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(54) **PROTEIN VARIANTS HAVING MODIFIED IMMUNOGENICITY**

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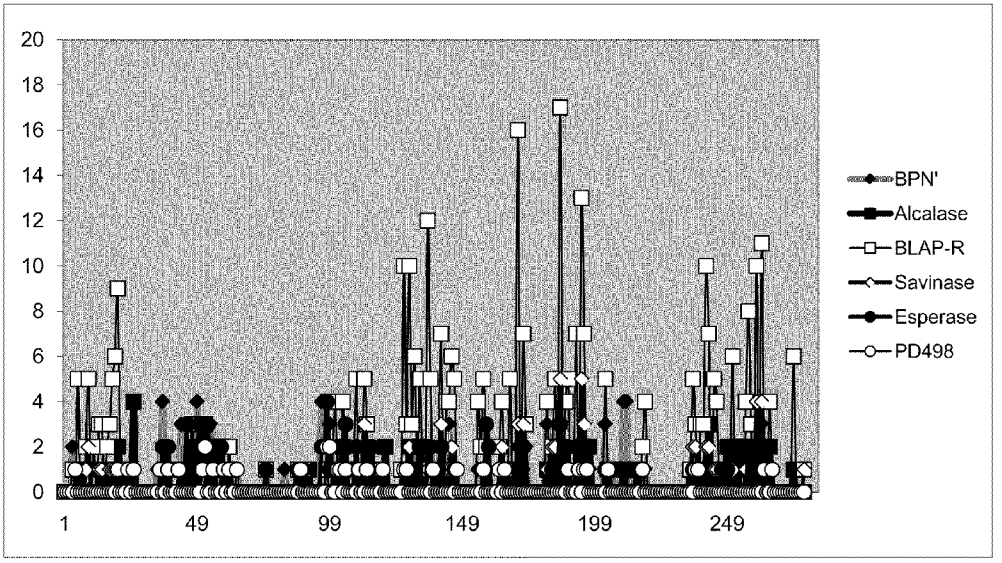
(57) **ABSTRACT**

The present invention relates to a method of selecting a protein variant having modified immunogenicity as compared to the parent protein comprising the steps obtaining antibody binding peptide sequences, using the sequences to localise epitope sequences on the 3-dimensional structure of parent protein, defining an epitope area including amino acids situated within 5 Å from the epitope amino acids constituting the epitope sequence, changing one or more of the amino acids defining the epitope area of the parent protein by genetical engineering mutations of a DNA sequence encoding the parent protein, introducing the mutated DNA sequence into a suitable host, culturing said host and expressing the protein variant, and evaluating the immunogenicity of the protein variant using the parent protein as reference. The invention further relates to the protein variant and use thereof, as well as to a method for producing said protein variant.

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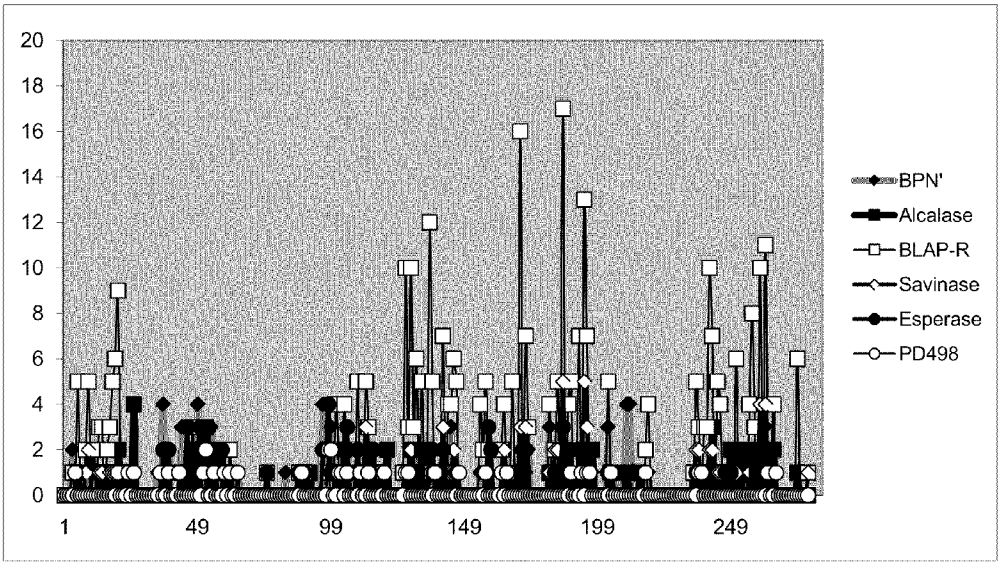
20
18
16
14
12
10
8
6
4
2
0
1
49
99
149
199
249
BPN'
Alcalase
BLAP-R
Savinase
Esperase
PD498

FIG. 1

# PROTEIN VARIANTS HAVING MODIFIED IMMUNOGENICITY

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application is a continuation of U.S. application Ser. No. 09/957,806 filed on Sep. 21, 2001, which is a continuation of PCT/DK01/00293 filed Apr. 30, 2001 and claims, under 35 U.S.C. 119, priority or the benefit of Danish application nos. PA 2000 00707 and PA 2001 00327 filed Apr. 28, 2000 and Feb. 28, 2001, respectively, and U.S. application Nos. 60/203,345 and 60/277,817 filed May 10, 2000 and Mar. 21, 2001, respectively, the contents of which are fully incorporated herein by reference.

## FIELD OF INVENTION

**[0002]** The present invention relates to a method of selecting a protein variant having modified immunogenicity as compared to the parent protein, to the protein variant and use thereof, as well as to a method for producing said protein variant.

## BACKGROUND OF THE INVENTION

**[0003]** An increasing number of proteins, including enzymes, are being produced industrially, for use in various industries, housekeeping and medicine. Being proteins they are likely to stimulate an immunological response in man and animals, including an allergic response.

**[0004]** Depending on the application, individuals get sensitised to the respective allergens by inhalation, direct contact with skin and eyes, or injection. The general mechanism behind an allergic response is divided in a sensitisation phase and a symptomatic phase. The sensitisation phase involves a first exposure of an individual to an allergen. This event activates specific T- and B-lymphocytes, and leads to the production of allergen specific IgE antibodies (in the present context the antibodies are denoted as usual, i.e. immunoglobulin E IgE etc.). These IgE antibodies eventually facilitate allergen capturing and presentation to T-lymphocytes at the onset of the symptomatic phase. This phase is initiated by a second exposure to the same or a resembling antigen. The specific IgE antibodies bind to the specific IgE receptors on mast cells and basophils, among others, and capture at the same time the allergen. The polyclonal nature of this process results in bridging and clustering of the IgE receptors, and subsequently in the activation of mast cells and basophils. This activation triggers the release of various chemical mediators involved in the early as well as late phase reactions of the symptomatic phase of allergy. Prevention of allergy in susceptible individuals is therefore a research area of great importance.

**[0005]** For certain forms of IgE-mediated allergies, a therapy exists, which comprises repeated administration of allergen preparations called 'allergen vaccines' (Int. Arch. Allergy Immunol., 1999, vol. 119, pp 1-5). This leads to reduction of the allergic symptoms, possibly due to a redirection of the immune response away from the allergic (Th2) pathway and towards the immunoprotective (Th1) pathway (Int. Arch. Allergy Immunol., 1999, vol. 119, pp 1-5).

**[0006]** Various attempts to reduce the immunogenicity of polypeptides and proteins have been conducted. It has been found that small changes in an epitope may affect the binding to an antibody. This may result in a reduced importance of such an epitope, maybe converting it from a high affinity to a low affinity epitope, or maybe even result in epitope loss, i.e. that the epitope cannot sufficiently bind an antibody to elicit an immunogenic response.

**[0007]** There is a need for methods to identify epitopes on proteins and alter these epitopes in order to modify the immunogenicity of proteins in a targeted manner. Such methods and kits for their execution can have at least four useful purposes:

**[0008]** 1) reduce the allergenicity of a commercial protein using protein engineering.

**[0009]** 2) reduce the potential of commercial proteins to cross-react with environmental allergens and hence cause allergic reactions in people sensitized to the environmental allergens (or vice versa).

**[0010]** 3) improve the immunotherapeutic effect of allergen vaccines.

**[0011]** 4) assist characterization of clinical allergies in order to select the appropriate treatment, including allergen vaccination.

**[0012]** In WO 99/53038 (Genencor Int.) as well as in prior references (Kammerer et al, Clin. Exp. Allergy, 1997, vol. 27, pp 1016-1026; Sakakibara et al, J. Vet. Med. Sci., 1998; vol. 60, pp. 599-605), methods are described, which identify linear T-cell epitopes among a library of known peptide sequences, each representing part of the primary sequence of the protein of interest. Further, several similar techniques for localization of B-cell epitopes are disclosed by Walshet et al, J. Immunol. Methods, vol. 121, 1275-280, (1989), and by Schoofs et al. J. Immunol. vol. 140, 611-616, (1987). All of these methods, however, only leads to identification of linear epitopes, not to identification of 'structural' or 'discontinuous' epitopes, which are found on the 3-dimensional surface of protein molecules and which comprise amino acids from several discrete sites of the primary sequence of the protein. For several allergens, it has been realized that the dominant epitopes are of such discontinuous nature (Collins et al., Clin. Exp. All. 1996, vol. 26, pp. 36-42).

**[0013]** Slootstra et al; Molecular Diversity, 2, pp. 156-164, 1996 disclose the screening of a semi-random library of synthetic peptides for their binding properties to three monoclonal antibodies by immobilizing the peptides on polyethylene pins and binding a dilution series of each antibody to the pins. This reference does not disclose any indication of how the antibody binding peptide sequences relate to any full protein antigens or allergens.

