pro + Thr212Gly + Tyr213Val
Gly201Pro + Tyr208Ser + Thr212Glu
Ala199Gln + Ser203Asp + Leu216Ser
Val202Gly + Asn211Glu + Tyr213Leu
Ser203Asp + Thr212Gly + Thr214Gln
Gly201Pro + Tyr205Gln + Thr207Gly
Gly201Asn + Tyr208Asn + Tyr213Pro
Pro200Ser + Tyr213Asp + Gly218Pro
Ala199Asn + Pro200Gly + Thr214Ser
Ser210Glu + Tyr213Gln + Gly218Asn
Ala199Pro + Tyr213Thr + Ser215Asp
Val202Pro + Tyr208Cys + Asn211Gln
Ala199Gln + Ser203Glu + Asn211Ser
Thr207Gln + Pro209Ser + Ser210Glu
Pro200Ser + Val202Asn + Gly218Ser
Tyr205Val + Tyr213Asn + Gly218Glu
Val202Glu + Val204His + Tyr205Gln
Ala199Gly + Thr207Pro + Asn211Glu
Val202Glu + Tyr208Met + Pro209Gly
Val204Ser + Tyr208Ile + Leu216Met
Tyr205Thr + Tyr208Ser + Asn217Ser
Thr207Ser + Pro209Ser + Leu216Asn
Gly201Asn + Pro209Asp + Tyr213Met
Gly201Asn + Pro209Gln + Thr214Asn
Pro200Asn + Val202Gln + Thr207Gln
Ala199Asn + Ser203Glu + Val204Gln
Ser203Asp + Pro209Gly + Leu216Ala
Ala199Thr + Pro200Asn + Ser210Glu
Ala199Thr + Tyr205Leu + Asn217Glu
Gly201Asn + Val202Met + Thr214Asp
Thr212Gln + Thr214Glu + Thr219Ser
Tyr205Glu + Tyr208Gly + Tyr213Leu
Pro200Ser + Asn217Glu + Gly218Gln
Ser210Glu + Thr214Pro + Leu216Asn
Tyr208Asn + Pro209Asn + Tyr213Ser
Ala199Pro + Gly201Pro + Ser210Asp
Gly201Gln + Thr207Gly + Gly218Glu
Pro200Gln + Tyr208Gly + Ser215Asp
Pro200Ser + Thr214Pro + Leu216Glu
Ala199Gln + Asn217Asp + Gly218Pro
Pro200Asn + Val202Asp + Pro209Ser
Thr212Gly + Tyr213Asn + Ser215Asp
Gly201Gln + Tyr205Met + Thr219Glu
Pro200Gly + Tyr213Gly + Thr219Glu
Pro200Gly + Ser203Glu + Thr214Gln
Tyr208Thr + Ser215Glu + Gly218Ser
Ser210Asp + Asn211Ser + Leu216Asn
Asn211Gln + Thr214Glu + Ser215Asp
Pro209Glu + Ser210Asp + Leu216Cys
Tyr208Pro + Asn217Asp + Gly218Asp

48

Tyr213Cys + Ser215Asp + Leu216Asp
 Thr212Asp + Tyr213Asp + Gly218Ser
 Ser210Asp + Asn211Glu + Tyr213Gln
 Val204Cys + Ser210Glu + Asn211Glu
 Tyr205Glu + Tyr208Ala + Ser215Glu
 Val202Glu + Ser203Glu + Asn217Asp
 Pro200Gln + Ser203Glu + Ser215Asp
 Ala199Asn + Ser203Glu + Asn217Glu
 Ser203Asp + Tyr208Cys + Asn217Glu
 Tyr205Asp + Asn211Gln + Thr214Glu

TABLE 25

 Loop 6 - Quadruple Mutation Variants

Gly201Asn + Tyr205Gly + Thr207Gln + Tyr213Ile
Val202Cys + Tyr205Ser + Thr212Pro + Gly218Asn
Ala199Ser + Tyr213His + Thr214Gln + Leu216Asn
Ala199Ser + Pro200Ser + Tyr208Ser + Thr214Ser
Ala199Gly + Gly201Ser + Val202Met + Ser210Glu
Gly201Asn + Tyr205His + Thr207Gln + Thr214Gln
Val202Cys + Thr207Gln + Tyr213Gln + Leu216Met
Val204Gln + Tyr205Asp + Tyr213Ala + Thr214Pro
Pro200Gly + Tyr213Leu + Thr214Pro + Asn217Ser
Pro200Gly + Leu216Val + Asn217Asp + Thr219Gln
Ala199Thr + Val204Ala + Asn217Gln + Gly218Gln
Gly201Gln + Val204Gln + Thr207Gln + Leu216His
Pro200Asn + Val202Pro + Thr207Gly + Ser215Asp
Ala199Ser + Gly201Pro + Ser215Asp + Leu216Ser
Ala199Thr + Tyr205Gly + Ser215Glu + Asn217Ser
Ala199Gly + Thr207Gln + Tyr208Thr + Pro209Gln
Ala199His + Pro200Gly + Gly218Gln + Thr219Gln
Ala199Gly + Thr207Asn + Asn211Ser + Thr212Ser
Pro200Ser + Val202Gln + Tyr208Ile + Thr219Pro
Pro200Gln + Tyr208Met + Pro209Asp + Leu216His
Ser203Asp + Asn211Gln + Leu216Thr + Gly218Gln
Ala199Gly + Gly201Ser + Tyr205Asp + Leu216Gly
Ala199Gln + Gly201Asn + Tyr205Gln + Thr214Asp
Pro200Ser + Gly201Pro + Val204Asn + Tyr208Pro
Gly201Pro + Val202Gly + Ser210Asp + Leu216Met
Pro200Gly + Val202Cys + Ser203Asp + Leu216Pro
Tyr205Gly + Thr207Pro + Asn211Gln + Asn217Ser
Gly201Pro + Thr207Gln + Thr212Ser + Asn217Asp
Pro200Gln + Tyr208Leu + Tyr213Met + Leu216Gln
Tyr205Met + Asn211Gln + Tyr213Met + Thr219Asp
Val202Met + Tyr205Leu + Tyr208Thr + Tyr213Gly
Ser203Asp + Val204Ser + Tyr213Gly + Gly218Pro
Gly201Gln + Val202Met + Ser215Asp + Leu216Ala
Val204Gln + Thr214Gln + Leu216Cys + Thr219Ser
Pro200Asn + Thr212Asp + Tyr213Ala + Thr219Pro
Ala199His + Val204Thr + Asn211Asp + Leu216Ser
Val202Thr + Ser203Glu + Val204Ala + Thr219Asn
Thr207Gly + Tyr208Gly + Thr212Gly + Leu216His

Gly201Asn + Ser203Glu + Thr207Gly + Asn211Gln
Tyr205His + Thr207Gln + Leu216Gly + Gly218Ser
Ala199Gln + Tyr205His + Tyr213Pro + Thr219Glu
Ala199Gly + Tyr205Met + Asn211Glu + Thr212Pro
Gly201Pro + Thr207Asn + Thr212Gln + Thr219Asn
Pro200Gln + Leu216Thr + Asn217Glu + Thr219Pro
Gly201Ser + Tyr205Cys + Thr214Asn + Leu216Thr
Gly201Gln + Val204Pro + Asn211Asp + Gly218Pro
Ser203Asp + Tyr205Val + Thr207Ser + Leu216Cys
Val202His + Pro209Gln + Ser215Asp + Leu216Gly
Pro200Asn + Gly201Gln + Val202Pro + Ser203Glu
Pro200Ser + Gly201Gln + Val202His + Leu216Thr
Pro200Asn + Gly201Asn + Leu216Ala + Gly218Pro
Ala199Pro + Val202Ser + Val204His + Thr214Glu
Gly201Gln + Ser203Glu + Tyr213Ile + Gly218Asn
Ala199His + Ser203Asp + Val204Gly + Asn211Gln
Pro200Gln + Val202Ala + Val204Pro + Thr207Pro
Gly201Asn + Thr212Ser + Tyr213Ala + Gly218Asp
Gly201Gln + Tyr213Cys + Thr214Gly + Gly218Glu
Ala199Gln + Pro200Ser + Gly201Asn + Thr214Asp
Ala199Ser + Val204Gly + Leu216Val + Thr219Pro
Pro200Gly + Val202His + Thr207Gln + Asn217Asp
Ala199Thr + Tyr208Gln + Thr212Ser + Thr219Pro
Pro200Ser + Thr207Ser + Tyr208Ile + Gly218Asp
Val204Pro + Tyr205Asp + Thr214Pro + Asn217Ser
Gly201Pro + Tyr205Met + Asn211Asp + Thr214Pro
Gly201Ser + Tyr213Val + Thr214Pro + Ser215Asp
Gly201Pro + Tyr208Ser + Thr212Gly + Tyr213Ala
Val202Asn + Asn211Glu + Tyr213Leu + Thr219Pro
Gly201Pro + Tyr205Gln + Thr207Gly + Thr212Gly
Pro200Gln + Tyr208Cys + Ser215Glu + Thr219Gly
Gly201Asn + Tyr208Asn + Tyr213Pro + Ser215Glu
Pro200Ser + Gly201Pro + Tyr213Asp + Gly218Pro
Ala199Pro + Pro200Ser + Thr212Asp + Thr219Ser
Pro200Asn + Ser203Asp + Thr207Gln + Thr212Gly
Val204Cys + Pro209Ser + Ser215Asp + Gly218Gln
Val202Gln + Val204Ser + Thr212Asp + Asn217Gln
Ala199Asn + Pro209Gly + Asn211Glu + Thr214Pro
Ala199Asn + Val204Thr + Ser210Asp + Tyr213Leu
Pro200Asn + Val204Met + Thr207Gln + Tyr213Asp
Val204Gln + Pro209Gly + Thr212Gly + Tyr213Cys
Pro209Asn + Tyr213Ile + Thr214Gly + Leu216Met
Pro200Gly + Ser203Asp + Leu216Cys + Asn217Ser
Val204Ser + Tyr208Ile + Asn211Asp + Leu216Met
Gly201Asn + Tyr205Ile + Pro209Gln + Leu216Val
Ser203Glu + Val204Gln + Tyr213Leu + Gly218Asn
Pro200Asn + Ser210Asp + Thr214Ser + Leu216Cys
Ala199Thr + Pro200Asn + Ser210Glu + Leu216Cys
Ala199Thr + Val202Met + Tyr205Leu + Thr214Asp
Gly201Asn + Thr212Gln + Thr214Glu + Thr219Ser
Gly201Pro + Tyr208Asn + Pro209Asn + Tyr213Ser
Ala199Pro + Gly201Gln + Thr207Gly + Ser210Asp
Ala199Gly + Pro200Gln + Tyr208Asn + Leu216Thr

50

Pro200Asn + Val202Asp + Pro209Ser + Gly218Pro
Pro200Gly + Thr207Asn + Leu216Asn + Thr219Asp
Ala199Ser + Pro200Gly + Val202Asp + Thr207Ser
Val204Ala + Thr207Asn + Ser210Glu + Tyr213Met
Pro200Gln + Gly201Ser + Tyr205Thr + Ser215Glu
Ala199His + Gly201Asn + Tyr208Ala + Ser210Asp
Pro200Gln + Val204Met + Tyr205Ala + Pro209Glu
Val202Ala + Tyr205Leu + Thr214Glu + Thr219Gln
Thr207Gln + Pro209Asn + Ser210Glu + Thr214Pro
Gly201Asn + Ser203Asp + Tyr208Val + Asn211Gln
Ala199Ser + Pro200Gly + Leu216Val + Gly218Pro
Gly201Gln + Asn211Gln + Thr212Glu + Leu216Ala
Val202Cys + Thr207Asn + Tyr213Pro + Leu216Asp
Ala199Gly + Val202His + Val204Ala + Ser215Glu
Ala199Gln + Thr212Pro + Tyr213Glu + Leu216Ala
Pro200Gln + Val202Gln + Thr214Gln + Gly218Glu
Gly201Gln + Thr207Pro + Tyr208Met + Thr219Pro
Ser203Asp + Val204Gln + Tyr208Ser + Leu216Pro
Ala199Asn + Ser203Glu + Val204Asn + Tyr208Pro
Pro200Gly + Tyr213Met + Leu216Val + Gly218Asn
Tyr205Ile + Tyr213Ile + Thr214Asn + Thr219Pro
Pro200Gln + Thr207Gly + Ser215Glu + Leu216Met
Ala199Ser + Tyr208Ala + Asn211Gln + Thr219Asp
Gly201Pro + Val204Ser + Tyr205His + Thr219Asp
Val202Met + Thr207Pro + Thr212Gly + Asn217Glu
Pro200Gln + Gly201Pro + Tyr208Thr + Ser215Glu
Val204Thr + Thr212Gly + Thr214Glu + Gly218Ser
Pro200Gln + Tyr208Ala + Tyr213Glu + Asn217Ser
Tyr213Met + Thr214Ser + Ser215Asp + Leu216Pro
Thr207Ser + Pro209Asp + Thr212Gly + Thr219Gln
Thr207Gln + Asn211Gln + Thr214Glu + Ser215Asp
Tyr205Ala + Tyr213Met + Thr214Glu + Ser215Asp
Tyr205Met + Thr214Asp + Ser215Glu + Asn217Gln
Thr207Asn + Thr214Gly + Leu216Glu + Asn217Asp
Val202Thr + Val204His + Asn211Glu + Thr212Asp
Pro200Gln + Asn211Asp + Thr212Glu + Thr214Ser
Asn211Glu + Thr212Glu + Tyr213Ile + Thr214Asn
Gly201Asn + Val202Glu + Ser203Glu + Thr212Gly
Thr212Asp + Tyr213Asp + Gly218Ser + Thr219Gly
Val204Ala + Thr212Glu + Tyr213Glu + Thr214Asn
Pro200Gly + Ser210Asp + Asn211Glu + Thr212Pro
Pro209Gln + Ser210Asp + Asn211Glu + Thr219Pro
Pro200Ser + Val204Met + Ser210Asp + Asn211Glu
Ala199Pro + Val202Ala + Ser210Glu + Asn211Glu
Pro209Asp + Ser210Asp + Asn211Glu + Thr212Pro
Val202Asp + Ser203Asp + Tyr213Gln + Asn217Glu
Ser203Asp + Ser215Asp + Leu216Glu + Thr219Asn
Pro200Ser + Ser203Glu + Leu216Glu + Asn217Glu
Ser203Asp + Pro209Gly + Ser215Glu + Leu216Ala
Ser203Glu + Thr214Gln + Ser215Glu + Gly218Ser
Gly201Asn + Ser203Asp + Tyr213Ala + Ser215Glu
Ser203Glu + Thr207Ser + Tyr213Gly + Ser215Asp
Ala199Gln + Ser203Glu + Thr214Pro + Asn217Asp

51

Ser203Glu + Val204Asn + Thr212Gly + Asn217Glu
 Ser203Asp + Thr207Gln + Tyr208Cys + Asn217Glu
 Gly201Ser + Ser203Glu + Thr214Pro + Asn217Asp
 Asn211Glu + Thr212Asp + Tyr213Glu + Thr219Gly
 Ala199Pro + Ser203Asp + Val204Pro + Leu216Glu
 Ala199Asn + Pro209Asp + Asn211Glu + Tyr213Ile
 Pro200Gln + Tyr205Leu + Pro209Glu + Thr212Asp
 Ser203Asp + Tyr205Glu + Tyr208Gly + Tyr213Leu
 Ala199Ser + Val202Glu + Leu216Cys + Gly218Asp
 Pro200Ser + Thr212Pro + Thr214Glu + Leu216Glu
 Ala199Asn + Val202Pro + Ser215Glu + Asn217Asp
 Pro200Gly + Thr207Ser + Ser215Glu + Asn217Glu
 Gly201Asn + Val202Glu + Thr207Ser + Leu216Glu
 Ala199Asn + Ser203Glu + Tyr205Glu + Asn217Glu
 Tyr205Glu + Thr214Pro + Ser215Asp + Asn217Glu
 Gly201Gln + Thr212Ser + Tyr213Glu + Ser215Glu
 Ala199Ser + Tyr213Asp + Thr214Pro + Ser215Glu
 Asn211Ser + Ser215Asp + Asn217Glu + Gly218Asp
 Ser203Asp + Thr212Gln + Ser215Asp + Gly218Asp
 Val202Glu + Thr212Gly + Ser215Asp + Leu216Gln
 Val202Asp + Val204Cys + Tyr208Pro + Thr219Glu
 Ser203Asp + Pro209Gln + Tyr213Thr + Thr214Asp
 Val202Asp + Tyr205Glu + Tyr208Gly + Leu216Ile
 Tyr208Gly + Thr214Asp + Asn217Glu + Gly218Asp
 Pro200Gln + Tyr208Gln + Tyr213Glu + Leu216Glu
 Gly201Gln + Tyr205Leu + Thr214Glu + Asn217Asp
 Ser203Asp + Val204Gly + Thr212Glu + Ser215Asp
 Gly201Gln + Val202Pro + Asn211Glu + Thr214Glu
 Thr207Gln + Pro209Ser + Asn211Asp + Thr214Glu
 Tyr205Ala + Ser210Glu + Thr212Asp + Ser215Asp
 Ala199Gln + Thr214Glu + Leu216Glu + Thr219Glu
 Thr212Asp + Thr214Glu + Leu216Asn + Asn217Glu
 Gly201Asn + Asn211Glu + Thr214Asp + Leu216Glu
 Pro209Asp + Ser215Asp + Leu216Asp + Gly218Gln
 Gly201Pro + Val202Cys + Ser203Asp + Thr219Asp
 Ser203Glu + Thr212Gln + Gly218Gln + Thr219Glu

TABLE 26

 Loop 6 - Quintuple Substitution Variants

Gly201Asn + Thr207Gln + Ser210Glu + Tyr213Ile +
 Leu216Ile
 Val202Cys + Tyr205Ser + Thr212Pro + Tyr213Leu +
 Gly218Asn
 Ala199Ser + Pro200Ser + Tyr213His + Thr214Gln +
 Leu216Asn
 Ala199Ser + Gly201Ser + Tyr208Ser + Ser210Glu +
 Thr214Ser
 Ala199Gly + Gly201Asn + Val202Met + Tyr205His +
 Thr214Gln
 Pro200Gly + Tyr213Leu + Thr214Pro + Asn217Ser +
 Thr219Glu

Ala199Thr	+	Tyr205Gln	+	Thr207Gln	+	Thr212Asp	+	Gly218Gln
Val204Gly	+	Tyr205Ala	+	Tyr208Gly	+	Asn211Gln	+	Gly218Asp
Ala199Ser	+	Gly201Pro	+	Tyr205Gly	+	Ser215Glu	+	Leu216Ser
Ala199His	+	Thr207Gln	+	Tyr208Thr	+	Gly218Gln	+	Thr219Gln
Ala199Gly	+	Pro209Gly	+	Asn211Ser	+	Thr212Ser	+	Tyr213Ser
Pro200Ser	+	Val202Gln	+	Thr207Asn	+	Tyr208Ile	+	Thr219Pro
Pro200Ser	+	Gly201Ser	+	Tyr208Gln	+	Ser210Glu	+	Asn217Gln
Ala199Gly	+	Gly201Ser	+	Val202Gly	+	Tyr205Asp	+	Leu216Gly
Ala199Gln	+	Pro200Ser	+	Gly201Ser	+	Tyr205Gln	+	Thr214Asp
Ala199Pro	+	Gly201Pro	+	Val204Asn	+	Tyr208Pro	+	Thr214Gly
Pro200Gly	+	Val202Gly	+	Ser203Asp	+	Asn211Ser	+	Leu216Pro
Tyr205Gly	+	Thr207Pro	+	Pro209Asp	+	Asn211Gln	+	Asn217Ser
Pro200Gln	+	Val204Ala	+	Tyr208Leu	+	Thr212Glu	+	Tyr213Met
Tyr205Met	+	Asn211Gln	+	Tyr213Met	+	Thr214Asn	+	Thr219Asp
Ser203Asp	+	Val204Ser	+	Tyr213Gly	+	Thr214Asn	+	Gly218Pro
Gly201Gln	+	Val204Gln	+	Ser215Asp	+	Leu216Ala	+	Thr219Ser
Gly201Asn	+	Thr207Gly	+	Tyr208Gly	+	Thr212Gly	+	Leu216His
Gly201Asn	+	Tyr205His	+	Thr207Ser	+	Leu216Glu	+	Gly218Ser
Ala199Gln	+	Pro200Ser	+	Tyr205His	+	Thr214Ser	+	Thr219Glu
Pro200Gln	+	Gly201Gln	+	Leu216His	+	Asn217Asp	+	Gly218Gln
Ala199Gly	+	Val204His	+	Tyr205Met	+	Asn211Glu	+	Thr212Pro
Val204His	+	Tyr205Pro	+	Tyr208Ile	+	Gly218Glu	+	Thr219Gly
Pro200Ser	+	Val202Gln	+	Val204Gly	+	Tyr213Ile	+	Ser215Glu
Ala199Ser	+	Pro200Asn	+	Asn211Glu	+	Tyr213Thr	+	Leu216Pro
Ser203Asp	+	Val204Gly	+	Asn211Gln	+	Tyr213Ile	+	Gly218Asn
Ala199Asn	+	Pro200Gln	+	Val202Ala	+	Val204Pro	+	Thr207Pro
Ala199Thr	+	Gly201Gln	+	Val202Gly	+	Ser215Asp	+	

Leu216Ala
 Thr212Gln + Tyr213Val + Leu216His + Gly218Gln +
 Thr219Glu
 Ala199Gln + Pro200Ser + Gly201Asn + Tyr205Ala +
 Thr214Gly
 Gly201Asn + Val204Gly + Thr212Gly + Leu216Val +
 Thr219Pro
 Pro200Gly + Val202His + Thr207Gln + Tyr208Gln +
 Asn217Asp
 Ala199Thr + Thr207Ser + Thr212Ser + Gly218Asp +
 Thr219Pro
 Pro200Ser + Val204Pro + Tyr208Ile + Thr214Pro +
 Asn217Ser
 Pro200Gln + Val202Ser + Tyr205Ser + Asn211Ser +
 Ser215Asp
 Val202Thr + Thr212Gln + Ser215Asp + Asn217Ser +
 Thr219Asn
 Val204Pro + Thr212Gly + Tyr213Val + Thr214Pro +
 Ser215Asp
 Gly201Ser + Val204His + Tyr208Leu + Thr212Ser +
 Gly218Glu
 Ser203Asp + Tyr205Gln + Thr207Gly + Thr212Gly +
 Thr214Gln
 Pro200Gln + Tyr208Cys + Thr212Gly + Ser215Glu +
 Thr219Gly
 Pro200Ser + Gly201Pro + Val202Ala + Tyr213Asp +
 Gly218Pro
 Val204Gly + Ser210Asp + Thr212Asn + Thr214Pro +
 Thr219Ser
 Pro200Gly + Ser203Asp + Pro209Asn + Leu216Cys +
 Asn217Ser
 Tyr205Thr + Thr207Ser + Tyr208Ser + Pro209Ser +
 Asn217Ser
 Gly201Asn + Tyr205Ile + Pro209Gln + Thr214Asn +
 Leu216Val
 Ala199Asn + Ser203Glu + Val204Gln + Tyr213Leu +
 Gly218Asn
 Ala199Thr + Val202Met + Tyr205Leu + Asn211Ser +
 Asn217Glu
 Ala199Pro + Gly201Pro + Pro209Asn + Ser210Asp +
 Tyr213Ser
 Ala199Ser + Gly201Pro + Pro209Gly + Tyr213Pro +
 Gly218Pro
 Pro200Ser + Pro209Gly + Thr212Pro + Thr214Pro +
 Leu216Glu
 Gly201Ser + Thr207Gly + Ser210Asp + Asn211Ser +
 Leu216Ile
 Ala199Ser + Gly201Gln + Val204Asn + Tyr205Leu +
 Thr219Asp
 Thr207Pro + Tyr208Thr + Ser210Glu + Thr212Gly +
 Asn217Ser
 Gly201Gln + Tyr205Asn + Thr207Asn + Pro209Asp +
 Thr212Gly

Pro200Asn + Tyr205Glu + Tyr208Met + Tyr213Val + Leu216Ile	
Tyr205Ala + Tyr213His + Thr214Ser + Asn217Gln + Thr219Gln	
Ala199Gln + Pro200Gly + Val202Met + Leu216Met + Gly218Pro	
Pro200Ser + Val204Ala + Ser210Glu + Asn211Gln + Gly218Ser	
Ala199Ser + Pro200Ser + Val202Asn + Leu216Val + Gly218Pro	
Pro200Gln + Val202Gln + Tyr213Leu + Thr214Pro + Gly218Glu	
Pro200Ser + Gly201Gln + Thr207Pro + Gly218Pro + Thr219Pro	
Ala199Asn + Ser203Asp + Val204Asn + Tyr208Pro + Leu216Pro	
Tyr205Ile + Tyr213Ile + Thr214Asn + Ser215Glu + Thr219Pro	
Gly201Ser + Val204His + Asn211Gln + Thr212Gln + Leu216Met	
Ala199Ser + Gly201Pro + Asn211Glu + Gly218Gln + Thr219Ser	
Ala199Ser + Thr207Ser + Thr214Gln + Ser215Glu + Leu216Asn	
Ala199His + Val204Cys + Asn211Ser + Thr214Gly + Thr219Glu	
Ala199Asn + Val202His + Tyr208Asn + Leu216Thr + Thr219Gly	
Ser203Glu + Thr207Pro + Tyr208Leu + Thr212Ser + Asn217Gln	
Pro200Ser + Val202Asp + Val204Asn + Tyr208Met + Thr212Gln	
Gly201Ser + Thr207Asn + Tyr208Leu + Ser210Asp + Asn217Ser	
Val202Asp + Val204Gln + Tyr208Cys + Pro209Gln + Tyr213Val	
Ala199Gly + Val202Thr + Thr207Gln + Thr212Glu + Thr214Gln	
Gly201Pro + Val202Cys + Ser210Asp + Thr212Pro + Tyr213Cys	
Ala199Asn + Gly201Ser + Val204Met + Pro209Asn + Thr214Pro	
Gly201Asn + Ser203Asp + Val204Thr + Thr207Ser + Leu216Ala	
Val202Thr + Tyr205Gly + Thr207Gln + Asn211Asp + Leu216Ala	
Ala199Gln + Val204Gly + Thr207Pro + Asn211Asp + Thr214Gly	
Gly201Pro + Val202Asn + Tyr205Ser + Asn211Glu + Gly218Asn	
Val204Gly + Pro209Asn + Asn211Asp + Asn217Gln + Thr219Gly	
Val202Gly + Val204Gly + Ser210Glu + Gly218Ser +	

55

Thr219Ser
 Ala199Ser + Asn211Ser + Tyr213Met + Thr214Asp +
 Thr219Pro
 Ala199Asn + Gly201Asn + Ser203Glu + Tyr213His +
 Leu216Ile
 Ala199Gln + Pro200Asn + Thr207Ser + Pro209Ser +
 Asn217Ser
 Tyr205Met + Thr214Asp + Ser215Glu + Leu216Cys +
 Asn217Gln
 Ala199Pro + Val202His + Tyr208Met + Thr214Glu +
 Ser215Asp
 Pro200Asn + Pro209Glu + Ser210Asp + Thr214Ser +
 Leu216Cys
 Val204Ser + Tyr208Pro + Leu216Met + Asn217Asp +
 Gly218Asp
 Tyr205Pro + Thr207Pro + Leu216Asn + Asn217Asp +
 Gly218Asp
 Pro200Gly + Tyr205Asn + Tyr208Gly + Asn217Glu +
 Gly218Glu
 Val202Thr + Val204His + Pro209Gly + Asn211Glu +
 Thr212Asp
 Gly201Gln + Val202Asn + Thr207Asn + Ser215Glu +
 Leu216Asp
 Ala199His + Pro200Gly + Ser210Asp + Asn211Glu +
 Thr212Pro
 Pro209Gln + Ser210Asp + Asn211Glu + Asn217Gln +
 Thr219Pro
 Ala199Pro + Val202Ala + Ser210Glu + Asn211Asp +
 Gly218Asn
 Ala199Asn + Thr207Asn + Ser210Asp + Asn211Asp +
 Thr212Gly
 Val204Ala + Tyr205Glu + Thr212Ser + Ser215Glu +
 Asn217Gln
 Gly201Pro + Val202Asp + Thr207Gln + Thr212Ser +
 Asn217Asp
 Thr207Ser + Pro209Asp + Ser210Glu + Asn211Asp +
 Tyr213Asn
 Gly201Ser + Val202Glu + Ser203Glu + Thr214Pro +
 Asn217Asp
 Ser203Glu + Thr207Asn + Thr214Gly + Leu216Glu +
 Asn217Asp
 Gly201Ser + Ser203Asp + Tyr208Ile + Leu216Asp +
 Asn217Asp
 Gly201Pro + Tyr208Gly + Leu216Asp + Asn217Glu +
 Gly218Asp
 Ser203Glu + Val204Pro + Thr207Gly + Tyr213Leu +
 Ser215Asp
 Gly201Asn + Ser203Asp + Tyr208Val + Asn211Gln +
 Ser215Asp
 Ala199Asn + Pro200Ser + Ser203Glu + Tyr213Met +
 Ser215Glu
 Ala199Ser + Ser203Glu + Asn211Gln + Ser215Glu +
 Leu216Cys

56

Ser203Glu + Val204Gln + Thr214Gln + Ser215Asp +
Leu216Gly
Ala199Pro + Gly201Pro + Ser203Asp + Tyr205Val +
Ser215Glu
Ala199Thr + Ser203Asp + Val204Gln + Tyr213Gly +
Ser215Asp
Val202Cys + Ser203Asp + Tyr205Met + Tyr213Gln +
Asn217Glu
Ser203Glu + Val204Asn + Thr212Gly + Asn217Glu +
Thr219Gln
Gly201Asn + Ser203Asp + Tyr208Val + Pro209Ser +
Asn217Glu
Ser203Asp + Thr212Pro + Tyr213Gly + Asn217Glu +
Gly218Ser
Val204Thr + Asn211Asp + Thr212Asp + Tyr213Asp +
Leu216Ser
Val202Ala + Ser203Asp + Val204Gln + Tyr205Met +
Leu216Glu
Ser203Glu + Val204Ser + Thr207Gly + Tyr208Val +
Leu216Asp
Pro200Gln + Pro209Gln + Ser210Glu + Thr212Glu +
Thr214Gly
Pro209Glu + Asn211Asp + Asn217Gln + Gly218Gln +
Thr219Pro
Pro200Ser + Tyr205Pro + Pro209Asp + Asn211Glu +
Tyr213Ile
Ala199Asn + Ser203Asp + Tyr205Glu + Thr207Asn +
Leu216Glu
Ser203Glu + Tyr205Glu + Tyr213Met + Leu216Ile +
Asn217Gln
Val202Glu + Ser203Glu + Asn211Gln + Leu216Met +
Gly218Glu
Ala199Ser + Val202Ser + Tyr205Asp + Tyr208Gly +
Tyr213Asp
Pro200Ser + Thr207Pro + Thr212Asp + Thr214Asp +
Thr219Ser
Pro200Gly + Thr207Ser + Thr214Gln + Ser215Glu +
Asn217Glu
Pro200Gln + Thr207Gly + Ser215Asp + Leu216Gln +
Asn217Asp
Ala199Asn + Tyr205Glu + Tyr208Ala + Ser215Glu +
Asn217Glu
Gly201Pro + Val204Ser + Tyr205His + Asn217Glu +
Thr219Asp
Ala199Ser + Tyr208Gln + Tyr213Asp + Thr214Pro +
Ser215Glu
Thr207Asn + Tyr208Gln + Thr212Ser + Tyr213Glu +
Ser215Glu
Gly201Gln + Tyr205Gly + Pro209Glu + Thr212Pro +
Tyr213Glu
Ser203Asp + Pro209Ser + Thr212Gln + Ser215Asp +
Gly218Asp
Ser203Asp + Pro209Gly + Ser215Glu + Leu216Ala +

57

Gly218Asp
Pro200Ser + Ser203Asp + Thr207Gln + Leu216Gln +
Gly218Glu
Pro200Asn + Gly201Pro + Ser203Glu + Thr207Gly +
Gly218Glu
Val202Asp + Val204Cys + Tyr208Ala + Gly218Gln +
Thr219Glu
Val202Glu + Val204Thr + Tyr208Gln + Asn217Ser +
Thr219Glu
Pro200Ser + Gly201Pro + Val202Asp + Asn211Gln +
Thr219Glu
Gly201Asn + Ser203Glu + Thr212Gln + Thr214Asp +
Thr219Ser
Gly201Asn + Ser203Asp + Thr214Glu + Leu216Cys +
Thr219Gly
Gly201Asn + Val202Cys + Ser203Asp + Thr214Asp +
Thr219Pro
Val202Cys + Ser203Asp + Tyr208Thr + Thr214Asp +
Thr219Pro
Val204Ser + Tyr208Leu + Ser210Asp + Tyr213Asp +
Thr214Asp
Ala199His + Ser203Asp + Tyr208Ser + Tyr213Glu +
Thr214Asp
Val204Cys + Ser210Asp + Asn211Ser + Tyr213Asp +
Thr219Gln
Ala199Asn + Ser210Glu + Thr212Asn + Tyr213Glu +
Thr214Gln
Ser203Asp + Tyr205Glu + Tyr208Gly + Tyr213Leu +
Gly218Asp
Val202Glu + Tyr205Glu + Pro209Asn + Leu216Thr +
Gly218Glu
Tyr208Val + Ser215Asp + Asn217Ser + Gly218Asp +
Thr219Glu
Tyr205Leu + Thr214Asp + Ser215Glu + Gly218Glu +
Thr219Asn
Ala199Pro + Pro209Asp + Thr212Asp + Ser215Asp +
Gly218Ser
Ser203Glu + Thr212Glu + Tyr213Ser + Thr214Glu +
Leu216Cys
Ser203Asp + Tyr208Cys + Thr212Asp + Tyr213Ile +
Thr214Asp
Val202Met + Val204Thr + Asn211Asp + Thr214Asp +
Ser215Asp
Pro209Asn + Asn211Asp + Thr214Glu + Ser215Glu +
Leu216Gly
Pro200Asn + Tyr205Glu + Thr212Asp + Tyr213Ala +
Thr219Pro
Pro200Asn + Ser203Asp + Tyr205His + Thr212Asp +
Ser215Asp
Ser203Asp + Val204Thr + Thr212Glu + Tyr213Ile +
Ser215Glu
Tyr205Gly + Thr207Gln + Tyr208Ile + Thr212Glu +
Ser215Glu