**[0014]** In WO 92/10755 a method for modifying proteins to obtain less immunogenic variants is described. Randomly constructed protein variants, revealing a reduced binding of antibodies to the parent enzyme as compared to the parent enzyme itself, are selected for the measurement in animal models in terms of allergenicity. Finally, it is assessed whether reduction in immunogenicity is due to true elimination of an epitope or a reduction in affinity for antibodies. This method targets the identification of amino acids that may be part of structural epitopes by using a complete protein for assessing antigen binding. The major drawbacks of this approach are the 'trial and error' character, which makes it a lengthy and expensive process, and the lack of general information on the epitope patterns. Without this information, the results obtained for one protein can not be applied on another protein.

**[0015]** WO 99/47680 (ALK-ABELLÓ) discloses the identification and modification of B-cell epitopes by protein engi-

neering. However, the method is based on crystal structures of Fab-antigen complexes, and B-cell epitopes are defined as "a section of the surface of the antigen comprising 15-25 amino acid residues, which are within a distance from the atoms of the antibody enabling direct interaction" (p. 3). This publication does not show how one selects which Fab fragment to use (e.g. to target the most dominant allergy epitopes) or how one selects the substitutions to be made. Further, their method cannot be used in the absence of such crystallographic data for antigen-antibody complexes, which are very cumbersome, sometimes impossible, to obtain—especially since one would need a separate crystal structure for each epitope to be changed.

**[0016]** Hence, it is of interest to establish a general and efficient method to identify structural epitopes on the 3-dimensional surface of commercial and environmental allergens.

## SUMMARY OF THE INVENTION

**[0017]** The present invention relates to a method of selecting a protein variant having modified immunogenicity as compared to a parent protein, comprising the steps of:

**[0018]** a) obtaining antibody binding peptide sequences,

**[0019]** b) using the sequences to localise epitope sequences on the 3-dimensional structure of parent protein,

**[0020]** c) defining an epitope area including amino acids situated within 5 Å from the epitope amino acids constituting the epitope sequence,

**[0021]** d) changing one or more of the amino acids defining the epitope area of the parent protein by genetic engineering mutations of a DNA sequence encoding the parent protein,

**[0022]** e) introducing the mutated DNA sequence into a suitable host, culturing said host and expressing the protein variant, and

**[0023]** f) evaluating the immunogenicity of the protein variant using the parent protein as reference.

**[0024]** A second aspect of the present invention is a protein variant having modified immunogenicity as compared to its parent protein. The amino acid sequence of the protein variant differs from the amino acid sequence of the parent protein with respect to at least one epitope pattern of the parent protein, such that the immunogenicity of the protein variant is modified as compared with the immunogenicity of the parent protein.

**[0025]** A further aspect of the present invention is a composition comprising a protein variant as defined above, as well as the use of the composition for industrial application, such as the production of a formulation for personal care products (for example shampoo; soap; skin, hand and face lotions; skin, hand and face crèmes; hair dyes; toothpaste), food (for example in the baking industry), detergents and for the production of pharmaceuticals, e.g. vaccines.

**[0026]** Yet another aspect is a DNA molecule encoding a protein variant as defined above.

**[0027]** Further aspects are a vector comprising a DNA molecule as described above as well a host cell comprising said DNA molecule.

**[0028]** Another aspect is a method of producing a protein variant having modified immunogenicity as compared to the parent protein as defined above.

## DEFINITIONS

**[0029]** Prior to a discussion of the detailed embodiments of the invention, a definition of specific terms related to the main aspects of the invention is provided.

**[0030]** In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (herein "Sambrook et al., 1989") DNA Cloning: A Practical Approach, Volumes I and II/D. N. Glover ed. 1985); Oligonucleotide Synthesis (M. J. Gait ed. 1984); Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds (1985)); Transcription And Translation (B. D. Hames & S. J. Higgins, eds. (1984)); Animal Cell Culture (R. I. Freshney, ed. (1986)); Immobilized Cells And Enzymes (IRL Press, (1986)); B. Perbal, A Practical Guide To Molecular Cloning (1984).

**[0031]** When applied to a protein, the term "isolated" indicates that the protein is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated protein is substantially free of other proteins, particularly other proteins of animal origin. It is preferred to provide the proteins in a highly purified form, i.e., greater than 95% pure, more preferably greater than 99% pure. When applied to a polynucleotide molecule, the term "isolated" indicates that the molecule is removed from its natural genetic milieu, and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems. Such isolated molecules are those that are separated from their natural environment and include cDNA and genomic clones. Isolated DNA molecules of the present invention are free of other genes with which they are ordinarily associated, and may include naturally occurring 5' and 3' untranslated regions such as promoters and terminators. The identification of associated regions will be evident to one of ordinary skill in the art (see for example, Dynan and Tijan, Nature 316: 774-78, 1985).

**[0032]** A "polynucleotide" is a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized in vitro, or prepared from a combination of natural and synthetic molecules.

**[0033]** A "nucleic acid molecule" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules") in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary or quaternary forms. Thus, this term includes double-stranded DNA found, inter alia, in linear or circular DNA molecules (e.g., restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.

**[0034]** A DNA "coding sequence" is a double-stranded DNA sequence, which is transcribed and translated into a polypeptide in a cell in vitro or in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

**[0035]** An "Expression vector" is a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments may include promoter and terminator sequences, and optionally one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, and the like. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

**[0036]** Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

**[0037]** A "secretory signal sequence" is a DNA sequence that encodes a polypeptide (a "secretory peptide" that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

**[0038]** The term "promoter" is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

**[0039]** "Operably linked", when referring to DNA segments, indicates that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initiates in the promoter and proceeds through the coding segment to the terminator.

**[0040]** A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then trans-RNA spliced and translated into the protein encoded by the coding sequence.

**[0041]** "Isolated polypeptide" is a polypeptide which is essentially free of other non-[enzyme]polypeptides, e.g., at least about 20% pure, preferably at least about 40% pure, more preferably about 60% pure, even more preferably about 80% pure, most preferably about 90% pure, and even most preferably about 95% pure, as determined by SDS-PAGE.

**[0042]** "Heterologous" DNA refers to DNA not naturally located in the cell, or in a chromosomal site of the cell. Preferably, the heterologous DNA includes a gene foreign to the cell.

**[0043]** A cell has been "transfected" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. A cell has been "transformed" by exogenous or heterologous DNA when the transfected DNA effects a phenotypic change. Preferably, the transforming DNA should be integrated (covalently linked) into chromosomal DNA making up the genome of the cell.

**[0044]** A "clone" is a population of cells derived from a single cell or common ancestor by mitosis.

**[0045]** "Homologous recombination" refers to the insertion of a foreign DNA sequence of a vector in a chromosome. Preferably, the vector targets a specific chromosomal site for homologous recombination. For specific homologous recombination, the vector will contain sufficiently long regions of homology to sequences of the chromosome to allow complementary binding and incorporation of the vector into the chromosome. Longer regions of homology, and greater degrees of sequence similarity, may increase the efficiency of homologous recombination.

Nucleic Acid Sequence

**[0046]** The techniques used to isolate or clone a nucleic acid sequence encoding a polypeptide are known in the art and include isolation from genomic DNA, preparation from cDNA, or a combination thereof. The cloning of the nucleic acid sequences of the present invention from such genomic DNA can be effected, e.g., by using the well known polymerase chain reaction (PCR) or antibody screening of expression libraries to detect cloned DNA fragments with shared structural features. See, e.g., Innis et al., 1990, A Guide to Methods and Application, Academic Press, New York. Other nucleic acid amplification procedures such as ligase chain reaction (LCR), ligated activated transcription (LAT) and nucleic acid sequence-based amplification (NASBA) may be used. The nucleic acid sequence may be cloned from a strain producing the polypeptide, or from another related organism and thus, for example, may be an allelic or species variant of the polypeptide encoding region of the nucleic acid sequence.

**[0047]** The term "isolated" nucleic acid sequence as used herein refers to a nucleic acid sequence which is essentially free of other nucleic acid sequences, e.g., at least about 20% pure, preferably at least about 40% pure, more preferably about 60% pure, even more preferably about 80% pure, most preferably about 90% pure, and even most preferably about 95% pure, as determined by agarose gel electorphoresis. For example, an isolated nucleic acid sequence can be obtained by standard cloning procedures used in genetic engineering to relocate the nucleic acid sequence from its natural location to a different site where it will be reproduced. The cloning procedures may involve excision and isolation of a desired nucleic acid fragment comprising the nucleic acid sequence encoding the polypeptide, insertion of the fragment into a vector molecule, and incorporation of the recombinant vector into a host cell where multiple copies or clones of the nucleic acid sequence will be replicated. The nucleic acid sequence may be of genomic, cDNA, RNA, semisynthetic, synthetic origin, or any combinations thereof.

Nucleic Acid Construct

**[0048]** As used herein the term "nucleic acid construct" is intended to indicate any nucleic acid molecule of cDNA, genomic DNA, synthetic DNA or RNA origin. The term "construct" is intended to indicate a nucleic acid segment which may be single- or double-stranded, and which may be based on a complete or partial naturally occurring nucleotide sequence encoding a polypeptide of interest. The construct may optionally contain other nucleic acid segments.

**[0049]** The DNA of interest may suitably be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the polypeptide by hybridization using synthetic oligonucleotide probes in accordance with standard techniques (cf. Sambrook et al., supra).

**[0050]** The nucleic acid construct may also be prepared synthetically by established standard methods, e.g. the phosphoamidite method described by Beaucage and Caruthers, Tetrahedron Letters 22 (1981), 1859-1869, or the method described by Matthes et al., EMBO Journal 3 (1984), 801-805. According to the phosphoamidite method, oligonucleotides are synthesized, e.g. in an automatic DNA synthesizer, purified, annealed, ligated and cloned in suitable vectors.

**[0051]** Furthermore, the nucleic acid construct may be of mixed synthetic and genomic, mixed synthetic and cDNA or mixed genomic and cDNA origin prepared by ligating fragments of synthetic, genomic or cDNA origin (as appropriate), the fragments corresponding to various parts of the entire nucleic acid construct, in accordance with standard techniques.

**[0052]** The nucleic acid construct may also be prepared by polymerase chain reaction using specific primers, for instance as described in U.S. Pat. No. 4,683,202 or Saiki et al., Science 239 (1988), 487-491.

**[0053]** The term nucleic acid construct may be synonymous with the term expression cassette when the nucleic acid construct contains all the control sequences required for expression of a coding sequence of the present invention. The term "coding sequence" as defined herein is a sequence which is transcribed into mRNA and translated into a polypeptide of the present invention when placed under the control of the above mentioned control sequences. The boundaries of the coding sequence are generally determined by a translation start codon ATG at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to, DNA, cDNA, and recombinant nucleic acid sequences.

**[0054]** The term "control sequences" is defined herein to include all components which are necessary or advantageous for expression of the coding sequence of the nucleic acid sequence. Each control sequence may be native or foreign to the nucleic acid sequence encoding the polypeptide. Such control sequences include, but are not limited to, a leader, a polyadenylation sequence, a propeptide sequence, a promoter, a signal sequence, and a transcription terminator. At a minimum, the control sequences include a promoter, and transcriptional and translational stop signals. The control sequences may be provided with linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences with the coding region of the nucleic acid sequence encoding a polypeptide.

**[0055]** The control sequence may be an appropriate promoter sequence, a nucleic acid sequence which is recognized by a host cell for expression of the nucleic acid sequence. The promoter sequence contains transcription and translation control sequences which mediate the expression of the polypeptide. The promoter may be any nucleic acid sequence which shows transcriptional activity in the host cell of choice and may be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell.

**[0056]** The control sequence may also be a suitable transcription terminator sequence, a sequence recognized by a host cell to terminate transcription. The terminator sequence is operably linked to the 3' terminus of the nucleic acid sequence encoding the polypeptide. Any terminator which is functional in the host cell of choice may be used in the present invention.

**[0057]** The control sequence may also be a polyadenylation sequence, a sequence which is operably linked to the 3' terminus of the nucleic acid sequence and which, when transcribed, is recognized by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence which is functional in the host cell of choice may be used in the present invention.

**[0058]** The control sequence may also be a signal peptide coding region, which codes for an amino acid sequence linked to the amino terminus of the polypeptide which can direct the expressed polypeptide into the cell's secretory pathway of the host cell. The 5' end of the coding sequence of the nucleic acid sequence may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region which encodes the secreted polypeptide.

**[0059]** Alternatively, the 5' end of the coding sequence may contain a signal peptide coding region which is foreign to that portion of the coding sequence which encodes the secreted polypeptide. A foreign signal peptide coding region may be required where the coding sequence does not normally contain a signal peptide coding region. Alternatively, the foreign signal peptide coding region may simply replace the natural signal peptide coding region in order to obtain enhanced secretion relative to the natural signal peptide coding region normally associated with the coding sequence. The signal peptide coding region may be obtained from a glucoamylase or an amylase gene from an *Aspergillus* species, a lipase or proteinase gene from a *Rhizomucor* species, the gene for the alpha-factor from *Saccharomyces cerevisiae*, an amylase or a protease gene from a *Bacillus* species, or the calf preprochymosin gene. However, any signal peptide coding region capable of directing the expressed polypeptide into the secretory pathway of a host cell of choice may be used in the present invention.

**[0060]** The control sequence may also be a propeptide coding region, which codes for an amino acid sequence positioned at the amino terminus of a polypeptide. The resultant polypeptide is known as a proenzyme or propolypeptide (or a zymogen in some cases). A propolypeptide is generally inactive and can be converted to mature active polypeptide by catalytic or autocatalytic cleavage of the propeptide from the propolypeptide. The propeptide coding region may be obtained from the *Bacillus subtilis* alkaline protease gene (aprE), the *Bacillus subtilis* neutral protease gene (nprT), the *Saccharomyces cerevisiae* alpha-factor gene, or the *Myceliophthora thermophilum* laccase gene (WO 95/33836).

**[0061]** The nucleic acid constructs of the present invention may also comprise one or more nucleic acid sequences which encode one or more factors that are advantageous in the expression of the polypeptide, e.g., an activator (e.g., a trans-acting factor), a chaperone, and a processing protease. Any factor that is functional in the host cell of choice may be used in the present invention. The nucleic acids encoding one or more of these factors are not necessarily in tandem with the nucleic acid sequence encoding the polypeptide.

**[0062]** An activator is a protein which activates transcription of a nucleic acid sequence encoding a polypeptide (Kudla et al., 1990, EMBO Journal 9:1355-1364; Jarai and Buxton,

1994, Current Genetics 26:2238-244; Verdier, 1990, Yeast 6:271-297). The nucleic acid sequence encoding an activator may be obtained from the genes encoding *Bacillus stearothermophilus* NprA (nprA), *Saccharomyces cerevisiae* heme activator protein 1 (hap1), *Saccharomyces cerevisiae* galactose metabolizing protein 4 (gal4), and *Aspergillus nidulans* ammonia regulation protein (areA). For further examples, see Verdier, 1990, supra and MacKenzie et al., 1993, Journal of General Microbiology 139:2295-2307.

**[0063]** A chaperone is a protein which assists another polypeptide in folding properly (Hartl et al., 1994, TIBS 19:20-25; Bergeron et al., 1994, TIBS 19:124-128; Demolder et al., 1994, Journal of Biotechnology 32:179-189; Craig, 1993, Science 260:1902-1903; Gething and Sambrook, 1992, Nature 355:33-45; Puig and Gilbert, 1994, Journal of Biological Chemistry 269:7764-7771; Wang and Tsou, 1993, The FASEB Journal 7:1515-11157; Robinson et al., 1994, Bio/Technology 1:381-384). The nucleic acid sequence encoding a chaperone may be obtained from the genes encoding *Bacillus subtilis* GroE proteins, *Aspergillus oryzae* protein disulphide isomerase, *Saccharomyces cerevisiae calnexin, Saccharomyces cerevisiae* BiP/GRP78, and *Saccharomyces cerevisiae* Hsp70. For further examples, see Gething and Sambrook, 1992, supra, and Hartl et al., 1994, supra.

**[0064]** A processing protease is a protease that cleaves a propeptide to generate a mature biochemically active polypeptide (Enderlin and Ogrydziak, 1994, Yeast 10:67-79; Fuller et al., 1989, Proceedings of the National Academy of Sciences USA 86:1434-1438; Julius et al., 1984, Cell 37:1075-1089; Julius et al., 1983, Cell 32:839-852). The nucleic acid sequence encoding a processing protease may be obtained from the genes encoding *Aspergillus niger* Kex2, *Saccharomyces cerevisiae* dipeptidylaminopeptidase, *Saccharomyces cerevisiae* Kex2, and *Yarrowia lipolytica* dibasic processing endoprotease (xpr6).

**[0065]** It may also be desirable to add regulatory sequences which allow the regulation of the expression of the polypeptide relative to the growth of the host cell. Examples of regulatory systems are those which cause the expression of the gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Regulatory systems in prokaryotic systems would include the lac, tac, and trp operator systems. In yeast, the ADH2 system or GAL1 system may be used. In filamentous fungi, the TAKA alpha-amylase promoter, *Aspergillus niger* glucoamylase promoter, and the *Aspergillus oryzae* glucoamylase promoter may be used as regulatory sequences. Other examples of regulatory sequences are those which allow for gene amplification. In eukaryotic systems, these include the dihydrofolate reductase gene which is amplified in the presence of methotrexate, and the metallothionein genes which are amplified with heavy metals. In these cases, the nucleic acid sequence encoding the polypeptide would be placed in tandem with the regulatory sequence.

Promoters

**[0066]** Examples of suitable promoters for directing the transcription of the nucleic acid constructs of the present invention, especially in a bacterial host cell, are the promoters obtained from the *E. coli* lac operon, the *Streptomyces coelicolor* agarase gene (dagA), the *Bacillus subtilis* levansucrase gene (sacB), the *Bacillus subtilis* alkaline protease gene, the *Bacillus licheniformis* alpha-amylase gene (amyL), the *Bacillus stearothermophilus* maltogenic amylase gene (amyM), the *Bacillus amyloliquefaciens* alpha-amylase gene (amyQ), the *Bacillus amyloliquefaciens* BAN amylase gene, the *Bacillus licheniformis* penicillinase gene (penP), the *Bacillus subtilis* xylA and xylB genes, and the prokaryotic beta-lactamase gene (Villa-Kamaroff et al., 1978, Proceedings of the National Academy of Sciences USA 75:3727-3731), as well as the tac promoter (DeBoer et al., 1983, Proceedings of the National Academy of Sciences USA 80:21-25), or the *Bacillus pumilus* xylosidase gene, or by the phage Lambda PR or PL promoters or the *E. coli* lac, trp or tac promoters. Further promoters are described in "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; and in Sambrook et al., 1989, supra.

**[0067]** Examples of suitable promoters for directing the transcription of the nucleic acid constructs of the present invention in a filamentous fungal host cell are promoters obtained from the genes encoding *Aspergillus oryzae* TAKA amylase, *Rhizomucor miehei* aspartic proteinase, *Aspergillus niger* neutral alpha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (glaA), *Rhizomucor miehei* lipase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Aspergillus nidulans* acetamidase, *Fusarium oxysporum* trypsin-like protease (as described in U.S. Pat. No. 4,288,627, which is incorporated herein by reference), and hybrids thereof. Particularly preferred promoters for use in filamentous fungal host cells are the TAKA amylase, NA2-tpi (a hybrid of the promoters from the genes encoding *Aspergillus niger* neutral alpha-amylase and *Aspergillus oryzae* triose phosphate isomerase), and glaA promoters. Further suitable promoters for use in filamentous fungus host cells are the ADH3 promoter (McKnight et al., The EMBO J. 4 (1985), 2093-2099) or the tpiA promoter.

**[0068]** Examples of suitable promoters for use in yeast host cells include promoters from yeast glycolytic genes (Hitzeman et al., J. Biol. Chem. 255 (1980), 12073-12080; Alber and Kawasaki, J. Mol. Appl. Gen. 1 (1982), 419-434) or alcohol dehydrogenase genes (Young et al., in Genetic Engineering of Microorganisms for Chemicals (Hollaender et al, eds.), Plenum Press, New York, 1982), or the TPI1 (U.S. Pat. No. 4,599,311) or ADH2-4-c (Russell et al., Nature 304 (1983), 652-654) promoters.