58

Ala199Asn + Tyr205Asp + Ser210Glu + Thr212Glu +
 Thr214Gly
 Val202Met + Tyr205Ala + Ser210Glu + Thr212Asp +
 Ser215Asp
 Thr207Gln + Tyr208Leu + Asn211Glu + Tyr213Glu +
 Leu216Glu
 Val204Met + Pro209Ser + Asn211Asp + Thr214Glu +
 Leu216Asp
 Pro200Ser + Ser203Glu + Tyr213Glu + Leu216Gly +
 Asn217Glu
 Ala199Gly + Tyr213Pro + Thr214Asp + Asn217Asp +
 Thr219Asp
 Ala199Gln + Tyr208Ala + Thr214Glu + Asn217Glu +
 Thr219Asp
 Ala199His + Val202His + Ser203Glu + Thr207Gly +
 Thr219Asp
 Gly201Ser + Val202Gly + Ser203Glu + Tyr205Thr +
 Thr219Asp
 Gly201Pro + Pro209Glu + Ser210Asp + Ser215Glu +
 Leu216Met
 Tyr205Glu + Ser215Glu + Leu216Cys + Gly218Gln +
 Thr219Glu
 Ser203Asp + Pro209Ser + Asn211Glu + Thr214Asp +
 Leu216Asn
 Ala199Thr + Ser203Glu + Thr207Gln + Asn211Asp +
 Thr214Glu
 Gly201Ser + Ser203Asp + Tyr205Gly + Pro209Asp +
 Ser215Asp
 Pro209Glu + Thr214Asn + Leu216Glu + Asn217Asp +
 Thr219Asn

TABLE 27

Loop 6 - Sextuple Substitution Variants

Gly201Asn + Tyr205Gly + Thr207Gln + Ser210Glu +
 Tyr213Ile + Thr219Gly
 Pro200Asn + Gly201Ser + Tyr208Gly + Asn211Asp +
 Thr212Ser + Thr219Asn
 Gly201Pro + Ser203Asp + Val204Gln + Tyr205Pro +
 Thr212Ser + Tyr213His
 Pro200Gln + Val204His + Thr207Ser + Thr214Pro +
 Ser215Asp + Thr219Asn
 Ala199Pro + Pro200Ser + Gly201Pro + Val204Asn +
 Tyr208Pro + Thr214Gly
 Pro200Gln + Val204Ala + Tyr208Leu + Thr212Glu +
 Tyr213Met + Leu216Gln
 Ala199Pro + Val202Met + Ser203Glu + Tyr205Leu +
 Asn211Gln + Tyr213Gly
 Gly201Gln + Val204Gln + Thr214Gln + Ser215Asp +
 Leu216Gly + Thr219Ser
 Val204Thr + Tyr208Met + Pro209Glu + Tyr213Gly +
 Thr214Pro + Gly218Ser

Ser203Asp + Val204Met + Thr207Gln + Pro209Gln +
Asn217Ser + Thr219Asn
Pro200Ser + Val202Met + Val204Gly + Pro209Gln +
Tyr213Ile + Ser215Glu
Ala199His + Ser203Asp + Val204Gly + Thr207Gly +
Asn211Gln + Thr214Ser
Ala199Gln + Pro200Gln + Gly201Gln + Pro209Glu +
Leu216Ile + Asn217Ser
Pro200Gln + Tyr208Ile + Pro209Gln + Thr212Gly +
Ser215Glu + Thr219Gly
Pro200Asn + Ser203Asp + Val204Cys + Thr207Gln +
Pro209Ser + Thr214Asn
Ala199Asn + Val202Gln + Val204Ser + Pro209Gly +
Thr212Asp + Asn217Gln
Pro200Asn + Val204Met + Thr207Gln + Pro209Gly +
Thr212Gly + Tyr213Asp
Pro200Gly + Val204Gln + Pro209Asn + Tyr213Ile +
Thr214Gly + Leu216Met
Ala199Asn + Tyr205Thr + Tyr208Ser + Pro209Ser +
Thr214Asp + Asn217Ser
Tyr205Ser + Thr207Gln + Tyr208Cys + Pro209Asn +
Thr212Asn + Leu216Glu
Ala199Asn + Pro200Ser + Ser203Asp + Tyr205Cys +
Tyr208Asn + Thr214Gln
Thr207Pro + Tyr213Ser + Thr214Asn + Asn217Ser +
Gly218Ser + Thr219Glu
Ala199Pro + Gly201Pro + Thr207Gly + Pro209Asn +
Ser210Asp + Tyr213Ser
Ala199Gln + Pro200Gly + Val202Met + Thr212Asp +
Leu216Met + Gly218Pro
Ala199Ser + Pro200Gly + Val202Asn + Asn211Gln +
Leu216Val + Gly218Pro
Ala199His + Pro200Gln + Val202Gln + Thr214Gln +
Leu216Thr + Gly218Glu
Pro200Ser + Gly201Gln + Thr207Pro + Tyr208Thr +
Gly218Pro + Thr219Pro
Pro200Gln + Val204Met + Thr207Gly + Ser215Glu +
Leu216Met + Thr219Pro
Thr207Ser + Pro209Asp + Thr212Gly + Thr214Ser +
Leu216Pro + Thr219Gln
Pro200Asn + Gly201Ser + Thr207Asn + Tyr208Leu +
Ser210Asp + Asn217Ser
Ala199Asn + Val202Ser + Tyr205Cys + Thr207Gln +
Tyr213Leu + Asn217Ser
Val202Ser + Pro209Gln + Tyr213Asp + Leu216Met +
Asn217Ser + Thr219Gly
Gly201Gln + Val204Gly + Pro209Asn + Asn211Asp +
Asn217Gln + Thr219Gly
Val202Gly + Ser203Glu + Val204Gly + Tyr213Met +
Gly218Ser + Thr219Ser
Ala199Gln + Pro200Asn + Tyr205Thr + Thr207Ser +
Thr214Gln + Asn217Ser
Ala199Ser + Gly201Asn + Val202Met + Val204Asn +

Asn211Gln + Gly218Glu
Pro200Asn + Val202Pro + Val204Gln + Tyr208Pro +
Gly218Gln + Thr219Glu
Thr207Gly + Tyr208Ile + Pro209Gly + Tyr213Gly +
Thr214Pro + Leu216Asn
Val202Met + Ser203Asp + Val204Met + Thr212Asn +
Thr214Asn + Leu216Gly
Ala199Ser + Val202Met + Val204Ala + Thr212Gly +
Tyr213Thr + Thr214Pro
Tyr208Val + Pro209Gln + Thr214Ser + Leu216Cys +
Asn217Glu + Thr219Asn
Pro200Gln + Gly201Gln + Tyr205Ala + Pro209Gln +
Leu216Pro + Thr219Glu
Ala199Gly + Pro200Gln + Gly201Ser + Tyr208Gln +
Ser215Asp + Gly218Asn
Ala199Asn + Pro200Asn + Val202Asn + Val204Gln +
Thr214Gly + Asn217Glu
Pro200Gly + Gly201Gln + Tyr213Gly + Thr214Asn +
Leu216Gln + Thr219Ser
Gly201Gln + Val202Gly + Thr207Gly + Asn211Gln +
Thr214Gly + Ser215Asp
Ala199Thr + Pro200Ser + Tyr208Ala + Thr212Ser +
Ser215Asp + Asn217Ser
Ala199His + Val202Asn + Ser203Glu + Val204His +
Tyr213Ile + Asn217Gln
Gly201Asn + Val202Cys + Tyr205Cys + Tyr213Ile +
Leu216Pro + Asn217Asp
Ala199Thr + Gly201Ser + Val202Gly + Tyr205Pro +
Leu216Thr + Gly218Glu
Ala199Ser + Val204Cys + Tyr208Cys + Pro209Gly +
Tyr213Ala + Leu216Asp
Ala199Gly + Gly201Asn + Val204Met + Thr207Pro +
Thr214Glu + Leu216Met
Pro200Gly + Tyr205Met + Tyr208Val + Thr212Gly +
Tyr213Ser + Leu216Val
Gly201Pro + Tyr208Val + Pro209Glu + Asn211Gln +
Tyr213Ala + Thr219Gly
Ala199Ser + Pro200Ser + Val202Thr + Tyr205Gln +
Gly218Asp + Thr219Pro
Ala199Gln + Ser203Glu + Val204Pro + Thr214Ser +
Gly218Gln + Thr219Pro
Ala199Thr + Pro200Gly + Gly201Gln + Thr212Ser +
Leu216Val + Gly218Ser
Pro200Ser + Val204Gln + Thr207Pro + Thr214Gly +
Ser215Glu + Gly218Gln
Ala199Gly + Gly201Gln + Val204His + Tyr205Asp +
Pro209Gln + Thr212Ser
Pro200Gln + Tyr208Asn + Asn211Ser + Thr212Ser +
Tyr213Thr + Leu216Asp
Ala199His + Tyr208Leu + Thr212Pro + Ser215Glu +
Leu216Ser + Gly218Gln
Gly201Pro + Val204Cys + Pro209Gln + Thr212Ser +
Tyr213Cys + Thr214Gln

Val204Ser + Tyr205Ser + Thr207Asn + Tyr208Cys +
Ser215Asp + Asn217Ser
Pro200Asn + Tyr208Ala + Tyr213Pro + Thr214Glu +
Ser215Asp + Thr219Gly
Val202His + Tyr208Met + Pro209Asn + Thr212Asn +
Thr214Glu + Ser215Asp
Pro200Gln + Gly201Ser + Val202Pro + Val204Ser +
Thr214Asp + Ser215Glu
Ala199Gln + Gly201Ser + Val204Gly + Thr207Ser +
Thr214Asp + Ser215Glu
Val202Asn + Tyr205Gly + Pro209Gln + Thr212Ser +
Thr214Glu + Ser215Glu
Pro200Asn + Pro209Glu + Ser210Asp + Asn211Ser +
Thr214Ser + Leu216Cys
Pro200Ser + Val204Pro + Tyr208Ile + Thr214Pro +
Asn217Asp + Gly218Asp
Gly201Gln + Tyr205Asn + Tyr208Gly + Thr212Gly +
Asn217Glu + Gly218Glu
Val202His + Thr207Asn + Tyr208Gln + Tyr213Pro +
Ser215Glu + Leu216Asp
Ala199Asn + Gly201Ser + Val202Pro + Tyr205Thr +
Ser215Asp + Leu216Asp
Pro200Gly + Val202Ala + Val204Ala + Ser210Glu +
Asn211Glu + Gly218Ser
Val204Asn + Pro209Ser + Ser210Asp + Asn211Asp +
Tyr213Asn + Leu216Met
Ala199Asn + Val204Thr + Ser210Asp + Asn211Glu +
Tyr213Leu + Thr214Pro
Val202Cys + Val204Ala + Tyr205Glu + Thr212Ser +
Ser215Glu + Asn217Gln
Thr207Pro + Ser210Asp + Asn211Glu + Thr212Glu +
Asn217Ser + Thr219Ser
Pro200Asn + Val202Asp + Tyr205Ala + Pro209Ser +
Asn217Asp + Gly218Pro
Val202Gln + Pro209Glu + Ser210Glu + Asn211Glu +
Thr212Gln + Asn217Gln
Val202Asp + Ser203Asp + Thr207Gln + Thr212Ser +
Tyr213Gln + Asn217Glu
Ala199Asn + Ser203Glu + Val204Asn + Tyr208Pro +
Leu216Glu + Asn217Asp
Val204Thr + Thr212Gly + Thr214Glu + Ser215Asp +
Leu216Glu + Gly218Ser
Ala199Gln + Ser203Asp + Val204Cys + Thr207Gly +
Ser215Glu + Leu216Gln
Ala199Pro + Pro200Gln + Ser203Asp + Asn211Ser +
Ser215Asp + Leu216Asn
Ser203Glu + Tyr208Thr + Tyr213Cys + Thr214Gln +
Ser215Glu + Gly218Ser
Ala199Thr + Pro200Gln + Gly201Gln + Ser203Asp +
Ser215Asp + Gly218Ser
Pro200Gly + Val202Cys + Ser203Asp + Asn211Ser +
Ser215Glu + Leu216Pro
Ala199Gln + Ser203Glu + Tyr205Gly + Tyr213Met +

Ser215Glu + Asn217Ser
Pro200Ser + Gly201Asn + Ser203Asp + Tyr208Val +
Pro209Ser + Asn217Glu
Pro200Ser + Gly201Ser + Val202His + Ser203Asp +
Asn217Glu + Gly218Pro
Val202Asn + Tyr205Glu + Thr207Gln + Tyr208Leu +
Thr214Asp + Gly218Pro
Ser203Asp + Val204Thr + Tyr205Glu + Thr212Ser +
Tyr213Ile + Ser215Glu
Ala199Asn + Pro200Ser + Tyr205Pro + Pro209Asp +
Asn211Glu + Tyr213Ile
Gly201Pro + Val202Cys + Val204Ser + Pro209Glu +
Thr212Glu + Thr214Ser
Ala199Asn + Val202Asp + Ser203Asp + Tyr208Cys +
Ser215Asp + Leu216Asn
Ala199Asn + Ser203Glu + Tyr205Cys + Thr212Gly +
Thr214Glu + Ser215Asp
Ala199Thr + Pro200Ser + Tyr208Met + Thr214Ser +
Ser215Glu + Asn217Asp
Ala199Pro + Tyr208Gly + Ser215Asp + Leu216Val +
Asn217Asp + Thr219Ser
Pro200Asn + Pro209Gly + Tyr213Pro + Ser215Asp +
Leu216Gly + Asn217Asp
Pro200Ser + Gly201Pro + Val202Ala + Asn211Glu +
Tyr213Asp + Gly218Pro
Val202Met + Val204Ser + Tyr205His + Thr212Gly +
Asn217Glu + Thr219Asp
Tyr205Val + Tyr208Pro + Ser215Glu + Leu216Met +
Asn217Asp + Gly218Asp
Val202Ser + Tyr208Ala + Asn211Ser + Thr214Glu +
Leu216Asp + Asn217Asp
Gly201Pro + Tyr208Gln + Thr212Asp + Tyr213Asp +
Ser215Asp + Asn217Ser
Ala199Gln + Gly201Gln + Tyr205Gly + Pro209Glu +
Thr212Pro + Tyr213Glu
Ser203Asp + Pro209Ser + Asn211Gln + Thr212Gln +
Ser215Asp + Gly218Asp
Ser203Asp + Pro209Gly + Tyr213Leu + Ser215Glu +
Leu216Ala + Gly218Asp
Val202Glu + Val204Cys + Thr212Gly + Ser215Asp +
Leu216Gln + Gly218Gln
Pro200Gly + Gly201Gln + Val202Glu + Val204Ala +
Thr207Pro + Ser215Asp
Ala199Gln + Pro200Ser + Gly201Ser + Ser203Asp +
Gly218Glu + Thr219Asp
Ala199Gln + Gly201Ser + Tyr205Gln + Ser210Asp +
Thr212Glu + Thr214Asp
Gly201Asn + Val202Pro + Ser210Glu + Thr212Glu +
Tyr213Thr + Thr214Glu
Pro200Gln + Val202Met + Ser203Asp + Val204Gly +
Tyr208Ile + Gly218Asp
Pro200Ser + Gly201Pro + Val202Asp + Tyr208Cys +
Asn211Gln + Thr219Glu

63

Ser203Asp + Tyr208Pro + Pro209Ser + Asn211Ser +
Thr214Asp + Thr219Pro
Ala199His + Gly201Asn + Ser203Glu + Tyr213His +
Thr214Glu + Leu216Ile
Ser203Glu + Tyr208Pro + Tyr213His + Thr214Asp +
Leu216Cys + Asn217Ser
Val202Asp + Tyr205Met + Thr214Asp + Ser215Glu +
Leu216Cys + Asn217Gln
Val202Cys + Val204Ser + Tyr208Leu + Ser210Asp +
Tyr213Asp + Thr214Asp
Gly201Asn + Tyr205Gln + Pro209Asn + Ser210Glu +
Tyr213Glu + Leu216Ala
Val202Cys + Pro209Asp + Tyr213Asp + Thr214Asn +
Ser215Glu + Asn217Gln
Ala199Gly + Gly201Gln + Ser215Asp + Leu216Asp +
Gly218Asn + Thr219Glu
Ala199His + Thr207Asn + Asn211Ser + Leu216Glu +
Asn217Gln + Thr219Asp
Val202Met + Thr207Gln + Pro209Gly + Thr214Glu +
Ser215Asp + Gly218Asp
Pro200Gln + Val202Ala + Val204Pro + Thr207Gln +
Pro209Glu + Thr214Glu
Gly201Pro + Tyr208Gly + Thr214Asp + Leu216Cys +
Asn217Glu + Gly218Asp
Ala199Gln + Thr207Pro + Tyr208Cys + Pro209Gln +
Tyr213Asp + Leu216Asp
Ser203Asp + Thr214Gly + Ser215Glu + Leu216Ala +
Asn217Ser + Thr219Glu
Pro200Gln + Ser203Glu + Val204His + Thr212Asp +
Thr214Asp + Gly218Gln
Ala199His + Gly201Ser + Ser203Glu + Tyr205Ser +
Thr214Glu + Gly218Glu
Gly201Pro + Ser203Glu + Thr207Gly + Tyr208Cys +
Thr214Asp + Gly218Glu
Ser203Glu + Tyr205Leu + Thr207Asn + Thr212Asp +
Tyr213Met + Ser215Glu
Ala199Pro + Ser203Asp + Pro209Ser + Thr212Glu +
Ser215Asp + Gly218Gln
Ser203Glu + Tyr205His + Thr212Asp + Tyr213Met +
Ser215Asp + Thr219Ser
Ser203Asp + Val204Gly + Thr212Glu + Thr214Pro +
Ser215Asp + Asn217Ser
Gly201Pro + Tyr205Gln + Thr207Gly + Asn211Glu +
Thr212Gly + Thr214Glu
Pro200Asn + Val202Gln + Thr207Gln + Pro209Ser +
Asn211Asp + Thr214Glu
Gly201Gln + Thr207Gly + Pro209Gln + Thr212Glu +
Ser215Glu + Gly218Gln
Val202Met + Tyr205Ala + Ser210Glu + Thr212Asp +
Ser215Asp + Leu216Ala
Gly201Gln + Pro209Gly + Asn211Asp + Tyr213Asp +
Thr214Gly + Leu216Asp
Ala199Gly + Val202Glu + Ser203Glu + Tyr213Glu +

Thr214Pro + Thr219Ser
Gly201Ser + Val202Cys + Ser203Asp + Tyr205Asp +
Leu216Gly + Thr219Asp
Pro200Ser + Ser203Glu + Tyr213Glu + Leu216Gly +
Asn217Glu + Thr219Gly
Tyr205Asn + Pro209Asn + Thr212Glu + Tyr213Met +
Ser215Glu + Asn217Asp
Ala199Gln + Tyr208Ala + Asn211Gln + Thr214Glu +
Asn217Glu + Thr219Asp
Gly201Pro + Val202Gly + Ser203Glu + Tyr205Thr +
Tyr208Ala + Thr219Asp
Pro200Asn + Gly201Pro + Ser203Asp + Thr207Gln +
Gly218Ser + Thr219Glu
Gly201Asn + Ser203Asp + Val204Gly + Tyr208Val +
Asn211Gln + Thr219Asp
Ala199His + Pro200Asn + Ser203Asp + Tyr205Thr +
Pro209Asp + Thr214Glu
Gly201Pro + Val202Gly + Pro209Glu + Ser210Asp +
Ser215Glu + Leu216Met
Ala199Gly + Val202His + Val204Ala + Pro209Asp +
Ser210Glu + Ser215Glu
Gly201Asn + Val204Gln + Tyr208Leu + Thr214Glu +
Ser215Glu + Thr219Glu
Ser203Glu + Tyr205Gln + Tyr208Gly + Asn211Glu +
Thr214Glu + Thr219Ser
Gly201Ser + Ser203Asp + Tyr205Ala + Pro209Asp +
Tyr213Met + Ser215Asp
Val202Asn + Pro209Asp + Asn211Glu + Thr212Ser +
Tyr213Leu + Ser215Glu
Ala199Pro + Val204Thr + Tyr205Glu + Ser210Glu +
Asn211Asp + Tyr213Leu
Val202Ala + Ser203Asp + Val204Gln + Tyr205Met +
Thr212Glu + Leu216Glu
Gly201Pro + Ser203Asp + Tyr208Thr + Thr214Asp +
Asn217Gln + Thr219Asp
Pro200Gln + Gly201Asn + Thr207Gln + Tyr213Glu +
Asn217Asp + Gly218Asp
Pro200Gln + Thr207Gly + Tyr208Ala + Thr212Glu +
Tyr213Glu + Asn217Glu
Thr207Ser + Tyr208Ser + Pro209Glu + Thr214Glu +
Asn217Glu + Thr219Asn
Ala199Gly + Tyr205Pro + Pro209Glu + Thr214Glu +
Leu216Asn + Asn217Asp
Ala199His + Gly201Asn + Tyr205Pro + Pro209Glu +
Ser215Glu + Asn217Glu
Pro200Gly + Pro209Asp + Tyr213Gln + Ser215Glu +
Leu216Ile + Asn217Asp
Ala199His + Val204Ser + Thr207Gln + Pro209Asp +
Ser210Asp + Thr219Asp
Ala199Thr + Thr207Pro + Tyr208Val + Pro209Glu +
Ser210Asp + Thr219Asp
Tyr208His + Pro209Glu + Ser210Glu + Tyr213Val +
Leu216Cys + Thr219Asp

65

Tyr205Met + Thr207Gly + Ser210Asp + Thr212Ser +
 Asn217Glu + Gly218Asp
 Ser210Glu + Thr212Pro + Thr214Gln + Asn217Asp +
 Gly218Glu + Thr219Ser
 Pro200Gln + Val204Thr + Tyr208Asn + Asn211Asp +
 Ser215Glu + Leu216Glu
 Gly201Pro + Tyr208Gln + Asn211Glu + Thr212Asp +
 Asn217Glu + Thr219Ser
 Val202Asp + Ser203Asp + Asn211Glu + Thr212Pro +
 Tyr213Leu + Thr214Asn
 Val202Glu + Ser203Asp + Tyr205Leu + Pro209Gly +
 Thr212Asp + Thr219Ser
 Ala199Gly + Pro200Asn + Tyr208Leu + Ser210Asp +
 Asn211Asp + Asn217Asp
 Pro200Ser + Gly201Ser + Val202Asp + Tyr208Gln +
 Ser210Glu + Asn211Asp
 Val202Asp + Thr207Gln + Tyr208Thr + Ser210Glu +
 Thr214Gln + Asn217Glu
 Thr207Gln + Asn211Asp + Thr214Glu + Leu216His +
 Asn217Asp + Gly218Gln
 Ser203Glu + Thr207Ser + Pro209Gly + Asn211Asp +
 Tyr213Gly + Ser215Asp
 Ser203Glu + Tyr205Val + Pro209Gly + Asn211Asp +
 Ser215Asp + Thr219Gly

TABLE 28

Loop 6 - Heptuple Substitution Mutation Variants

Ala199Ser + Pro200Ser + Val204Met + Tyr208Ser +
 Tyr213His + Thr214Gln + Leu216Ala
 Ala199Ser + Gly201Ser + Val202Met + Thr207Gln +
 Ser210Glu + Thr214Ser + Asn217Gln
 Gly201Gln + Val204Gly + Tyr205Ala + Thr207Gln +
 Tyr208Gly + Asn211Gln + Gly218Asp
 Ala199Gly + Pro200Gly + Thr207Gln + Tyr208Thr +
 Pro209Gln + Gly218Gln + Thr219Gln
 Ala199Gly + Pro200Ser + Val202Gln + Thr207Asn +
 Asn211Ser + Thr212Ser + Thr219Pro
 Pro200Gln + Val204Ala + Tyr208Leu + Thr212Glu +
 Tyr213Met + Thr214Asn + Leu216Gln
 Ser203Asp + Val204Met + Thr207Gln + Tyr208Met +
 Pro209Gln + Asn217Ser + Thr219Asn
 Ala199Gln + Pro200Ser + Gly201Gln + Tyr205Ala +
 Tyr213Cys + Thr214Gly + Gly218Glu
 Ala199Thr + Pro200Ser + Thr207Gln + Tyr208Gln +
 Thr212Ser + Gly218Asp + Thr219Pro
 Gly201Ser + Val204Pro + Tyr208Ser + Thr212Gly +
 Tyr213Val + Thr214Pro + Ser215Asp
 Pro200Gln + Tyr208Cys + Pro209Gln + Thr212Gly +
 Tyr213Pro + Ser215Glu + Thr219Gly
 Val204Met + Thr207Gln + Pro209Gly + Thr212Gly +
 Tyr213Cys + Thr214Gly + Leu216Met

Pro200Gly + Ser203Asp + Thr207Asn + Pro209Asn +
Tyr213Ile + Leu216Cys + Asn217Ser
Pro200Asn + Gly201Asn + Tyr205Ile + Pro209Asp +
Tyr213Met + Thr214Asn + Leu216Val
Ala199Asn + Ser203Glu + Val204Met + Pro209Gly +
Tyr213Leu + Leu216Ala + Gly218Asn
Ala199His + Thr207Pro + Tyr213Ser + Thr214Asn +
Asn217Ser + Gly218Ser + Thr219Glu
Ala199Ser + Gly201Pro + Val204Gln + Thr207Ser +
Thr212Asn + Tyr213Pro + Gly218Glu
Ala199Ser + Pro200Asn + Tyr205Met + Tyr208Met +
Thr212Asn + Thr214Gly + Leu216Met
Gly201Gln + Tyr205Asn + Thr207Asn + Pro209Asp +
Thr212Gly + Tyr213Val + Leu216Ile
Pro200Asn + Tyr205Glu + Tyr208Met + Tyr213His +
Thr214Ser + Asn217Gln + Thr219Gln
Ala199Gln + Pro200Gly + Val202Met + Tyr208Ile +
Thr212Asp + Leu216Met + Gly218Pro
Ala199Ser + Pro200Gly + Gly201Gln + Val202Asn +
Asn211Gln + Leu216Val + Gly218Pro
Ala199Asn + Val202Ser + Tyr205Cys + Thr207Gly +
Tyr213Leu + Thr214Asp + Asn217Ser
Gly201Pro + Val202Asn + Val204Asn + Tyr205Ser +
Asn211Glu + Thr212Ser + Gly218Asn
Ala199Ser + Val204Cys + Thr207Ser + Asn211Ser +
Thr214Gln + Ser215Glu + Asn217Gln
Pro200Ser + Val202Met + Tyr205Gly + Tyr208Pro +
Ser210Glu + Thr212Ser + Tyr213Gly
Pro200Ser + Val202Gln + Ser203Glu + Tyr205Cys +
Thr207Gln + Tyr208Ile + Asn211Ser
Ala199Pro + Gly201Ser + Val204Ala + Thr207Ser +
Pro209Gln + Tyr213Asp + Leu216His
Gly201Ser + Ser203Asp + Val204Gln + Tyr208Thr +
Asn211Gln + Leu216Ser + Thr219Ser
Ala199Thr + Gly201Ser + Tyr205Pro + Tyr213Pro +
Thr214Gly + Leu216Thr + Gly218Glu
Pro200Asn + Gly201Gln + Tyr208Val + Thr214Gln +
Ser215Asp + Gly218Ser + Thr219Asn
Pro200Gln + Val202Met + Val204Gly + Tyr208Gly +
Asn211Ser + Tyr213Pro + Gly218Asp
Ala199Asn + Pro200Gly + Tyr205Thr + Tyr208Val +
Thr212Gly + Tyr213Ser + Leu216Val
Ala199Ser + Gly201Ser + Val202Gly + Tyr205Leu +
Thr214Asn + Leu216Ala + Gly218Glu
Ala199Ser + Gly201Gln + Val204Ser + Tyr208Asn +
Pro209Ser + Thr214Asn + Ser215Glu
Ala199Pro + Gly201Asn + Tyr208Cys + Tyr213Gly +
Thr214Glu + Asn217Ser + Gly218Gln
Ala199Pro + Val202His + Tyr208Met + Pro209Asn +
Thr212Asn + Thr214Glu + Ser215Asp
Ala199His + Val202Gly + Val204Ser + Thr207Gln +
Pro209Asp + Ser210Asp + Asn211Ser
Ala199Gly + Pro209Glu + Ser210Glu + Thr212Gln +

Tyr213Ala + Leu216Pro + Thr219Asn
Ala199His + Gly201Gln + Val202Met + Val204His +
Thr207Gly + Pro209Glu + Ser210Asp
Ala199His + Val202Gln + Thr207Gly + Thr214Gln +
Leu216Thr + Asn217Asp + Gly218Glu
Gly201Gln + Val204Thr + Tyr205Pro + Thr207Gly +
Leu216Asn + Asn217Asp + Gly218Asp
Gly201Ser + Tyr208Thr + Pro209Gln + Tyr213Ser +
Asn217Asp + Gly218Glu + Thr219Gly
Gly201Ser + Val202Cys + Tyr205Val + Thr207Gln +
Tyr213Ile + Leu216Asp + Asn217Asp
Gly201Asn + Val202Glu + Ser203Glu + Val204Gly +
Thr212Gly + Leu216Val + Thr219Pro
Val202Asp + Ser203Glu + Val204Asn + Thr207Pro +
Tyr208Leu + Thr212Ser + Asn217Gln
Gly201Gln + Tyr208Ser + Pro209Asn + Asn211Gln +
Tyr213Asp + Thr214Asp + Asn217Gln
Ala199Asn + Pro200Asn + Val204Thr + Ser210Asp +
Asn211Glu + Tyr213Leu + Thr214Pro
Gly201Ser + Val202Ala + Tyr208Met + Ser210Asp +
Asn211Asp + Thr212Asp + Asn217Ser
Ala199Ser + Pro200Gly + Gly201Asn + Val202Glu +
Leu216Cys + Asn217Asp + Gly218Asn
Ala199Asn + Pro200Ser + Ser203Asp + Tyr205Cys +
Tyr208Asn + Thr214Gln + Ser215Glu
Ala199Gln + Val202Gly + Ser203Glu + Tyr205Gly +
Tyr213Met + Ser215Glu + Asn217Ser
Gly201Asn + Val202Gly + Ser203Asp + Tyr208Ile +
Tyr213Pro + Ser215Glu + Asn217Ser
Val202Met + Ser203Glu + Thr207Asn + Thr214Gly +
Ser215Asp + Leu216Glu + Asn217Asp
Ala199Gln + Ser203Glu + Val204Cys + Asn211Ser +
Thr214Gly + Asn217Glu + Thr219Pro
Ala199Gln + Ser203Asp + Tyr205Thr + Thr207Ser +
Thr212Asn + Asn217Asp + Gly218Asn
Pro200Ser + Val202Ser + Ser203Asp + Val204Gly +
Thr212Asn + Tyr213Met + Asn217Glu
Ser203Glu + Tyr205Met + Tyr208Thr + Pro209Gly +
Asn217Glu + Gly218Asn + Thr219Ser
Ala199Gln + Tyr205Leu + Tyr208Pro + Asn211Glu +
Thr212Asp + Tyr213Asp + Thr214Gly
Gly201Pro + Val202Thr + Val204His + Tyr205Met +
Asn211Glu + Thr212Asp + Tyr213Asp
Gly201Pro + Ser203Glu + Tyr205Asp + Thr207Ser +
Ser215Asp + Leu216His + Thr219Ser
Ala199Asn + Val202Gln + Val204Ser + Pro209Gly +
Ser210Glu + Thr212Asp + Asn217Gln
Pro200Gly + Gly201Pro + Tyr208Val + Pro209Glu +
Asn211Asp + Thr212Gln + Thr219Ser
Ala199Asn + Pro200Gln + Val202Ala + Val204Pro +
Thr207Asn + Pro209Glu + Asn211Glu
Ala199Asn + Ser203Asp + Thr207Gly + Asn211Ser +
Ser215Glu + Asn217Asp + Gly218Gln

68

Pro200Gln + Ser203Glu + Val204Gly + Tyr213Pro +
Ser215Glu + Asn217Glu + Thr219Gln
Pro200Gly + Val202Asp + Ser203Asp + Thr207Ser +
Thr212Gly + Ser215Glu + Gly218Ser
Val202Glu + Ser203Asp + Tyr208Ser + Asn211Gln +
Tyr213Ala + Ser215Asp + Thr219Ser
Ala199Gln + Ser203Asp + Tyr205Met + Thr214Asp +
Ser215Glu + Leu216Cys + Asn217Gln
Gly201Gln + Ser203Glu + Tyr205Glu + Pro209Gln +
Asn211Ser + Thr214Glu + Leu216Glu
Gly201Asn + Val202Cys + Ser203Asp + Tyr205Asp +
Thr214Asp + Leu216Gly + Thr219Pro
Ala199Gly + Val202Met + Ser203Glu + Val204Pro +
Tyr205Glu + Thr207Ser + Thr214Asp
Gly201Ser + Val204Met + Pro209Gly + Asn211Ser +
Tyr213Cys + Thr214Glu + Leu216Asp
Gly201Pro + Val202Asp + Thr207Gln + Thr212Ser +
Asn217Asp + Gly218Asn + Thr219Asp
Pro200Ser + Val204Gly + Tyr205Gly + Tyr208Cys +
Thr214Ser + Ser215Glu + Asn217Glu
Gly201Asn + Tyr205Ile + Tyr213Ile + Thr214Asn +
Ser215Glu + Asn217Asp + Thr219Pro
Pro200Ser + Val202Gln + Tyr208His + Thr212Pro +
Thr214Ser + Ser215Glu + Asn217Glu
Pro200Gly + Val204Gln + Thr207Ser + Tyr213Leu +
Thr214Gln + Ser215Glu + Asn217Glu
Thr207Gly + Ser210Asp + Asn211Asp + Thr212Gly +
Tyr213Asp + Asn217Gln + Thr219Gln
Ser203Glu + Thr207Asn + Tyr208His + Asn211Gln +
Thr214Asp + Leu216Glu + Asn217Glu
Gly201Pro + Val202Met + Val204Ser + Tyr205His +
Thr212Gly + Asn217Glu + Thr219Asp
Ala199Asn + Val204Cys + Tyr208Cys + Tyr213Ala +
Thr214Glu + Leu216Asp + Asn217Glu
Pro200Gln + Thr207Gly + Tyr208Cys + Thr214Asp +
Ser215Asp + Leu216Gln + Asn217Asp
Ala199Gln + Val204His + Asn211Ser + Thr212Ser +
Thr214Glu + Ser215Glu + Asn217Glu
Val202Asp + Ser203Asp + Tyr205Ala + Thr207Gln +
Tyr213Met + Thr214Glu + Ser215Asp
Ala199Gly + Pro200Gln + Val204Cys + Thr212Glu +
Thr214Glu + Ser215Glu + Leu216Gln
Val204Ala + Thr207Gly + Ser210Asp + Asn211Asp +
Thr212Glu + Thr214Glu + Asn217Ser
Gly201Pro + Pro209Ser + Ser215Glu + Leu216Glu +
Asn217Asp + Gly218Ser + Thr219Asp
Pro200Asn + Gly201Pro + Ser203Asp + Thr207Gln +
Asn217Asp + Gly218Ser + Thr219Glu
Ala199Pro + Val202Gly + Ser203Asp + Pro209Gln +
Thr212Asn + Ser215Glu + Gly218Asp
Ala199Gln + Pro200Ser + Gly201Ser + Tyr205Gln +
Ser210Asp + Thr212Glu + Thr214Asp
Ala199Pro + Pro200Ser + Thr207Gln + Ser210Glu +