**[0069]** Further useful promoters are obtained from the *Saccharomyces cerevisiae* enolase (ENO-1) gene, the *Saccharomyces cerevisiae* galactokinase gene (GAL1), the *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase genes (ADH2/GAP), and the *Saccharomyces cerevisiae* 3-phosphoglycerate kinase gene. Other useful promoters for yeast host cells are described by Romanos et al., 1992, Yeast 8:423-488. In a mammalian host cell, useful promoters include viral promoters such as those from Simian Virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus, and bovine papilloma virus (BPV).

**[0070]** Examples of suitable promoters for directing the transcription of the DNA encoding the polypeptide of the invention in mammalian cells are the SV40 promoter (Subramani et al., Mol. Cell. Biol. 1 (1981), 854-864), the MT-1 (metallothionein gene) promoter (Palmiter et al., Science 222 (1983), 809-814) or the adenovirus 2 major late promoter.

**[0071]** An example of a suitable promoter for use in insect cells is the polyhedrin promoter (U.S. Pat. No. 4,745,051; Vasuvedan et al., FEBS Lett. 311, (1992) 7-11), the P10 promoter (J. M. Vlak et al., J. Gen. Virology 69, 1988, pp.

765-776), the *Autographa californica* polyhedrosis virus basic protein promoter (EP 397 485), the baculovirus immediate early gene 1 promoter (U.S. Pat. No. 5,155,037; U.S. Pat. No. 5,162,222), or the baculovirus 39K delayed-early gene promoter (U.S. Pat. No. 5,155,037; U.S. Pat. No. 5,162, 222).

Terminators

**[0072]** Preferred terminators for filamentous fungal host cells are obtained from the genes encoding *Aspergillus oryzae* TAKA amylase, *Aspergillus niger glucoamylase, Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* alpha-glucosidase, and *Fusarium oxysporum* trypsin-like protease. for fungal hosts) the TPI1 (Alber and Kawasaki, op. cit.) or ADH3 (McKnight et al., op. cit.) terminators.

**[0073]** Preferred terminators for yeast host cells are obtained from the genes encoding *Saccharomyces cerevisiae enolase, Saccharomyces cerevisiae* cytochrome C (CYC1), or *Saccharomyces cerevisiae* glyceraldehyde-3-phosphate dehydrogenase. Other useful terminators for yeast host cells are described by Romanos et al., 1992, supra.

Polyadenylation Signals

**[0074]** Preferred polyadenylation sequences for filamentous fungal host cells are obtained from the genes encoding *Aspergillus oryzae* TAKA amylase, *Aspergillus niger* glucoamylase, *Aspergillus nidulans* anthranilate synthase, and *Aspergillus niger* alpha-glucosidase.

**[0075]** Useful polyadenylation sequences for yeast host cells are described by Guo and Sherman, 1995, Molecular Cellular Biology 15:5983-5990.

**[0076]** Polyadenylation sequences are well known in the art for mammalian host cells such as SV40 or the adenovirus 5 Elb region.

Signal Sequences

**[0077]** An effective signal peptide coding region for bacterial host cells is the signal peptide coding region obtained from the maltogenic amylase gene from *Bacillus* NCIB 11837, the *Bacillus stearothermophilus* alpha-amylase gene, the *Bacillus licheniformis* subtilisin gene, the *Bacillus licheniformis* beta-lactamase gene, the *Bacillus stearothermophilus* neutral proteases genes (nprT, nprS, nprM), and the *Bacillus subtilis* PrsA gene. Further signal peptides are described by Simonen and Palva, 1993, Microbiological Reviews 57:109-137.

**[0078]** An effective signal peptide coding region for filamentous fungal host cells is the signal peptide coding region obtained from *Aspergillus oryzae* TAKA amylase gene, *Aspergillus niger* neutral amylase gene, the *Rhizomucor miehei* aspartic proteinase gene, the *Humicola lanuginosa* cellulase or lipase gene, or the *Rhizomucor miehei* lipase or protease gene, *Aspergillus* sp. amylase or glucoamylase, a gene encoding a *Rhizomucor miehei* lipase or protease. The signal peptide is preferably derived from a gene encoding *A. oryzae* TAKA amylase, *A. niger* neutral alpha-amylase, *A. niger* acid-stable amylase, or *A. niger* glucoamylase.

**[0079]** Useful signal peptides for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* alpha-factor and *Saccharomyces cerevisiae* invertase. Other useful signal peptide coding regions are described by Romanos et al., 1992, supra.

**[0080]** For secretion from yeast cells, the secretory signal sequence may encode any signal peptide which ensures efficient direction of the expressed polypeptide into the secretory pathway of the cell. The signal peptide may be naturally occurring signal peptide, or a functional part thereof, or it may be a synthetic peptide. Suitable signal peptides have been found to be the a-factor signal peptide (cf. U.S. Pat. No. 4,870,008), the signal peptide of mouse salivary amylase (cf. O. Hagenbuchle et al., Nature 289, 1981, pp. 643-646), a modified carboxypeptidase signal peptide (cf. L. A. Valls et al., Cell 48, 1987, pp. 887-897), the yeast BAR1 signal peptide (cf. WO 87/02670), or the yeast aspartic protease 3 (YAP3) signal peptide (cf. M. Egel-Mitani et al., Yeast 6, 1990, pp. 127-137).

**[0081]** For efficient secretion in yeast, a sequence encoding a leader peptide may also be inserted downstream of the signal sequence and upstream of the DNA sequence encoding the polypeptide. The function of the leader peptide is to allow the expressed polypeptide to be directed from the endoplasmic reticulum to the Golgi apparatus and further to a secretory vesicle for secretion into the culture medium (i.e. exportation of the polypeptide across the cell wall or at least through the cellular membrane into the periplasmic space of the yeast cell). The leader peptide may be the yeast a-factor leader (the use of which is described in e.g. U.S. Pat. No. 4,546,082, EP 16 201, EP 123 294, EP 123 544 and EP 163 529). Alternatively, the leader peptide may be a synthetic leader peptide, which is to say a leader peptide not found in nature. Synthetic leader peptides may, for instance, be constructed as described in WO 89/02463 or WO 92/11378.

**[0082]** For use in insect cells, the signal peptide may conveniently be derived from an insect gene (cf. WO 90/05783), such as the lepidopteran *Manduca sexta* adipokinetic hormone precursor signal peptide (cf. U.S. Pat. No. 5,023,328).

Expression Vectors

**[0083]** The present invention also relates to recombinant expression vectors comprising a nucleic acid sequence of the present invention, a promoter, and transcriptional and translational stop signals. The various nucleic acid and control sequences described above may be joined together to produce a recombinant expression vector which may include one or more convenient restriction sites to allow for insertion or substitution of the nucleic acid sequence encoding the polypeptide at such sites. Alternatively, the nucleic acid sequence of the present invention may be expressed by inserting the nucleic acid sequence or a nucleic acid construct comprising the sequence into an appropriate vector for expression. In creating the expression vector, the coding sequence is located in the vector so that the coding sequence is operably linked with the appropriate control sequences for expression, and possibly secretion.

**[0084]** The recombinant expression vector may be any vector (e.g., a plasmid or virus) which can be conveniently subjected to recombinant DNA procedures and can bring about the expression of the nucleic acid sequence. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vectors may be linear or closed circular plasmids. The vector may be an autonomously replicating vector, i.e., a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g., a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may con-

tain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. The vector system may be a single vector or plasmid or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the host cell, or a transposon.

**[0085]** The vectors of the present invention preferably contain one or more selectable markers which permit easy selection of transformed cells. A selectable marker is a gene the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like. Examples of bacterial selectable markers are the dal genes from *Bacillus subtilis* or *Bacillus licheniformis*, or markers which confer antibiotic resistance such as ampicillin, kanamycin, chloramphenicol, tetracycline, neomycin, hygromycin or methotrexate resistance. A frequently used mammalian marker is the dihydrofolate reductase gene (DHFR). Suitable markers for yeast host cells are ADE2, HIS3, LEU2, LYS2, MET3, TRP1, and URA3. A selectable marker for use in a filamentous fungal host cell may be selected from the group including, but not limited to, amdS (acetamidase), argB (ornithine carbamoyltransferase), bar (phosphinothricin acetyltransferase), hygB (hygromycin phosphotransferase), niaD (nitrate reductase), pyrG (orotidine-5'-phosphate decarboxylase), sC (sulfate adenyltransferase), trpC (anthranilate synthase), and glufosinate resistance markers, as well as equivalents from other species. Preferred for use in an *Aspergillus* cell are the amdS and pyrG markers of *Aspergillus nidulans* or *Aspergillus oryzae* and the bar marker of *Streptomyces hygroscopicus*. Furthermore, selection may be accomplished by co-transformation, e.g., as described in WO 91/17243, where the selectable marker is on a separate vector.

**[0086]** The vectors of the present invention preferably contain an element(s) that permits stable integration of the vector into the host cell genome or autonomous replication of the vector in the cell independent of the genome of the cell.

**[0087]** The vectors of the present invention may be integrated into the host cell genome when introduced into a host cell. For integration, the vector may rely on the nucleic acid sequence encoding the polypeptide or any other element of the vector for stable integration of the vector into the genome by homologous or nonhomologous recombination. Alternatively, the vector may contain additional nucleic acid sequences for directing integration by homologous recombination into the genome of the host cell. The additional nucleic acid sequences enable the vector to be integrated into the host cell genome at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, the integrational elements should preferably contain a sufficient number of nucleic acids, such as 100 to 1,500 base pairs, preferably 400 to 1,500 base pairs, and most preferably 800 to 1,500 base pairs, which are highly homologous with the corresponding target sequence to enhance the probability of homologous recombination. The integrational elements may be any sequence that is homologous with the target sequence in the genome of the host cell. Furthermore, the integrational elements may be non-encoding or encoding nucleic acid sequences. On the other hand, the vector may be integrated into the genome of the host cell by non-homologous recombination. These nucleic acid sequences may be any sequence that is homologous with a target sequence in the genome of the host cell, and, furthermore, may be non-encoding or encoding sequences.

**[0088]** For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. Examples of bacterial origins of replication are the origins of replication of plasmids pBR322, pUC19, pACYC177, pACYC184, pUB110, pE194, pTA1060, and pAMβ1. Examples of origin of replications for use in a yeast host cell are the 2 micron origin of replication, the combination of CEN6 and ARS4, and the combination of CEN3 and ARS1. The origin of replication may be one having a mutation which makes its functioning temperature-sensitive in the host cell (see, e.g., Ehrlich, 1978, Proceedings of the National Academy of Sciences USA 75:1433).

**[0089]** More than one copy of a nucleic acid sequence encoding a polypeptide of the present invention may be inserted into the host cell to amplify expression of the nucleic acid sequence. Stable amplification of the nucleic acid sequence can be obtained by integrating at least one additional copy of the sequence into the host cell genome using methods well known in the art and selecting for transformants.

**[0090]** The procedures used to ligate the elements described above to construct the recombinant expression vectors of the present invention are well known to one skilled in the art (see, e.g., Sambrook et al., 1989, supra).

Host Cells

**[0091]** The present invention also relates to recombinant host cells, comprising a nucleic acid sequence of the invention, which are advantageously used in the recombinant production of the polypeptides. The term "host cell" encompasses any progeny of a parent cell which is not identical to the parent cell due to mutations that occur during replication.

**[0092]** The cell is preferably transformed with a vector comprising a nucleic acid sequence of the invention followed by integration of the vector into the host chromosome. "Transformation" means introducing a vector comprising a nucleic acid sequence of the present invention into a host cell so that the vector is maintained as a chromosomal integrant or as a self-replicating extra-chromosomal vector. Integration is generally considered to be an advantage as the nucleic acid sequence is more likely to be stably maintained in the cell. Integration of the vector into the host chromosome may occur by homologous or non-homologous recombination as described above.

**[0093]** The choice of a host cell will to a large extent depend upon the gene encoding the polypeptide and its source. The host cell may be a unicellular microorganism, e.g., a prokaryote, or a non-unicellular microorganism, e.g., a eukaryote. Useful unicellular cells are bacterial cells such as gram positive bacteria including, but not limited to, a *Bacillus* cell, e.g., *Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus coagulans, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus stearothermophilus, Bacillus subtilis*, and *Bacillus thuringiensis*; or a *Streptomyces* cell, e.g., *Streptomyces lividans* or *Streptomyces murinus*, or gram negative bacteria such as *E. coli* and *Pseudomonas* sp. In a preferred embodiment, the bacterial host cell is a *Bacillus lentus, Bacillus licheniformis, Bacillus stearothermophilus* or *Bacillus subtilis* cell. The transformation of a bacterial host cell may,

for instance, be effected by protoplast transformation (see, e.g., Chang and Cohen, 1979, Molecular General Genetics 168:111-115), by using competent cells (see, e.g., Young and Spizizin, 1961, Journal of Bacteriology 81:823-829, or Dubnar and Davidoff-Abelson, 1971, Journal of Molecular Biology 56:209-221), by electroporation (see, e.g., Shigekawa and Dower, 1988, Biotechniques 6:742-751), or by conjugation (see, e.g., Koehler and Thorne, 1987, Journal of Bacteriology 169:5771-5278).

**[0094]** The host cell may be a eukaryote, such as a mammalian cell, an insect cell, a plant cell or a fungal cell.

**[0095]** Useful mammalian cells include Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, COS cells, or any number of other immortalized cell lines available, e.g., from the American Type Culture Collection.

**[0096]** Examples of suitable mammalian cell lines are the COS (ATCC CRL 1650 and 1651), BHK (ATCC CRL 1632, 10314 and 1573, ATCC CCL 10), CHL (ATCC CCL39) or CHO (ATCC CCL 61) cell lines. Methods of transfecting mammalian cells and expressing DNA sequences introduced in the cells are described in e.g. Kaufman and Sharp, J. Mol. Biol. 159 (1982), 601-621; Southern and Berg, J. Mol. Appl. Genet. 1 (1982), 327-341; Loyter et al., Proc. Natl. Acad. Sci. USA 79 (1982), 422-426; Wigler et al., Cell 14 (1978), 725; Corsaro and Pearson, Somatic Cell Genetics 7 (1981), 603, Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., N.Y., 1987, Hawley-Nelson et al., Focus 15 (1993), 73; Ciccarone et al., Focus 15 (1993), 80; Graham and van der Eb, Virology 52 (1973), 456; and Neumann et al., EMBO J. 1 (1982), 841-845.

**[0097]** In a preferred embodiment, the host cell is a fungal cell. "Fungi" as used herein includes the phyla Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota (as defined by Hawksworth et al., In, Ainsworth and Bisby's Dictionary of The Fungi, 8th edition, 1995, CAB International, University Press, Cambridge, UK) as well as the Oomycota (as cited in Hawksworth et al., 1995, supra, page 171) and all mitosporic fungi (Hawksworth et al., 1995, supra). Representative groups of Ascomycota include, e.g., *Neurospora, Eupenicillium* (=*Penicillium*), *Emericella* (=*Aspergillus*), *Eurotium* (=*Aspergillus*), and the true yeasts listed above. Examples of Basidiomycota include mushrooms, rusts, and smuts. Representative groups of Chytridiomycota include, e.g., Allomyces, Blastocladiella, Coelomomyces, and aquatic fungi. Representative groups of Oomycota include, e.g., Saprolegniomycetous aquatic fungi (water molds) such as *Achlya*. Examples of mitosporic fungi include *Aspergillus, Penicillium, Candida*, and *Alternaria*. Representative groups of Zygomycota include, e.g., *Rhizopus* and *Mucor*.

**[0098]** In a preferred embodiment, the fungal host cell is a yeast cell. "Yeast" as used herein includes ascosporogenous yeast (Endomycetales), basidiosporogenous yeast, and yeast belonging to the Fungi Imperfecti (Blastomycetes). The ascosporogenous yeasts are divided into the families Spermophthoraceae and Saccharomycetaceae. The latter is comprised of four subfamilies, Schizosaccharomycoideae (e.g., genus *Schizosaccharomyces*), Nadsonioideae, Lipomycoideae, and Saccharomycoideae (e.g., genera *Pichia, Kluyveromyces* and *Saccharomyces*). The basidiosporogenous yeasts include the genera *Leucosporidim, Rhodosporidium, Sporidiobolus, Filobasidium*, and *Filobasidiella*. Yeast belonging to the Fungi Imperfecti are divided into two families, Sporobolomycetaceae (e.g., genera *Sorobolomyces* and *Bullera*) and Cryptococcaceae (e.g., genus *Candida*). Since the classification of yeast may change in the future, for the purposes of this invention, yeast shall be defined as described in Biology and Activities of Yeast (Skinner, F. A., Passmore, S. M., and Davenport, R. R., eds, Soc. App. Bacteriol. Symposium Series No. 9, 1980. The biology of yeast and manipulation of yeast genetics are well known in the art (see, e.g., Biochemistry and Genetics of Yeast, Bacil, M., Horecker, B. J., and Stopani, A. O. M., editors, 2nd edition, 1987; The Yeasts, Rose, A. H., and Harrison, J. S., editors, 2nd edition, 1987; and The Molecular Biology of the Yeast *Saccharomyces, Strathern et al., editors,* 1981).

**[0099]** The yeast host cell may be selected from a cell of a species of *Candida, Kluyveromyces, Saccharomyces, Schizosaccharomyces, Candida, Pichia, Hansenula*, or *Yarrowia*. In a preferred embodiment, the yeast host cell is a *Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces diastaticus, Saccharomyces douglasii, Saccharomyces kluyveri, Saccharomyces norbensis* or *Saccharomyces oviformis* cell. Other useful yeast host cells are a *Kluyveromyces lactis, Kluyveromyces fragilis, Hansenula polymorpha, Pichia pastoris, Yarrowia lipolytica, Schizosaccharomyces pombe, Ustilgo maylis, Candida maltose, Pichia guillermondii* and *Pichia methanolio* cell (cf. Gleeson et al., J. Gen. Microbiol. 132, 1986, pp. 3459-3465; U.S. Pat. No. 4,882,279 and U.S. Pat. No. 4,879,231).

**[0100]** In a preferred embodiment, the fungal host cell is a filamentous fungal cell. "Filamentous fungi" include all filamentous forms of the subdivision Eumycota and Oomycota (as defined by Hawksworth et al., 1995, supra). The filamentous fungi are characterized by a vegetative mycelium composed of chitin, cellulose, glucan, chitosan, mannan, and other complex polysaccharides. Vegetative growth is by hyphal elongation and carbon catabolism is obligately aerobic. In contrast, vegetative growth by yeasts such as *Saccharomyces cerevisiae* is by budding of a unicellular thallus and carbon catabolism may be fermentative. In a more preferred embodiment, the filamentous fungal host cell is a cell of a species of, but not limited to, *Acremonium, Aspergillus, Fusarium, Humicola, Mucor, Myceliophthora, Neurospora, Penicillium, Thielavia, Tolypocladium*, and *Trichoderma* or a teleomorph or synonym thereof. In an even more preferred embodiment, the filamentous fungal host cell is an *Aspergillus* cell. In another even more preferred embodiment, the filamentous fungal host cell is an *Acremonium* cell. In another even more preferred embodiment, the filamentous fungal host cell is a *Fusarium* cell. In another even more preferred embodiment, the filamentous fungal host cell is a *Humicola* cell. In another even more preferred embodiment, the filamentous fungal host cell is a *Mucor* cell. In another even more preferred embodiment, the filamentous fungal host cell is a *Myceliophthora* cell. In another even more preferred embodiment, the filamentous fungal host cell is a *Neurospora* cell. In another even more preferred embodiment, the filamentous fungal host cell is a *Penicillium* cell. In another even more preferred embodiment, the filamentous fungal host cell is a *Thielavia* cell. In another even more preferred embodiment, the filamentous fungal host cell is a *Tolypocladium* cell. In another even more preferred embodiment, the filamentous fungal host cell is a *Trichoderma* cell. In a most preferred embodiment, the filamentous fungal host cell is an *Aspergillus awamori, Aspergillus foetidus, Aspergillus japonicus, Aspergillus niger, Aspergillus nidulans* or *Aspergillus oryzae*