Thr212Asp + Thr214Asp + Thr219Ser
Ala199His + Ser203Asp + Val204Gly + Thr207Gly +
Asn211Gln + Thr214Ser + Gly218Glu
Gly201Asn + Val202Asp + Val204Gly + Tyr208Val +
Asn211Gln + Asn217Ser + Thr219Asp
Val202Asn + Tyr205Glu + Thr207Gln + Tyr208Leu +
Thr214Asp + Asn217Asp + Gly218Pro
Ala199Asn + Gly201Asn + Ser203Asp + Tyr208Asn +
Thr214Glu + Leu216Cys + Thr219Gly
Val202Cys + Ser203Asp + Val204Ser + Tyr208Thr +
Thr214Asp + Asn217Gln + Thr219Pro
Ser203Asp + Tyr208Pro + Pro209Ser + Asn211Ser +
Tyr213Met + Thr214Asp + Thr219Pro
Ala199Asn + Pro200Gln + Ser203Asp + Thr212Glu +
Thr214Asp + Ser215Glu + Asn217Ser
Pro200Asn + Gly201Asn + Val202Glu + Ser203Asp +
Thr212Gly + Ser215Glu + Thr219Asp
Ala199Gln + Gly201Gln + Pro209Gly + Ser210Glu +
Thr212Pro + Tyr213Glu + Leu216Ala
Pro200Ser + Val204Ser + Thr207Asn + Tyr208Leu +
Ser210Asp + Asn211Ser + Tyr213Asp
Ala199His + Ser203Asp + Tyr205Asp + Thr207Asn +
Tyr208Ile + Leu216Ser + Gly218Asp
Ala199Gln + Gly201Pro + Val202Glu + Tyr205Glu +
Pro209Gly + Thr214Asp + Leu216Gln
Gly201Asn + Val204Pro + Tyr208Cys + Ser210Glu +
Asn211Glu + Thr212Pro + Thr214Asp
Ala199His + Pro200Asn + Tyr208Val + Thr214Glu +
Ser215Glu + Leu216Glu + Thr219Asp
Pro200Gly + Val202Glu + Tyr205Pro + Tyr208Ala +
Thr214Glu + Asn217Asp + Gly218Asn
Val202Glu + Tyr205His + Pro209Gln + Thr214Glu +
Ser215Asp + Asn217Ser + Gly218Asp
Pro200Ser + Tyr205Leu + Thr212Gln + Thr214Asp +
Ser215Glu + Gly218Glu + Thr219Asn
Ser203Asp + Val204Gly + Tyr205Ser + Thr212Glu +
Thr214Pro + Ser215Asp + Asn217Glu
Pro200Asn + Val204Gln + Pro209Glu + Ser210Glu +
Asn211Glu + Thr212Asn + Ser215Asp
Ala199Gln + Thr207Pro + Tyr208Cys + Pro209Gln +
Tyr213Asp + Thr214Asn + Leu216Asp
Ala199Gly + Pro209Glu + Ser210Asp + Thr214Glu +
Ser215Glu + Gly218Pro + Thr219Gly
Ala199His + Thr207Ser + Tyr208Thr + Pro209Gly +
Ser215Asp + Asn217Gln + Gly218Glu
Ala199Ser + Ser203Glu + Tyr208Ala + Asn211Gln +
Ser215Glu + Leu216Cys + Thr219Asp
Gly201Pro + Val202Gly + Ser203Glu + Tyr205Thr +
Tyr208Ala + Ser215Asp + Thr219Asp
Pro200Gln + Val202His + Ser203Glu + Asn211Gln +
Thr212Asp + Thr214Asp + Thr219Gly
Gly201Pro + Ser203Asp + Thr207Gln + Ser210Glu +
Thr214Glu + Ser215Asp + Thr219Ser

Ala199Ser + Tyr205Gly + Thr207Ser + Ser210Asp +
Tyr213Glu + Ser215Glu + Leu216Glu
Val204Thr + Thr207Pro + Ser210Glu + Asn211Glu +
Thr212Glu + Gly218Asp + Thr219Ser
Val202Asp + Ser203Asp + Thr207Gly + Pro209Asn +
Asn211Glu + Asn217Asp + Gly218Ser
Pro200Ser + Ser203Asp + Val204Pro + Tyr208Ile +
Thr214Asp + Asn217Ser + Gly218Asp
Pro200Ser + Gly201Pro + Val202Gly + Ser203Glu +
Tyr205Glu + Pro209Asp + Tyr213Glu
Ala199Gly + Gly201Gln + Val202Ser + Asn211Asp +
Ser215Glu + Leu216Asp + Asn217Glu
Ala199Thr + Tyr205Gln + Thr207Gln + Pro209Gln +
Thr212Asp + Ser215Glu + Gly218Gln
Ala199Gln + Val204Ser + Tyr205Glu + Thr207Asn +
Ser210Glu + Thr212Glu + Asn217Gln
Tyr205Val + Tyr208Pro + Pro209Gln + Ser210Asp +
Thr212Glu + Ser215Glu + Leu216Gly
Ala199Pro + Gly201Ser + Thr214Glu + Ser215Glu +
Asn217Gln + Gly218Glu + Thr219Asp
Ser203Glu + Ser210Asp + Asn211Gln + Thr212Glu +
Tyr213Ser + Thr214Glu + Leu216Cys
Ala199His + Pro200Gln + Pro209Asn + Tyr213Gln +
Thr214Asp + Leu216Glu + Thr219Asp
Ala199Asn + Ser203Asp + Tyr205Glu + Thr207Asn +
Pro209Asn + Asn211Glu + Leu216Glu
Ala199Thr + Gly201Pro + Ser210Glu + Thr214Asp +
Ser215Asp + Asn217Ser + Thr219Ser
Ser203Glu + Thr207Pro + Tyr208Ala + Ser210Asp +
Ser215Glu + Asn217Asp + Thr219Pro
Val202Asp + Val204Cys + Tyr205Asp + Tyr208Ala +
Pro209Gly + Gly218Gln + Thr219Glu
Val202Ala + Val204Thr + Tyr208Leu + Asn211Glu +
Thr212Glu + Ser215Asp + Leu216Glu
Pro200Asn + Val202Gln + Thr207Gln + Pro209Ser +
Asn211Asp + Thr214Glu + Leu216Asp
Ala199Asn + Val202Met + Thr207Asn + Pro209Glu +
Tyr213Glu + Ser215Glu + Asn217Asp
Ala199Gly + Val202Cys + Val204Gln + Tyr205Asp +
Ser210Asp + Asn211Gln + Thr214Glu
Ala199His + Tyr208Thr + Pro209Asp + Ser215Asp +
Leu216Met + Asn217Asp + Gly218Asp
Ala199Asn + Pro200Asn + Gly201Gln + Pro209Asp +
Thr214Ser + Ser215Asp + Leu216Asp
Ala199Asn + Pro200Gln + Gly201Ser + Val202Asp +
Asn211Asp + Ser215Glu + Leu216Asp
Ala199Thr + Val202Asp + Val204Cys + Ser210Asp +
Tyr213Ile + Ser215Asp + Leu216Asp
Gly201Ser + Val202Glu + Tyr205Gly + Tyr208Leu +
Ser210Asp + Ser215Glu + Asn217Asp
Gly201Gln + Thr207Ser + Tyr208His + Pro209Glu +
Asn211Glu + Tyr213Asp + Asn217Glu
Pro200Gly + Gly201Asn + Ser203Glu + Tyr208Cys +

Thr212Asn + Asn217Gln + Thr219Glu
Ala199Gly + Gly201Asn + Val202His + Val204Ala +
Pro209Asp + Ser210Glu + Ser215Glu
Gly201Asn + Val202Glu + Pro209Gly + Ser210Glu +
Thr212Glu + Tyr213Thr + Thr214Glu
Val202Cys + Val204Ala + Tyr205Glu + Thr212Ser +
Ser215Glu + Asn217Gln + Thr219Asp
Gly201Ser + Ser203Glu + Ser210Glu + Asn211Ser +
Thr212Glu + Ser215Glu + Leu216Pro
Ala199His + Ser203Glu + Thr207Gln + Ser210Asp +
Thr212Asp + Ser215Asp + Gly218Gln
Ala199Gln + Pro200Asn + Gly201Gln + Val204Asn +
Thr214Asp + Ser215Glu + Thr219Glu
Ala199Pro + Gly201Pro + Ser203Asp + Tyr205Val +
Pro209Glu + Ser215Glu + Thr219Pro
Pro200Gly + Ser203Asp + Thr207Gln + Asn211Glu +
Tyr213Glu + Leu216Thr + Asn217Gln
Val202Asp + Tyr205Glu + Tyr208Gly + Thr212Asp +
Tyr213Ala + Leu216Ile + Thr219Pro
Pro200Ser + Val202Glu + Tyr205Asp + Asn211Glu +
Thr212Asp + Tyr213Cys + Thr219Gly
Val204Pro + Tyr205Asp + Asn211Asp + Thr214Pro +
Ser215Asp + Asn217Ser + Thr219Pro
Gly201Asn + Ser203Asp + Pro209Gln + Tyr213Thr +
Thr214Asp + Gly218Asn + Thr219Glu
Pro200Gln + Val204Thr + Thr212Asn + Tyr213Glu +
Thr214Pro + Asn217Asp + Gly218Asp
Ala199Gln + Gly201Gln + Val204Asn + Tyr208Met +
Ser210Glu + Thr214Glu + Asn217Ser
Gly201Pro + Val204Thr + Tyr208Gly + Asn211Asp +
Thr214Asp + Asn217Glu + Gly218Asp
Pro200Asn + Pro209Glu + Ser210Asp + Asn211Ser +
Thr214Ser + Leu216Cys + Thr219Asp
Ser203Asp + Thr207Ser + Tyr208Asn + Pro209Asp +
Ser210Asp + Thr214Gln + Asn217Gln
Val204Ser + Tyr208Pro + Pro209Ser + Asn211Asp +
Leu216Met + Asn217Asp + Gly218Asp
Tyr205Ser + Tyr208Cys + Pro209Asn + Asn211Asp +
Thr212Asn + Leu216Glu + Asn217Asp
Pro200Gly + Ser210Asp + Thr212Pro + Tyr213Val +
Asn217Ser + Gly218Asp + Thr219Asp
Val202Cys + Tyr205Ala + Thr207Asn + Asn211Asp +
Tyr213Val + Ser215Asp + Leu216Asp
Ala199Gln + Val202Glu + Ser203Asp + Val204Thr +
Pro209Gln + Asn211Asp + Tyr213Pro
Gly201Pro + Val202Glu + Ser203Glu + Val204Gly +
Pro209Gln + Thr212Glu + Leu216Ala
Pro200Gln + Val202Asp + Ser203Asp + Tyr208Ala +
Thr212Glu + Leu216Val + Gly218Gln
Ala199Thr + Gly201Gln + Val202Gly + Ser210Glu +
Asn211Asp + Ser215Asp + Leu216Ala
Ala199Gly + Gly201Gln + Val204Asn + Ser210Asp +
Asn211Asp + Ser215Glu + Thr219Asn

Ala199Asn + Val202Ala + Ser203Asp + Val204Gln +
 Ser210Glu + Thr212Glu + Leu216Glu
 Val202Asp + Val204Asn + Pro209Ser + Ser210Asp +
 Asn211Asp + Tyr213Asn + Leu216Met
 Val202Pro + Ser203Asp + Val204Ser + Thr212Glu +
 Tyr213Asp + Thr214Asn + Gly218Asp
 Ser203Glu + Val204Pro + Tyr205Asp + Pro209Glu +
 Thr212Gln + Tyr213Asn + Gly218Asp
 Pro200Ser + Thr207Gly + Ser210Glu + Thr212Gly +
 Thr214Glu + Ser215Asp + Gly218Glu
 Val202Met + Val204His + Ser210Glu + Thr212Asp +
 Tyr213Ile + Leu216Asp + Gly218Glu
 Ser203Glu + Tyr208Ser + Asn211Asp + Thr212Pro +
 Thr214Asn + Ser215Glu + Gly218Pro
 Ala199Thr + Gly201Pro + Ser203Asp + Tyr205Gly +
 Asn211Asp + Ser215Glu + Asn217Ser
 Pro200Gln + Gly201Asn + Ser203Glu + Pro209Asn +
 Asn211Asp + Thr212Gly + Ser215Glu

TABLE 29

Multi-loop Double Mutation Variants

Leu 95Gly + Ser203Glu
 Thr 58Gln + Asp 59Glu
 Tyr103Cys + Leu216Gln
 Val202Gln + Thr212Gly
 Gly126Gln + Gly156Glu
 Thr214Gly + Ser215Glu
 Asn 96Gln + Ile106Ala
 Gly101Asp + Tyr205Pro
 Asn 61Ser + Gly165Gln
 Gly 64Gln + Thr132Gly
 Asp 59Glu + Tyr205Asn
 Ser100Glu + Tyr205His
 Gln161Glu + Tyr205Pro
 Thr 58Asp + Tyr205Ile
 Asn 61Asp + Asn162Ser
 Gln161Glu + Pro209Gln
 Gly 62Asp + Pro209Asn
 Ile106Leu + Asn211Asp
 Tyr166Pro + Ser215Glu
 Ile106Leu + Pro209Glu
 Thr163Pro + Val202Cys
 Thr 65Pro + Leu216Ser
 Thr132Gly + Phe188Val
 Gly156Glu + Ala186Thr
 Pro128Ser + Ser160Asp
 Gly 62Gln + Gly165Asp
 Leu 95Gln + Pro128Glu
 Ser 97Glu + Asn211Ser
 Leu 95Asn + Phe188Leu
 Leu125Gln + Gly159Glu

73

Ser187Glu + Tyr213Ala
Tyr205Cys + Asn211Asp
Asn162Gln + Tyr213Pro
Ser158Asp + Leu216Gln
Gly156Asp + Val202Ala
Ser100Asp + Tyr205Ile
Gly 99Asn + Thr214Glu
Thr 58Asp + Gly130Gln
Gly156Glu + Tyr208Pro
Ala105Pro + Leu216Ile
Ser187Asp + Tyr205Gln
Leu125Asn + Ser187Glu
Thr 65Gln + Thr102Glu
Tyr103Ile + Tyr205Glu
Thr132Asp + Gly218Gln
Asn211Ser + Thr212Asp
Thr212Gln + Ser215Glu
Thr132Pro + Tyr205Gly
Tyr103Glu + Thr132Ser
Gly165Gln + Ser203Glu
Gly 60Pro + Thr 65Pro
Gly 99Asn + Gly153Asn
Pro128Asn + Val202His
Ala105Glu + Gly130Ser
Thr212Gly + Gly218Glu
Phe188Val + Tyr205Ser
Asn217Glu + Thr219Gly
Leu 95Gln + Ser215Asp
Val 94Gln + Thr212Glu
Asn 61Ser + Ser203Asp
Ser129Glu + Asn154Gln
Gly 62Glu + Thr219Asn
Ser 97Glu + Tyr213His
Gly 99Asp + Ile164Thr
Gly101Pro + Ser215Asp
Thr 58Pro + Tyr205Gln
Gly101Gln + Tyr208Gln
Asn 61Gln + Thr212Asp
Thr212Asp + Leu216Ile
Asn211Glu + Leu216His
Gly153Glu + Thr163Gln
Thr132Asn + Tyr205Asp
Asn 61Ser + Ser203Glu
Ser215Asp + Leu216Ser
Gly 60Asp + Met198Gln
Ser158Glu + Tyr205Met
Phe188Leu + Thr212Glu
Gly130Pro + Ser190Glu
Gly101Asp + Gly126Ser
Ala105Gly + Ser157Asp
Asn 61Ser + Ser131Asp
Asn 61Ser + Phe188Ser
Ser100Glu + Asn162Gln

74

Thr 58Gly + Pro128Asp
Ser100Glu + Tyr205Ala
Gly127Glu + Tyr213Thr
Asn154Glu + Leu216Gln
Ser 97Asp + Ala199Pro
Ser203Glu + Leu216Ser
Gly126Gln + Ser215Glu
Gly 60Ser + Asn154Glu
Thr 65Ser + Gly156Asp
Pro128Ser + Thr163Gln
Ser215Asp + Leu216Val
Ser129Glu + Leu216Ala
Asn211Asp + Tyr213Leu
Val 94Ser + Phe188Val
Ser158Glu + Thr212Gln
Gly 99Pro + Asn154Gln
Val202Ser + Tyr205Glu
Tyr205Val + Asn217Glu
Val 94Gly + Ser215Asp
Tyr205Glu + Tyr213Pro
Asn 96Ser + Gly127Glu
Tyr205Thr + Thr214Gly
Gly 62Ser + Thr 65Ser
Thr 65Glu + Pro128Asn
Ser215Asp + Leu216Gln
Asp 59Glu + Gly 60Pro
Val 94Thr + Leu216Met
Ser 97Glu + Thr212Gln
Gly101Asp + Val202Asn
Ser129Asp + Val202Gly
Tyr103Ala + Thr132Ser
Leu 95Met + Leu216Asp
Ser100Asp + Gly101Pro
Ser100Asp + Thr219Pro
Val 94Asn + Ser215Asp
Gly153Asn + Tyr205Asp
Thr 58Glu + Ile106Cys
Thr 65Glu + Tyr103Pro
Val 94Met + Ser 98Glu
Ser210Glu + Gly218Ser
Leu125Pro + Ala199His
Ile106Gly + Thr212Glu
Tyr205Ile + Thr214Pro
Gly130Glu + Tyr208His
Ser187Asp + Leu216Val
Thr102Glu + Thr219Gln
Leu125Ser + Gly153Glu
Gly153Asn + Val202Asp
Thr163Glu + Tyr205Gly
Ser129Glu + Tyr166Pro
Gly153Gln + Pro209Gln
Thr212Ser + Ser215Glu
Thr163Asp + Tyr205His

75

Asn154Glu + Tyr166Thr
Asn 96Ser + Ala105Gly
Gly101Ser + Phe188Gly
Gly153Glu + Gly218Asn
Ser100Asp + Thr212Pro
Gly126Pro + Gly156Asp
Leu125Ser + Ser215Glu
Asp 59Glu + Thr 65Gly
Gly156Pro + Gly159Asp
Val202Cys + Tyr213Ser
Leu 95Met + Ser131Glu
Ser 97Asp + Gly 99Ser
Asn 61Gln + Leu 95Asp
Gly126Asn + Tyr205Glu
Gly159Pro + Gly218Asn
Leu 95Thr + Leu216Ala
Ala105Glu + Leu216Thr
Thr102Glu + Gly130Pro
Gly 64Gln + Ser215Glu
Tyr103Asp + Gly126Asn
Gly101Glu + Gly127Gln
Thr132Asn + Ser160Asp
Thr 58Pro + Gly153Asn
Ser203Glu + Thr214Ser
Tyr103His + Ser129Asp
Ser 98Asp + Pro209Ser
Asp 59Glu + Tyr205Ser
Gly 60Gln + Ser215Asp
Thr132Asp + Val202Asn
Gly218Ser + Thr219Gly
Ser190Asp + Val202Thr
Asp 59Glu + Gln161Asn
Leu216Asn + Asn217Asp
Val 94Asp + Tyr205Asn
Tyr103Ser + Ser203Asp
Gly 99Pro + Phe188Gln
Ser 97Asp + Leu216His
Thr163Asp + Leu216Asn
Asn 61Asp + Thr132Ser
Leu 95Asn + Ser215Asp
Ser100Asp + Thr132Gly
Tyr166Thr + Asn211Gln
Thr207Pro + Leu216Thr
Ile106Ala + Gly165Asp
Tyr205Asn + Ser215Glu
Leu 95Asn + Val202Gln
Ser160Asp + Phe188Gly
Gly 64Ser + Asn 96Gln
Gly101Asp + Asn154Gln
Gly 64Asn + Ser157Glu
Phe188Asp + Tyr213Asn
Ser215Glu + Leu216Ile
Tyr103His + Ser187Glu

76

Asn 96Ser + Thr214Asn
Gly 62Asn + Ser 98Asp
Ile106Ser + Ser157Glu
Thr 65Glu + Thr212Gly
Gly127Ser + Ser215Asp
Gly156Ser + Asn217Glu
Ser 98Asp + Tyr103Thr
Gly127Asn + Gly165Pro
Gly153Glu + Leu216Met
Ser129Asp + Gly165Pro
Asn 61Asp + Thr163Gln
Gly126Asn + Tyr205Met
Gly165Gln + Ser215Glu
Thr 65Glu + Gly101Pro
Val 94Gln + Gly165Glu
Tyr103Ile + Ser190Glu
Asn217Gln + Gly218Ser
Ser 98Glu + Gly 99Asn
Thr102Asp + Thr212Ser
Thr212Ser + Ser215Asp
Gly130Glu + Pro209Asn
Pro128Glu + Gly165Pro
Thr 58Asn + Leu125Glu
Leu125His + Thr163Ser
Thr212Asp + Tyr213Ala
Thr 65Pro + Ser210Glu
Val 94Thr + Thr132Gly
Tyr205Cys + Thr214Gln
Gly127Gln + Ala186Gln
Gly127Glu + Tyr205Cys
Ser 98Glu + Gly130Ser
Gly127Glu + Phe188Tyr
Phe188Asp + Asn211Ser
Gly 64Asn + Leu216Asp
Ser203Glu + Thr212Ser
Tyr166Val + Ser210Asp
Gly165Glu + Tyr205Met
Asn 96Gln + Thr132Glu
Ser 98Glu + Gly101Pro
Leu125Cys + Ser215Asp
Leu125Cys + Gly126Ser
Gly 99Gln + Ser210Asp
Ser160Asp + Phe188Leu
Val204Gln + Ser215Glu
Asn 61Gln + Leu216Asp
Leu125Gly + Ser155Asp
Ala186Pro + Val204Gln
Ile106Met + Ser160Asp
Leu125Ile + Leu216Ser
Gly 60Ser + Ser 97Glu
Gly165Asp + Tyr213Gln
Pro128Glu + Asn154Gln
Gly 60Asp + Gly126Pro

77

Ser203Glu + Leu216Ala
Gly126Glu + Val202Gly
Thr132Asp + Tyr213Ser
Ser157Asp + Thr212Ser
Gly130Ser + Ser203Glu
Gly 99Asn + Gly130Ser
Asp 59Glu + Gly159Asn
Gly153Asn + Thr212Asp
Gly153Asp + Tyr205Ser
Ser100Glu + Tyr205Thr
Gly126Asn + Asn154Glu
Ser190Asp + Tyr205Val
Thr102Glu + Tyr166Thr
Ser157Glu + Pro209Gly
Ser100Asp + Tyr205Leu
Ser131Glu + Thr132Asn
Gly127Asp + Leu216Thr
Ser210Glu + Asn217Gln
Asn 61Asp + Ile106Gln
Ala105Pro + Tyr205Pro
Ser190Asp + Leu216Cys
Ser203Glu + Tyr205Thr
Leu 95Asp + Asn 96Ser
Tyr205Ala + Thr212Glu
Thr163Gln + Phe188Glu
Tyr103Ala + Thr212Asp
Ser155Glu + Gly218Asn
Val 94Thr + Thr163Ser
Thr102Glu + Asn217Gln
Gly159Ser + Val202Glu
Asn154Ser + Tyr166Ala
Asp 59Glu + Phe188Gly
Thr163Gln + Gly218Ser
Gly153Asn + Tyr205Val
Ser129Glu + Leu216Pro
Gly126Ser + Thr212Glu
Ser203Asp + Leu216Asn
Gly126Glu + Thr212Pro
Val 94Pro + Ala186Glu
Ser129Asp + Gly153Asn
Gly165Ser + Ser215Asp
Gly 60Gln + Ser187Glu
Ser203Asp + Leu216His
Gly101Glu + Ile106Met
Val 94Glu + Gly127Gln
Val202Gly + Leu216His
Asp 59Glu + Thr132Asn
Val202Asn + Tyr205Pro
Leu 95Ile + Ser215Glu
Pro128Glu + Leu216Pro
Ser 97Asp + Tyr213Met
Ser187Glu + Leu216Cys
Asn154Ser + Gly156Gln

78

Leu125Gly + Gly126Ser
Asn154Ser + Leu216Asn
Gly126Asn + Val202Asn
Asn 96Gln + Gly127Ser
Gly 64Ser + Thr212Gly
Ser160Asp + Asn211Ser
Ser210Glu + Gly218Gln
Tyr205Ala + Thr212Asp
Ser158Asp + Leu216Ser
Thr212Gly + Ser215Glu
Ala199Ser + Val202Ala
Ser129Glu + Tyr205Asn
Asn154Gln + Gly201Gln
Ser190Glu + Thr212Ser
Thr 58Pro + Ser160Asp
Gly127Glu + Gly130Ser
Asn154Glu + Thr212Gly
Gly153Ser + Gln161Asn
Tyr205Thr + Ser210Glu
Leu125Gly + Pro200Gln
Tyr103Val + Thr132Gly
Gly126Asp + Leu216Ala
Ser100Glu + Gly127Asn
Ser129Asp + Ala186Ser
Ser160Glu + Tyr205Ser
Thr163Glu + Ile164His
Gly159Ser + Gln161Glu
Asp 59Glu + Gly159Pro
Gly101Asp + Tyr166Cys
Thr 58Asn + Ser129Glu
Asn 61Glu + Thr214Ser
Gly127Ser + Ser203Glu
Gly127Pro + Gly130Asp
Gly127Glu + Val202Gly
Phe188Met + Ser203Glu
Ser 97Glu + Tyr166Asn
Gly 60Glu + Gly 99Asn
Asn162Glu + Asn217Ser
Thr102Asp + Leu216Gly
Gly 99Gln + Ala105Thr
Gly153Ser + Ser203Glu
Tyr103Pro + Ser158Glu
Ser187Glu + Tyr205Pro
Ser160Asp + Val204Thr
Ser100Asp + Leu216His
Gly101Asn + Ser203Asp
Asn 96Ser + Tyr205Pro
Thr132Gly + Thr212Pro
Ile106Pro + Val202Cys
Gly 62Ser + Pro209Glu
Phe188Cys + Leu216Asp
Leu 95Cys + Ser104Glu
Ser104Asp + Tyr205Met

Tyr205Glu + Thr214Gly
 Gly153Glu + Leu216Asn
 Val 94Thr + Ser100Glu
 Thr 65Asp + Val202His
 Val202Gln + Ser215Asp
 Val 94Ser + Ala105Glu
 Thr 58Pro + Gly153Asp
 Ala105Asn + Ser203Glu
 Ser104Asp + Ala105Gly
 Gly126Gln + Ile164Val
 Gly153Ser + Ser215Glu
 Pro200Gly + Ser215Glu

TABLE 33

Multi-loop Triple Mutation Variants

Thr 58Gln + Leu 95Gly + Ser203Glu
 Asp 59Glu + Tyr103Cys + Leu216Gln
 Gly156Glu + Val202Gln + Thr212Gly
 Gly126Gln + Thr214Gly + Ser215Glu
 Asn 96Gln + Ile106Ala + Gly156Glu
 Asn 61Ser + Gly101Asp + Tyr205Pro
 Gly 64Gln + Thr132Gly + Gly165Gln
 Thr 65Pro + Thr163Pro + Val202Cys
 Thr132Gly + Phe188Val + Leu216Ser
 Leu 95Asn + Ser 97Glu + Asn211Ser
 Asn162Gln + Ser187Asp + Tyr213Pro
 Ser100Asp + Val202Ala + Tyr205Ile
 Thr 58Asp + Gly 99Asn + Gly130Gln
 Gly156Glu + Tyr208Pro + Leu216Ile
 Thr 65Gln + Leu125Asn + Ser187Glu
 Asn211Ser + Thr212Asp + Gly218Gln
 Tyr205Gly + Thr212Gln + Ser215Glu
 Thr 58Glu + Gly 60Pro + Thr 65Pro
 Asn 96Gln + Gly153Pro + Leu216Val
 Gly 99Asn + Gly153Asn + Pro209Glu
 Ala105Glu + Pro128Asn + Val202His
 Tyr205Ser + Thr212Gly + Gly218Glu
 Gly 99Gln + Asn217Glu + Thr219Gly
 Leu 95Gln + Val202Thr + Ser215Asp
 Gly101Pro + Ile164Thr + Ser215Asp
 Thr 58Pro + Gly130Asp + Tyr205Gln
 Gly101Gln + Ser190Glu + Tyr208Gln
 Asn 61Gln + Thr212Asp + Leu216Ile
 Asn 61Ser + Thr132Asn + Tyr205Asp
 Gly130Pro + Phe188Leu + Thr212Glu
 Asn 61Ser + Ala105Gly + Ser131Asp
 Asn 61Ser + Asn162Gln + Phe188Ser
 Gly126Gln + Ser203Glu + Leu216Ser
 Thr 65Ser + Gly156Asp + Thr163Gln
 Pro128Ser + Ser215Asp + Leu216Val
 Val 94Ser + Ser100Asp + Phe188Val

Gly 99Pro + Asn154Gln + Ser203Glu
Tyr166Asp + Tyr205Thr + Thr214Gly
Gly 62Ser + Thr 65Ser + Asn211Glu
Thr 65Glu + Pro128Asn + Leu216Gln
Val 94Thr + Thr212Gln + Leu216Met
Ser129Asp + Thr132Ser + Val202Gly
Leu 95Met + Tyr103Ala + Leu216Asp
Val 94Met + Ser 98Glu + Tyr103Pro
Leu125Pro + Ala199His + Ser203Glu
Ile106Gly + Tyr205Ile + Thr214Pro
Ser129Glu + Tyr166Pro + Tyr205Gly
Pro209Gln + Thr212Ser + Ser215Glu
Asn154Glu + Tyr166Thr + Tyr205His
Asn 96Ser + Gly101Ser + Ala105Gly
Gly153Glu + Phe188Gly + Gly218Asn
Gly126Pro + Gly156Asp + Thr212Pro
Gly156Pro + Gly159Asp + Val202Cys
Leu 95Met + Ser 97Asp + Gly 99Ser
Gly126Asn + Gly159Pro + Gly218Asn
Thr 65Gln + Leu 95Thr + Leu216Ala
Tyr103Asp + Gly126Asn + Gly127Gln
Thr 58Pro + Ala105Glu + Gly153Asn
Gly 60Gln + Tyr205Ser + Ser215Asp
Thr132Asp + Gly218Ser + Thr219Gly
Tyr103Ser + Phe188Gln + Ser203Asp
Ser 97Asp + Gly 99Pro + Leu216His
Asn 61Asp + Thr132Ser + Leu216Asn
Leu 95Asn + Thr132Gly + Ser215Asp
Tyr166Thr + Asn211Gln + Leu216Thr
Ile106Ala + Gly165Asp + Thr207Pro
Leu 95Asn + Val202Gln + Ser215Glu
Gly 64Ser + Gly101Asp + Asn154Gln
Thr 58Asn + Gly 64Asn + Ser157Glu
Gly 62Asn + Asn 96Ser + Thr214Asn
Gly130Asp + Phe188Asn + Gly218Pro
Gly 99Ser + Pro128Glu + Leu216Gly
Val 94Ala + Pro209Ser + Ser215Asp
Leu 95Thr + Gly126Gln + Leu216Gly
Asp 59Glu + Gly159Ser + Tyr205Pro
Val202Gln + Ser203Glu + Tyr205Asn
Thr 58Ser + Ser190Glu + Tyr205Pro
Leu 95Asn + Tyr205Ala + Pro209Glu
Thr 58Asn + Gly153Pro + Ser210Glu
Ser131Glu + Thr132Asn + Gly165Asn
Gly127Ser + Thr212Asn + Ser215Asp
Thr 65Gly + Thr102Asp + Gly127Asn
Gly 60Asn + Tyr205Thr + Asn217Gln
Ala199Ser + Val202Ala + Thr212Gly
Thr 58Pro + Gly130Ser + Ser160Asp
Gly153Ser + Asn154Glu + Thr212Gly
Leu125Gly + Tyr205Thr + Ser210Glu
Tyr103Val + Thr132Gly + Pro200Gln
Ser100Glu + Gly127Asn + Leu216Ala

Ser160Glu + Ala186Ser + Tyr205Ser
Gly159Ser + Thr163Glu + Ile164His
Phe188Met + Val202Gly + Ser203Glu
Thr102Asp + Ala105Thr + Leu216Gly
Thr 58Asp + Asn 96Ser + Tyr205Pro
Ser 97Asp + Thr132Gly + Thr212Pro
Ile106Pro + Val202Cys + Ser215Asp
Leu 95Cys + Phe188Cys + Leu216Asp
Thr 65Asp + Val 94Thr + Val202His
Thr 58Pro + Ala105Asn + Gly153Asp
Ser104Asp + Ala105Gly + Ile164Val
Gly126Gln + Ser203Asp + Leu216His
Ser160Asp + Gly218Gln + Thr219Asn
Gly 62Ser + Ala105Glu + Pro128Ser
Thr 58Gly + Ile106Gly + Leu216Asp
Ser 97Asp + Thr163Gln + Ala186Ser
Thr 65Glu + Gly126Gln + Leu216His
Phe188Ile + Ser203Glu + Leu216Thr
Thr132Gln + Gly165Asp + Thr212Ser
Ser129Asp + Asn211Ser + Leu216Thr
Leu125Ile + Asn154Ser + Leu216Met
Thr 65Pro + Gly101Pro + Thr212Asp
Thr102Asn + Thr212Gly + Leu216Asp
Thr 65Gln + Tyr205Gln + Leu216Met
Leu125Asn + Pro209Gly + Thr214Glu
Leu125Pro + Val202His + Ser215Asp
Ser160Glu + Gln161Asn + Tyr205Val
Gly126Ser + Thr212Glu + Gly218Asn
Val 94Thr + Ser155Asp + Thr207Gly
Ser157Glu + Tyr208Gln + Thr212Gln
Tyr205Met + Thr212Gln + Ser215Glu
Pro128Gln + Thr132Pro + Tyr213Asn
Gly 60Ser + Gly156Asp + Pro209Asn
Asn162Asp + Thr163Asn + Ala199Gly
Gly165Ser + Tyr205Thr + Ser215Glu
Ser100Asp + Gly156Pro + Tyr205Gln
Ala105His + Ser157Glu + Ala186Asn
Asn162Gln + Ser190Glu + Val202Ser
Gly 99Gln + Gly126Gln + Gln161Asp
Gly126Asp + Gly159Ser + Thr212Gly
Gly 62Glu + Asn154Gln + Leu216Gln
Asn154Asp + Tyr205Ser + Tyr213Met
Asn 61Asp + Leu 95Ser + Val204Gln
Thr102Gln + Gly159Gln + Ser190Glu
Pro128Asn + Gly159Asn + Asn211Ser
Gly159Ser + Thr163Gly + Ala199Thr
Asn211Gln + Tyr213Gln + Ser215Glu
Gly 99Ser + Leu125Gly + Ser215Glu
Thr 65Gly + Ser190Glu + Thr219Ser
Val 94Met + Asn162Ser + Leu216Met
Leu 95Ser + Tyr103His + Gly156Glu
Thr 58Gly + Asn211Glu + Leu216Ala
Ala105Gly + Thr212Pro + Gly218Glu