cell. In another most preferred embodiment, the filamentous fungal host cell is a *Fusarium* cell of the section Discolor (also known as the section *Fusarium*). For example, the filamentous fungal parent cell may be a *Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium reticulaturn, Fusarium roseum, Fusarium sambucinum, Fusarium sarcochroum, Fusarium sulphureum*, or *Fusarium trichothecioides* cell. In another preferred embodiment, the filamentous fungal parent cell is a *Fusarium* strain of the section Elegans, e.g., *Fusarium oxysporum*. In another most preferred embodiment, the filamentous fungal host cell is a *Humicola insolens* or *Humicola lanuginosa* cell. In another most preferred embodiment, the filamentous fungal host cell is a *Mucor miehei* cell. In another most preferred embodiment, the filamentous fungal host cell is a *Myceliophthora thermophilum* cell. In another most preferred embodiment, the filamentous fungal host cell is a *Neurospora crassa* cell. In another most preferred embodiment, the filamentous fungal host cell is a *Penicillium purpurogenum* cell. In another most preferred embodiment, the filamentous fungal host cell is a *Thielavia terrestris* cell or an *Acremonium chtysogenum* cell. In another most preferred embodiment, the *Trichoderma* cell is a *Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei* or *Trichoderma viride* cell. The use of *Aspergillus* spp. for the expression of proteins is described in, e.g., EP 272 277, EP 230 023.

Transformation

**[0101]** Fungal cells may be transformed by a process involving protoplast formation, transformation of the protoplasts, and regeneration of the cell wall in a manner known per se. Suitable procedures for transformation of *Aspergillus* host cells are described in EP 238 023 and Yelton et al., 1984, Proceedings of the National Academy of Sciences USA 81:1470-1474. A suitable method of transforming *Fusarium* species is described by Malardier et al., 1989, Gene 78:147-156 or in copending U.S. Ser. No. 08/269,449. Examples of other fungal cells are cells of filamentous fungi, e.g. *Aspergillus* spp., *Neurospora* spp., *Fusarium* spp. or *Trichoderma* spp., in particular strains of *A. oryzae, A. nidulans* or *A. niger*. The use of *Aspergillus* spp. for the expression of proteins is described in, e.g., EP 272 277, EP 230 023. The transformation of *F. oxysporum* may, for instance, be carried out as described by Malardier et al., 1989, Gene 78: 147-156.

**[0102]** Yeast may be transformed using the procedures described by Becker and Guarente, In Abelson, J. N. and Simon, M. I., editors, Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology, Volume 194, pp 182-187, Academic Press, Inc., New York; Ito et al., 1983, Journal of Bacteriology 153:163; and Hinnen et al., 1978, Proceedings of the National Academy of Sciences USA 75:1920. Mammalian cells may be transformed by direct uptake using the calcium phosphate precipitation method of Graham and Van der Eb (1978, Virology 52:546).

**[0103]** Transformation of insect cells and production of heterologous polypeptides therein may be performed as described in U.S. Pat. No. 4,745,051; U.S. Pat. No. 4,775,624; U.S. Pat. No. 4,879,236; U.S. Pat. No. 5,155,037; U.S. Pat. No. 5,162,222; EP 397,485) all of which are incorporated herein by reference. The insect cell line used as the host may suitably be a *Lepidoptera* cell line, such as *Spodoptera frugiperda* cells or *Trichoplusia ni* cells (cf. U.S. Pat. No. 5,077,214). Culture conditions may suitably be as described in, for instance, WO 89/01029 or WO 89/01028, or any of the aforementioned references.

Methods of Production

**[0104]** The transformed or transfected host cells described above are cultured in a suitable nutrient medium under conditions permitting the production of the desired molecules, after which these are recovered from the cells, or the culture broth.

**[0105]** The medium used to culture the cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection). The media are prepared using procedures known in the art (see, e.g., references for bacteria and yeast; Bennett, J. W. and LaSure, L., editors, More Gene Manipulations in Fungi, Academic Press, CA, 1991).

**[0106]** If the molecules are secreted into the nutrient medium, they can be recovered directly from the medium. If they are not secreted, they can be recovered from cell lysates. The molecules are recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. ion exchange chromatography, gelfiltration chromatography, affinity chromatography, or the like, dependent on the type of molecule in question.

**[0107]** The molecules of interest may be detected using methods known in the art that are specific for the molecules. These detection methods may include use of specific antibodies, formation of a product, or disappearance of a substrate. For example, an enzyme assay may be used to determine the activity of the molecule. Procedures for determining various kinds of activity are known in the art.

**[0108]** The molecules of the present invention may be purified by a variety of procedures known in the art including, but not limited to, chromatography (e.g., ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (e.g., preparative isoelectric focusing (IEF), differential solubility (e.g., ammonium sulfate precipitation), or extraction (see, e.g., Protein Purification, J-C Janson and Lars Ryden, editors, VCH Publishers, New York, 1989).

**[0109]** The term "immunological response", used in connection with the present invention, is the response of an organism to a compound, which involves the immune system according to any of the four standard reactions (Type I, II, III and IV according to Coombs & Gell).

**[0110]** Correspondingly, the "immunogenicity" of a compound used in connection with the present invention refers to the ability of this compound to induce an 'immunological response' in animals including man.

**[0111]** The term "allergic response", used in connection with the present invention, is the response of an organism to a compound, which involves IgE mediated responses (Type I reaction according to Coombs & Gell). It is to be understood that sensibilization (i.e. development of compound-specific IgE antibodies) upon exposure to the compound is included in the definition of "allergic response".

**[0112]** Correspondingly, the "allergenicity" of a compound used in connection with the present invention refers to the ability of this compound to induce an 'allergic response' in animals including man.

**[0113]** The term "parent protein" refer to the polypeptide to be modified by creating a library of diversified mutants. The "parent protein" may be a naturally occurring (or wild-type) polypeptide or it may be a variant thereof prepared by any suitable means. For instance, the "parent protein" may be a variant of a naturally occurring polypeptide which has been modified by substitution, deletion or truncation of one or more amino acid residues or by addition or insertion of one or more amino acid residues to the amino acid sequence of a naturally-occurring polypeptide.

**[0114]** The term "enzyme variants" or "protein variants" refer to a polypeptide of the invention comprising one or more substitutions of the specified amino acid residues. The total number of such substitutions is typically not more than 10, e.g. one, two, three, four, five or six of said substitutions. In addition, the enzyme variant or protein variant of the invention may optionally include other modifications of the parent enzyme, typically not more than 10, e.g. not more than 5 such modifications. The variant generally has a homology with the parent enzyme of at least 80%, e.g. at least 85%, typically at least 90% or at least 95%.

**[0115]** The term "randomized library" of protein variants refers to a library with at least partially randomized composition of the members, e.g. protein variants.

**[0116]** An "epitope" is a set of amino acids on a protein that are involved in an immunological response, such as antibody binding or T-cell activation. One particularly useful method of identifying epitopes involved in antibody binding is to screen a library of peptide-phage membrane protein fusions and selecting those that bind to relevant antigen-specific antibodies, sequencing the randomized part of the fusion gene, aligning the sequences involved in binding, defining consensus sequences based on these alignments, and mapping these consensus sequences on the surface or the sequence and/or structure of the antigen, to identify epitopes involved in antibody binding.

**[0117]** By the term "epitope pattern" is meant such a consensus sequence of antibody binding peptides. An example is the epitope pattern A R R<R. The sign "<" in this notation indicates that the aligned antibody binding peptides included a non-consensus amino acid between the second and the third arginine.

**[0118]** An "epitope area" is defined as the amino acids situated close to the epitope sequence amino acids. Preferably, the amino acids of an epitope area are located <5 Å from the epitope sequence. Hence, an epitope area also includes the corresponding epitope sequence itself. Modifications of amino acids of the 'epitope area' can possibly affect the immunogenic function of the corresponding epitope.

**[0119]** By the term "epitope sequence" is meant the amino acid residues of a parent protein, which have been identified to belong to an epitope by the methods of the present invention (an example of an epitope sequence is E271 Q12 I8 in Savinase).

**[0120]** The term 'antibody binding peptide' denotes a peptide that bind with sufficiently high affinity to antibodies. Identification of 'antibody binding peptides' and their sequences constitute the first step of the method of this invention.

**[0121]** "Anchor amino acids" are the individual amino acids of an epitope pattern.

**[0122]** "Hot spot amino acids" are amino acids of parent protein, which are particularly likely to result in modified immunogenecity if they are mutated. Amino acids, which appear in three or more epitope sequences or which correspond to anchor amino acids are hot spot amino acids.

**[0123]** "Environmental allergens" are protein allergens that are present naturally. They include pollen, dust mite allergens, pet allergens, food allergens, venoms, etc.

**[0124]** "Commercial allergens" are protein allergens that are being brought to the market commercially. They include enzymes, pharmaceutical proteins, antimicrobial peptides, as well as allergens of transgenic plants.

**[0125]** The "donor protein" is the protein that was used to raise antibodies used to identify antibody binding sequences, hence the donor protein provides the information that leads to the epitope patterns.

**[0126]** The "acceptor protein" is the protein, whose structure is used to fit the identified epitope patterns and/or to fit the antibody binding sequences. Hence the acceptor protein is also the parent protein.

**[0127]** An "autoepitope" is one that has been identified using antibodies raised against the parent protein, i.e. the acceptor and the donor proteins are identical.

**[0128]** A "heteroepitope" is one that has been identified with distinct donor and acceptor proteins.

**[0129]** The term "functionality" of protein variants refers to e.g. enzymatic activity; binding to a ligand or receptor; stimulation of a cellular response (e.g. $^{3}$H-thymidine incorporation as response to a mitogenic factor); or anti-microbial activity.