Ser158Asp + Gly159Ser + Tyr205Pro
Thr 58Gln + Ser158Glu + Thr163Gly
Thr 65Pro + Leu125Met + Thr163Gly
Gly 62Asn + Gly126Pro + Ser210Glu
Leu 95Met + Thr132Asp + Asn211Ser
Tyr103Ile + Gly159Asp + Val202Gly
Gly101Gln + Tyr166Pro + Asn211Gln
Pro128Gly + Thr132Pro + Thr214Gln
Pro200Ser + Thr212Asp + Leu216Gly
Thr212Asp + Leu216Met + Gly218Gln
Thr163Gln + Tyr205Cys + Thr212Pro
Thr 58Gln + Ser 98Glu + Thr212Pro
Thr102Pro + Ser155Asp + Tyr205Asn
Gly 62Ser + Gly 99Glu + Val202Ser
Leu 95Asp + Ile106Gln + Gly126Asn
Ser100Glu + Gly101Gln + Val202Ala
Thr 58Ser + Ser100Glu + Ile164Leu
Gly101Ser + Phe188Asp + Tyr213Gly
Ser104Glu + Gly156Gln + Thr212Gly
Thr 65Asp + Val 94Gly + Tyr205Met
Ser203Glu + Thr212Ser + Tyr213Val
Thr 65Ser + Leu125Glu + Gln161Ser
Thr 65Gly + Tyr205Asp + Tyr213Thr
Gly101Pro + Ser104Asp + Thr163Gln
Ala105Gly + Asn154Asp + Pro209Gly
Gly126Gln + Gln161Ser + Leu216Ala
Gly153Gln + Tyr166Cys + Gly218Asp
Gly 99Gln + Val204Pro + Thr219Glu
Thr 58Ser + Ser 97Asp + Thr212Pro
Asn162Ser + Met198Gly + Val202Thr
Val 94Ser + Leu125Asp + Tyr205Thr
Ser157Asp + Tyr205Ser + Leu216Thr
Gly165Asn + Ser215Glu + Leu216Pro
Gly156Asn + Thr163Glu + Ala199Ser
Pro128Ser + Gly156Glu + Thr219Gly
Asn 96Gln + Gly126Gln + Ser203Glu
Gly159Asp + Gly165Pro + Tyr213Ile
Thr 58Gln + Gly153Ser + Asn217Gln
Pro128Ser + Thr132Glu + Thr212Gly
Gly156Gln + Tyr205Pro + Ser215Glu
Gly 62Ser + Tyr205Ile + Leu216Asp
Leu125Pro + Gly159Glu + Gly165Pro
Thr 58Gln + Ser203Asp + Thr212Ser
Thr102Gln + Val202His + Thr214Ser
Gly 99Glu + Ile106Gly + Gly218Asn
Ser 97Asp + Leu125Val + Tyr213Ile
Ile106Leu + Phe188Glu + Thr212Asn
Thr 58Pro + Gly153Pro + Asn162Gln
Gly127Ser + Pro128Gln + Thr212Pro
Tyr103Pro + Ser203Asp + Tyr205Ile
Thr 58Gln + Thr163Ser + Tyr205Ser
Val 94Cys + Ser215Glu + Gly218Pro
Thr102Pro + Gly165Pro + Val202Thr

Thr 58Glu + Tyr103Asn + Pro200Gln
Phe188Thr + Val202Asn + Asn217Ser
Gly 99Asn + Tyr205Ser + Ser215Glu
Thr102Gly + Gly165Asp + Gly218Gln
Gly 60Asp + Ala186Pro + Tyr205Asn
Thr102Asn + Ile106Asn + Ser158Glu
Gly 62Ser + Ser 97Glu + Gly218Asn
Ile106His + Ser129Glu + Thr212Asn
Gly126Glu + Pro128Asn + Val202Asn
Asp 59Glu + Gly 64Asn + Tyr213Ser
Thr163Pro + Val204Thr + Tyr205Cys
Ser129Glu + Asn162Ser + Leu216Cys
Gly156Asn + Phe188Ala + Tyr205His
Val 94Ser + Gly101Ser + Leu125Asp
Asn 96Glu + Tyr166Ala + Leu216Cys
Tyr103Gln + Phe188Met + Ser210Glu
Gly101Ser + Thr212Pro + Thr219Pro
Ser104Glu + Gly159Gln + Thr212Pro
Gly 62Asp + Gly126Ser + Leu216Pro
Gly101Asp + Gly218Pro + Thr219Asn
Ser157Glu + Tyr205Pro + Thr212Ser
Pro209Glu + Ser210Glu + Gly218Ser
Asn154Glu + Ser155Glu + Gly218Pro
Asn211Glu + Thr212Asp + Leu216His
Tyr205Gly + Ser215Glu + Leu216Asp
Val202Ser + Ser215Asp + Leu216Asp
Thr 58Asp + Asp 59Glu + Thr212Ser
Tyr205Glu + Tyr213Pro + Ser215Asp
Gly156Ser + Tyr205Asp + Ser215Glu
Gly 60Glu + Ser 97Glu + Gly 99Asn
Ser155Glu + Asn162Ser + Ser190Glu
Leu 95Asn + Ser203Asp + Ser215Asp
Ser203Asp + Ser215Asp + Thr219Gln
Gly 62Asn + Ser203Glu + Ser215Glu
Ser203Glu + Tyr205Pro + Ser215Asp
Phe188Asn + Ser203Asp + Ser215Asp
Gly 62Asn + Ser203Glu + Ser215Asp
Ser203Glu + Ser215Asp + Leu216Ser
Gly156Gln + Ser203Glu + Ser215Asp
Ser203Asp + Tyr205Ala + Asn217Glu
Tyr205Ala + Ser210Glu + Thr212Asp
Ile164Cys + Ser210Glu + Thr212Asp
Ser158Asp + Gln161Glu + Tyr205Pro
Asp 59Glu + Asn 61Glu + Gly130Ser
Gly 64Gln + Ser 98Glu + Ser100Asp
Ser 97Glu + Gly 99Asp + Tyr213His
Asn154Gln + Ser157Glu + Ser190Asp
Ser158Asp + Ser160Glu + Tyr205Cys
Gly201Pro + Ser203Glu + Tyr205Asp
Gly 99Gln + Ser129Asp + Tyr166Glu
Ser160Asp + Asn162Glu + Phe188Gly
Ser129Asp + Ser131Asp + Gly218Asn
Asp 59Glu + Gly165Asn + Pro209Glu

84

Val 94Gly + Ser215Asp + Asn217Glu
Val202Glu + Tyr205Gln + Leu216Glu
Gly156Glu + Ser158Glu + Tyr205Met
Asn 61Asp + Asn 96Glu + Gly127Ser
Gly159Asn + Phe188Asp + Gly218Asp
Tyr103His + Asn162Glu + Ser190Asp
Ser 97Asp + Ser100Glu + Gly201Asn
Ser157Glu + Ser160Glu + Tyr205Pro
Gly153Asp + Val202Ser + Gly218Asp
Ser157Glu + Gly159Glu + Ser190Asp
Asp 59Glu + Asn 96Glu + Tyr205Ser
Gly153Ser + Asn154Asp + Gly165Asp
Asn154Glu + Gly165Asp + Asn211Ser
Ser155Asp + Gly156Asp + Thr219Asp
Ser210Asp + Tyr213Asp + Thr214Glu
Asn 61Glu + Ala186Gly + Pro209Asp
Asn154Asp + Ser157Glu + Thr214Ser
Ser100Glu + Leu125Glu + Tyr213His
Gly 60Glu + Leu 95Glu + Ile106Leu
Gly 99Glu + Gly101Glu + Tyr103Glu
Thr 58Glu + Thr 65Glu + Ile106Cys
Ser 97Glu + Gly101Asp + Val202Asn
Gly 62Asp + Ser210Asp + Leu216Ala
Asn 61Asp + Ser100Glu + Asn162Ser
Gly127Glu + Asn162Glu + Gly165Glu
Ile106Asn + Gly156Glu + Gly159Glu
Ser100Glu + Gly126Glu + Ala186Gln
Gly 62Asp + Phe188Leu + Thr212Glu
Ile106Gln + Ser215Glu + Gly218Glu
Tyr103His + Ser155Glu + Gly218Glu
Ala186Glu + Val202Glu + Ser215Asp
Gly127Glu + Asn154Glu + Tyr213Thr
Ser157Glu + Ser160Asp + Ser187Asp
Gly 62Asp + Leu216Glu + Thr219Gln
Thr 58Asp + Ser210Asp + Thr212Glu
Ser155Glu + Ser157Asp + Gly218Asp
Pro128Asp + Asn162Glu + Asn217Ser
Gly156Asp + Gly165Glu + Tyr205Asn
Ser158Glu + Phe188Asp + Val202Met
Thr102Glu + Ser104Asp + Leu125Glu
Ser 98Asp + Ser100Asp + Ser104Glu
Gly 62Asp + Pro209Asn + Asn211Asp
Ser160Glu + Ser187Asp + Ser190Asp
Gly159Asp + Val202Asp + Ser203Asp
Pro128Glu + Thr212Asp + Tyr213Asp
Leu 95Glu + Tyr205Asp + Ser215Asp
Asp 59Glu + Tyr205Glu + Ser215Glu
Gly 60Ser + Thr 65Glu + Leu216Asp
Gly101Ser + Asn154Glu + Ser158Glu
Pro128Asp + Ser203Asp + Ser215Asp
Ser203Asp + Ser210Glu + Ser215Glu
Ser104Glu + Ser203Asp + Asn217Glu
Ser160Asp + Ser203Asp + Leu216Asp

85

Ser203Glu + Ser210Asp + Leu216Asp
Gly127Glu + Ser129Glu + Ser215Glu
Pro128Glu + Ala186Glu + Phe188Asp
Asp 59Glu + Asn 61Glu + Ser203Asp
Ser 97Asp + Gly 99Asp + Ser210Asp
Asn 96Glu + Ser100Asp + Thr212Glu
Thr 65Asp + Thr163Gln + Ser215Asp
Ser 98Asp + Thr102Glu + Leu216Gln
Thr132Glu + Ser157Asp + Ser190Glu
Ser129Glu + Ser157Asp + Ser190Glu
Ser190Glu + Ser203Glu + Tyr205Glu
Asn 61Asp + Ser 98Asp + Tyr205Asp
Ser129Asp + Phe188Glu + Ser190Asp
Ser100Glu + Ser104Glu + Tyr205Met
Gly127Glu + Ser190Asp + Tyr205Leu
Asp 59Glu + Ser203Glu + Pro209Asp
Gln161Glu + Ser215Glu + Asn217Asp
Asp 59Glu + Ser 97Glu + Ser157Asp
Leu125Glu + Gly153Glu + Ser203Glu
Ser104Glu + Ser157Asp + Ser160Asp
Asp 59Glu + Asn 96Glu + Ser187Glu
Ser129Asp + Thr132Glu + Asn162Glu
Ser131Glu + Ser203Glu + Gly218Glu
Gly156Asp + Ser203Asp + Thr214Asp
Ser160Glu + Thr163Asp + Ser215Asp
Thr 58Asn + Gly 99Glu + Ala105Glu
Asp 59Glu + Phe188Leu + Thr212Asp
Ser104Asp + Ser131Glu + Tyr205Glu
Gly 62Asp + Ser104Asp + Ser131Asp
Ser104Glu + Ser131Glu + Gly156Glu
Gly 60Asp + Gly101Asp + Met198Gln
Ser 98Glu + Ser215Glu + Gly218Asp
Tyr103Asp + Ser129Asp + Tyr205Glu
Ser100Asp + Ile106Asp + Ser215Asp
Thr 65Asp + Ser100Glu + Ala105Glu
Asp 59Glu + Ser100Asp + Tyr205Ile
Gly153Glu + Asn162Asp + Ser203Glu
Ser 98Glu + Thr212Glu + Ser215Glu
Ser131Asp + Thr212Glu + Ser215Asp
Ala105Asp + Thr212Glu + Ser215Asp
Ser158Glu + Thr212Asp + Ser215Asp
Ser157Asp + Ser203Glu + Thr219Asp
Gly 62Gln + Thr212Glu + Leu216Asp
Ser 97Glu + Ser187Glu + Asn217Asp
Ser155Glu + Asn217Asp + Gly218Asn
Gly159Glu + Ser190Glu + Asn211Glu
Pro128Asp + Ser190Glu + Ser215Asp
Asp 59Glu + Gly156Asp + Ser160Glu
Gly 60Asp + Ser100Glu + Ser215Glu
Gly130Glu + Gly153Glu + Tyr205Asn
Thr102Glu + Gly153Glu + Asn217Asp
Ser129Glu + Ser155Glu + Gln161Asn
Tyr205Glu + Asn211Glu + Leu216Cys

Gly153Glu + Gln161Asp + Ala199Asn
 Gly 62Asn + Gly153Glu + Val202Asp
 Ser129Asp + Asn162Glu + Asn211Glu
 Gly153Glu + Ser158Asp + Leu216Asp
 Ser157Glu + Leu216Ile + Thr219Asp
 Ser158Glu + Ser215Asp + Thr219Asp

TABLE 34

Multi-loop Quadruple Mutation Variants

Tyr103Cys + Val202Gln + Thr212Gly + Leu216Gln
Asn 61Gln + Gly101Asp + Ile106Ala + Tyr205Pro
Asn 61Ser + Gly 64Gln + Thr132Gly + Gly165Gln
Leu 95Ile + Tyr205Glu + Tyr213Ala + Thr219Asn
Gly126Ser + Val202Ser + Tyr205Cys + Asn217Glu
Asn 96Ser + Asn154Glu + Tyr166Thr + Tyr205His
Gly101Ser + Ala105Gly + Phe188Gly + Gly218Asn
Gly165Ser + Val202Gln + Tyr205Thr + Thr212Ser
Gly126Asn + Ser190Glu + Pro209Asn + Leu216Val
Ile106Glu + Gly127Ser + Asn154Ser + Tyr205Leu
Thr 58Pro + Ser203Asp + Tyr213Met + Leu216Val
Gly 62Gln + Asn 96Glu + Ile164Asn + Leu216Ile
Ser 98Glu + Tyr166Gln + Thr212Asn + Leu216Val
Gly156Asp + Tyr166Ala + Tyr208Pro + Leu216Ile
Thr 58Pro + Ser 98Asp + Gly130Pro + Tyr205Val
Gly130Asp + Phe188Asn + Leu216Gly + Gly218Pro
Leu 95Thr + Gly126Gln + Pro209Ser + Leu216Gly
Val202Gln + Ser203Glu + Tyr205Asn + Gly218Asn
Thr 58Asn + Leu 95Asn + Gly153Pro + Ser210Glu
Gly127Ser + Ser131Glu + Thr132Asn + Gly165Asn
Thr 65Gly + Gly127Asn + Thr212Gln + Thr214Glu
Asn 96Gln + Gly126Asn + Gly127Ser + Val202Asn
Gly 64Ser + Ser160Asp + Asn211Ser + Thr212Gly
Asp 59Glu + Ala199Ser + Val202Ala + Tyr205Ser
Leu125Gly + Thr132Gly + Pro200Gln + Tyr205Thr
Gly159Ser + Thr163Glu + Ile164His + Tyr205Ser
Gly127Pro + Phe188Met + Val202Gly + Ser215Glu
Gly101Gln + Asn211Gln + Thr212Pro + Ser215Asp
Pro128Gly + Pro200Ser + Thr214Gln + Leu216Gly
Tyr205Cys + Thr212Pro + Leu216Met + Gly218Gln
Thr102Pro + Val202Ser + Tyr205Asn + Gly218Asp
Leu 95Asp + Ile106Gln + Gly126Asn + Val202Ala
Tyr103Gln + Ser203Asp + Thr212Asn + Leu216Asn
Phe188Met + Ser210Glu + Thr212Pro + Thr219Pro
Ser104Glu + Gly159Gln + Thr212Pro + Leu216Pro
Gly 60Gln + Ala186Gly + Thr212Gly + Ser215Asp
Thr102Glu + Thr132Pro + Leu216Pro + Asn217Gln
Leu 95Ala + Phe188Thr + Tyr205Gln + Ser215Asp
Thr 58Gly + Gly 60Asp + Val202Cys + Thr212Pro
Gly156Asn + Val202Asp + Thr212Ser + Thr214Gly
Thr 58Pro + Gly165Glu + Tyr205Ser + Tyr213His
Gly 99Asn + Tyr166Asn + Ser203Asp + Leu216His

Gly 62Glu + Leu 95Met + Ala105Ser + Gly126Ser
Ile106Gln + Ser187Glu + Tyr205Gly + Thr219Pro
Ser160Glu + Tyr205Ser + Thr212Pro + Leu216Thr
Gly130Gln + Ser131Glu + Asn162Gln + Leu216Asn
Gly156Glu + Thr163Ser + Asn211Ser + Thr212Asn
Gly165Gln + Phe188Leu + Val202His + Thr212Glu
Val 94Cys + Pro128Asp + Val202Thr + Tyr205Ala
Leu 95Thr + Gly156Ser + Thr214Glu + Leu216Ala
Gly 99Pro + Tyr103Glu + Gly165Pro + Leu216Cys
Gly 64Asn + Thr 65Glu + Tyr166Ala + Phe188Tyr
Tyr103Gln + Gly156Pro + Tyr166Asn + Ser215Glu
Gly126Gln + Pro128Gln + Val202Thr + Thr212Glu
Gly 64Ser + Val 94Met + Gly130Asp + Tyr213Gly
Gly 99Asn + Val202Pro + Thr212Asn + Ser215Glu
Gly101Pro + Asn154Ser + Leu216His + Asn217Glu
Tyr103Asp + Gly153Pro + Ala186Asn + Val202Ser
Leu125Thr + Gln161Asn + Thr212Gly + Leu216Asn
Gly 64Pro + Val 94His + Ser210Asp + Thr212Gly
Asn 61Ser + Thr 65Gln + Ser190Asp + Val202Gly
Ala105Thr + Gly153Gln + Gly156Gln + Asn211Gln
Asn 61Gln + Tyr166Ile + Ala186Asn + Ser215Asp
Gly 60Gln + Tyr103Gly + Gly130Asn + Asn217Asp
Leu 95Pro + Tyr205Leu + Asn211Gln + Ser215Glu
Thr102Asp + Gly126Gln + Val202Asn + Thr212Gly
Val 94Thr + Gly153Pro + Ala186Pro + Tyr205Glu
Ser104Asp + Ala105Asn + Thr163Ser + Tyr205Ala
Gly 64Ser + Val 94Asn + Gly130Asp + Leu216Cys
Gly101Asn + Leu125Asn + Gly201Pro + Leu216Ser
Gly159Pro + Gly165Pro + Ala186Thr + Thr212Pro
Pro128Gln + Ser129Asp + Ala186Ser + Asn217Ser
Gly 60Asn + Gly159Ser + Phe188Val + Ser203Glu
Ser 98Glu + Gly 99Asn + Thr102Asn + Ala186Pro
Thr 65Asn + Gly 99Gln + Thr212Ser + Leu216Asp
Val202Gln + Tyr205Thr + Tyr213Val + Ser215Glu
Thr132Ser + Gly156Ser + Phe188Thr + Asn211Glu
Leu125His + Gly156Ser + Thr212Glu + Leu216Asn
Gly126Ser + Tyr205Asp + Thr214Gln + Leu216Asn
Ala186Gly + Ser215Glu + Gly218Pro + Thr219Gln
Thr 58Gly + Gly 60Asn + Leu125His + Leu216Gly
Asp 59Glu + Val 94Gly + Tyr205Gln + Tyr213Ile
Ser155Glu + Met198Thr + Val202Met + Tyr205Thr
Gly127Asn + Tyr166Pro + Phe188Leu + Thr214Glu
Leu 95Cys + Gly130Gln + Thr212Ser + Leu216Val
Asp 59Glu + Thr 65Gly + Leu 95Gly + Leu216Asn
Ser131Glu + Gly201Ser + Val202Gln + Tyr205Gly
Leu125Val + Tyr205Gln + Ser215Asp + Gly218Gln
Val 94Gly + Thr102Ser + Ser203Glu + Leu216Asn
Asp 59Glu + Gly 60Gln + Thr212Gly + Leu216Cys
Ala186Gln + Gly201Gln + Val202Pro + Ser203Glu
Thr 65Ser + Ser 98Asp + Gln161Ser + Tyr205Cys
Tyr166Ala + Ser187Glu + Tyr205Gly + Leu216Val
Gly 99Asp + Met198Cys + Pro209Gly + Thr212Asn
Thr 65Pro + Ile106Gly + Tyr205Gln + Leu216Glu

Leu 95Ser + Thr102Asn + Leu216Asp + Asn217Ser
 Thr 58Gly + Gly127Ser + Tyr166Glu + Val202Asn
 Val 94Pro + Tyr213Thr + Thr214Glu + Asn217Gln
 Ser 97Glu + Gln161Asn + Tyr166Gly + Val202Ser
 Tyr103Met + Thr132Gly + Tyr166Met + Ser210Asp
 Leu 95Met + Gly156Asn + Val202Glu + Leu216Gln
 Ser 97Asp + Met198Ala + Val204Thr + Tyr205Thr
 Gly101Ser + Gly153Pro + Asn217Glu + Thr219Pro
 Ile106Met + Thr163Glu + Tyr166Ile + Thr212Ser
 Gly165Ser + Ser190Asp + Pro200Ser + Val202Met
 Gly 62Ser + Gly153Asp + Thr163Ser + Gly201Gln
 Gly101Gln + Thr102Ser + Gly153Ser + Val202Asp
 Val 94Ala + Leu 95Gly + Leu125Thr + Gly130Asp
 Asn 96Asp + Tyr205Ala + Thr212Pro + Thr219Gly
 Gly153Gln + Tyr205Ile + Thr212Gly + Ser215Glu
 Ile106Cys + Asn154Glu + Tyr205Ala + Leu216Ala
 Asn 96Asp + Gly 99Pro + Tyr205Val + Asn217Gln
 Ala105Thr + Leu125Thr + Val204Ala + Tyr205Met
 Thr102Pro + Gly156Pro + Tyr208Val + Thr212Asn
 Gly 60Ser + Phe188Ile + Thr212Ser + Leu216Asp
 Ser100Asp + Tyr103Gln + Gly159Gln + Leu216Val
 Pro128Gly + Gly165Gln + Ser203Glu + Leu216Ser
 Ser129Glu + Thr132Gln + Met198Thr + Thr219Gln
 Val 94Pro + Gly 99Asn + Thr132Gly + Gly159Asp
 Ser155Asp + Gly165Ser + Tyr205His + Thr212Gly
 Gly 99Asp + Phe188Val + Thr207Asn + Leu216Pro
 Thr102Gln + Gly126Asn + Gly159Asn + Leu216Asp
 Asp 59Glu + Thr132Pro + Thr163Pro + Phe188Thr
 Gly101Ser + Ala105Glu + Ala186Pro + Thr212Gly
 Ser129Asp + Gly153Asn + Gly159Pro + Val202Met
 Gly 60Pro + Asn 96Asp + Gly126Gln + Tyr166Ala
 Thr 65Gln + Gly 99Pro + Tyr205Ala + Ser215Asp
 Val 94Gln + Gln161Asp + Tyr205Pro + Leu216Met
 Asp 59Glu + Gly 60Glu + Ala186Gly + Gly218Asn
 Ser 98Asp + Gly 99Glu + Phe188Met + Tyr213Ala
 Ser160Asp + Gln161Asp + Tyr205Ser + Thr219Gln
 Ala105Gly + Ile106Cys + Tyr205Asp + Ser215Asp
 Pro200Asn + Tyr205Asp + Asn211Gln + Ser215Glu
 Tyr205Asp + Thr207Ser + Thr214Ser + Ser215Asp
 Asn 61Asp + Thr 65Gln + Ser 97Asp + Tyr205Ala
 Leu125His + Ser203Glu + Ser215Glu + Leu216Asp
 Thr 65Asn + Ser203Glu + Tyr205Cys + Ser215Asp
 Gly 64Pro + Ala186Ser + Ser203Asp + Ser215Asp
 Pro128Ser + Ser203Asp + Tyr213Ala + Asn217Glu
 Val202His + Tyr205Val + Ser210Glu + Thr212Asp
 Gly 99Asn + Gly130Ser + Ser210Glu + Thr212Asp
 Tyr205Ala + Ser210Glu + Thr212Asp + Gly218Gln
 Phe188Ala + Ser203Asp + Tyr205Asp + Thr212Ser
 Asn154Glu + Ser155Asp + Thr163Asp + Tyr213Thr
 Gly 99Gln + Ser129Asp + Tyr166Glu + Leu216Thr
 Thr102Gly + Ala105Thr + Ser155Glu + Ser157Asp
 Gly127Pro + Ser157Glu + Gly159Asp + Gln161Asp
 Gly101Asn + Asn154Asp + Thr163Gly + Gly218Glu

Pro128Ser + Ser158Asp + Thr163Asp + Asn217Ser
Ile106Val + Thr214Asn + Ser215Asp + Asn217Asp
Thr 58Gly + Asn154Asp + Ser157Asp + Ser190Glu
Leu125Asn + Gly153Glu + Thr163Asp + Thr214Pro
Gly 64Pro + Ser157Glu + Gln161Glu + Tyr205Cys
Ser155Asp + Ser187Asp + Ser190Glu + Thr212Gln
Val 94Pro + Ser155Asp + Ser157Asp + Ser187Glu
Gly126Gln + Ser203Asp + Ser215Glu + Gly218Asp
Gly156Ser + Ser187Asp + Ser190Glu + Thr212Ser
Asn154Asp + Ser187Glu + Val202Asn + Leu216Ser
Asn162Asp + Gly165Asp + Thr212Gln + Leu216Cys
Ser155Glu + Ser187Asp + Pro200Ser + Tyr213Val
Asn 96Glu + Thr102Asp + Ala105Pro + Tyr213Val
Asn154Ser + Gly156Glu + Asn162Asp + Thr212Ser
Asn 61Glu + Ser100Asp + Tyr205Val + Leu216Gly
Thr 65Gly + Asn154Glu + Ser157Asp + Asn162Glu
Tyr205Leu + Thr212Glu + Ser215Glu + Leu216Glu
Asn 61Gln + Gly 62Glu + Ser 98Asp + Thr214Gln
Gly 62Glu + Ser 98Glu + Gly130Ser + Val202Pro
Thr 65Glu + Tyr205Met + Thr212Asp + Thr214Gly
Ala105His + Phe188Asp + Val202Asp + Asn211Ser
Asp 59Glu + Pro209Ser + Asn211Asp + Thr212Glu
Leu 95Gln + Gly126Gln + Gly127Asp + Ser155Asp
Gly 60Asp + Val 94His + Asn154Ser + Ser210Glu
Thr 58Gly + Gly126Glu + Ser155Asp + Gly201Pro
Gly156Asn + Tyr166Pro + Thr212Glu + Ser215Glu
Thr212Asp + Ser215Glu + Leu216Ala + Thr219Ser
Thr 58Asp + Gly101Ser + Ser210Asp + Thr212Glu
Ile106Glu + Gly126Asp + Gly127Asn + Leu216Pro
Asn 61Ser + Gly 99Asp + Thr102Asp + Ser104Asp
Ser210Asp + Thr212Asp + Tyr213Pro + Ser215Glu
Gly127Pro + Pro128Ser + Ala186Glu + Ser203Asp
Gly101Glu + Thr102Asp + Ser129Asp + Leu216Asn
Gly 62Glu + Leu 95Asp + Gly159Asn + Leu216Gly
Asn 61Asp + Ser 97Asp + Ser210Glu + Thr212Gln
Ser203Glu + Tyr205Pro + Thr212Ser + Thr219Glu
Thr 65Glu + Tyr103Pro + Ser215Asp + Asn217Asp
Gln161Asp + Thr163Glu + Ser187Glu + Asn211Gln
Gly153Glu + Tyr166Gly + Ser187Glu + Leu216Met
Asp 59Glu + Gly159Glu + Ser160Asp + Pro209Gln
Gly 60Asn + Ser129Glu + Ser158Asp + Gly159Asp
Gly130Glu + Ser131Asp + Ser155Glu + Thr212Asn
Gly130Asp + Asn154Glu + Ser155Asp + Gln161Asn
Ser129Asp + Gly130Glu + Gly156Glu + Gln161Ser
Gly 99Glu + Ser100Asp + Ser203Glu + Leu216Ser
Pro128Asp + Ser129Asp + Val202Asn + Ser215Glu
Tyr103Asp + Ser104Glu + Thr212Pro + Ser215Glu
Thr 65Pro + Ser157Glu + Ser158Asp + Ser215Asp
Thr 58Asn + Asn 96Asp + Ser 97Asp + Ser187Asp
Ser104Asp + Ile164Gly + Ser215Asp + Leu216Asp
Ser157Asp + Ser215Asp + Leu216Glu + Gly218Gln
Gln161Ser + Ser187Asp + Ser215Glu + Leu216Glu
Thr 58Pro + Ser104Asp + Thr212Glu + Tyr213Glu

Thr102Glu + Ser160Glu + Gln161Asp + Leu216Val
 Asn 61Glu + Gly 62Glu + Ser203Glu + Thr212Gly
 Tyr205Val + Ser210Asp + Asn211Glu + Leu216Glu
 Asp 59Glu + Gly126Ser + Tyr205Glu + Ser215Glu
 Pro128Asn + Ser129Asp + Tyr205Asp + Ser215Asp
 Gly101Glu + Tyr205Glu + Ser215Asp + Leu216His
 Ser155Glu + Ile164Ser + Tyr205Asp + Ser215Asp
 Tyr103Ala + Tyr166Glu + Tyr205Glu + Ser215Glu
 Gly 60Glu + Ser 97Glu + Gly 99Asn + Asn162Glu
 Ser187Glu + Ser203Glu + Tyr205Asp + Tyr213Pro
 Ser157Glu + Ser203Asp + Tyr208Pro + Ser215Glu
 Ser 98Glu + Ser203Glu + Tyr205Val + Ser215Glu
 Gly127Gln + Ser160Glu + Ser203Asp + Ser215Glu
 Val 94Glu + Ser203Asp + Ser215Asp + Leu216Pro
 Ser100Asp + Ser203Glu + Ser215Glu + Leu216Thr
 Ser157Glu + Phe188Thr + Ser203Asp + Ser215Asp
 Val 94Gly + Leu 95Glu + Ser203Glu + Ser215Asp
 Phe188Val + Ser190Asp + Ser203Asp + Ser215Glu
 Ser158Asp + Ser203Asp + Ser215Asp + Asn217Ser
 Ser157Asp + Ser203Asp + Ser215Asp + Thr219Gln
 Gly126Pro + Gly153Glu + Ser203Glu + Ser215Glu
 Ser131Glu + Gly165Asn + Ser203Glu + Ser215Asp
 Tyr103Glu + Gly153Asp + Ser190Glu + Tyr205Val
 Gly159Glu + Ser203Glu + Leu216Gln + Asn217Glu
 Ser100Asp + Gly153Gln + Thr163Asp + Ser190Asp
 Thr102Asp + Gly127Glu + Ser155Asp + Tyr213Pro
 Asn 61Glu + Asn154Glu + Ser190Asp + Val204Met
 Asn154Asp + Gln161Ser + Ser190Asp + Ser203Glu
 Thr 65Gly + Gly127Asp + Gly165Asp + Ser203Asp
 Asn 96Asp + Gly 99Glu + Gly101Asn + Ser187Glu
 Thr102Asp + Ala105Glu + Gln161Ser + Pro209Asp
 Gly127Glu + Ser129Glu + Gly130Ser + Ser160Asp
 Thr102Glu + Ser104Glu + Pro200Asn + Thr212Glu
 Ser 98Asp + Ser100Glu + Pro128Gly + Gly159Asp
 Gly159Asp + Gln161Asp + Val204Gly + Ser215Glu
 Ser155Glu + Phe188Glu + Tyr205Asn + Ser215Glu
 Ser129Glu + Thr132Asn + Gly165Asp + Gly218Asp
 Ser100Glu + Gly153Asp + Gly165Gln + Thr219Asp
 Gly126Asp + Ser131Glu + Thr207Gly + Gly218Gln
 Asn 61Asp + Gly 99Glu + Tyr103Thr + Ser131Asp
 Gly 99Asp + Ser203Asp + Tyr205Asp + Thr212Pro
 Ser190Glu + Ser203Glu + Tyr205Asp + Leu216His
 Gly 60Pro + Ser157Asp + Ser203Glu + Tyr205Asp
 Ser157Glu + Thr163Glu + Pro209Ser + Ser215Glu
 Gly 60Asn + Ser157Asp + Thr163Glu + Asn211Asp
 Thr102Glu + Gly127Asn + Gly130Glu + Tyr205Gln
 Asn 61Glu + Gly153Ser + Pro209Asp + Ser215Glu
 Asp 59Glu + Asn 61Gln + Leu216Asp + Gly218Asp
 Leu125His + Ser129Asp + Ser131Asp + Val202Glu
 Ile106Cys + Tyr205Asp + Ser210Glu + Leu216Asp
 Ser 97Glu + Gly156Asp + Thr163Asp + Thr212Gln
 Ser131Asp + Asn154Gln + Ser215Glu + Asn217Asp
 Ser104Glu + Thr212Ser + Ser215Asp + Asn217Glu

91

Ser157Asp + Ser187Asp + Ser203Asp + Thr212Gly
Asn 61Asp + Asn 96Asp + Ala105His + Asn162Asp
Asn 61Asp + Asn 96Glu + Gly127Ser + Gly130Asp
Thr 58Gly + Ser158Glu + Asn211Asp + Tyr213Asp
Ser158Asp + Asn162Asp + Thr212Asp + Leu216Thr
Gly 99Ser + Ser157Asp + Asn162Glu + Leu216Asp
Gly159Gln + Asn162Glu + Ser190Asp + Ser215Glu
Ser 97Asp + Ser100Asp + Gly126Asn + Ser160Asp
Gly 60Glu + Ser 98Glu + Gly153Gln + Ala186Asp
Ser157Asp + Ser160Asp + Thr212Asn + Ser215Glu
Gly 62Pro + Ser131Glu + Gly165Glu + Thr219Asp
Ser190Asp + Val202Glu + Ser215Glu + Leu216Ala
Asn 96Glu + Gly153Asp + Gly156Asp + Gln161Asn
Tyr103Glu + Ser131Asp + Ser203Asp + Leu216Cys
Gly130Glu + Ser157Asp + Ser187Asp + Tyr208His
Asn 61Asp + Thr163Gln + Ser203Asp + Thr214Glu
Ser155Asp + Ser158Asp + Ser203Asp + Tyr205Thr
Ser155Asp + Gly156Ser + Ser215Glu + Thr219Asp
Gly 60Gln + Thr 65Gln + Asn 96Asp + Leu125Glu
Asp 59Glu + Asn 96Glu + Ser203Glu + Thr214Asn
Asp 59Glu + Asn 96Asp + Ser215Asp + Leu216Ile
Gly 62Asp + Ser 97Glu + Ala105His + Ser131Glu
Leu125Asp + Gly165Asp + Ser203Asp + Tyr213Gln
Leu 95Glu + Tyr166Asn + Ser203Glu + Thr214Glu
Gly101Asp + Asn162Asp + Gly165Glu + Leu216Asn
Gly 99Glu + Tyr103Glu + Ser129Glu + Tyr205Asn
Asp 59Glu + Asn 96Ser + Thr212Glu + Ser215Asp
Asn 61Glu + Ser100Asp + Ala186Gly + Thr212Glu
Thr 65Gly + Ser 97Glu + Gly101Glu + Gly130Glu
Ala186Glu + Phe188Tyr + Thr212Asp + Asn217Asp
Asn 61Gln + Gly156Glu + Ser215Glu + Thr219Glu
Gly 99Gln + Ser129Glu + Ser155Glu + Gly218Glu
Ser160Glu + Thr163Asp + Thr212Ser + Ser215Asp
Asn 96Asp + Thr102Asp + Ser157Glu + Ala186His
Gly 99Glu + Ala105Asp + Thr214Asn + Leu216Val
Asn162Gln + Ala186Asp + Tyr205Ser + Leu216Asp
Ser 97Glu + Gly156Glu + Gly159Asp + Val202Asn
Ile106Cys + Ser129Glu + Ser155Glu + Ala186Glu
Ser100Glu + Leu125Gly + Gly127Glu + Ser203Asp
Thr 65Asp + Ser131Asp + Ile164Asn + Thr212Asp
Ser 97Glu + Thr102Glu + Gln161Ser + Tyr205Leu
Asp 59Glu + Gly 99Glu + Ser203Asp + Tyr205Asn
Asp 59Glu + Gly 99Asp + Gly126Ser + Thr214Glu
Ser160Asp + Pro209Asn + Ser215Asp + Gly218Glu
Ser155Glu + Gly159Asn + Pro209Glu + Gly218Glu
Ser155Asp + Asn211Asp + Tyr213Met + Gly218Asp
Ser129Asp + Thr132Glu + Ser160Glu + Ala186Ser
Val 94Glu + Ser 97Asp + Gly101Pro + Ser203Glu
Gly101Asp + Gly126Asp + Thr132Asp + Thr212Asn
Ser 98Glu + Gly127Glu + Thr163Asp + Thr212Asn
Gly 99Asp + Gly101Pro + Thr102Glu + Ser203Glu
Gly159Asp + Thr212Asp + Ser215Asp + Asn217Ser
Ser158Glu + Asn162Gln + Thr212Glu + Ser215Asp

Val 94Pro + Ser155Asp + Thr212Glu + Ser215Glu
 Gly 99Gln + Ile106Asp + Thr212Glu + Ser215Glu
 Thr 58Asp + Tyr205Pro + Thr212Glu + Ser215Asp
 Asp 59Glu + Ser 98Glu + Ser203Asp + Tyr205Gly
 Asp 59Glu + Ser100Asp + Ser104Asp + Ala105Pro
 Asp 59Glu + Ser100Asp + Ser104Asp + Tyr205Ile
 Pro128Glu + Asn162Asp + Thr212Glu + Leu216Cys
 Asn 96Gln + Thr102Asp + Ser129Glu + Asn162Glu
 Ser 97Glu + Gly130Asn + Ser155Asp + Ala186Glu
 Ala105Glu + Ser155Glu + Gln161Asp + Tyr205Thr
 Gly 62Asp + Ser158Asp + Ser215Asp + Leu216Asn
 Thr102Asp + Gly126Asp + Ser158Asp + Thr212Ser
 Thr 58Asn + Gly 64Pro + Thr 65Glu + Val 94Asp
 Ser190Glu + Ser203Glu + Tyr213Glu + Leu216His
 Gly159Asp + Ser203Asp + Tyr205Cys + Tyr213Asp
 Ile106Asp + Gly130Asp + Ser215Asp + Asn217Gln
 Ser 98Glu + Thr102Asp + Ser203Asp + Thr214Gly
 Gly 99Gln + Ser104Asp + Gly130Asp + Asn154Glu
 Ser104Glu + Gly126Ser + Gly130Asp + Asn217Glu
 Asn 96Gln + Ser100Glu + Ser104Glu + Tyr205Asp
 Asn 61Gln + Gly101Asp + Ser157Asp + Gly165Glu
 Thr102Ser + Val202Cys + Thr214Glu + Gly218Glu
 Asn 61Gln + Ser104Glu + Gly127Glu + Gly165Pro
 Thr 65Gly + Tyr205Glu + Asn211Glu + Leu216Cys
 Gly 99Gln + Gly159Ser + Pro209Asp + Leu216Asp
 Asp 59Glu + Ser158Asp + Tyr205Pro + Thr212Glu
 Asp 59Glu + Ser131Glu + Thr212Glu + Leu216Ile
 Ala105Asp + Leu125Glu + Asn211Glu + Leu216Ile
 Thr 58Asp + Gly156Ser + Asn211Asp + Ser215Glu
 Ser157Glu + Gly159Ser + Gly165Pro + Thr219Glu
 Asn162Asp + Tyr205Gln + Thr212Glu + Leu216Asp
 Ser100Glu + Met198Gln + Thr212Asp + Leu216Glu
 Gly 64Gln + Pro128Glu + Thr132Asp + Thr163Gly
 Gly 62Gln + Pro128Asp + Thr132Asp + Leu216Thr
 Gly101Asp + Ile106Met + Leu125Gly + Pro128Glu
 Thr 65Glu + Asn 96Glu + Gly156Asp + Leu216Cys
 Ser104Asp + Leu125Gln + Ser129Asp + Asn162Gln
 Asp 59Glu + Gly130Glu + Gly153Glu + Tyr205Asn
 Ser129Asp + Ser155Asp + Tyr205Val + Leu216Asp
 Ser129Glu + Ser155Glu + Gln161Asn + Ser210Glu
 Gly 60Asp + Gly127Asp + Asn162Glu + Phe188Asn
 Gly 62Gln + Ser 98Glu + Ser187Asp + Ser203Glu
 Ser104Asp + Gly130Gln + Ser187Asp + Ser203Glu
 Asn 61Asp + Tyr103Ser + Thr212Asp + Thr219Asp
 Asp 59Glu + Gly101Asp + Gly159Pro + Gln161Glu
 Gly 60Glu + Leu125Ser + Gly153Glu + Val202Asp
 Thr132Glu + Ser157Glu + Thr212Gly + Thr219Asp
 Gly101Glu + Leu125Cys + Gly130Glu + Thr219Asp
 Gly 60Asp + Pro128Glu + Ser157Asp + Tyr205Cys
 Pro128Glu + Asn154Gln + Ser157Asp + Ser203Asp
 Ser 97Asp + Ala105Asp + Ser203Asp + Thr212Gln
 Ser100Asp + Ser158Glu + Tyr166Asp + Phe188Pro
 Tyr205Thr + Asn211Glu + Ser215Glu + Leu216Asn

TABLE 35

Multi-loop Quintuple Mutation Variants	
Tyr103Cys + Gly126Gln + Gly156Glu + Val202Gln + Thr212Gly	
Val 94Met + Gly101Asn + Thr102Asn + Tyr205Leu + Gly218Asp	
Ser 98Asp + Tyr103His + Leu125Cys + Gly159Asn + Met198Cys	
Leu 95Met + Gly101Pro + Tyr103Ala + Gly165Ser + Leu216Asp	
Thr 65Asp + Val 94Thr + Thr132Gly + Asn154Ser + Thr219Gly	
Val 94Thr + Thr132Gly + Ala186Gln + Tyr205Cys + Thr214Gln	
Thr163Gln + Tyr205Cys + Thr212Pro + Leu216Met + Gly218Gln	
Leu 95Asp + Gly101Gln + Ile106Gln + Gly126Asn + Val202Ala	
Gly 99Glu + Gly126Ser + Tyr166Ala + Asn211Gln + Leu216Asn	
Gly 60Asn + Gly159Glu + Gln161Ser + Asn162Gln + Leu216Ser	
Ala186His + Val202His + Thr212Pro + Ser215Glu + Leu216Thr	
Gly 99Gln + Leu125Ile + Gly127Ser + Tyr205Val + Thr212Asp	
Thr 58Gly + Val 94Gly + Tyr166Cys + Ser203Asp + Tyr205His	
Val 94Asp + Gly 99Asn + Gln161Ser + Gly201Ser + Thr212Asn	
Gly126Asn + Gly127Pro + Gly153Asp + Gly159Pro + Ile164Ala	
Asn 61Glu + Thr102Asn + Gly165Gln + Phe188Leu + Val202His	
Val 94Cys + Gly156Ser + Val202Thr + Tyr205Ala + Leu216Ala	
Tyr103Gln + Gly126Gln + Gly156Pro + Tyr166Asn + Ser215Glu	
Gly 99Asn + Val202Pro + Thr212Asn + Tyr213Gly + Ser215Glu	
Gly101Asp + Leu125Thr + Gln161Asn + Thr212Gly + Leu216Asn	
Asn 61Ser + Gly153Gln + Gly156Gln + Val202Gly + Asn211Gln	
Asn 61Gln + Ala105Thr + Tyr166Ile + Ala186Asn + Ser215Asp	
Ala105Gln + Leu125Gly + Thr163Ser + Val202Gln + Asn217Asp	
Pro200Ser + Val202Ser + Ser203Glu + Thr207Gly + Thr214Gly	

94

Val 94Asn + Gly130Pro + Tyr205Ser + Ser215Asp +
 Thr219Pro
 Thr163Asn + Ala199Gln + Tyr205Asn + Thr212Ser +
 Ser215Glu
 Leu 95Met + Gly156Asn + Val202Cys + Leu216Gln +
 Gly218Glu
 Val 94His + Gly 99Glu + Asn211Ser + Tyr213His +
 Leu216Met
 Gly 64Ser + Val 94Gln + Ser157Asp + Tyr205Ser +
 Thr207Pro
 Ile106Ala + Gly165Asn + Ser190Glu + Ala199Asn +
 Tyr205Asn
 Asp 59Glu + Gly159Ser + Gly165Asn + Phe188Thr +
 Asn217Gln
 Gly130Pro + Ser155Glu + Gly159Asn + Tyr205Pro +
 Thr212Ser
 Tyr103Gln + Ser155Glu + Val202Asn + Thr212Ser +
 Thr219Asn
 Gly 60Pro + Val 94Ser + Ser100Glu + Tyr103Leu +
 Tyr205Ser
 Gly 60Gln + Leu 95Val + Pro128Gly + Ser158Asp +
 Tyr205Thr
 Thr132Gln + Asn162Ser + Gly165Asp + Tyr205Gly +
 Tyr208Cys
 Gly 62Asn + Val 94Thr + Ala105His + Pro128Glu +
 Tyr205His
 Thr 58Pro + Gly156Pro + Val202Pro + Thr212Glu +
 Leu216Thr
 Leu 95Met + Gly 99Gln + Tyr103Cys + Asn154Gln +
 Thr212Gln
 Ser 98Glu + Tyr205Asn + Pro209Gln + Tyr213His +
 Thr219Ser
 Gly 62Ser + Val 94His + Tyr103Gln + Ser187Glu +
 Phe188Tyr
 Gly 62Gln + Asn 96Ser + Gly165Glu + Val202Gly +
 Tyr205Thr
 Gly 60Pro + Ile106Thr + Ser160Glu + Thr214Gln +
 Leu216Ile
 Ser100Asp + Ala105Thr + Tyr205Ile + Thr214Gly +
 Leu216Cys
 Leu 95Pro + Ala105Asn + Phe188Leu + Thr212Pro +
 Leu216Val
 Asp 59Glu + Gly 60Ser + Gly127Pro + Tyr205Thr +
 Leu216Ala
 Ala105Asn + Ser131Asp + Thr163Pro + Leu216His +
 Gly218Asn
 Leu 95Cys + Tyr103Met + Ile106Cys + Ala186Asp +
 Tyr205Met
 Leu125Cys + Tyr205His + Leu216Asn + Asn217Ser +
 Thr219Asn
 Asn 96Ser + Gly153Glu + Asn154Gln + Ala186Gln +
 Thr212Asn
 Gly101Gln + Ala105Pro + Gly130Asp + Asn154Gln +

95

Gly156Asn
 Thr 58Gly + Gly159Pro + Thr163Ser + Tyr205Gln +
 Thr212Asn
 Tyr205Pro + Thr212Asn + Tyr213Gln + Ser215Asp +
 Leu216Gln
 Gly130Pro + Gly153Pro + Gln161Glu + Tyr205Met +
 Thr212Gln
 Ala105Gly + Ser157Asp + Tyr166Asn + Val202Gln +
 Thr212Ser
 Thr 58Gln + Asn 96Ser + Ser203Asp + Thr207Gly +
 Leu216Ser
 Ser158Glu + Val202Ser + Thr212Asn + Thr214Gln +
 Thr219Asn
 Asn 61Ser + Ser100Glu + Tyr205Leu + Thr212Ser +
 Asn217Ser
 Gly 60Gln + Gly 99Glu + Thr212Ser + Tyr213Gln +
 Leu216Thr
 Leu125Asn + Ser129Asp + Asn162Ser + Ala186His +
 Tyr205Gln
 Gly 64Gln + Leu 95Glu + Ala199Asn + Val204His +
 Leu216Met
 Leu 95His + Gly101Pro + Val204His + Tyr205Asn +
 Leu216Gln
 Thr163Ser + Gly165Asn + Pro209Gly + Tyr213His +
 Leu216Asp
 Gly127Gln + Ser131Asp + Thr132Pro + Tyr205Cys +
 Leu216Asn
 Asn 61Gln + Gly101Pro + Ser190Asp + Tyr205Pro +
 Thr214Gly
 Gly126Asn + Pro200Gly + Val204Gly + Tyr205Asp +
 Thr212Asn
 Asn 61Gln + Val 94Gln + Gly126Asp + Tyr205Val +
 Thr212Gln
 Gly 60Gln + Ile106Pro + Ser131Glu + Ile164Ala +
 Val202Pro
 Gly101Ser + Tyr103Glu + Ser104Asp + Gly165Asn +
 Pro209Gln
 Thr 58Glu + Asp 59Glu + Tyr166His + Val202Cys +
 Thr219Asn
 Gly126Pro + Ala186His + Tyr205Asp + Tyr213Val +
 Ser215Asp
 Gly 60Asp + Ser 97Glu + Ala105Pro + Leu125Cys +
 Gly153Pro
 Asn154Ser + Pro209Ser + Thr214Glu + Ser215Asp +
 Leu216Glu
 Thr163Pro + Ser203Glu + Val204Thr + Tyr205Cys +
 Ser215Asp
 Gly 62Ser + Met198Gln + Ser203Glu + Ser215Asp +
 Leu216His
 Gly156Asn + Ser203Asp + Tyr205Met + Ser215Asp +
 Gly218Pro
 Gly 60Gln + Pro128Ser + Ser203Glu + Ser215Glu +
 Leu216Pro

96

Tyr166Cys + Tyr205Glu + Pro209Asn + Ser215Glu +
 Leu216Asp
 Tyr103Leu + Leu125Gly + Gly153Glu + Ser190Glu +
 Leu216Ile
 Gly 99Pro + Ile106Thr + Gly153Asp + Ser155Asp +
 Ser190Asp
 Thr 65Pro + Leu125His + Ser210Glu + Thr212Asp +
 Tyr213Ala
 Gly 64Ser + Thr 65Ser + Gly 99Asn + Pro209Glu +
 Asn211Glu
 Leu 95Asn + Gly101Asn + Asn154Asp + Gly156Glu +
 Tyr166Gln
 Ser155Glu + Thr163Asp + Tyr205Cys + Thr212Pro +
 Leu216Val
 Gly159Pro + Thr163Ser + Ser203Asp + Tyr205Asp +
 Thr212Ser
 Thr102Gln + Gly126Asp + Gly153Asp + Phe188Thr +
 Tyr205His
 Asn 61Ser + Val202Glu + Ser203Glu + Tyr205Asp +
 Thr212Pro
 Thr 58Gln + Thr102Pro + Tyr213Cys + Ser215Glu +
 Asn217Asp
 Gly 60Asp + Gly 62Glu + Thr 65Glu + Gly126Asn +
 Leu216Thr
 Ala105Gly + Gly126Pro + Gly153Glu + Leu216Thr +
 Gly218Asp
 Tyr103Gln + Ala105Thr + Val202Asp + Tyr205His +
 Ser215Glu
 Val202Glu + Tyr205Thr + Pro209Asn + Ser215Asp +
 Leu216Met
 Val 94Asn + Ala105Asn + Ile106Val + Ser203Glu +
 Gly218Asp
 Asn 96Ser + Ser203Asp + Tyr205Ile + Thr212Gln +
 Gly218Asp
 Asn 61Asp + Gly 62Gln + Thr 65Asp + Gly126Asn +
 Gly159Pro
 Gly153Gln + Ala186Glu + Tyr205Thr + Leu216Thr +
 Asn217Asp
 Leu125Asp + Gly126Asp + Ala186Ser + Gly218Glu +
 Thr219Pro
 Thr 58Glu + Thr 65Glu + Val 94Gln + Gly101Pro +
 Gly165Gln
 Gly153Glu + Ser158Glu + Thr163Glu + Gly165Pro +
 Leu216Met
 Gly159Asp + Thr163Glu + Tyr166Thr + Ala186His +
 Pro200Ser
 Leu125Thr + Gly130Ser + Asn154Asp + Ser157Asp +
 Ala186Glu
 Asp 59Glu + Asn 96Glu + Gly101Glu + Pro128Ser +
 Thr212Asn
 Asp 59Glu + Asn 96Asp + Gly101Asp + Tyr166Asn +
 Thr219Asn
 Gly 99Glu + Gly101Glu + Gly127Glu + Tyr166Leu +

Asn217Gln
 Ala186Gln + Ser187Asp + Leu216Pro + Gly218Glu +
 Thr219Gln
 Thr 58Pro + Leu 95Ile + Gly126Glu + Gly153Glu +
 Phe188Asp
 Thr 58Glu + Asn 96Glu + Gly 99Asp + Phe188Met +
 Gly218Gln
 Gly 62Glu + Asn162Ser + Val202Met + Thr212Asp +
 Leu216Met
 Gly 62Pro + Gly 99Gln + Ser104Glu + Ser131Asp +
 Tyr213Thr
 Gly 62Glu + Gly130Ser + Tyr205Glu + Ser215Glu +
 Leu216Asn
 Gly126Gln + Gly127Glu + Asn154Glu + Tyr213Pro +
 Leu216His
 Ser129Glu + Asn154Glu + Asn162Ser + Gly165Asp +
 Leu216Cys
 Thr 58Gly + Leu125Asn + Gly156Asp + Gln161Glu +
 Tyr205Gly
 Gly126Ser + Gly153Gln + Gly156Glu + Gln161Asp +
 Tyr205Pro
 Thr163Gln + Val202His + Tyr205Asp + Pro209Gly +
 Thr212Asp
 Gly 62Gln + Val202Met + Asn211Asp + Thr212Asn +
 Thr214Glu
 Thr102Gln + Gly126Pro + Thr212Asp + Ser215Glu +
 Leu216Asn
 Gly101Ser + Gln161Asn + Thr212Glu + Ser215Asp +
 Leu216Gln
 Leu125Gln + Ala186Glu + Val202Cys + Ser203Asp +
 Ser215Asp
 Ser129Glu + Ser155Glu + Thr163Asp + Ala186Ser +
 Tyr208Ser
 Thr 58Asn + Leu125Glu + Pro128Glu + Thr163Ser +
 Gly165Pro
 Asn 61Glu + Gly 62Asp + Gly130Ser + Asn211Asp +
 Thr212Gly
 Thr 65Gln + Asn154Asp + Ser215Glu + Leu216His +
 Asn217Glu
 Asp 59Glu + Ser 97Glu + Thr102Gln + Ser210Asp +
 Leu216Thr
 Gly153Glu + Ile164Thr + Val202Gln + Ser203Glu +
 Asn217Glu
 Val 94His + Leu125Cys + Gly126Ser + Ser155Glu +
 Gln161Glu
 Ser187Glu + Tyr205Thr + Thr212Asn + Tyr213Gly +
 Asn217Glu
 Gly 99Ser + Gly130Glu + Asn162Asp + Thr163Asp +
 Tyr205Asn
 Pro128Gln + Gly156Asp + Ala186Glu + Ser203Glu +
 Tyr213Gln
 Gly 62Asp + Gly156Asn + Ala186Pro + Thr212Glu +
 Leu216Asp

98

Gly126Ser + Tyr166Gly + Ser203Asp + Pro209Asp +
Ser215Asp
Tyr103Val + Pro128Asp + Gly153Asn + Asn162Ser +
Ser190Asp
Gly130Pro + Gly156Asn + Gln161Asp + Thr163Glu +
Ser187Glu
Leu125Pro + Ser203Asp + Pro209Gln + Thr212Glu +
Leu216Asp
Leu 95Asp + Gly126Glu + Pro128Gly + Gly159Gln +
Ala186His
Gly 62Ser + Ser190Asp + Thr214Gly + Ser215Asp +
Gly218Asp
Gly 64Asn + Tyr103Ile + Leu125Asp + Ser203Asp +
Asn217Glu
Gly126Ser + Gln161Asp + Asn162Ser + Thr214Glu +
Ser215Glu
Ile106Leu + Gly126Asp + Gly127Glu + Gly130Gln +
Val202Glu
Asp 59Glu + Thr102Ser + Ala105Gly + Gly159Glu +
Ser160Glu
Leu 95Asp + Asn 96Asp + Tyr166Leu + Val204Ala +
Gly218Glu
Gly130Glu + Ser131Asp + Gly153Gln + Ser158Glu +
Ala199Pro
Gly 60Glu + Asn 61Asp + Gln161Asn + Pro209Gln +
Ser215Glu
Thr 65Asn + Gly 99Gln + Asn154Asp + Pro209Asp +
Ser210Glu
Asn154Glu + Ser155Asp + Tyr213Thr + Ser215Glu +
Gly218Pro
Ser100Asp + Asn154Glu + Ser155Asp + Pro209Ser +
Thr214Gly
Gly 62Asn + Gly153Asp + Asn211Glu + Thr212Asp +
Gly218Gln
Tyr103His + Ser129Glu + Tyr205Pro + Asn211Asp +
Thr212Glu
Ser129Asp + Asn162Gln + Tyr205Leu + Asn211Asp +
Thr212Asp
Ser100Glu + Gly153Pro + Thr163Pro + Val202Glu +
Ser203Glu
Thr 58Asp + Thr 65Pro + Val202Glu + Ser203Asp +
Leu216Asn
Thr 65Pro + Gly126Asn + Ser157Glu + Ser158Asp +
Ser215Asp
Gly101Asn + Ser157Asp + Ser158Glu + Ser215Glu +
Leu216Ile
Asn 96Glu + Ser 97Glu + Gly159Asn + Phe188His +
Ser210Glu
Thr 65Asn + Ser 98Asp + Gly159Asn + Ser215Asp +
Leu216Asp
Asn 96Glu + Pro128Ser + Tyr205Cys + Ser215Glu +
Leu216Glu
Ile106Gly + Ser155Glu + Thr212Asn + Ser215Glu +

Leu216Asp
 Leu125Glu + Thr163Asn + Val202Thr + Ser215Asp +
 Leu216Glu
 Gly101Gln + Gly130Glu + Tyr205Ala + Ser215Glu +
 Leu216Glu
 Pro128Gly + Ser210Asp + Thr212Ser + Ser215Glu +
 Leu216Glu
 Ser104Asp + Ala105Glu + Met198Pro + Ser203Glu +
 Leu216Ser
 Ser 97Glu + Ser 98Glu + Ala105Gly + Gly153Asp +
 Asn217Gln
 Gly153Gln + Ser210Asp + Asn211Asp + Thr212Ser +
 Ser215Asp
 Thr 65Glu + Leu125Cys + Gly126Ser + Val202Glu +
 Ser215Asp
 Thr102Gln + Gly165Asn + Ser203Glu + Tyr205Glu +
 Pro209Asp
 Leu 95Glu + Asn 96Gln + Tyr205Glu + Pro209Ser +
 Ser215Glu
 Gly126Asn + Tyr205Asp + Ser210Asp + Ser215Glu +
 Leu216Met
 Gly 62Gln + Leu 95Pro + Ser158Asp + Tyr205Asp +
 Ser215Glu
 Asn 61Glu + Gly101Glu + Gly126Ser + Leu216Met +
 Thr219Ser
 Gly156Asp + Ala186Gln + Ser190Glu + Ser203Glu +
 Thr212Pro
 Gly 60Glu + Ser 97Glu + Gly 99Asn + Asn162Glu +
 Asn217Ser
 Thr 58Pro + Ser160Asp + Ser190Asp + Val202Asn +
 Thr212Asn
 Gln161Asn + Ser203Asp + Asn211Glu + Tyr213Cys +
 Ser215Glu
 Pro128Glu + Gly165Gln + Ala186His + Ser203Glu +
 Ser215Glu
 Gly101Pro + Thr132Ser + Ser158Glu + Ser203Glu +
 Ser215Glu
 Pro128Ser + Ala186Gly + Ser203Glu + Ser210Glu +
 Ser215Asp
 Asn 61Gln + Ser158Asp + Ala186Ser + Ser203Asp +
 Ser215Glu
 Leu 95Val + Ser100Asp + Tyr166Met + Ser203Glu +
 Ser215Asp
 Gly153Glu + Ala186Gln + Ser203Glu + Ser215Asp +
 Thr219Gly
 Ser160Asp + Ser203Glu + Val204Gln + Thr212Pro +
 Ser215Glu
 Thr 58Pro + Leu 95His + Ser155Asp + Ser203Asp +
 Ser215Asp
 Asn154Gln + Ser157Asp + Ser203Glu + Thr212Ser +
 Ser215Glu
 Leu 95Gln + Ser100Asp + Ser203Glu + Tyr205His +
 Ser215Glu

100

Asn154Asp + Tyr166His + Ser203Asp + Tyr205Ala +
Ser215Glu
Ser 98Asp + Ser203Glu + Asn211Ser + Ser215Asp +
Leu216Ser
Asn162Glu + Ser203Asp + Tyr208Asn + Thr212Ser +
Ser215Asp
Gln161Asp + Thr163Gln + Ser203Glu + Ser215Glu +
Leu216Thr
Leu 95Cys + Ser129Glu + Ser203Asp + Asn211Gln +
Ser215Asp
Thr132Asn + Asn154Glu + Ser203Asp + Thr212Asn +
Ser215Asp
Pro128Gln + Ser131Asp + Gly156Asn + Ser203Glu +
Ser215Asp
Thr 58Glu + Asn 61Ser + Gly 99Pro + Ser203Glu +
Ser215Asp
Ser100Asp + Gly153Ser + Ser203Asp + Tyr205Val +
Ser215Glu
Ala105Glu + Ser203Asp + Thr212Asn + Ser215Glu +
Gly218Asn
Thr102Asn + Ser104Asp + Gly127Asn + Ser203Asp +
Ser215Asp
Gly 62Asp + Leu125Thr + Gly153Glu + Gly165Ser +
Ser190Glu
Gly153Glu + Phe188His + Ser190Glu + Ser203Glu +
Leu216Gly
Thr 58Asp + Phe188Asn + Ser203Glu + Tyr205Leu +
Asn217Asp
Thr 65Gly + Thr102Ser + Gly126Asp + Thr212Ser +
Gly218Asp
Asp 59Glu + Thr163Glu + Ser190Glu + Thr212Gln +
Leu216Ser
Tyr166Val + Ser203Glu + Ser210Asp + Thr212Ser +
Leu216Asp
Gly159Asp + Ser203Glu + Thr207Pro + Thr212Asn +
Leu216Asp
Ser157Asp + Asn162Gln + Gly165Gln + Ser203Glu +
Leu216Asp
Leu 95Thr + Ser131Glu + Val202Ser + Ser203Asp +
Leu216Glu
Ser100Asp + Gly127Asn + Ser203Asp + Tyr208Val +
Leu216Asp
Thr102Gly + Ser129Glu + Ser203Asp + Tyr205Ala +
Leu216Asp
Thr 65Gln + Ser 98Asp + Thr102Pro + Gly126Asp +
Gly165Asp
Thr 65Asn + Gly127Glu + Ser129Asp + Tyr205Ile +
Ser215Glu
Gly 60Asn + Ser203Glu + Tyr205Val + Ser210Asp +
Thr212Glu
Val 94Cys + Ser 98Glu + Pro209Ser + Ser210Asp +
Thr212Glu
Gly 60Gln + Tyr103Asn + Pro128Glu + Ser157Asp +

101

Gly159Asp
 Ser 98Glu + Ser100Asp + Ser203Glu + Thr212Gln +
 Leu216Thr
 Gly 60Glu + Thr 65Asp + Thr132Gln + Asn154Ser +
 Leu216Asp
 Asn 96Asp + Ser100Asp + Thr102Pro + Tyr205Asp +
 Thr212Gln
 Thr102Gly + Phe188His + Gly201Asn + Tyr205Glu +
 Gly218Asp
 Asn154Gln + Ser155Asp + Tyr205Gln + Pro209Glu +
 Thr212Asp
 Tyr103Val + Gly153Asn + Pro209Asp + Thr212Asp +
 Gly218Glu
 Thr 65Glu + Ile106Met + Leu125His + Tyr205Leu +
 Ser215Asp
 Leu125Met + Gly156Asp + Tyr166Ser + Ser187Glu +
 Ser215Asp
 Thr 58Glu + Gly 60Glu + Ser131Glu + Tyr205Val +
 Gly218Pro
 Thr 58Asp + Gly 60Asp + Asn 61Ser + Asn162Gln +
 Ser203Asp
 Ser157Glu + Ser203Asp + Tyr205Asp + Thr212Ser +
 Leu216Cys
 Thr 58Asn + Thr102Gln + Ser155Glu + Ser157Asp +
 Thr212Glu
 Ala105Gln + Ser155Asp + Ser157Asp + Ser203Asp +
 Thr212Gly
 Thr163Glu + Val202Asp + Tyr205Cys + Leu216Pro +
 Gly218Glu
 Thr 58Gln + Asn 61Glu + Ile106Thr + Pro209Asp +
 Ser215Asp
 Gly 62Glu + Ser 97Glu + Asn154Ser + Tyr205Asp +
 Tyr213Val
 Leu125Gln + Gly130Asp + Leu216Glu + Gly218Asp +
 Thr219Gly
 Gly126Ser + Ser131Glu + Tyr205Ala + Leu216Asp +
 Gly218Glu
 Ile106Ala + Ser160Glu + Asn162Glu + Ala186Gly +
 Ser203Asp
 Asp 59Glu + Thr 65Asp + Asn162Asp + Leu216Ile +
 Asn217Ser
 Asn 96Gln + Thr102Pro + Gly127Glu + Ala186Pro +
 Ser190Asp
 Gly 99Asn + Leu125Thr + Ser157Glu + Gly165Glu +
 Thr212Gln
 Val 94His + Gly126Asn + Ser157Glu + Gly165Glu +
 Tyr205Val
 Asp 59Glu + Asn 61Ser + Val202Asn + Thr207Pro +
 Asn211Asp
 Asp 59Glu + Tyr103Ala + Ser155Glu + Pro209Glu +
 Thr212Asn
 Gly101Gln + Gly127Glu + Gly153Ser + Asn154Glu +
 Ser158Asp

Ser 97Glu + Thr212Ser + Ser215Asp + Leu216Cys + Asn217Glu
Gly 99Gln + Ser157Glu + Ser215Asp + Leu216Ser + Asn217Asp
Ser131Glu + Gly159Asn + Tyr205Met + Ser215Glu + Asn217Glu
Val 94Ala + Gly101Asp + Gly156Gln + Ser215Asp + Asn217Asp
Asn 61Asp + Asn 96Glu + Pro209Ser + Ser215Glu + Leu216Ala
Asn 61Glu + Asn 96Glu + Gly127Ser + Ser203Glu + Thr214Ser
Gly 99Pro + Ser100Asp + Thr102Glu + Ala105Gly + Asn211Glu
Ser155Glu + Ala186Pro + Tyr205Asp + Leu216Cys + Gly218Glu
Phe188Asp + Ser203Asp + Tyr213Ser + Thr214Glu + Gly218Asn
Ser129Asp + Thr163Gly + Gly165Glu + Ser203Asp + Tyr205Ile
Ser104Asp + Ser158Glu + Asn162Glu + Val202Gln + Leu216Val
Ser 98Asp + Ser158Asp + Asn162Glu + Leu216Ile + Thr219Gly
Pro128Gly + Ser158Asp + Asn162Glu + Val202Gln + Asn217Glu
Thr102Glu + Ala105Asn + Ser129Asp + Tyr208Thr + Leu216Gln
Thr 65Asn + Ser157Asp + Asn162Glu + Ser203Asp + Tyr205Gly
Ala105Gly + Ser157Asp + Ser160Glu + Ala186His + Ser210Asp
Ser 98Glu + Gly153Asp + Val202Ser + Tyr205Asn + Gly218Asp
Gly 62Asp + Gly 64Asn + Gly 99Pro + Gly127Asp + Gly153Glu
Thr 58Ser + Val 94Met + Ser104Glu + Gly153Asp + Tyr166Asp
Asn 61Asp + Thr 65Gly + Ala186Gln + Ser210Asp + Thr214Asp
Gly127Asn + Val202Asp + Ser210Glu + Ser215Glu + Asn217Ser
Gly 62Asn + Gly101Asp + Tyr103Met + Ser104Glu + Ser203Glu
Gly159Asp + Asn162Glu + Ile164Ala + Thr212Asn + Ser215Asp
Ser 98Asp + Gly101Glu + Asn154Gln + Tyr205Gln + Ser210Asp
Leu 95Glu + Tyr166Asn + Phe188Met + Ser203Glu + Thr214Glu
Gly101Pro + Ser129Glu + Ser203Asp + Thr214Glu + Gly218Gln
Asn 96Gln + Asn162Glu + Gly165Glu + Val202Asp + Thr214Glu

103

Tyr205Met
 Thr 65Gln + Val 94Asp + Ser104Glu + Leu125Asn +
 Thr212Asp
 Thr102Pro + Ser155Asp + Ser187Glu + Tyr205Val +
 Ser215Glu
 Asn 96Glu + Thr102Gln + Ala186Asp + Phe188Gln +
 Ser190Asp
 Thr 58Glu + Asn 61Glu + Gly 64Asn + Ala105Pro +
 Asn217Glu
 Val 94Ala + Gly126Asp + Asn154Glu + Tyr166Pro +
 Thr212Glu
 Thr 58Asn + Asn 61Ser + Gly130Glu + Phe188Glu +
 Asn217Asp
 Thr 58Asp + Ser 97Asp + Ile106Leu + Gly153Gln +
 Gly156Asp
 Thr 58Asp + Ser 97Glu + Thr102Pro + Ala105Ser +
 Ser158Asp
 Tyr103Glu + Gly127Glu + Asn154Gln + Ser157Asp +
 Thr163Ser
 Asn 96Gln + Pro128Glu + Gly130Gln + Ser131Glu +
 Ser157Asp
 Gly127Glu + Gly153Asn + Pro209Asp + Thr214Glu +
 Thr219Ser
 Ser100Asp + Ile106Met + Pro128Asn + Ser190Asp +
 Gly218Glu
 Thr 58Asp + Asn 96Asp + Gly127Asp + Tyr166Ala +
 Ala186Ser
 Asn 96Glu + Thr163Pro + Phe188Asp + Gly201Gln +
 Val202Glu
 Ser 98Glu + Gly127Asp + Ser131Glu + Val202Ala +
 Thr214Ser
 Gly 99Asn + Gly127Glu + Ser131Glu + Gly156Gln +
 Asn211Glu
 Val 94Asp + Pro128Asp + Ser131Asp + Tyr205Thr +
 Leu216Ser
 Gly101Glu + Gly126Ser + Gly127Asp + Tyr205His +
 Ser215Asp
 Ser160Asp + Tyr205Glu + Asn211Gln + Thr212Asp +
 Thr219Gly
 Gly 62Asp + Gly127Gln + Gly153Pro + Ser187Glu +
 Leu216Glu
 Gly 60Asp + Gly127Asp + Gly159Pro + Ser210Asp +
 Gly218Asn
 Asn 61Ser + Gly 99Asp + Thr102Asp + Ser203Asp +
 Thr212Gln
 Gly126Pro + Ser160Asp + Tyr205Met + Thr212Glu +
 Ser215Asp
 Thr 65Ser + Gly127Asp + Thr132Ser + Thr212Asp +
 Ser215Asp
 Asn154Ser + Ser187Glu + Thr212Glu + Tyr213Gln +
 Ser215Glu
 Ser129Asp + Gly153Glu + Ser158Glu + Gly165Asn +
 Leu216Thr