**[0130]** By the term "specific polyclonal antibodies" is meant polyclonal antibodies isolated according to their specificity for a certain antigen, e.g. the protein backbone.

**[0131]** By the term "monospecific antibodies" is meant polyclonal antibodies isolated according to their specificity for a certain epitope. Such monospecific antibodies will bind to the same epitope, but with different affinity, as they are produced by a number of antibody producing cells recognizing overlapping but not necessarily identical epitopes.

**[0132]** The term "randomized library" of protein variants refers to a library with at least partially randomized composition of the members, e.g. protein variants.

**[0133]** 'Spiked mutagenesis' is a form of site-directed mutagenesis, in which the primers used have been synthesized using mixtures of oligonucleotides at one or more positions.

**[0134]** By the term "a protein variant having modified immunogenicity as compared to the parent protein" is meant a protein variant which differs from the parent protein in one or more amino acids whereby the immunogenicity of the variant is modified. The modification of immunogenicity may be confirmed by testing the ability of the protein variant to elicit an IgE/IgG response.

**[0135]** In the present context the term "protein" is intended to cover oligopeptides, polypeptides as well as proteins as such.

## DETAILED DESCRIPTION OF THE INVENTION

**[0136]** The present invention relates to a method of selecting a protein variant having modified immunogenicity as compared to a parent protein, comprising the steps of:

**[0137]** a) obtaining antibody binding peptide sequences,

**[0138]** b) using the sequences to localise epitope sequences on the 3-dimensional structure of parent protein,

**[0139]** c) defining an epitope area including amino acids situated within 5 Å from the epitope amino acids constituting the epitope sequence,

**[0140]** d) changing one or more of the amino acids defining the epitope area of the parent protein by genetic engineering mutations of a DNA sequence encoding the parent protein,

**[0141]** e) introducing the mutated DNA sequence into a suitable host, culturing said host and expressing the protein variant, and

**[0142]** f) evaluating the immunogenicity of the protein variant using the parent protein as reference.

A) How to Find Antibody Binding Peptide Sequences and Epitope Patterns

**[0143]** A first step of the method is to identify peptide sequences, which bind specifically to antibodies.

**[0144]** Antibody binding peptide sequences can be found by testing a set of known peptide sequences for binding to antibodies raised against the donor protein. These sequences are typically selected, such that each represents a segment of the donor protein sequence (Mol. Immunol., 1992, vol. 29, pp. 1383-1389; Am. J. Resp. Cell. Mol. Biol. 2000, vol. 22, pp. 344-351). Also, randomized synthetic peptide libraries can be used to find antibody binding sequences (Slootstra et al; Molecular Diversity, 1996, vol. 2, pp. 156-164).

**[0145]** In a preferred method, the identification of antibody binding sequences may be achieved by screening of a display package library, preferably a phage display library. The principle behind phage display is that a heterologous DNA sequence can be inserted in the gene coding for a coat protein of the phage (WO 92/15679). The phage will make and display the hybrid protein on its surface where it can interact with specific target agents. Such target agent may be antigen-specific antibodies. It is therefore possible to select specific phages that display antibody-binding peptide sequences. The displayed peptides can be of predetermined lengths, for example 9 amino acids long, with randomized sequences, resulting in a random peptide display package library. Thus, by screening for antibody binding, one can isolate the peptide sequences that have sufficiently high affinity for the particular antibody used. The peptides of the hybrid proteins of the specific phages which bind protein-specific antibodies characterize epitopes that are recognized by the immune system.

**[0146]** The antibodies used for reacting with the display package are preferably IgE antibodies to ensure that the epitopes identified are IgE epitopes, i.e. epitopes inducing and binding IgE. In a preferred embodiment the antibodies are polyclonal antibodies, optionally monospecific antibodies.

**[0147]** For the purpose of the present invention polyclonal antibodies are preferred in order to obtain a broader knowledge about the epitopes of a protein.

**[0148]** It is of great importance that the amino acid sequence of the peptides presented by the display packages is long enough to represent a significant part of the epitope to be identified. In a preferred embodiment of the invention the peptides of the peptide display package library are oligopeptides having from 5 to 25 amino acids, preferably at least 8 amino acids, such as 9 amino acids. For a given length of peptide sequences (n), the theoretical number of different possible sequences can be calculated as $20^n$. The diversity of the package library used must be large enough to provide a suitable representation of the theoretical number of different sequences. In a phage-display library, each phage has one specific sequence of a determined length. Hence an average phage display library can express $10^8$-$10^{12}$ different random sequences, and is therefore well-suited to represent the theoretical number of different sequences.

**[0149]** The antibody binding peptide sequences can be further analysed by consensus alignment e.g. by the methods described by Feng and Doolittle, Meth. Enzymol., 1996, vol. 266, pp. 368-382; Feng and Doolittle, J. Mol. Evol., 1987, vol. 25, pp. 351-360; and Taylor, Meth. Enzymol., 1996, vol. 266, pp. 343-367.

**[0150]** This leads to identification of epitope patterns, which can assist the comparison of the linear information obtained from the antibody binding peptide sequences to the 3-o dimensional structure of the acceptor protein in order to identify epitope sequences at the surface of the acceptor protein.

B) How to Identify Epitope Sequences and Epitope Areas.

**[0151]** Given a number of antibody binding peptide sequences and possibly the corresponding epitope patterns, one need the 3-dimensional structure coordinates of an acceptor protein to find the epitope sequences on its surface.

**[0152]** These coordinates can be found in databases (NCBI: http://www.ncbi.nlm.nih.gov/), determined experimentally using conventional methods (Ducruix and Giege: Crystallization of Nucleic Acids and Proteins, IRL PRess, Oxford, 1992, ISBN 0-19-963245-6), or they can be deduced from the coordinates of a homologous protein. Typical actions required for the construction of a model structure are: alignment of homologous sequences for which 3-dimensional structures exist, definition of Structurally Conserved Regions (SCRs), assignment of coordinates to SCRs, search for structural fragments/loops in structure databases to replace Variable Regions, assignment of coordinates to these regions, and structural refinement by energy minimization. Regions containing large inserts (>3 residues) relative to the known 3-dimensional structures are known to be quite difficult to model, and structural predictions must be considered with care.

**[0153]** Using the coordinates and the several methods of mapping the linear information on the 3-dimensional surface are possible, as described in the examples below.

**[0154]** One can match each amino acid residue of the antibody binding peptide to an identical or homologous amino acid on the 3-D surface of the acceptor protein, such that amino acids that are adjacent in the primary sequence are close on the surface of the acceptor protein, with close being <5 Å, preferably <3 Å between any two atoms of the two amino acids.

**[0155]** Alternatively, one can define a geometric body (e.g. an ellipsoid, a sphere, or a box) of a size that matches a possible binding interface between antibody and antigen and look for a positioning of this body where it will contain most of or all the anchor amino acids.

**[0156]** Also, one can use the epitope patterns to facilitate identification of epitope sequences. This can be done, by first matching the anchor amino acids on the 3-D structure and subsequently looking for other elements of the antibody binding peptide sequences, which provide additional matches. If there are many residues to be matched, it is only necessary that a suitable number can be found on the 3-D structure. For

example if an epitope pattern comprises 4, 5, 6, or 7 amino acids, it is only necessary that 3 matches surface elements of the acceptor protein.

**[0157]** In all cases, it is desirable that amino acids of the epitope sequence are surface exposed (as described below in Examples).

**[0158]** It is known, that amino acids that surround binding sequences can affect binding of a ligand without participating actively in the binding process. Based on this knowledge, areas covered by amino acids with potential steric effects on the epitope-antibody interaction, were defined around the identified epitope sequences. These areas are called 'epitope areas'. Practically, all amino acids situated within 5 Å from the amino acids defining the epitope sequence were included. Preferably, the epitope area equals the epitope sequence. The accessibility criterium was not used as hidden amino acids of an epitope area also can have an effect on the adjacent amino acids of the epitope sequence.

C) How to Use the Epitope Information.

**[0159]** There are at least four ways to utilize the information about epitope sequences, which has been derived by the methods of this invention:

**[0160]** 1) reduce the allergenicity of a commercial protein using protein engineering.

**[0161]** 2) reduce the potential of commercial proteins to cross-react with environmental allergens and hence cause allergic reactions in people sensitized to the environmental allergens (or vice versa).

**[0162]** 3) improve the immunotherapeutic effect of allergen vaccines.

**[0163]** 4) assist characterization of clinical allergies in order to select the appropriate allergen vaccine.

Protein Engineering to Reduce the Allergenicity, Cross-Reactivity and/or Immunotherapeutic Effect of Proteins.

**[0164]** The methods described thus far have led to identification of epitope areas on an acceptor protein, each containing epitope sequences. These subsets of amino acids, are preferred for introducing mutations that are meant to modify the immunogenecity of the acceptor protein. An even more preferred subset of amino acids to target by mutagenesis are 'hot spot amino acids', which appear in several different epitope sequences, or which corresponds to anchor amino acids of the epitope patterns.

**[0165]** Thus, genetic engineering mutations should be designed in the epitope areas, preferably in epitope sequences, and more preferably in the 'hot spot amino acids'.

Substitution, Deletion, Insertion

**[0166]** When the epitope area(s) have been identified, a protein variant exhibiting a modified immunogenicity may be produced by changing the identified epitope area of the parent protein by genetic engineering mutation of a DNA sequence encoding the parent protein.

**[0167]** The epitope identified may be changed by substituting at least one amino acid of the epitope area. In a preferred embodiment at least one anchor amino acid or hot spot amino acid is changed. The change will often be substituting to an amino acid of different size, hydrophilicity, and/or polarity, such as a small amino acid versus a large amino acid, a hydrophilic amino acid versus a hydrophobic amino acid, a polar amino acid versus a non-polar amino acid and a basic versus an acidic amino acid.