104

Ile106His + Leu125Pro + Ser129Glu + Ala186Glu +
Ser203Glu
Gly 60Pro + Gly 62Gln + Tyr205Thr + Ser215Glu +
Thr219Glu
Asp 59Glu + Val 94Pro + Gly130Gln + Ser187Asp +
Thr219Asp
Gly153Ser + Ser155Glu + Ser160Asp + Asn162Ser +
Thr212Pro
Ser155Asp + Gln161Glu + Ala186Thr + Thr214Gly +
Ser215Glu
Ser104Asp + Gly130Asn + Ser187Glu + Thr212Gln +
Asn217Glu
Ala105Asn + Ser203Glu + Thr212Glu + Asn217Ser +
Thr219Asp
Leu125His + Gly165Gln + Phe188Asp + Ser203Glu +
Tyr205Leu
Asn 96Asp + Ser104Asp + Gly201Pro + Val202His +
Leu216Ser
Gly 62Asp + Tyr103Val + Ser190Glu + Asn211Glu +
Thr219Ser
Gly 62Asp + Ser190Glu + Val202Ser + Asn211Glu +
Thr214Gln
Ser100Asp + Pro128Asp + Tyr205Ile + Thr212Gln +
Leu216His
Thr132Gly + Gly156Asn + Gly159Glu + Ser190Asp +
Ser203Glu
Asn 96Ser + Ser 98Asp + Gly159Asp + Ser190Asp +
Leu216Met
Val 94Asp + Ser 98Glu + Thr132Asn + Phe188Cys +
Thr219Asp
Gly101Ser + Tyr103Glu + Gly153Glu + Tyr166His +
Ser187Asp
Gly 99Ser + Ser160Asp + Ser190Glu + Val202Glu +
Tyr205Ser
Asn 61Asp + Gly 99Gln + Ala186Glu + Thr212Gln +
Leu216Asp
Ala186His + Phe188Gln + Ser190Glu + Pro200Ser +
Asn217Asp
Asn 61Ser + Thr 65Asp + Ser129Glu + Tyr205Glu +
Tyr213Gly
Asn 61Asp + Gly 62Ser + Ser203Asp + Asn211Gln +
Tyr213Glu
Thr102Asn + Gly130Asp + Tyr166Ser + Tyr205Asp +
Gly218Asp
Asn 61Glu + Asn154Gln + Gln161Asn + Tyr205Asp +
Gly218Glu
Gly 99Glu + Gly127Asp + Thr163Pro + Tyr213Ile +
Asn217Glu
Gly101Gln + Gly126Asp + Ser131Asp + Gly156Asn +
Ser203Glu
Thr102Asp + Gly130Glu + Pro209Asn + Thr212Ser +
Ser215Asp
Thr 58Ser + Ser100Glu + Ser104Glu + Ile164Leu +

105

Ser215Glu
 Asp 59Glu + Ile106Val + Gly127Glu + Tyr166Asn +
 Ser190Glu
 Gly 99Ser + Pro128Asp + Gly156Asp + Tyr205Cys +
 Asn217Asp
 Thr 65Asp + Leu 95Glu + Gly 99Pro + Ser158Asp +
 Tyr205Gln
 Ser 97Asp + Ala105His + Ser155Glu + Tyr205Asn +
 Pro209Glu
 Gly 62Asn + Thr 65Asp + Ser 97Glu + Ser131Glu +
 Leu216Gly
 Gly130Pro + Gly153Glu + Asn154Gln + Gln161Asp +
 Pro209Asn
 Gly159Ser + Tyr166Glu + Tyr205Asp + Thr212Pro +
 Thr219Glu
 Gly 62Asp + Leu 95Cys + Ser100Glu + Thr163Pro +
 Ser187Asp
 Thr 58Gly + Gly159Glu + Ser187Asp + Ser203Glu +
 Thr219Asn
 Thr 58Pro + Tyr166Asp + Val202Met + Pro209Asp +
 Ser215Asp
 Leu 95Asp + Thr102Pro + Ala105Gly + Gly127Glu +
 Leu216Glu
 Pro128Glu + Gln161Asp + Phe188Gln + Ser203Glu +
 Asn217Gln
 Leu125Pro + Pro128Glu + Thr132Asn + Gln161Glu +
 Leu216Asp
 Ser 97Glu + Thr102Asp + Val202Thr + Ser215Glu +
 Gly218Ser
 Asp 59Glu + Ser100Asp + Phe188Gln + Thr212Ser +
 Ser215Glu
 Gly101Gln + Thr102Asn + Gly130Glu + Thr163Glu +
 Ser210Asp
 Asn 61Glu + Gly101Asn + Ser129Glu + Tyr205Thr +
 Leu216Glu
 Leu 95Glu + Ile164Val + Thr212Glu + Tyr213Gly +
 Leu216Asp
 Thr 58Gln + Gly126Glu + Gly156Gln + Thr212Asp +
 Leu216Asp
 Leu125Met + Gly126Ser + Ser131Asp + Thr212Asp +
 Leu216Asp
 Gly 60Asn + Asn 96Gln + Ser104Glu + Thr212Asp +
 Leu216Glu
 Thr 65Asp + Gly126Gln + Ser158Asp + Gly165Asp +
 Leu216Val
 Ser158Asp + Gly165Glu + Ser203Asp + Leu216Ser +
 Gly218Gln
 Gly101Asp + Leu125Gly + Pro128Glu + Ala186Gly +
 Thr219Gln
 Thr 65Glu + Asn 96Glu + Tyr103Val + Ser155Glu +
 Met198Ala
 Thr 65Asp + Asn 96Glu + Leu125His + Asn162Glu +
 Tyr166His

Asn 61Ser + Ser100Asp + Phe188Asp + Ser203Glu + Gly218Ser
Ser104Glu + Gly156Gln + Phe188Asp + Ser203Glu + Thr212Gly
Gly 99Asp + Tyr205Asp + Pro209Asp + Thr212Pro + Tyr213Met
Thr102Asp + Asn154Ser + Tyr166Glu + Ser203Asp + Gly218Ser
Gly101Glu + Ser129Asp + Ser157Asp + Thr214Ser + Leu216Asn
Asp 59Glu + Gly101Asp + Gly127Ser + Gly159Pro + Gln161Glu
Gly 60Asn + Gly127Asp + Thr132Asp + Asn162Gln + Ser203Glu
Thr 58Asn + Gly 60Asp + Gly130Glu + Val202Ser + Thr212Glu
Ser100Asp + Tyr166Asp + Ala186His + Tyr205His + Leu216Thr
Ser 97Asp + Gly101Ser + Ala105Glu + Tyr205Met + Ser215Glu
Ser 97Glu + Ala105Glu + Leu125Ala + Ser158Asp + Tyr205Cys
Ser100Glu + Ile106His + Gly127Ser + Ser158Glu + Tyr166Glu
Thr102Asn + Gly130Ser + Ser131Asp + Asn211Asp + Ser215Glu
Gly127Gln + Ser131Glu + Asn211Asp + Tyr213His + Ser215Glu
Ser129Glu + Ser158Glu + Val202Gly + Ser215Asp + Leu216Gln
Leu125Pro + Pro128Ser + Ser157Glu + Ser203Glu + Gly218Ser
Thr 65Gln + Gln161Glu + Ser210Glu + Thr212Gln + Ser215Glu
Val 94Met + Gly101Glu + Ala105Asn + Ala186His + Thr212Asp

Multi-loop Sextuple Mutation Variants	
Asn 61Ser + Gly 64Gln + Gly101Asp + Thr132Gly + Gly165Gln + Tyr205Pro	
Asn 96Ser + Gly101Ser + Ala105Gly + Asn154Glu + Tyr166Thr + Tyr205His	
Val 94Ala + Leu 95Thr + Gly126Gln + Pro209Ser + Ser215Asp + Leu216Gly	
Thr 65Gln + Leu125Asn + Asn162Asp + Tyr205Gln + Pro209Gly + Leu216Met	
Thr163Gln + Tyr205Cys + Thr212Pro + Leu216Met + Asn217Gln + Gly218Gln	
Gly 60Gln + Val 94Cys + Asn 96Ser + Gly127Pro + Gly159Asp + Asn162Ser	

Gly 99Ser + Gly126Asn + Gly127Pro + Gly153Asp +
Gly159Pro + Ile164Ala
Val 94Cys + Pro128Asp + Gly156Ser + Val202Thr +
Tyr205Ala + Leu216Ala
Asn 61Gln + Ala105Thr + Gly153Gln + Gly156Gln +
Tyr166Ile + Asn211Gln
Gly 60Asn + Ser104Asp + Ala105Asn + Gln161Ser +
Thr163Ser + Tyr205Ala
Gly 64Ser + Val 94Asn + Gly101Asn + Leu125Asn +
Gly130Asp + Leu216Cys
Gly159Pro + Gly165Pro + Ala186Thr + Gly201Pro +
Thr212Pro + Leu216Ser
Gly 99Asn + Gly159Pro + Ser203Glu + Tyr205Leu +
Asn211Gln + Thr212Ser
Thr 58Gln + Leu125Pro + Thr132Asp + Gly153Asn +
Tyr213Ala + Leu216Gly
Ile106Ala + Asn154Glu + Asn162Gln + Met198Thr +
Ala199Asn + Tyr205Asn
Asp 59Glu + Gly159Ser + Gly165Asn + Phe188Thr +
Pro200Gln + Asn217Gln
Gly 99Ser + Gly126Asn + Thr132Gly + Ser187Asp +
Val202Ser + Tyr205His
Tyr103Gln + Gly130Pro + Ser155Glu + Gly159Asn +
Tyr205Pro + Thr212Ser
Gly 60Pro + Val 94Ser + Ser100Glu + Tyr103Leu +
Tyr205Ser + Thr219Pro
Thr 58Gly + Leu125Ala + Val202Cys + Tyr205Asn +
Ser215Asp + Leu216Ala
Val 94Thr + Ser104Glu + Gly130Pro + Thr212Pro +
Leu216Asn + Gly218Ser
Ser 97Glu + Thr132Gly + Pro200Gly + Tyr205Ala +
Asn211Ser + Tyr213Gln
Gly 62Asn + Leu 95Asp + Tyr166Val + Tyr205Cys +
Asn211Ser + Leu216Val
Gly156Asn + Gly165Pro + Gly201Asn + Tyr213Ala +
Thr214Gln + Asn217Glu
Gly 62Ser + Ser 98Asp + Gly126Ser + Tyr205Pro +
Thr212Ser + Tyr213Thr
Ala105Gln + Gly127Ser + Gln161Asp + Thr163Gly +
Tyr205Cys + Leu216Pro
Val 94Ala + Gly130Gln + Ala186His + Ser203Asp +
Tyr205Cys + Thr214Gln
Thr 65Gln + Leu125Gly + Ser187Asp + Phe188Gln +
Val202Asn + Thr212Asn
Thr 58Ser + Ile106Pro + Thr132Gly + Gln161Asn +
Tyr205Asn + Thr212Asp
Ser100Asp + Ala105Thr + Leu125Pro + Tyr205Ile +
Thr214Gly + Leu216Cys
Thr102Ser + Gly127Pro + Pro128Gly + Ser203Glu +
Tyr208His + Tyr213Thr
Thr102Ser + Gln161Glu + Asn162Asp + Thr163Gln +
Ala186His + Phe188Ile
Gly 62Pro + Pro128Asp + Ser129Asp + Gly156Asn +

Tyr213Cys + Gly218Asn
Thr 58Gln + Gly127Pro + Ala186Gln + Val202Asp +
Ser203Asp + Leu216Val
Gly156Ser + Phe188Gly + Ser203Glu + Tyr213Gln +
Ser215Asp + Leu216Asp
Gly101Gln + Ala105His + Asn162Gln + Ala186Gly +
Ser203Glu + Ser215Glu
Thr 58Pro + Thr163Asn + Ser203Glu + Tyr205Ile +
Ser215Glu + Leu216Gln
Thr 65Asn + Gly127Glu + Gly156Asn + Tyr166Glu +
Phe188Met + Thr212Pro
Leu 95Asp + Ser100Glu + Gly101Gln + Ile106Gln +
Ile164Leu + Val202Ala
Tyr103Pro + Gly127Glu + Ser129Asp + Gly130Glu +
Asn162Gln + Leu216His
Gly 60Gln + Gly165Ser + Ser203Glu + Tyr205Asp +
Thr214Glu + Ser215Asp
Leu 95Asn + Leu125Pro + Ser203Asp + Tyr205Thr +
Thr214Glu + Ser215Asp
Gly 62Asn + Tyr103Ile + Leu125Cys + Gly126Glu +
Gly153Asp + Tyr166Cys
Gly 99Ser + Pro128Glu + Gly130Asp + Phe188Asn +
Leu216Gly + Gly218Pro
Asp 59Glu + Gly 60Asp + Thr 65Asp + Asn162Ser +
Thr163Gln + Tyr166Gln
Phe188Glu + Tyr205Thr + Thr212Asn + Tyr213Gly +
Asn217Glu + Gly218Asp
Gly 62Gln + Ser203Glu + Tyr205Ser + Thr212Ser +
Ser215Asp + Gly218Asp
Ser187Glu + Ser190Asp + Tyr205Val + Pro209Ser +
Thr214Ser + Thr219Ser
Thr 58Ser + Asp 59Glu + Asn 96Asp + Asn154Ser +
Ala186His + Leu216Ser
Thr 65Gly + Thr102Gly + Leu125Asp + Gly165Asp +
Ala199Ser + Val202Thr
Leu125Asn + Ser158Glu + Gly159Asp + Ser190Glu +
Thr212Gly + Leu216Val
Thr 58Pro + Asn 61Asp + Val 94Glu + Asn 96Asp +
Gly126Ser + Gly165Pro
Ala186Glu + Val202Glu + Tyr205Ala + Tyr213Val +
Ser215Asp + Asn217Glu
Tyr103Val + Ser203Glu + Tyr205Asp + Thr212Glu +
Ser215Asp + Leu216Ile
Gly 62Ser + Ile106Leu + Pro128Ser + Asn154Asp +
Ser157Glu + Thr214Ser
Val 94Asp + Gly 99Asp + Pro128Gln + Ala199Gln +
Tyr205Gln + Leu216Ile
Asn 61Gln + Thr102Gln + Gly126Asp + Ser155Glu +
Ser190Asp + Val202Ser
Leu125Asp + Gly126Asp + Ala186Ser + Tyr205Gln +
Gly218Glu + Thr219Pro
Asn 61Glu + Ser100Asp + Thr102Asn + Gly165Gln +
Phe188Leu + Val202His

Tyr103Gly + Tyr205Leu + Thr212Glu + Ser215Glu +
 Leu216Glu + Asn217Asp
 Gly 99Pro + Ser104Glu + Ile106Thr + Ser131Asp +
 Gly201Gln + Thr214Ser
 Leu 95Ile + Gly126Glu + Gly153Glu + Ser187Asp +
 Phe188Asp + Asn211Gln
 Gly 62Asn + Leu 95Ser + Gly153Ser + Tyr205Asp +
 Thr212Glu + Leu216Asp
 Ala105Gly + Ile106Cys + Ser155Asp + Tyr205Asp +
 Ser215Asp + Leu216Asp
 Tyr103Ala + Ser155Glu + Tyr166Pro + Ser203Glu +
 Tyr205Glu + Ser215Glu
 Pro128Gly + Asn162Glu + Gly165Asn + Ser203Glu +
 Tyr205Glu + Ser215Glu
 Gly 99Pro + Leu125Gln + Gly130Asn + Ala186Asp +
 Phe188Ser + Ser203Glu
 Ile106Asn + Gly156Glu + Gly159Asn + Phe188Asp +
 Ser215Glu + Gly218Asp
 Gly156Pro + Asn162Asp + Ser203Glu + Tyr205Gln +
 Ser215Asp + Asn217Glu
 Gly153Asn + Ser155Glu + Ser157Asp + Ser190Glu +
 Thr212Asp + Leu216Ile
 Leu 95Glu + Gly 99Glu + Leu125Glu + Pro128Gly +
 Asn154Glu + Thr219Gly
 Gly 60Asp + Ile106Met + Asn162Ser + Ser203Glu +
 Tyr205Asp + Leu216Asp
 Ser 97Asp + Asn162Ser + Thr163Gln + Ser203Glu +
 Tyr205Asp + Leu216Asp
 Leu125Ile + Gly159Glu + Ser203Glu + Thr214Glu +
 Ser215Glu + Leu216Gln
 Gly156Ser + Ser158Glu + Tyr205Asp + Tyr213Glu +
 Ser215Asp + Leu216Cys
 Gly 62Gln + Asn154Asp + Ser155Asp + Ser160Asp +
 Gly165Glu + Tyr205Met
 Gly 64Pro + Ala186Glu + Ser187Asp + Ser203Glu +
 Tyr205His + Ser215Glu
 Tyr103Asp + Tyr166Ser + Ala186Asn + Val202Glu +
 Ser203Asp + Tyr205Glu
 Leu 95Gln + Gly126Gln + Gly127Asp + Pro128Ser +
 Ser155Asp + Ser158Glu
 Asp 59Glu + Ile106His + Tyr205Ala + Tyr213Glu +
 Ser215Glu + Leu216Asp
 Ser158Asp + Ser160Glu + Thr163Asp + Pro209Gln +
 Thr212Ser + Ser215Glu
 Gly 62Asp + Tyr103Met + Val202Ser + Asn211Glu +
 Thr214Gln + Leu216Ala
 Asp 59Glu + Ser 97Asp + Ser 98Asp + Gly153Ser +
 Gln161Asn + Ser203Glu
 Leu125Asn + Gly153Glu + Ser157Asp + Thr163Glu +
 Val202Asp + Thr214Pro
 Leu 95Glu + Ser100Asp + Leu125Asp + Pro128Asn +
 Ser131Asp + Tyr205Gly
 Gly126Gln + Ser158Glu + Ser203Asp + Ser215Glu +

Leu216Thr + Gly218Asp
Gly101Ser + Pro128Asp + Ser158Glu + Gly159Asp +
Ala186Gly + Phe188Val
Ser187Glu + Ser203Asp + Tyr205Cys + Pro209Gln +
Ser215Asp + Leu216Ser
Gly 62Ser + Ser187Glu + Met198Gln + Ser203Glu +
Ser215Asp + Leu216His
Thr132Asn + Gly165Pro + Ser187Glu + Ser203Glu +
Tyr205His + Leu216Asp
Asn 96Ser + Ser131Glu + Gly159Asp + Ser160Asp +
Thr214Gln + Leu216Gly
Gly130Glu + Ser158Asp + Gly159Glu + Thr163Gln +
Tyr205Thr + Thr207Asn
Gly130Asp + Ser131Glu + Gly165Pro + Ser190Glu +
Val202Gly + Asn217Ser
Leu 95Thr + Gly130Glu + Ser131Glu + Tyr205Ala +
Ser210Glu + Gly218Gln
Thr 58Gly + Ala105Glu + Ile106Gly + Tyr205Val +
Ser215Glu + Leu216Asp
Thr 58Asp + Asp 59Glu + Leu125Asn + Ser203Glu +
Tyr205Cys + Thr212Ser
Thr 58Glu + Asp 59Glu + Tyr103Asn + Pro209Gln +
Thr212Asn + Ser215Glu
Thr 65Ser + Ser 97Asp + Ser 98Glu + Ser104Glu +
Thr163Gln + Leu216Ser
Ser 97Glu + Ser 98Glu + Gly101Gln + Thr212Asn +
Leu216His + Gly218Glu
Gly101Asp + Thr102Glu + Ile106Val + Thr163Pro +
Tyr205Glu + Ser215Asp
Gly 60Pro + Ser129Glu + Val202Glu + Thr214Asp +
Ser215Asp + Leu216Ile
Gly 62Asp + Gly 99Glu + Tyr205Asn + Asn211Asp +
Tyr213Met + Thr214Asp
Thr 58Gly + Gly127Glu + Ser131Glu + Ser155Asp +
Gly165Pro + Tyr205Cys
Gly159Asp + Tyr166Gly + Tyr205Asp + Tyr213Asn +
Ser215Asp + Leu216Thr
Gly 62Glu + Gly101Ser + Thr132Gly + Ser203Glu +
Asn211Asp + Tyr213Glu
Asn 96Asp + Ser 97Asp + Leu125Pro + Gly127Gln +
Ser203Asp + Ser215Asp
Asp 59Glu + Val 94Glu + Ser 98Asp + Ser104Glu +
Leu125Asn + Tyr213Gly
Ser155Asp + Gly156Gln + Ser190Asp + Tyr205Glu +
Thr212Ser + Leu216Ala
Val 94Met + Gly 99Pro + Ile106Thr + Ser155Asp +
Ser190Asp + Thr212Glu
Gln161Ser + Thr163Glu + Val202Ala + Ser203Glu +
Asn211Ser + Ser215Asp
Gly156Glu + Ala186Asn + Ser203Asp + Thr212Gln +
Ser215Glu + Leu216Gln
Ser 98Glu + Ser203Asp + Val204Cys + Ser215Asp +
Asn217Ser + Thr219Gln

111

Asn 61Gln + Thr 65Ser + Ser131Asp + Ser203Asp +
 Ser215Glu + Leu216Cys
 Ile106Val + Thr132Ser + Ser158Glu + Ser203Glu +
 Thr214Ser + Ser215Glu
 Val 94Thr + Gly153Glu + Ala186Gln + Ser203Glu +
 Ser215Asp + Thr219Gly
 Val 94Met + Thr102Asp + Pro128Glu + Ser131Asp +
 Gly165Asn + Thr219Ser
 Ser155Asp + Gln161Asp + Ser190Glu + Ser203Glu +
 Thr212Ser + Leu216Cys
 Asn 61Glu + Ser100Asp + Thr132Pro + Ala186Gly +
 Ser210Glu + Thr212Glu
 Ala105Glu + Thr132Ser + Gly201Ser + Ser203Glu +
 Thr212Pro + Asn217Asp
 Thr 58Gly + Gly 62Pro + Ala105Asp + Ser203Asp +
 Tyr208Ile + Leu216Glu
 Asn 96Asp + Gly 99Asp + Ile106Gly + Asn154Gln +
 Gln161Asn + Thr214Glu
 Asn 96Glu + Gly 99Glu + Thr102Asn + Gly127Ser +
 Ser203Glu + Asn217Gln
 Asp 59Glu + Gly127Asp + Ser129Asp + Tyr213His +
 Leu216Val + Thr219Gly
 Thr 65Gln + Tyr205Ser + Pro209Gln + Ser210Glu +
 Thr212Glu + Thr219Asp
 Thr 58Asp + Asn 96Ser + Ser158Glu + Gln161Asp +
 Gly201Gln + Tyr205Val
 Asp 59Glu + Asn 61Glu + Tyr166His + Phe188Val +
 Thr212Gly + Ser215Asp
 Thr102Glu + Gly126Pro + Gly127Asn + Val202Asn +
 Pro209Asp + Asn211Glu
 Asn 61Gln + Gly159Gln + Ser187Asp + Pro209Asp +
 Asn211Asp + Gly218Ser
 Ser 97Asp + Ser 98Glu + Gly156Asp + Ser158Glu +
 Thr163Gln + Pro209Gln
 Gly 60Gln + Gly130Asn + Ser155Glu + Phe188Glu +
 Tyr205Asn + Ser215Glu
 Val 94Asp + Ile106Glu + Pro128Asp + Ser131Asp +
 Tyr205Thr + Leu216Ser
 Gly 60Pro + Ser 98Asp + Ser100Glu + Pro128Gly +
 Ser157Asp + Gly159Asp
 Thr 58Asn + Gly 99Asp + Thr102Gln + Pro209Asp +
 Thr212Glu + Tyr213Met
 Thr 65Glu + Tyr103Gln + Gly156Pro + Tyr166Asn +
 Phe188Tyr + Ser215Glu
 Val 94Asp + Gly101Glu + Ile164Gly + Ala186Pro +
 Ser203Asp + Leu216Pro
 Gly 60Asn + Asn 61Glu + Gly153Ser + Pro209Asp +
 Asn211Asp + Ser215Glu
 Thr132Gly + Ser157Glu + Ser187Asp + Ser203Asp +
 Tyr205Met + Ser215Glu
 Asn 61Asp + Asn 96Glu + Gly156Asn + Ser203Glu +
 Thr212Gln + Ser215Glu
 Gly 99Pro + Ser158Glu + Ser160Glu + Tyr205His +

112

Thr212Ser + Ser215Glu
 Gly101Pro + Ser157Glu + Ser203Asp + Tyr205Asp +
 Thr212Ser + Leu216Cys
 Val 94Ala + Pro128Ser + Gly156Gln + Ser190Asp +
 Ser203Asp + Tyr205Glu
 Ile106Met + Leu125Ile + Ser160Asp + Ser203Asp +
 Tyr205Glu + Leu216Ser
 Leu 95Glu + Ser 97Glu + Thr102Glu + Gly159Asp +
 Tyr205Leu + Leu216Ala
 Leu125Cys + Gly126Ser + Gly159Asp + Gln161Glu +
 Ser215Glu + Asn217Glu
 Ser100Glu + Thr102Gly + Ala105Thr + Ser155Glu +
 Ser157Asp + Asn162Ser
 Ser 98Glu + Gly127Asn + Ser157Asp + Gln161Asn +
 Asn162Asp + Gly165Glu
 Asn 61Gln + Ser100Asp + Ser104Glu + Ile106Asp +
 Ser215Asp + Leu216Ala
 Ser131Glu + Gly159Asn + Val202Cys + Ser215Asp +
 Leu216Asp + Thr219Glu
 Leu125Cys + Gln161Asp + Asn162Glu + Ser203Glu +
 Leu216Thr + Gly218Glu
 Gly101Asp + Ala105Asp + Gly126Asn + Gln161Ser +
 Ala186Pro + Thr214Glu
 Ser100Glu + Thr102Glu + Tyr103Thr + Gly126Asp +
 Ser160Glu + Leu216Met
 Gly127Gln + Asn154Ser + Ser157Glu + Thr163Glu +
 Ser215Glu + Asn217Glu
 Leu 95Ile + Asn 96Glu + Thr102Gly + Tyr103Glu +
 Gly126Glu + Gly165Glu
 Ser129Asp + Ser131Asp + Thr132Asn + Gly159Ser +
 Tyr213Gln + Thr214Glu
 Gly101Pro + Gly126Glu + Ser129Asp + Tyr205Glu +
 Ser215Glu + Leu216Gly
 Leu 95Met + Pro128Gly + Ser129Glu + Gly153Glu +
 Thr163Gly + Leu216Ser
 Gly 60Glu + Val202Met + Tyr205Ile + Thr212Glu +
 Ser215Glu + Leu216Asp
 Thr 58Ser + Ser158Asp + Gln161Asn + Thr163Asp +
 Leu216Asp + Gly218Asp
 Ile106Ser + Ser158Asp + Ser187Asp + Ser190Asp +
 Tyr205Met + Ser215Asp
 Ser187Glu + Val202Gln + Tyr205Asn + Thr214Glu +
 Leu216Glu + Gly218Ser
 Asp 59Glu + Ser100Asp + Gly126Pro + Gly127Asp +
 Tyr166Glu + Thr212Asn
 Asp 59Glu + Gly 62Pro + Gly127Asn + Ala186Pro +
 Val202Glu + Pro209Asp
 Asp 59Glu + Asn 96Glu + Gly101Glu + Thr102Gln +
 Pro128Ser + Leu216Glu
 Gly159Asp + Asn162Asp + Ala186Gln + Val202Gln +
 Ser203Glu + Ser215Asp
 Gly159Ser + Ala186Asn + Ser210Asp + Tyr213Pro +
 Ser215Asp + Asn217Asp

113

Ser104Glu + Ser131Asp + Thr132Gln + Asn162Ser +
Gly165Asp + Tyr208Cys
Gly156Glu + Ser158Asp + Thr163Gly + Ser203Asp +
Thr212Asn + Leu216Ser
Gly 64Pro + Thr 65Glu + Ala105Asp + Val202Thr +
Ser215Asp + Leu216Glu
Thr 58Pro + Gly 99Ser + Ser100Asp + Gly101Ser +
Thr102Asp + Asn211Glu
Gly 62Glu + Asn 96Ser + Ser100Asp + Gly101Ser +
Ser210Asp + Tyr213Ala
Gly 60Pro + Gln161Asp + Gly165Asp + Phe188Val +
Ser190Glu + Ser210Glu
Gly153Gln + Ser155Asp + Gly159Asn + Pro209Glu +
Thr212Asp + Ser215Asp
Thr 58Gln + Thr102Glu + Ser129Glu + Gly130Asp +
Tyr205Ala + Ser215Asp
Tyr103Ser + Asn154Asp + Ser187Glu + Val202Asn +
Ser203Glu + Leu216Ser
Leu125Pro + Pro128Ser + Ser157Glu + Gln161Glu +
Ser203Glu + Gly218Ser
Ser 97Glu + Gly 99Asp + Ala105Ser + Leu125Asp +
Gly165Gln + Tyr205Asp
Thr 65Asp + Leu 95Cys + Ser 97Asp + Asn162Ser +
Thr212Glu + Ser215Asp
Ser 97Glu + Ser100Glu + Gly156Asn + Ser157Glu +
Ser160Glu + Tyr205Pro
Leu 95Asp + Ala105Asp + Ser157Glu + Val202His +
Tyr205Ile + Leu216Met
Leu 95His + Gly156Pro + Ser157Asp + Asn162Glu +
Ser187Glu + Leu216Asp
Ser100Glu + Leu125Gly + Gly127Glu + Ser203Asp +
Tyr208Val + Leu216Asp
Asn154Ser + Gly165Asp + Ser190Glu + Thr212Gly +
Ser215Asp + Gly218Pro
Thr 58Gln + Ser 98Glu + Gly153Asp + Val202Ser +
Tyr205Asn + Gly218Asp
Asn 61Gln + Thr132Gly + Gly156Glu + Ser158Glu +
Ser215Asp + Thr219Glu
Val 94Glu + Ser 97Glu + Ala105Asp + Gly165Pro +
Leu216Met + Thr219Asp
Val 94Glu + Ser 97Glu + Ala105Asp + Asn154Gln +
Gln161Asp + Ala186Asn
Gly130Gln + Ser155Glu + Ser158Glu + Ala186Gln +
Ser203Asp + Leu216Cys
Asn 61Gln + Ser160Asp + Ser203Glu + Val204Gln +
Thr212Glu + Ser215Glu
Thr102Glu + Ala105Asp + Ser131Glu + Ser203Glu +
Tyr205Ala + Thr214Asn
Thr 58Pro + Ser155Glu + Gly159Asn + Pro209Glu +
Thr212Glu + Gly218Glu
Asp 59Glu + Gly 99Asp + Gly126Ser + Met198Thr +
Thr212Asp + Gly218Pro
Thr132Ser + Asn154Ser + Ser157Asp + Val202Glu +

114

Ser215Glu + Leu216Gly
 Val 94Asp + Gly 99Asn + Ser100Asp + Gly130Ser +
 Asn154Gln + Ser215Asp
 Pro128Glu + Gly156Ser + Ser160Glu + Ser187Asp +
 Ser190Glu + Thr212Ser
 Asn 61Gln + Ser 98Glu + Ile106Cys + Ser203Asp +
 Tyr205Gly + Gly218Asp
 Ala105His + Ser158Glu + Ser187Glu + Ser203Glu +
 Tyr205Leu + Ser215Glu
 Asn 96Ser + Gly 99Gln + Gly153Glu + Ala186Glu +
 Tyr205Glu + Ser215Glu
 Asp 59Glu + Ser 98Glu + Gly153Glu + Ser155Glu +
 Phe188Pro + Leu216His
 Asn162Asp + Gly165Asp + Val204Gln + Tyr205Thr +
 Ser210Asp + Thr212Pro
 Gly101Pro + Gly156Asp + Tyr166Asp + Ser190Glu +
 Tyr205Asp + Thr212Asn
 Gly126Glu + Thr132Asp + Ser155Asp + Ser157Asp +
 Val202Gly + Tyr213Ser
 Gly 60Asp + Asn 61Asp + Asn154Glu + Ser158Glu +
 Gly165Gln + Tyr213Gln
 Gly 62Glu + Leu125His + Ser131Asp + Tyr166Leu +
 Ser203Glu + Ser215Asp
 Asp 59Glu + Val 94Asp + Thr102Gly + Ser203Glu +
 Tyr205Asp + Thr219Gly
 Ser104Glu + Tyr166Cys + Val202Thr + Thr212Asp +
 Ser215Asp + Asn217Glu
 Ser100Asp + Gly126Pro + Asn154Asp + Gly201Ser +
 Ser203Glu + Thr219Asp
 Gly101Asp + Pro128Asp + Ser155Asp + Ser187Asp +
 Thr207Gly + Thr212Gln
 Ile106Ser + Ser160Glu + Ser190Asp + Ser203Asp +
 Ser215Glu + Leu216Val
 Gly 99Gln + Gly153Asp + Ser160Asp + Phe188Leu +
 Ser190Asp + Ser210Asp
 Ser158Asp + Gly165Asn + Ser190Glu + Val202His +
 Tyr205Asp + Tyr213Leu
 Thr 65Asp + Leu 95Thr + Ser131Glu + Val202Ser +
 Ser203Asp + Leu216Glu
 Asn 61Glu + Gly165Ser + Met198Gln + Val202Glu +
 Tyr205Glu + Leu216Asn
 Val 94Ala + Gly126Asp + Asn154Glu + Tyr166Pro +
 Val202Gln + Thr212Glu
 Ser 97Glu + Leu125Glu + Ser155Glu + Gly159Asn +
 Tyr166Asp + Leu216Val
 Asn 96Glu + Ser 97Asp + Thr102Gln + Leu125Asp +
 Leu216Glu + Thr219Gln
 Tyr103Cys + Ser160Glu + Thr163Asp + Tyr208Ser +
 Leu216Met + Gly218Glu
 Val 94Cys + Leu125Glu + Ser155Asp + Gly156Asp +
 Gly201Gln + Ser215Glu
 Asn 61Ser + Thr 65Glu + Val 94Asp + Tyr103Pro +
 Ser215Asp + Asn217Asp

115

Gly 99Gln + Gly127Asp + Thr132Pro + Ser155Glu +
Ser187Glu + Ser203Asp
Ser 98Glu + Ser129Glu + Gly153Glu + Ser190Asp +
Tyr213Val + Thr214Pro
Asn 61Ser + Gly130Glu + Phe188Glu + Tyr205Asp +
Thr212Pro + Asn217Asp
Ser158Glu + Gly159Glu + Val202Ser + Tyr205Met +
Pro209Glu + Ser215Glu
Asn 61Asp + Gly 99Asn + Gly101Asp + Ser203Asp +
Tyr205Asp + Thr212Pro
Ser 98Asp + Ser100Asp + Ser104Glu + Gly153Glu +
Phe188Gly + Gly218Asn
Leu 95Val + Gly 99Asp + Tyr103Val + Ala105Asp +
Tyr205Asp + Ser215Asp
Asp 59Glu + Asn 61Asp + Ser160Glu + Pro200Asn +
Asn211Ser + Thr212Asp
Gly 62Glu + Thr 65Gly + Ser 97Asp + Thr102Pro +
Ser131Glu + Leu216Asp
Gly126Pro + Pro128Glu + Gly156Glu + Ser158Asp +
Tyr205Asn + Asn217Asp
Asp 59Glu + Gly130Asn + Ser155Asp + Ser210Asp +
Tyr213Gly + Gly218Asn
Asn 96Gln + Gly 99Glu + Ser100Asp + Pro128Asp +
Tyr205Cys + Asn217Glu
Pro128Gly + Ser129Asp + Asn162Glu + Gly165Asn +
Ser203Asp + Ser215Asp
Asn 96Ser + Ser100Glu + Tyr103Glu + Tyr205His +
Ser215Glu + Leu216Pro
Asn 96Glu + Ser 98Glu + Ala186Asp + Val202Asn +
Tyr213Met + Leu216Glu
Gly 99Asp + Ser158Asp + Asn162Asp + Thr163Asn +
Ser187Glu + Ala199Gly
Leu 95Ser + Ser 97Asp + Ser 98Glu + Gly165Glu +
Leu216Ile + Gly218Glu
Gly101Asp + Leu125Asp + Gln161Asn + Tyr205Asp +
Thr212Gly + Leu216Asn
Ser160Asp + Val202His + Ser203Asp + Thr212Asp +
Tyr213Val + Leu216Asp
Leu 95Val + Tyr103Glu + Ala105Glu + Thr163Ser +
Thr212Asp + Leu216Glu
Ser129Asp + Gly130Ser + Ser157Asp + Gly165Asp +
Tyr205Glu + Leu216Gln
Thr132Asn + Gly156Glu + Tyr166Thr + Ser203Glu +
Thr212Glu + Asn217Glu
Asn 61Gln + Ser 97Glu + Ser187Glu + Ser203Glu +
Pro209Gln + Ser215Glu
Gly101Gln + Ala105Asp + Ser187Glu + Ser203Asp +
Ser215Glu + Leu216His
Gly 99Asn + Ser104Asp + Thr132Gly + Ser187Asp +
Ser203Glu + Ser215Asp
Gly 62Asp + Asn 96Gln + Ser104Glu + Tyr205Gln +
Thr212Asp + Tyr213Val
Val 94Gly + Asn 96Asp + Ala105Glu + Asn162Asp +

Asn211Gln + Thr212Gln
Ser 97Glu + Ser187Asp + Ser203Glu + Tyr213Gln +
Leu216Glu + Asn217Gln
Gly 64Pro + Ser104Glu + Gly126Glu + Tyr166Ser +
Tyr205Glu + Ser215Glu
Gly130Asn + Thr132Asp + Ser187Glu + Pro209Gln +
Thr214Glu + Ser215Glu
Gly126Glu + Ser160Asp + Tyr166Met + Val202Thr +
Thr214Asp + Ser215Glu
Ser 98Asp + Gly101Ser + Gly130Asp + Ser158Glu +
Gly159Asp + Leu216Ala
Thr 58Glu + Tyr103Pro + Gly130Glu + Ser131Asp +
Ser158Glu + Ala199Pro
Gly 60Asp + Asn 61Asp + Ser131Asp + Gly153Ser +
Thr163Glu + Val202Cys
Gly 99Asp + Pro128Asn + Asn162Glu + Thr212Ser +
Leu216Glu + Asn217Glu
Gly 60Ser + Ser 98Asp + Asn154Asp + Ser155Glu +
Gly159Pro + Asn211Glu
Tyr103Pro + Ser104Glu + Leu125Cys + Val202Glu +
Ser203Glu + Pro209Glu
Gly101Gln + Ser104Asp + Leu125Val + Val202Asp +
Ser203Asp + Thr212Asp
Thr 65Asn + Ser 98Asp + Gly159Asn + Gly165Asp +
Ser215Asp + Leu216Asp
Gly127Glu + Gly165Pro + Tyr205Cys + Ser210Asp +
Ser215Glu + Leu216Glu
Val 94Ser + Ser 98Glu + Gly 99Asp + Gly156Asp +
Phe188Leu + Ser203Glu
Gly 62Asp + Gly101Glu + Ser160Glu + Gln161Asp +
Tyr205Met + Thr212Pro
Ser 97Asp + Ser 98Glu + Ala105His + Ser157Glu +
Thr212Ser + Ser215Asp
Gly 62Gln + Ser 97Glu + Ser 98Asp + Gly126Glu +
Gly130Pro + Thr212Glu
Val 94Glu + Ser 97Asp + Leu125Asn + Thr163Ser +
Ser210Asp + Leu216Ile
Leu 95Glu + Asn 96Gln + Gly159Glu + Tyr205Glu +
Pro209Ser + Ser215Glu
Ser129Glu + Asn154Ser + Thr212Glu + Ser215Asp +
Gly218Asp + Thr219Ser
Asp 59Glu + Gly127Gln + Val202Glu + Asn211Glu +
Thr212Ser + Ser215Asp
Ser104Asp + Ala105Pro + Ser187Glu + Ser203Glu +
Tyr205Asp + Tyr213Pro
Thr 58Pro + Asn 96Asp + Gly127Glu + Ser155Glu +
Asn162Ser + Thr212Ser
Thr 58Gly + Leu125Asn + Gly156Asp + Gln161Glu +
Tyr205Gly + Ser215Asp
Gly 60Ser + Gly156Asp + Pro209Ser + Thr214Asp +
Leu216Thr + Thr219Asp
Leu125His + Gly127Gln + Ser131Glu + Ser203Asp +
Asn211Asp + Ser215Glu

Thr 58Glu + Pro128Glu + Gly165Gln + Ala186His +
Ser203Glu + Ser215Glu
Ser131Asp + Ser158Glu + Ser203Asp + Tyr205His +
Ser215Asp + Leu216Gln
Thr 65Asn + Gly130Asp + Ser158Asp + Ser203Asp +
Pro209Ser + Ser215Glu
Gly 60Gln + Thr102Glu + Ser157Asp + Ser203Asp +
Ser215Asp + Thr219Gln
Gly 64Gln + Gly127Asp + Ser160Glu + Ser203Asp +
Ser215Glu + Gly218Gln
Ile106Leu + Thr132Asp + Thr163Asp + Ser203Glu +
Ser215Asp + Leu216Ile
Gly126Ser + Ser160Asp + Thr212Glu + Ser215Glu +
Leu216Val + Thr219Ser
Gly 60Pro + Gly101Asn + Ser158Glu + Tyr205Ile +
Thr212Asp + Ser215Glu
Gly 62Glu + Gly 99Ser + Val202Ser + Tyr205Asp +
Thr212Asn + Thr214Ser
Val 94His + Thr132Glu + Gly165Asp + Tyr166Cys +
Ser203Asp + Leu216Glu
Gly 62Asn + Ser131Asp + Ser157Glu + Gln161Asn +
Ser203Glu + Leu216Glu
Gly 60Pro + Val 94Asp + Ala105Glu + Gly127Pro +
Gln161Asp + Ser210Asp
Gly 62Gln + Ser 97Asp + Asn154Asp + Ser190Asp +
Ser203Glu + Thr212Ser
Ser 97Glu + Asn154Glu + Gly159Asn + Ser190Asp +
Tyr205Thr + Asn211Glu
Thr 65Glu + Ser 98Asp + Thr102Pro + Gly126Asp +
Gly165Asp + Gly218Gln
Asn 96Glu + Tyr103Asp + Gly159Asp + Asn162Asp +
Tyr208Leu + Thr212Pro
Gly 62Asp + Ser104Glu + Ile106Asp + Ser157Asp +
Gly218Ser + Thr219Pro
Asn 96Glu + Gly 99Asp + Ser160Asp + Gly201Ser +
Asn211Gln + Ser215Glu
Ile106Pro + Gly153Asp + Gly165Glu + Tyr166Ser +
Ser203Asp + Thr212Asp
Ser 97Asp + Gly 99Asn + Ser158Asp + Ser210Glu +
Thr212Glu + Leu216Thr
Asp 59Glu + Ser 98Asp + Ser160Glu + Gly201Ser +
Thr212Gly + Tyr213Asn
Val 94Ser + Ser 97Asp + Ser187Glu + Ser190Asp +
Pro209Asp + Leu216Asn
Gly101Gln + Gly126Asp + Ser131Asp + Asn154Glu +
Gly156Asn + Ser203Asp
Gly 64Ser + Ser 98Glu + Ser100Glu + Ala186Glu +
Thr212Glu + Leu216Pro
Ser 97Asp + Gly 99Asp + Val202Asp + Thr207Asn +
Asn211Glu + Leu216Met
Thr 65Pro + Thr132Gly + Ala186Asp + Val202Ala +
Ser203Glu + Thr212Asp
Ile106Asp + Gly127Ser + Asn154Glu + Gly156Glu +

Thr214Pro + Ser215Glu
Thr 58Asp + Ser129Glu + Thr132Asn + Gly165Asp +
Leu216Val + Gly218Asp
Thr 58Pro + Gly 60Glu + Gly 62Glu + Thr102Asp +
Ser158Glu + Leu216Ala
Pro128Asp + Asn154Glu + Phe188Val + Val202Cys +
Ser203Asp + Tyr205Asn
Gly101Gln + Ala105His + Ser155Asp + Tyr166Val +
Ala186Asp + Pro209Glu
Leu 95Val + Ser155Glu + Tyr166Ala + Ala186Glu +
Ser210Asp + Leu216Met
Asn 96Asp + Gly101Glu + Asn162Glu + Val202Thr +
Ser210Asp + Leu216Asn
Ser129Glu + Ser157Asp + Phe188Ala + Ser190Glu +
Val202Ser + Tyr205Glu
Ser131Asp + Ser158Asp + Ser160Asp + Tyr205Ile +
Ser215Asp + Leu216Met
Gly 62Glu + Leu 95Glu + Tyr103Pro + Ala186His +
Ser203Glu + Thr207Gly
Ser100Glu + Leu125Ala + Ser157Glu + Ser203Asp +
Tyr205Asp + Leu216Cys
Thr102Gln + Ser104Asp + Gly153Pro + Ser158Glu +
Ser203Glu + Tyr205Asp
Asn 61Glu + Asn 96Gln + Ser 98Asp + Ser158Asp +
Thr212Asn + Thr219Asp
Val 94Thr + Thr132Asp + Ser155Asp + Ser157Glu +
Thr207Gly + Ser215Asp
Pro128Gly + Gly130Pro + Ser158Glu + Ser187Glu +
Phe188Cys + Ser203Asp
Thr 65Glu + Gly130Pro + Gly159Glu + Gly165Asp +
Phe188Met + Pro209Asp
Thr163Glu + Val202Asp + Tyr205Cys + Thr212Asp +
Leu216Pro + Gly218Glu
Ser 97Glu + Gly101Asn + Gly130Glu + Asn154Asp +
Thr163Gly + Gly218Glu
Thr 65Gly + Ala105Glu + Ser155Asp + Gln161Glu +
Phe188Val + Asn217Gln
Thr 65Gln + Tyr103Asp + Pro128Glu + Ser155Asp +
Ser215Asp + Leu216Ser
Thr132Glu + Ala186Gln + Ser187Glu + Val202Pro +
Thr207Gly + Asn217Asp
Gly 60Glu + Ser129Glu + Ser131Glu + Gly165Asn +
Ser203Glu + Pro209Gln
Gly101Pro + Pro128Asp + Gly130Asp + Ser203Glu +
Tyr205Ile + Thr212Asp
Val 94Glu + Asn 96Glu + Asn154Asp + Tyr205Ala +
Thr212Glu + Leu216Cys
Ser 97Glu + Thr102Ser + Ser157Asp + Ser215Asp +
Leu216Ser + Asn217Glu
Ser 98Asp + Gly156Glu + Ser158Glu + Gln161Ser +
Ser203Asp + Gly218Asn
Gly130Ser + Gly156Asp + Ser158Glu + Ser210Asp +
Thr212Gln + Ser215Asp

119

Ser100Glu + Thr102Asp + Gly126Pro + Gly127Ser +
Ser187Glu + Ser215Glu
Asn 96Glu + Gly127Ser + Pro128Gln + Ser190Asp +
Tyr213Asp + Ser215Glu
Pro128Glu + Gly153Ser + Ser158Glu + Gly165Ser +
Ala186Gly + Ser187Glu
Thr 65Glu + Gly153Asp + Thr163Asp + Gly165Asn +
Met198His + Ser203Glu
Leu125Gly + Ser129Glu + Thr163Glu + Ala186His +
Ser203Glu + Tyr205Leu
Thr 58Glu + Asn 61Ser + Thr 65Gly + Ser158Asp +
Asn162Asp + Ser203Glu
Thr 65Asn + Val 94Thr + Gly126Glu + Ser157Asp +
Asn162Glu + Leu216Asp
Asp 59Glu + Ser 97Glu + Pro128Asn + Thr132Glu +
Ser157Asp + Tyr205Gly
Thr 58Glu + Gly 60Gln + Leu 95Glu + Val202Gln +
Tyr205Gln + Ser215Asp
Asp 59Glu + Ser157Asp + Asn162Asp + Tyr166Asn +
Leu216Ile + Asn217Glu
Ser157Glu + Asn162Glu + Phe188Gly + Ser203Asp +
Asn211Asp + Thr212Ser
Ser157Glu + Asn162Asp + Ser210Asp + Thr212Ser +
Tyr213Leu + Leu216Asp
Gly 60Pro + Ser 97Glu + Ser100Asp + Ala105His +
Ser203Glu + Asn211Asp
Asn 61Asp + Leu125His + Gly156Asn + Pro200Gln +
Thr212Glu + Leu216Asp
Thr 65Asp + Ser 97Asp + Leu125Asn + Ser157Glu +
Thr163Gly + Thr212Asp
Gly 62Glu + Thr 65Asn + Asn162Glu + Thr163Ser +
Asn211Asp + Ser215Glu
Val 94Glu + Asn 96Ser + Gly101Pro + Thr102Asp +
Ser155Glu + Thr212Asp
Gly 60Glu + Ser 98Glu + Gly 99Gln + Ser104Glu +
Ala105Thr + Thr214Asp
Ser155Asp + Ser187Asp + Ala199Gln + Ser203Asp +
Tyr205Val + Thr212Glu
Ser104Glu + Ile106Cys + Thr163Ser + Gly165Glu +
Ser190Glu + Thr212Asp
Val 94Thr + Ile106Asp + Gly130Asn + Ser131Asp +
Ser203Glu + Tyr205Pro
Ser 98Glu + Pro128Glu + Ser155Asp + Ser160Glu +
Ala186Ser + Pro209Asn
Thr 58Glu + Gly159Ser + Tyr166Glu + Ser190Glu +
Thr212Pro + Leu216Ser
Leu 95Glu + Leu125His + Gly126Asp + Tyr205Gln +
Ser215Asp + Thr219Asn
Asp 59Glu + Gly 99Asp + Ala105Glu + Ala186Pro +
Ser187Asp + Thr212Pro
Asn 96Gln + Ser 98Glu + Gly101Pro + Thr132Glu +
Val202Asp + Ser215Asp
Gly 99Asp + Pro128Asp + Thr132Asn + Val202Asp +

Ser215Asp + Leu216His
 Val 94Gln + Gly101Asp + Ser104Asp + Thr163Pro +
 Ser187Asp + Ser215Asp
 Leu 95Met + Gly101Asp + Ser104Glu + Gly153Glu +
 Tyr205Ala + Ser215Glu
 Gly101Glu + Ser104Glu + Ser203Asp + Tyr205Ile +
 Ser210Glu + Thr212Pro
 Val 94Glu + Ser100Glu + Ile106Met + Gly130Glu +
 Val202Glu + Thr214Asn
 Ser 98Asp + Ile106Asp + Asn154Asp + Gly165Asp +
 Val202Met + Leu216Gln
 Leu 95Met + Thr102Ser + Gly127Asn + Ser190Glu +
 Val202Gly + Asn217Asp
 Gly 62Pro + Ile106Gln + Gly126Asp + Ser129Glu +
 Ser160Asp + Ser215Asp
 Gly 62Asp + Asn154Glu + Ser160Glu + Ala186Thr +
 Ser187Asp + Leu216Thr
 Gly 62Ser + Thr 65Ser + Ser155Asp + Gly159Glu +
 Ser203Glu + Thr219Gln

II. Cleaning Compositions

In another embodiment of the present invention, an effective amount of one or more of the enzyme variants are included in compositions useful for cleaning a variety of surfaces in need of proteinaceous stain removal. Such cleaning compositions include detergent compositions for cleaning hard surfaces, unlimited in form (e.g., liquid and granular); detergent compositions for cleaning fabrics, unlimited in form (e.g., granular, liquid and bar formulations); dishwashing compositions (unlimited in form); oral cleaning compositions, unlimited in form (e.g., dentifrice, toothpaste and mouthwash formulations); denture cleaning compositions, unlimited in form (e.g., liquid, tablet); and contact lens cleaning compositions, unlimited in form (e.g., liquid, tablet).

The cleaning compositions also comprise, in addition to the Subtilisin DY variants described hereinbefore, one or more cleaning composition materials compatible with the protease enzyme. the term "cleaning composition material", as used herein, means any liquid, solid or gaseous material selected for the particular type of cleaning composition desired and the form of the product (e.g., liquid, granule, bar, spray, stick, paste, gel), which materials are also compatible with the Subtilisin DY variant used in the composition. the specific selection of cleaning composition materials are readily made by considering the surface material to be cleaned, the desired form of the composition for the cleaning condition during use (e.g., through the wash detergent use). The term "compatible", as used herein,

means the cleaning composition materials do not reduce the proteolytic activity of the Subtilisin DY variant to such an extent that the protease is not effective as desired during normal use situations. Specific cleaning composition materials are exemplified in detail hereinafter.

As used herein, "effective amount of enzyme variant" refers to the quantity of enzyme variant necessary to achieve the enzymatic activity necessary in the specific cleaning composition. Such effective amounts are readily ascertained by one of ordinary skill in the art and is based on many factors, such as the particular enzyme variant used, the cleaning application, the specific composition of the cleaning composition, and whether a liquid or dry (e.g., granular, bar) composition is required, and the like. Preferably the cleaning compositions comprise from about 0.0001% to about 10% of one or more enzyme variants of the present invention, more preferably from about 0.001% to about 1%, more preferably still from about 0.01% to about 0.1%. Several examples of various cleaning compositions wherein the enzyme variants may be employed are discussed in further detail below. All parts, percentages and ratios used herein are by weight unless otherwise specified.

As used herein, "non-fabric cleaning compositions" include hard surface cleaning compositions, dishwashing compositions, oral cleaning compositions, denture cleaning compositions and contact lens cleaning compositions.

A. Cleaning Compositions for Hard Surfaces, Dishes and Fabrics

The enzyme variants of the present invention can be used in a variety of detergent compositions where high sudsing and good insoluble substrate removal are desired. Thus the enzyme variants can be used with various conventional ingredients to provide fully-formulated hard-surface cleaners, dishwashing compositions, fabric laundering compositions and the like. Such compositions can be in the form of liquids, granules, bars and the like. Such compositions can be formulated as modern "concentrated" detergents which contain as much as 30%-60% by weight of surfactants.

The cleaning compositions herein can optionally, and preferably, contain various anionic, nonionic, zwitterionic, etc., surfactants. Such surfactants are typically present at levels of from about 5% to about 35% of the compositions.

Nonlimiting examples of surfactants useful herein include the conventional C₁₁-C₁₈ alkyl benzene sulfonates and primary and random

alkyl sulfates, the C₁₀-C₁₈ secondary (2,3) alkyl sulfates of the formulas $\text{CH}_3(\text{CH}_2)_x(\text{CHOSO}_3^- \text{M}^+)\text{CH}_3$ and $\text{CH}_3(\text{CH}_2)_y(\text{CHOSO}_3^- \text{M}^+)\text{CH}_2\text{CH}_3$ wherein x and (y+1) are integers of at least about 7, preferably at least about 9, and M is a water-solubilizing cation, especially sodium, the C₁₀-C₁₈ alkyl alkoxy sulfates (especially EO 1-5 ethoxy sulfates), C₁₀-C₁₈ alkyl alkoxy carboxylates (especially the EO 1-5 ethoxycarboxylates), the C₁₀-C₁₈ alkyl polyglycosides, and their corresponding sulfated polyglycosides, C₁₂-C₁₈ alpha-sulfonated fatty acid esters, C₁₂-C₁₈ alkyl and alkyl phenol alkoxyates (especially ethoxylates and mixed ethoxy/propoxy), C₁₂-C₁₈ betaines and sulfobetaines ("sultaines"), C₁₀-C₁₈ amine oxides, and the like. The alkyl alkoxy sulfates (AES) and alkyl alkoxy carboxylates (AEC) are preferred herein. (Use of such surfactants in combination with the aforesaid amine oxide and/or betaine or sultaine surfactants is also preferred, depending on the desires of the formulator.) Other conventional useful surfactants are listed in standard texts. Particularly useful surfactants include the C₁₀-C₁₈ N-methyl glucamides disclosed in US Patent 5, 194,639, Connor et al., issued March 16, 1993.

A wide variety of other ingredients useful in detergent cleaning compositions can be included in the compositions herein, including other active ingredients, carriers, hydrotropes, processing aids, dyes or pigments, solvents for liquid formulations, etc. If an additional increment of sudsing is desired, suds boosters such as the C₁₀-C₁₆ alkalamides can be incorporated into the compositions, typically at about 1% to about 10% levels. The C₁₀-C₁₄ monoethanol and diethanol amides illustrate a typical class of such suds boosters. Use of such suds boosters with high sudsing adjunct surfactants such as the amine oxides, betaines and sultaines noted above is also advantageous. If desired, soluble magnesium salts such as MgCl₂, MgSO₄, and the like, can be added at levels of, typically, from about 0.1% to about 2%, to provide additionally sudsing.

The liquid detergent compositions herein can contain water and other solvents as carriers. Low molecular weight primary or secondary alcohols exemplified by methanol, ethanol, propanol, and isopropanol are suitable. Monohydric alcohols are preferred for solubilizing surfactants, but polyols such as those containing from about 2 to about 6 carbon atoms and from about 2 to about 6 hydroxy groups (e.g., 1,3-propanediol, ethylene glycol, glycerine, and 1,2-propanediol) can also be used. The compositions may

contain from about 5% to about 90%, typically from about 10% to about 50% of such carriers.

The detergent compositions herein will preferably be formulated such that during use in aqueous cleaning operations, the wash water will have a pH between about 6.8 and about 11.0. Finished products thus are typically formulated at this range. Techniques for controlling pH at recommended usage levels include the use of buffers, alkalis, acids, etc., and are well known to those skilled in the art.

When formulating the hard surface cleaning compositions and fabric cleaning compositions of the present invention, the formulator may wish to employ various builders at levels from about 5% to about 50% by weight. Typical builders include the 1-10 micron zeolites, polycarboxylates such as citrate and oxydisuccinates, layered silicates, phosphates, and the like. Other conventional builders are listed in standard formularies.

Likewise, the formulator may wish to employ various additional enzymes, such as cellulases, lipases, amylases and proteases in such compositions, typically at levels of from about 0.001% to about 1% by weight. Various detergent and fabric care enzymes are well-known in the laundry detergent art.

Various bleaching compounds, such as the percarbonates, perborates and the like, can be used in such compositions, typically at levels from about 1% to about 15% by weight. If desired, such compositions can also contain bleach activators such as tetraacetyl ethylenediamine, nonanoyloxybenzene sulfonate, and the like, which are also known in the art. Usage levels typically range from about 1% to about 10% by weight.

Various soil release agents, especially of the anionic oligoester type, various chelating agents, especially the aminophosphonates and ethylenediaminedisuccinates, various clay soil removal agents, especially ethoxylated tetraethylene pentamine, various dispersing agents, especially polyacrylates and polyaspartates, various brighteners, especially anionic brighteners, various suds suppressors, especially silicones and secondary alcohols, various fabric softeners, especially smectite clays, and the like can all be used in such compositions at levels ranging from about 1% to about 35% by weight. Standard formularies and published patents contain multiple, detailed descriptions of such conventional materials.

Enzyme stabilizers may also be used in the cleaning compositions. Such enzyme stabilizers include propylene glycol (preferably from about 1%

to about 10%), sodium formate (preferably from about 0.1% to about 1%) and calcium formate (preferably from about 0.1% to about 1%).

1. Hard surface cleaning compositions

As used herein "hard surface cleaning composition" refers to liquid and granular detergent compositions for cleaning hard surfaces such as floors, walls, bathroom tile, and the like. Hard surface cleaning compositions of the present invention comprise an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from about .01% to about 5%, more preferably still from about .05% to about 1% by weight of active enzyme of the composition. In addition to comprising one or more of the enzyme variants, such hard surface cleaning compositions typically comprise a surfactant and a water-soluble sequestering builder. In certain specialized products such as spray window cleaners, however, the surfactants are sometimes not used since they may produce a filmy/streaky residue on the glass surface.

The surfactant component, when present, may comprise as little as 0.1% of the compositions herein, but typically the compositions will contain from about 0.25% to about 10%, more preferably from about 1% to about 5% of surfactant.

Typically the compositions will contain from about 0.5% to about 50% of a detergency builder, preferably from about 1% to about 10%.

Preferably the pH should be in the range of about 8 to 12. Conventional pH adjustment agents such as sodium hydroxide, sodium carbonate or hydrochloric acid can be used if adjustment is necessary.

Solvents may be included in the compositions. Useful solvents include, but are not limited to, glycol ethers such as diethyleneglycol monohexyl ether, diethyleneglycol monobutyl ether, ethyleneglycol monobutyl ether, ethyleneglycol monohexyl ether, propyleneglycol monobutyl ether, dipropyleneglycol monobutyl ether, and diols such as 2,2,4-trimethyl-1,3-pentanediol and 2-ethyl-1,3-hexanediol. When used, such solvents are typically present at levels of from about 0.5% to about 15%, preferably from about 3% to about 11%.

Additionally, highly volatile solvents such as isopropanol or ethanol can be used in the present compositions to facilitate faster evaporation of the composition from surfaces when the surface is not rinsed after "full strength" application of the composition to the surface. When used, volatile

solvents are typically present at levels of from about 2% to about 12% in the compositions.

The hard surface cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 7-12

Liquid Hard Surface Cleaning Compositions						
Component	Example No.					
	7	8	9	10	11	12
Phe188Asp	0.05	0.50	0.02	0.03	0.10	0.03
Val202Ala	—	—	—	—	0.20	0.02
Na ₂ DIDA*						
EDTA**	—	—	2.90	2.90	—	—
Na Citrate	—	—	—	—	2.90	2.90
NaC ₁₂ Alkyl-benzene sulfonate	1.95	—	1.95	—	1.95	—
NaC ₁₂ Alkylsulfate	—	2.20	—	2.20	—	2.20
NaC ₁₂ (ethoxy)*** sulfate	—	2.20	—	2.20	—	2.20
C ₁₂ Dimethylamine oxide	—	0.50	—	0.50	—	0.50
Na Cumene sulfonate	1.30	—	1.30	—	1.30	—
Hexyl Carbitol***	6.30	6.30	6.30	6.30	6.30	6.30
Water****	balance to 100%					

*Disodium N-diethyleneglycol-N,N-iminodiacetate

**Na₄ ethylenediamine diacetic acid

***Diethyleneglycol monohexyl ether

****All formulas adjusted to pH 7

In Examples 7-10, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Phe188Asp, with substantially similar results.

In Examples 11-12, any combination of the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Phe188Asp and Val202Ala, with substantially similar results.

Examples 13-18
Spray Compositions for Cleaning Hard Surfaces
and Removing Household Mildew

Component	Example No.					
	13	14	15	16	17	18
Asn 96Asp + Tyr103His	0.50	0.05	0.60	0.30	0.20	0.30
Pro200Gly + Gly201Ser + Ser210Asp	-	-	-	-	0.30	0.10
Sodium octyl sulfate	2.00	2.00	2.00	2.00	2.00	2.00
Sodium dodecyl sulfate	4.00	4.00	4.00	4.00	4.00	4.00
Sodium hydroxide	0.80	0.80	0.80	0.80	0.80	0.80
Silicate (Na)	0.04	0.04	0.04	0.04	0.04	0.04
Perfume	0.35	0.35	0.35	0.35	0.35	0.35
Water	balance to 100%					

Product pH is about 7.

In Examples 13-16, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Asn 96Asp + Tyr103His, with substantially similar results.

In Examples 17-18, any combination of the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Asn 96Asp + Tyr103His and Pro200Gly + Gly201Ser + Ser210Asp, with substantially similar results.

2. Dishwashing Compositions

In another embodiment of the present invention, dishwashing compositions comprise one or more enzyme variants of the present invention. As used herein, "dishwashing composition" refers to all forms for compositions for cleaning dishes, including but not limited to, granular and liquid forms. The dishwashing composition embodiment of the present invention is illustrated by the following examples.

Examples 19-24
Dishwashing Composition

Component	Example No.					
	19	20	21	22	23	24
Gly127Asn	0.05	0.50	0.02	0.40	0.10	0.03
Gly201Asn + Tyr205Gly + Thr207Gln + Tyr213Ile	-	-	-	-	0.40	0.02
C ₁₂ -C ₁₄ N-methyl- glucamide	0.90	0.90	0.90	0.90	0.90	0.90
C ₁₂ ethoxy (1) sulfate	12.00	12.00	12.00	12.00	12.00	12.00
2-methyl undecanoic acid	4.50	4.50	4.50	4.50	4.50	4.50
C ₁₂ ethoxy (2) carboxylate	4.50	4.50	4.50	4.50	4.50	4.50
C ₁₂ alcohol ethoxylate (4)	3.00	3.00	3.00	3.00	3.00	3.00
C ₁₂ amine oxide	3.00	3.00	3.00	3.00	3.00	3.00
Sodium cumene sulfonate	2.00	2.00	2.00	2.00	2.00	2.00
Ethanol	4.00	4.00	4.00	4.00	4.00	4.00
Mg ⁺⁺ (as MgCl ₂)	0.20	0.20	0.20	0.20	0.20	0.20
Ca ⁺⁺ (as CaCl ₂)	0.40	0.40	0.40	0.40	0.40	0.40
Water	balance to 100%					

Product pH is adjusted to 7.

In Examples 19-22, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Gly127Asn, with substantially similar results.

In Examples 23-24, any combination of the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Gly127Asn and Gly201Asn + Tyr205Gly + Thr207Gln + Tyr213Ile, with substantially similar results.

3. Fabric cleaning compositions

In another embodiment of the present invention, fabric cleaning compositions comprise one or more enzyme variants of the present invention. As used herein, "fabric cleaning composition" refers to all forms for detergent compositions for cleaning fabrics, including but not limited to, granular, liquid and bar forms. Preferred fabric cleaning compositions are those in the liquid form.

a. Granular fabric cleaning compositions

The granular fabric cleaning compositions of the present invention contain an effective amount of one or more enzyme variants of the present

invention, preferably from about 0.001% to about 10%, more preferably from about 0.005% to about 5%, more preferably from about 0.01% to about 1% by weight of active enzyme of the composition. In addition to one or more enzyme variants, the granular fabric cleaning compositions typically comprise at least one surfactant, one or more builders, and, in some cases, a bleaching agent.

The granular fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 25-28

Granular Fabric Cleaning Composition

Component	Example No.			
	25	26	27	28
Gly156Glu + Asn162Gln	0.10	0.20	0.03	0.05
Leu 95Glu	-	-	0.02	0.05
C ₁₃ linear alkyl benzene sulfonate	22.00	22.00	22.00	22.00
Phosphate (as sodium tripolyphosphates)	23.00	23.00	23.00	23.00
Sodium carbonate	23.00	23.00	23.00	23.00
Sodium silicate	14.00	14.00	14.00	14.00
Zeolite	8.20	8.20	8.20	8.20
Chelant (diethylenetriamine-pentaacetic acid)	0.40	0.40	0.40	0.40
Sodium sulfate	5.50	5.50	5.50	5.50
Water	balance to 100%			

In Examples 25-26, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Gly156Glu + Asn162Gln, with substantially similar results.

In Examples 27-28, any combination of the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Gly156Glu + Asn162Gln and Leu 95Glu, with substantially similar results.

Examples 29-32
Granular Fabric Cleaning Composition

Component	Example No.			
	29	30	31	32
Gln161Glu	0.10	0.20	0.03	0.05
Ala186Ser + Phe188Ala + Ser190Glu	-	-	0.02	0.05
C ₁₂ alkyl benzene sulfonate	12.00	12.00	12.00	12.00
Zeolite A (1-10 micrometer)	26.00	26.00	26.00	26.00
2-butyl octanoic acid	4.00	4.00	4.00	4.00
C ₁₂ -C ₁₄ secondary (2,3) alkyl sulfate, Na salt	5.00	5.00	5.00	5.00
Sodium citrate	5.00	5.00	5.00	5.00
Optical brightener	0.10	0.10	0.10	0.10
Sodium sulfate	17.00	17.00	17.00	17.00
Water and minors	balance to 100%			

In Examples 29-30, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Gln161Glu, with substantially similar results.

In Examples 31-32, any combination of the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Gln161Glu and Ala186Ser + Phe188Ala + Ser190Glu, with substantially similar results.

Examples 33-36
Granular Fabric Cleaning Composition

Component	Example No.			
	33	34	35	36
Asn 96Gln + Ile106Ala	0.10	0.20	0.03	0.05
Gly 60Asn + Asn 61Glu + Gly153Ser + Pro209Asp + Asn211Asp + Ser215Glu	-	-	0.02	0.05
C ₁₃ linear alkyl benzene sulfonate	22.00	22.00	22.00	22.00
Phosphate (as sodium tripolyphosphates)	23.00	23.00	23.00	23.00
Sodium carbonate	23.00	23.00	23.00	23.00
Sodium silicate	14.00	14.00	14.00	14.00
Zeolite	8.20	8.20	8.20	8.20
Chelant (diethylenetriamine- pentaacetic acid)	0.40	0.40	0.40	0.40
Sodium sulfate	5.50	5.50	5.50	5.50
Water	balance to 100%			

In Examples 33-34, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Asn 96Gln + Ile106Ala, with substantially similar results.

In Examples 35-36, any combination of the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Asn 96Gln + Ile106Ala and Gly 60Asn + Asn 61Glu + Gly153Ser + Pro209Asp + Asn211Asp + Ser215Glu, with substantially similar results.

Examples 37-40
Granular Fabric Cleaning Composition

Component	Example No.			
	37	38	39	40
Gly60Asn	0.10	0.20	0.03	0.05
Ala186Asn + Ser187Asp + Phe188Gln + Ser190Glu	-	-	0.02	0.05
C ₁₂ alkyl benzene sulfonate	12.00	12.00	12.00	12.00
Zeolite A (1-10 micrometer)	26.00	26.00	26.00	26.00
2-butyl octanoic acid	4.00	4.00	4.00	4.00
C ₁₂ -C ₁₄ secondary (2,3) alkyl sulfate, Na salt	5.00	5.00	5.00	5.00
Sodium citrate	5.00	5.00	5.00	5.00
Optical brightener	0.10	0.10	0.10	0.10
Sodium sulfate	17.00	17.00	17.00	17.00
Water and minors	balance to 100%			

In Examples 37-38, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Gly60Asn, with substantially similar results.

In Examples 39-40, any combination of the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Gly60Asn and Ala186Asn + Ser187Asp + Phe188Gln + Ser190Glu, with substantially similar results.

Examples 41-42

Granular Fabric Cleaning Composition

Component	Example No.	
	41	42
Linear alkyl benzene sulphonate	11.4	10.70
Tallow alkyl sulphate	1.80	2.40
C ₁₄₋₁₅ alkyl sulphate	3.00	3.10
C ₁₄₋₁₅ alcohol 7 times ethoxylated	4.00	4.00
Tallow alcohol 11 times ethoxylated	1.80	1.80
Dispersant	0.07	0.1
Silicone fluid	0.80	0.80
Trisodium citrate	14.00	15.00
Citric acid	3.00	2.50
Zeolite	32.50	32.10
Maleic acid acrylic acid copolymer	5.00	5.00
Diethylene triamine penta methylene phosphonic acid	1.00	0.20
Ser215Asp	0.30	0.30
Lipase	0.36	0.40
Amylase	0.30	0.30
Sodium silicate	2.00	2.50
Sodium sulphate	3.50	5.20
Polyvinyl pyrrolidone	0.30	0.50
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Peroxidase	0.1	0.1
Minors	Up to 100	Up to 100

Examples 43-44

Granular Fabric Cleaning Composition

Component	Example No.	
	43	44
Sodium linear C ₁₂ alkyl benzene-sulfonate	6.5	8.0
Sodium sulfate	15.0	18.0
Zeolite A	26.0	22.0
Sodium nitrilotriacetate	5.0	5.0
Polyvinyl pyrrolidone	0.5	0.7
Tetraacetylene diamine	3.0	3.0
Boric acid	4.0	-
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Val202Met + Thr212Pro	0.4	0.4
Fillers (e.g., silicates; carbonates; perfumes; water)	Up to 100	Up to 100

Example 45

Compact Granular Fabric Cleaning Composition

Component	Weight %
Alkyl Sulphate	8.0
Alkyl Ethoxy Sulphate	2.0
Mixture of C ₂₅ and C ₄₅ alcohol 3 and 7 times ethoxylated	6.0
Polyhydroxy fatty acid amide	2.5
Zeolite	17.0
Layered silicate/citrate	16.0
Carbonate	7.0
Maleic acid acrylic acid copolymer	5.0
Soil release polymer	0.4
Carboxymethyl cellulose	0.4
Poly (4-vinylpyridine) -N-oxide	0.1
Copolymer of vinylimidazole and vinylpyrrolidone	0.1
PEG2000	0.2
Thr 65Glu + Ile106Met + Leu125His + Tyr205Leu + Ser215Asp	0.5
Lipase	0.2
Cellulase	0.2
Tetracetylene diamine	6.0

134

Percarbonate	22.0
Ethylene diamine disuccinic acid	0.3
Suds suppressor	3.5
Disodium-4,4'-bis (2-morpholino -4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulphonate	0.25
Disodium-4,4'-bis (2-sulfostyryl) biphenyl	0.05
Water, Perfume and Minors	Up to 100

Example 46

Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulphonate	7.6
C ₁₆ -C ₁₈ alkyl sulfate	1.3
C ₁₄ -15 alcohol 7 times ethoxylated	4.0
Coco-alkyl-dimethyl hydroxyethyl ammonium chloride	1.4
Dispersant	0.07
Silicone fluid	0.8
Trisodium citrate	5.0
Zeolite 4A	15.0
Maleic acid acrylic acid copolymer	4.0
Diethylene triamine penta methylene phosphonic acid	0.4
Perborate	15.0
Tetraacetylene diamine	5.0
Smectite clay	10.0
Poly (oxy ethylene) (MW 300,000)	0.3
Thr207Asn + Tyr208Gln + Thr212Ser + Tyr213Glu + Ser215Glu	0.4
Lipase	0.2
Amylase	0.3
Cellulase	0.2
Sodium silicate	3.0
Sodium carbonate	10.0
Carboxymethyl cellulose	0.2
Brighteners	0.2
Water, perfume and minors	Up to 100

Example 47

Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulfonate	6.92
Tallow alkyl sulfate	2.05
C ₁₄₋₁₅ alcohol 7 times ethoxylated	4.4
C ₁₂₋₁₅ alkyl ethoxy sulfate - 3 times ethoxylated	0.16
Zeolite	20.2
Citrate	5.5
Carbonate	15.4
Silicate	3.0
Maleic acid acrylic acid copolymer	4.0
Carboxymethyl cellulase	0.31
Soil release polymer	0.30
Gly 60Asp + Asn 61Asp + Gly 62Asp + Thr 65Gln	0.2
Lipase	0.36
Cellulase	0.13
Perborate tetrahydrate	11.64
Perborate monohydrate	8.7
Tetraacetylene diamine	5.0
Diethylene tramine penta methyl phosphonic acid	0.38
Magnesium sulfate	0.40
Brightener	0.19
Perfume, silicone, suds suppressors	0.85
Minors	Up to 100

b. Liquid fabric cleaning compositions

Liquid fabric cleaning compositions of the present invention comprise an effective amount of one or more enzyme variants of the present invention, preferably from about 0.005% to about 5%, more preferably from about 0.01% to about 1%, by weight of active enzyme of the composition. Such liquid fabric cleaning compositions typically additionally comprise an anionic surfactant, a fatty acid, a water-soluble detergency builder and water.

The liquid fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 48-52

Liquid Fabric Cleaning Compositions

Component	Example No.				
	48	49	50	51	52
Val94Gly + Gly99Pro + Ile106Val	0.05	0.03	0.30	0.03	0.10
Gly218Pro	-	-	-	0.01	0.20
C ₁₂ - C ₁₄ alkyl sulfate, Na	20.00	20.00	20.00	20.00	20.00
2-butyl octanoic acid	5.00	5.00	5.00	5.00	5.00
Sodium citrate	1.00	1.00	1.00	1.00	1.00
C ₁₀ alcohol ethoxylate (3)	13.00	13.00	13.00	13.00	13.00
Monethanolamine	2.50	2.50	2.50	2.50	2.50
Water/propylene glycol/ethanol (100:1:1)	balance to 100%				

In Examples 48-50 the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Val94Gly + Gly99Pro + Ile106Val, with substantially similar results.

In Examples 51-52, any combination of the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Val94Gly + Gly99Pro + Ile106Val and Gly218Pro, with substantially similar results.

Examples 53-57

Liquid Fabric Cleaning Compositions

Component	Example No.				
	53	54	55	56	57
Gly156Ser	0.05	0.03	0.30	0.03	0.10
Ser187Glu	-	-	-	0.01	0.20
C ₁₂ - C ₁₄ alkyl sulfate, Na	20.00	20.00	20.00	20.00	20.00
2-butyl octanoic acid	5.00	5.00	5.00	5.00	5.00
Sodium citrate	1.00	1.00	1.00	1.00	1.00
C ₁₀ alcohol ethoxylate (3)	13.00	13.00	13.00	13.00	13.00
Monethanolamine	2.50	2.50	2.50	2.50	2.50
Water/propylene glycol/ethanol (100:1:1)	balance to 100%				

In Examples 53-55 the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Gly156Ser, with substantially similar results.

In Examples 56-57, any combination of the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Gly156Ser and Ser187Glu, with substantially similar results.

Examples 58-59
Liquid Fabric Cleaning Composition

Component	Example No.	
	58	59
C ₁₂₋₁₄ alkenyl succinic acid	3.0	8.0
Citric acid monohydrate	10.0	15.0
Sodium C ₁₂₋₁₅ alkyl sulphate	8.0	8.0
Sodium sulfate of C ₁₂₋₁₅ alcohol 2 times ethoxylated	-	3.0
C ₁₂₋₁₅ alcohol 7 times ethoxylated	-	8.0
C ₁₂₋₁₅ alcohol 5 times ethoxylated	8.0	-
Diethylene triamine penta (methylene phosphonic acid)	0.2	-
Oleic acid	1.8	-
Ethanol	4.0	4.0
Propanediol	2.0	2.0
Gly101Glu + Ser104Glu + Ser203Asp + Tyr205Ile + Ser210Glu + Thr212Pro	0.2	0.2
Polyvinyl pyrrolidone	1.0	2.0
Suds suppressor	0.15	0.15
NaOH	up to pH 7.5	
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Peroxidase	0.4	0.1
Waters and minors	up to 100 parts	

In each of Examples 58 and 59 herein, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Gly101Glu + Ser104Glu + Ser203Asp + Tyr205Ile + Ser210Glu + Thr212Pro, with substantially similar results.

Examples 60-62
Liquid Fabric Cleaning Composition

Component	Example No.		
	60	61	62
Citric Acid	7.10	3.00	3.00
Fatty Acid	2.00	-	2.00
Ethanol	1.93	3.20	3.20
Boric Acid	2.22	3.50	3.50
Monoethanolamine	0.71	1.09	1.09
1,2 Propanediol	7.89	8.00	8.00
NaCumene Sulfonate	1.80	3.00	3.00
NaFormate	0.08	0.08	0.08
NaOH	6.70	3.80	3.80
Silicon anti-foam agent	1.16	1.18	1.18
Asn 61Glu + Gly 64Ser + Thr 65Gly	0.0145	-	-
Ser129Asp + Gly130Asn	-	0.0145	-
Leu216Asn	-	-	0.0145
Lipase	0.200	0.200	0.200
Cellulase	-	7.50	7.50
Soil release polymer	0.29	0.15	0.15
Anti-foaming agents	0.06	0.085	0.085
Brightener 36	0.095	-	-
Brightener 3	-	0.05	0.05
C ₁₂ alkyl benzenesulfonic acid	9.86	-	-
C ₁₂₋₁₅ alkyl polyethoxylate (2.5) sulfate	13.80	18.00	18.00
C ₁₂ glucose amide	-	5.00	5.00
C ₁₂₋₁₃ alkyl polyethoxylate (9)	2.00	2.00	2.00
Water, perfume and minors	balance to 100%		

c. Bar fabric cleaning compositions

Bar fabric cleaning compositions of the present invention suitable for hand-washing soiled fabrics contain an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from about 0.01% to about 1% by weight of the composition.

The bar fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 63-66
Bar Fabric Cleaning Compositions

Component	Example No.			
	63	64	65	66
Val202Ser + Asn211Asp	0.3	-	0.1	0.02
Gly156Gln + Ile164Thr	-	-	0.4	0.03
C ₁₂ -C ₁₆ alkyl sulfate, Na	20.0	20.0	20.0	20.00
C ₁₂ -C ₁₄ N-methyl glucamide	5.0	5.0	5.0	5.00
C ₁₁ -C ₁₃ alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00
Sodium carbonate	25.0	25.0	25.0	25.00
Sodium pyrophosphate	7.0	7.0	7.0	7.00
Sodium tripolyphosphate	7.0	7.0	7.0	7.00
Zeolite A (0.1-.10 μ)	5.0	5.0	5.0	5.00
Carboxymethylcellulose	0.2	0.2	0.2	0.20
Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20
Coconut monethanolamide	5.0	5.0	5.0	5.00
Brightener, perfume	0.2	0.2	0.2	0.20
CaSO ₄	1.0	1.0	1.0	1.00
MgSO ₄	1.0	1.0	1.0	1.00
Water	4.0	4.0	4.0	4.00
Filler*	balance to 100%			

*Can be selected from convenient materials such as CaCO₃, talc, clay, silicates, and the like.

In Examples 63-64 the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Val202Ser + Asn211Asp, with substantially similar results.

In Examples 65-66, any combination of the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Val202Ser + Asn211Asp and Gly156Gln + Ile164Thr, with substantially similar results.

Examples 67-70
Bar Fabric Cleaning Compositions

Component	Example No.			
	67	68	69	70
Val204Thr + Pro209Asp + Tyr213Cys	0.3	-	0.1	0.02
Gly126Gln + Thr214Gly + Ser215Glu	-	0.3	0.4	0.03
C ₁₂ -C ₁₆ alkyl sulfate, Na	20.0	20.0	20.0	20.00
C ₁₂ -C ₁₄ N-methyl glucamide	5.0	5.0	5.0	5.00
C ₁₁ -C ₁₃ alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00
Sodium carbonate	25.0	25.0	25.0	25.00
Sodium pyrophosphate	7.0	7.0	7.0	7.00
Sodium tripolyphosphate	7.0	7.0	7.0	7.00
Zeolite A (0.1-.10 μ)	5.0	5.0	5.0	5.00
Carboxymethylcellulose	0.2	0.2	0.2	0.20
Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20
Coconut monethanolamide	5.0	5.0	5.0	5.00
Brightener, perfume	0.2	0.2	0.2	0.20
CaSO ₄	1.0	1.0	1.0	1.00
MgSO ₄	1.0	1.0	1.0	1.00
Water	4.0	4.0	4.0	4.00
Filler*	balance to 100%			

*Can be selected from convenient materials such as CaCO₃, talc, clay, silicates, and the like.

In Example 67, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Val204Thr + Pro209Asp + Tyr213Cys, with substantially similar results.

In Example 68, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Gly126Gln + Thr214Gly + Ser215Glu, with substantially similar results.

In Examples 69-70, any combination of the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Val204Thr + Pro209Asp + Tyr213Cys and Gly126Gln + Thr214Gly + Ser215Glu, with substantially similar results.

B. Additional Cleaning Compositions

In addition to the hard surface cleaning, dishwashing and fabric cleaning compositions discussed above, one or more enzyme variants of the present invention may be incorporated into a variety of other cleaning

compositions where hydrolysis of an insoluble substrate is desired. Such additional cleaning compositions include but are not limited to, oral cleaning compositions, denture cleaning compositions, and contact lens cleaning compositions.

1. Oral cleaning compositions

In another embodiment of the present invention, a pharmaceutically-acceptable amount of one or more enzyme variants of the present invention are included in compositions useful for removing proteinaceous stains from teeth or dentures. As used herein, "oral cleaning compositions" refers to dentifrices, toothpastes, toothgels, toothpowders, mouthwashes, mouth sprays, mouth gels, chewing gums, lozenges, sachets, tablets, biogels, prophylaxis pastes, dental treatment solutions, and the like. Preferably, the oral cleaning compositions comprise from about 0.0001% to about 20% of one or more enzyme variants of the present invention, more preferably from about 0.001% to about 10%, more preferably still from about 0.01% to about 5%, by weight of the composition, and a pharmaceutically-acceptable carrier. As used herein, "pharmaceutically-acceptable" means that drugs, medicaments or inert ingredients which the term describes are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, incompatibility, instability, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio.

Typically, the pharmaceutically-acceptable oral cleaning carrier components of the oral cleaning compositions will generally comprise from about 50% to about 99.99%, preferably from about 65% to about 99.99%, more preferably from about 65% to about 99%, by weight of the composition.

The pharmaceutically-acceptable carrier components and optional components which may be included in the oral cleaning compositions of the present invention are well known to those skilled in the art. A wide variety of composition types, carrier components and optional components useful in the oral cleaning compositions are disclosed in U.S. Patent 5,096,700, Seibel, issued March 17, 1992; U.S. Patent 5,028,414, Sampathkumar, issued July 2, 1991; and U.S. Patent 5,028,415, Benedict, Bush and Sunberg, issued July 2, 1991.

The oral cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 71-74
Dentifrice Composition

Component	Example No.			
	71	72	73	74
Thr214Asp	2.000	3.500	1.500	2.000
Sorbitol (70% aqueous solution)	35.000	35.000	35.000	35.000
PEG-6*	1.000	1.000	1.000	1.000
Silica dental abrasive**	20.000	20.000	20.000	20.000
Sodium fluoride	0.243	0.243	0.243	0.243
Titanium dioxide	0.500	0.500	0.500	0.500
Sodium saccharin	0.286	0.286	0.286	0.286
Sodium alkyl sulfate (27.9% aqueous solution)	4.000	4.000	4.000	4.000
Flavor	1.040	1.040	1.040	1.040
Carboxyvinyl Polymer***	0.300	0.300	0.300	0.300
Carrageenan****	0.800	0.800	0.800	0.800
Water	balance to 100%			

*PEG-6 = Polyethylene glycol having a molecular weight of 600.

**Precipitated silica identified as ZeodentTM 119 offered by J.M. Huber.

***CarbopolTM offered by B.F. Goodrich Chemical Company.

****Iota Carrageenan offered by Hercules Chemical Company.

In Examples 71-74 the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Thr214Asp, with substantially similar results.

Examples 75-78
Mouthwash Composition

Component	Example No.			
	75	76	77	78
Thr132Glu	3.00	7.50	1.00	5.00
SDA 40 Alcohol	8.00	8.00	8.00	8.00
Flavor	0.08	0.08	0.08	0.08
Emulsifier	0.08	0.08	0.08	0.08
Sodium Fluoride	0.05	0.05	0.05	0.05
Glycerin	10.00	10.00	10.00	10.00
Sweetener	0.02	0.02	0.02	0.02
Benzoic acid	0.05	0.05	0.05	0.05
Sodium hydroxide	0.20	0.20	0.20	0.20
Dye	0.04	0.04	0.04	0.04
Water	balance to 100%			

In Examples 75-78, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Thr132Glu, with substantially similar results.

Examples 79-82
Lozenge Composition

Component	Example No.			
	79	80	81	82
Ser 97Glu + Ile106Leu	0.01	0.03	0.10	0.02
Sorbitol	17.50	17.50	17.50	17.50
Mannitol	17.50	17.50	17.50	17.50
Starch	13.60	13.60	13.60	13.60
Sweetener	1.20	1.20	1.20	1.20
Flavor	11.70	11.70	11.70	11.70
Color	0.10	0.10	0.10	0.10
Corn Syrup	balance to 100%			

In Examples 79-82, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Ser 97Glu + Ile106Leu, with substantially similar results.

Examples 83-86
Chewing Gum Composition

Component	Example No.			
	83	84	85	86
Leu125Ala + Ser129Glu + Gly130Pro + Ser131Asp	0.03	0.02	0.10	0.05
Sorbitol crystals	38.44	38.40	38.40	38.40
Paloja-T gum base*	20.00	20.00	20.00	20.00
Sorbitol (70% aqueous solution)	22.00	22.00	22.00	22.00
Mannitol	10.00	10.00	10.00	10.00
Glycerine	7.56	7.56	7.56	7.56
Flavor	1.00	1.00	1.00	1.00

*Supplied by L.A. Dreyfus Company.

In Examples 83-86, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Leu125Ala + Ser129Glu + Gly130Pro + Ser131Asp, with substantially similar results.

2. Denture cleaning compositions

In another embodiment of the present invention, denture cleaning compositions for cleaning dentures outside of the oral cavity comprise one or more enzyme variants of the present invention. Such denture cleaning compositions comprise an effective amount of one or more of the enzyme variants, preferably from about 0.0001% to about 50% of one or more of the enzyme variants, more preferably from about 0.001% to about 35%, more preferably still from about 0.01% to about 20%, by weight of the composition, and a denture cleansing carrier. Various denture cleansing composition formats such as effervescent tablets and the like are well known in the art (see for example U.S. Patent 5,055,305, Young), and are generally appropriate for incorporation of one or more of the enzyme variants for removing proteinaceous stains from dentures.

The denture cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 87-90

Two-layer Effervescent Denture Cleansing Tablet

Component	Example No.			
	87	88	89	90
<u>Acidic Layer</u>				
Thr212Pro + Thr219Pro	1.0	1.5	0.01	0.05
Tartaric acid	24.0	24.0	24.00	24.00
Sodium carbonate	4.0	4.0	4.00	4.00
Sulphamic acid	10.0	10.0	10.00	10.00
PEG 20,000	4.0	4.0	4.00	4.00
Sodium bicarbonate	24.5	24.5	24.50	24.50
Potassium persulfate	15.0	15.0	15.00	15.00
Sodium acid pyrophosphate	7.0	7.0	7.00	7.00
Pyrogenic silica	2.0	2.0	2.00	2.00
TAED*	7.0	7.0	7.00	7.00
Ricinoleylsulfosuccinate	0.5	0.5	0.50	0.50
Flavor	1.0	1.0	1.00	1.00
<u>Alkaline Layer</u>				
Sodium perborate monohydrate	32.0	32.0	32.00	32.00
Sodium bicarbonate	19.0	19.0	19.00	19.00
EDTA	3.0	3.0	3.00	3.00
Sodium tripolyphosphate	12.0	12.0	12.00	12.00
PEG 20,000	2.0	2.0	2.00	2.00
Potassium persulfate	26.0	26.0	26.00	26.00
Sodium carbonate	2.0	2.0	2.00	2.00
Pyrogenic silica	2.0	2.0	2.00	2.00
Dye/flavor	2.0	2.0	2.00	2.00

*Tetraacetylene diamine

In Examples 87-90, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Thr212Pro + Thr219Pro, with substantially similar results.

3. Contact Lens Cleaning Compositions

In another embodiment of the present invention, contact lens cleaning compositions comprise one or more enzyme variants of the present invention. Such contact lens cleaning compositions comprise an effective amount of one or more of the enzyme variants, preferably from about 0.01% to about 50% of one or more of the enzyme variants, more preferably from

about 0.01% to about 20%, more preferably still from about 1% to about 5%, by weight of the composition, and a contact lens cleaning carrier. Various contact lens cleaning composition formats such as tablets, liquids and the like are well known in the art (see for example U.S. Patent 4,863,627, Davies, Meaken and Rees, issued September 5, 1989; U.S. Patent Re. 32,672, Huth, Lam and Kirai, reissued May 24, 1988; U.S. Patent 4,609,493, Schäfer, issued September 2, 1986; U.S. Patent, 4,690,793, Ogunbiyi and Smith, issued September 1, 1987; U.S. Patent 4,614,549, Ogunbiyi, Riedhammer and Smith, issued September 30, 1986; and U.S. Patent 4,285,738, Ogata, issued August 25, 1981), and are generally appropriate for incorporation of one or more enzyme variants of the present invention for removing proteinaceous stains from contact lens.

The contact lens cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 91-94

Enzymatic Contact Lens Cleaning Solution

Component	Example No.			
	91	92	93	94
Gly156Glu + Thr163Ser + Asn211Ser + Thr212Asn	0.01	0.5	0.1	2.0
Glucose	50.00	50.0	50.0	50.0
Nonionic surfactant (polyoxyethylene-polyoxypropylene copolymer)	2.00	2.0	2.0	2.0
Anionic surfactant (polyoxyethylene-alkylphenylether sodium sulfricester)	1.00	1.0	1.0	1.0
Sodium chloride	1.00	1.0	1.0	1.0
Borax	0.30	0.3	0.3	0.3
Water	balance to 100%			

In Examples 91-94, the Subtilisin DY variants recited in Tables 2-36, among others, are substituted for Gly156Glu + Thr163Ser + Asn211Ser + Thr212Asn, with substantially similar results.

While particular embodiments of the subject invention have been described, it will be obvious to those skilled in the art that various changes and modifications of the subject invention can be made without departing

from the spirit and scope of the invention. It is intended to cover, in the appended claims, all such modifications that are within the scope of the invention.

148

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT: BRODE, PHILIP F.
BARNETT, BOBBY L.
RUBINGH, DONN N.

10 (ii) TITLE OF INVENTION: SUBTILISIN DY VARIANTS HAVING DECREASED
ADSORPTION AND INCREASED HYDROLYSIS

(iii) NUMBER OF SEQUENCES: 1

15 (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: THE PROCTER & GAMBLE COMPANY
(B) STREET: 11810 East Miami River Road
(C) CITY: Ross
(D) STATE: OH
20 (E) COUNTRY: USA
(F) ZIP: 45061

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
25 (B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
30 (A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
35 (A) NAME: HAKE, RICHARD A.
(B) REGISTRATION NUMBER: 37,343
(C) REFERENCE/DOCKET NUMBER: 5606

(ix) TELECOMMUNICATION INFORMATION:
40 (A) TELEPHONE: (513) 627-0087
(B) TELEFAX: (513) 627-0260

(2) INFORMATION FOR SEQ ID NO:1:

45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 274 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
50 (D) TOPOLOGY: linear

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala	Gln	Thr	Val	Pro	Tyr	Gly	Ile	Pro	Leu	Ile	Lys	Ala	Asp	Lys	Val
1				5				10						15	
Gln	Ala	Gln	Gly	Tyr	Lys	Gly	Ala	Asn	Val	Lys	Val	Gly	Ile	Ile	Asp
		20					25						30		
Thr	Gly	Ile	Ala	Ala	Ser	His	Thr	Asp	Leu	Lys	Val	Val	Gly	Gly	Ala
65		35					40					45			

PCT/US96/03006

[illegible]

Claims:

1. A Subtilisin DY variant having a modified amino acid sequence identified as SEQ ID NO: 1 of Subtilisin DY wild-type amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region, a fifth loop region, and a sixth loop region; wherein the modified amino acid sequence comprises a substitution at one or more positions in one or more of the loop regions; wherein

- A. when a substitution occurs in the first loop region, the substitution occurs at one or more of positions 60, 64 or 65; wherein
 - a. when a substitution occurs at position 60, the substituting amino acid is Asn, Gln, Pro or Ser; with the proviso that when position 60 is substituted, the variant is not a single mutation variant wherein the substituting amino acid is Asn;
 - b. when a substitution occurs at position 64, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; and
 - c. when a substitution occurs at position 65, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser;
- B. when a substitution occurs in the second loop region, the substitution occurs at one or more of positions 94, 95, 96, 99, 101, 102, 103, 105 or 106; wherein
 - a. when a substitution occurs at position 94, the substituting amino acid is Ala, Cys, Gln, Gly, His, Met, Pro or Ser;
 - b. when a substitution occurs at position 95, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val;
 - c. when a substitution occurs at position 96, the substituting amino acid is Gln or Ser;
 - f. when a substitution occurs at position 99, the substituting amino acid is Asn, Gln, Pro or Ser; with the proviso that when position 99 is substituted, the variant is not a single mutation variant wherein the substituting amino acid is Ser;
 - h. when a substitution occurs at position 101, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
 - i. when a substitution occurs at position 102, the substituting amino acid is Asn, Gln, Gly, Pro or Ser;

- j. when a substitution occurs at position 103, the substituting amino acid is Ala, Asn, Cys, Gln, Gly, His, Ile, Leu, Met, Pro or Ser;
 - l. when a substitution occurs at position 105, the substituting amino acid is Asn, Gln, Gly, His, Pro, Ser or Thr; and
 - m. when a substitution occurs at position 106, the substituting amino acid is Ala, Cys, Gln, Gly, His, Met, Pro, Ser or Val;
- C. when a substitution occurs in the third loop region, the substitution occurs at one or more of positions 125, 126, 128, 131 or 132; wherein
- a. when a substitution occurs at position 125, the substituting amino acid is Ala, Asn, Cys, Gln, Gly, His, Met, Pro, Ser, Thr or Val;
 - b. when a substitution occurs at position 126, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
 - d. when a substitution occurs at position 128, the substituting amino acid is Asn, Gln or Ser;
 - g. when a substitution occurs at position 131, the substituting amino acid is Asp or Glu; and
 - h. when a substitution occurs at position 132, the substituting amino acid is Asn, Gln, Gly, Pro or Ser;
- D. when a substitution occurs in the fourth loop region, the substitution occurs at one or more of positions 154, 156, or 166; wherein
- b. when a substitution occurs at position 154, the substituting amino acid is Gln or Ser;
 - d. when a substitution occurs at position 156, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; with the proviso that when position 156 is substituted, the variant is not a single mutation variant wherein the substituting amino acid is Glu; and
 - n. when a substitution occurs at position 166, the substituting amino acid is Ala, Gly, His, Ile, Leu, Met or Pro;
- E. when a substitution occurs in the fifth loop region, the substitution occurs at one or more of positions 186, 187, or 188; wherein
- a. when a substitution occurs at position 186, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr; with the proviso that when position 186 is substituted, the variant is not a single mutation variant wherein the substituting amino acid is Pro;
 - b. when a substitution occurs at position 187, the substituting amino acid is Asp or Glu;

- c. when a substitution occurs at position 188, the substituting amino acid is
Ala, Asn, Cys, Gln, Gly, His, Ile, Leu, Met, Pro, Ser, Thr, Tyr or Val;
- F. when a substitution occurs in the sixth loop region, the substitution occurs at one or more of positions 199, 200, 201, 202, 204, 205, 206, 207, 208, 209, 211, 212, 213, 214, 216, or 219; wherein
 - a. when a substitution occurs at position 199, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;
 - b. when a substitution occurs at position 200, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;
 - c. when a substitution occurs at position 201, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;
 - d. when a substitution occurs at position 202, the substituting amino acid is Ala, Cys, Gln, Gly, His, Met, Pro or Ser;
 - f. when a substitution occurs at position 204, the substituting amino acid is Ala, Cys, Gln, Gly, His, Met, Pro or Ser;
 - g. when a substitution occurs at position 205, the substituting amino acid is Ala, Asn, Gln, Gly, His, Ile, Leu, Met, Pro or Ser;
 - h. when a substitution occurs at position 206, the substituting amino acid is Asp or Glu; with the proviso that when position 206 is substituted, the variant is not a single mutation variant;
 - i. when a substitution occurs at position 207, the substituting amino acid is Asn, Gln, Gly, Pro or Ser;
 - j. when a substitution occurs at position 208, the substituting amino acid is Ala, Asn, Cys, Gln, Gly, His, Ile, Met, Pro or Ser;
 - k. when a substitution occurs at position 209, the substituting amino acid is Asn, Gln, Gly or Ser;
 - m. when a substitution occurs at position 211, the substituting amino acid is Gln or Ser;
 - n. when a substitution occurs at position 212, the substituting amino acid is Asn, Gln, Gly, Pro or Ser;
 - o. when a substitution occurs at position 213, the substituting amino acid is Ala, Asn, Cys, Gln, Gly, His, Ile, Leu, Met or Pro;
 - p. when a substitution occurs at position 214, the substituting amino acid is Asn, Gln, Gly, Pro or Ser; with the proviso that when

position 214 is substituted, the variant is not a single mutation variant wherein the substituting amino acid is Ser;

- r. when a substitution occurs at position 216, the substituting amino acid is Ala, Asn, Cys, Gln, Gly, His, Ile, Met, Pro, Ser, Thr or Val; and
- u. when a substitution occurs at position 219, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser;

whereby the Subtilisin DY variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type Subtilisin DY.

2. The Subtilisin DY variant of Claim 1, wherein one or more substitutions occur in the first loop region.
3. The Subtilisin DY variant of Claim 1, wherein one or more substitutions occur in the second loop region.
4. The Subtilisin DY variant of Claim 1, wherein one or more substitutions occur in the third loop region.
5. The Subtilisin DY variant of Claim 1, wherein one or more substitutions occur in the fourth loop region.
6. The Subtilisin DY variant of Claim 1, wherein one or more substitutions occur in the fifth loop region.
7. The Subtilisin DY variant of Claim 1, wherein one or more substitutions occur in the sixth loop region.
8. A cleaning composition selected from the group consisting of a hard surface cleaning composition, a dishwashing composition, an oral

cleaning composition, a denture cleansing composition, a contact lens cleaning composition and a fabric cleaning composition, wherein the cleaning composition comprises the Subtilisin DY variant of Claim 1 and a cleaning composition carrier.

9. The cleaning composition of Claim 8, wherein the cleaning composition is a hard surface cleaning composition.
10. The cleaning composition of Claim 8, wherein the cleaning composition is a fabric cleaning composition.
11. The fabric cleaning composition of Claim 10, wherein the composition is in the form of a liquid.
12. The fabric cleaning composition of Claim 11, wherein the composition comprises at least about 5% surfactant and at least about 5% builder, by weight of the composition.
13. The fabric cleaning composition of Claim 12 further comprising cleaning composition materials selected from the group consisting of solvents, buffers, enzymes, soil release agents, clay soil removal agents, dispersing agents, brighteners, suds suppressors, fabric softeners, suds boosters, enzyme stabilizers, bleaching agents, dyes, perfumes, and mixtures thereof.
14. The fabric cleaning composition of Claim 12 further comprising at least one bleaching agent.
15. A cleaning composition selected from the group consisting of a hard surface cleaning composition, a dishwashing composition, an oral cleaning composition, a denture cleansing composition, a contact lens cleaning composition and a fabric cleaning composition, wherein the cleaning composition comprises the Subtilisin DY variant of Claim 2 and a cleaning composition carrier.
16. A cleaning composition selected from the group consisting of a hard surface cleaning composition, a dishwashing composition, an oral

cleaning composition, a denture cleansing composition, a contact lens cleaning composition and a fabric cleaning composition, wherein the cleaning composition comprises the Subtilisin DY variant of Claim 3 and a cleaning composition carrier.

17. A cleaning composition selected from the group consisting of a hard surface cleaning composition, a dishwashing composition, an oral cleaning composition, a denture cleansing composition, a contact lens cleaning composition and a fabric cleaning composition, wherein the cleaning composition comprises the Subtilisin DY variant of Claim 4 and a cleaning composition carrier.
18. A cleaning composition selected from the group consisting of a hard surface cleaning composition, a dishwashing composition, an oral cleaning composition, a denture cleansing composition, a contact lens cleaning composition and a fabric cleaning composition, wherein the cleaning composition comprises the Subtilisin DY variant of Claim 5 and a cleaning composition carrier.
19. A cleaning composition selected from the group consisting of a hard surface cleaning composition, a dishwashing composition, an oral cleaning composition, a denture cleansing composition, a contact lens cleaning composition and a fabric cleaning composition, wherein the cleaning composition comprises the Subtilisin DY variant of Claim 6 and a cleaning composition carrier.
20. A cleaning composition selected from the group consisting of a hard surface cleaning composition, a dishwashing composition, an oral cleaning composition, a denture cleansing composition, a contact lens cleaning composition and a fabric cleaning composition, wherein the cleaning composition comprises the Subtilisin DY variant of Claim 7 and a cleaning composition carrier.
21. A DNA sequence encoding the Subtilisin DY variant of Claim 1.