**[0168]** Other changes may be the addition/insertion or deletion of at least one amino acid of the epitope sequence, preferably deleting an anchor amino acid or a hot spot amino acid. Furthermore, an epitope pattern may be changed by substituting some amino acids, and deleting/adding other.

**[0169]** In the claims a position to be changed by substitution, insertion, deletion will be indicated by: "Position xx to aaa, bbb, ccc, insertion, deletion", meaning that position xx can be substituted by the amino acid aaa, bbb, ccc or that any amino acid can be inserted after position xx or that position xx can be deleted, e.g. "Position 27 to A, D, E, insertion, deletion" means that in position 27 the amino acid can be substituted by A, D or E, or that any amino acid can be inserted after position 27, or that the amino acid in position 27 can be deleted.

**[0170]** When one uses protein engineering to eliminate epitopes, it is indeed possible that new epitopes are created, or existing epitopes are duplicated. To reduce this risk, one can map the planned mutations at a given position on the 3-dimensional structure of the protein of interest, and control the emerging amino acid constellation against a database of known epitope patterns, to rule out those possible replacement amino acids, which are predicted to result in creation or duplication of epitopes. Thus, risk mutations can be identified and eliminated by this procedure, thereby reducing the risk of making mutations that lead to increased rather than decreased allergenicity.

Introduction of Residues for Chemical Derivatization in Epitope Areas

**[0171]** In yet another embodiment, one can design the mutation, such that amino acids suitable for chemical modification are substituted for existing ones in the epitope areas. The protein variant can then be conjugated to activated polymers. Which amino acids to substitute and/or insert, depends in principle on the coupling chemistry to be applied. The chemistry for preparation of covalent bioconjugates can be found in "Bioconjugate Techniques", Hermanson, G. T. (1996), Academic Press Inc., which is hereby incorporated as reference (see below). It is preferred to make conservative substitutions in the polypeptide when the polypeptide has to be conjugated, as conservative substitutions secure that the impact of the substitution on the polypeptide structure is limited. In the case of providing additional amino groups this may be done by substitution of arginine to lysine, both residues being positively charged, but only the lysine having a free amino group suitable as an attachment groups. In the case of providing additional carboxylic acid groups the conservative substitution may for instance be an asparagine to aspartic acid or glutamine to glutamic acid substitution. These residues resemble each other in size and shape, except from the carboxylic groups being present on the acidic residues. In the case of providing SH-groups the conservative substitution may be done by changing threonine or serine to cysteine.

Chemical Conjugation

**[0172]** For chemical conjugation, the protein variant needs to be incubate with an active or activated polymer and subsequently separated from the unreacted polymer. This can be done in solution followed by purification or it can conveniently be done using the immobilized protein variants, which can easily be exposed to different reaction environments and washes.

**[0173]** In the case were polymeric molecules are to be conjugated with the polypeptide in question and the polymeric molecules are not active they must be activated by the use of a suitable technique. It is also contemplated according to the invention to couple the polymeric molecules to the polypeptide through a linker. Suitable linkers are well-known to the skilled person. Methods and chemistry for activation of polymeric molecules as well as for conjugation of polypeptides are intensively described in the literature. Commonly used methods for activation of insoluble polymers include activation of functional groups with cyanogen bromide, periodate, glutaraldehyde, biepoxides, epichlorohydrin, divinylsulfone, carbodiimide, sulfonyl halides, trichlorotriazine etc. (see R. F. Taylor, (1991), "Protein immobilisation. Fundamental and applications", Marcel Dekker, N.Y.; S. S. Wong, (1992), "Chemistry of Protein Conjugation and Crosslinking", CRC Press, Boca Raton; G. T. Hermanson et al., (1993), "Immobilized Affinity Ligand Techniques", Academic Press, N.Y.). Some of the methods concern activation of insoluble polymers but are also applicable to activation of soluble polymers e.g. periodate, trichlorotriazine, sulfonylhalides, divinylsulfone, carbodiimide etc. The functional groups being amino, hydroxyl, thiol, carboxyl, aldehyde or sulfydryl on the polymer and the chosen attachment group on the protein must be considered in choosing the activation and conjugation chemistry which normally consist of i) activation of polymer, ii) conjugation, and iii) blocking of residual active groups.

**[0174]** In the following a number of suitable polymer activation methods will be described shortly. However, it is to be understood that also other methods may be used.

**[0175]** Coupling polymeric molecules to the free acid groups of polypeptides may be performed with the aid of diimide and for example amino-PEG or hydrazino-PEG (Pollak et al., (1976), J. Am. Chem. Soc., 98, 289-291) or diazoacetate/amide (Wong et al., (1992), "Chemistry of Protein Conjugation and Crosslinking", CRC Press).

**[0176]** Coupling polymeric molecules to hydroxy groups is generally very difficult as it must be performed in water. Usually hydrolysis predominates over reaction with hydroxyl groups.

**[0177]** Coupling polymeric molecules to free sulfhydryl groups can be achieved with special groups like maleimido or the ortho-pyridyl disulfide. Also vinylsulfone (U.S. Pat. No. 5,414,135, (1995), Snow et al.) has a preference for sulfhydryl groups but is not as selective as the other mentioned.

**[0178]** Accessible arginine residues in the polypeptide chain may be targeted by groups comprising two vicinal carbonyl groups.

**[0179]** Techniques involving coupling of electrophilically activated PEGs to the amino groups of Lysines may also be useful. Many of the usual leaving groups for alcohols give rise to an amine linkage. For instance, alkyl sulfonates, such as tresylates (Nilsson et al., (1984), Methods in Enzymology vol. 104, Jacoby, W. B., Ed., Academic Press: Orlando, p. 56-66; Nilsson et al., (1987), Methods in Enzymology vol. 135; Mosbach, K., Ed.; Academic Press: Orlando, pp. 65-79; Scouten et al., (1987), Methods in Enzymology vol. 135, Mosbach, K., Ed., Academic Press: Orlando, 1987; pp 79-84; Crossland et al., (1971), J. Amr. Chem. Soc. 1971, 93, pp. 4217-4219), mesylates (Harris, (1985), supra; Harris et al., (1984), J. Polym. Sci. Polym. Chem. Ed. 22, pp 341-352), aryl sulfonates like tosylates, and para-nitrobenzene sulfonates can be used.

**[0180]** Organic sulfonyl chlorides, e.g. Tresyl chloride, effectively converts hydroxy groups in a number of polymers, e.g. PEG, into good leaving groups (sulfonates) that, when reacted with nucleophiles like amino groups in polypeptides allow stable linkages to be formed between polymer and polypeptide. In addition to high conjugation yields, the reaction conditions are in general mild (neutral or slightly alkaline pH, to avoid denaturation and little or no disruption of activity), and satisfy the non-destructive requirements to the polypeptide.

**[0181]** Tosylate is more reactive than the mesylate but also less stable decomposing into PEG, dioxane, and sulfonic acid (Zalipsky, (1995), Bioconjugate Chem., 6, 150-165). Epoxides may also been used for creating amine bonds but are much less reactive than the abovementioned groups.

**[0182]** Converting PEG into a chloroformate with phosgene gives rise to carbamate linkages to Lysines. Essentially the same reaction can be carried out in many variants substituting the chlorine with N-hydroxy succinimide (U.S. Pat. No. 5,122,614, (1992); Zalipsky et al., (1992), Biotechnol. Appl. Biochem., 15, p. 100-114; Monfardini et al., (1995), Bioconjugate Chem., 6, 62-69, with imidazole (Allen et al., (1991), Carbohydr. Res., 213, pp 309-319), with para-nitrophenol, DMAP (EP 632 082 A1, (1993), Looze, Y.) etc. The derivatives are usually made by reacting the chloroformate with the desired leaving group. All these groups give rise to carbamate linkages to the peptide.

**[0183]** Furthermore, isocyanates and isothiocyanates may be employed, yielding ureas and thioureas, respectively.

**[0184]** Amides may be obtained from PEG acids using the same leaving groups as mentioned above and cyclic imid thrones (U.S. Pat. No. 5,349,001, (1994), Greenwald et al.). The reactivity of these compounds are very high but may make the hydrolysis to fast.

**[0185]** PEG succinate made from reaction with succinic anhydride can also be used. The hereby comprised ester group make the conjugate much more susceptible to hydrolysis (U.S. Pat. No. 5,122,614, (1992), Zalipsky). This group may be activated with N-hydroxy succinimide.

**[0186]** Furthermore, a special linker can be introduced. The most well studied being cyanuric chloride (Abuchowski et al., (1977), J. Biol. Chem., 252, 3578-3581; U.S. Pat. No. 4,179, 337, (1979), Davis et al.; Shafer et al., (1986), J. Polym. Sci. Polym. Chem. Ed., 24, 375-378.

**[0187]** Coupling of PEG to an aromatic amine followed by diazotation yields a very reactive diazonium salt, which can be reacted with a peptide in situ. An amide linkage may also be obtained by reacting an azlactone derivative of PEG (U.S. Pat. No. 5,321,095, (1994), Greenwald, R. B.) thus introducing an additional amide linkage.

**[0188]** As some peptides do not comprise many Lysines it may be advantageous to attach more than one PEG to the same Lysine. This can be done e.g. by the use of 1,3-diamino-2-propanol.

**[0189]** PEGs may also be attached to the amino-groups of the enzyme with carbamate linkages (WO 95/11924, Greenwald et al.). Lysine residues may also be used as the backbone.

**[0190]** The coupling technique used in the examples is the N-succinimidyl carbonate conjugation technique descried in WO 90/13590 (Enzon).

**[0191]** In a preferred embodiment, the activated polymer is methyl-PEG which has been activated by N-succinimidyl

carbonate as described WO 90/13590. The coupling can be carried out at alkaline conditions in high yields.

**[0192]** For coupling of polymers to the protein variants, it is preferred to use conditions similar to those described in WO 96/17929 and WO 99/00489 (Novo Nordisk A/S) e.g. mono or bis activated PEG's of molecular weight ranging from 100 to 5000 Da. For instance, a methyl-PEG 350 could be activated with N-succinimidyl carbonate and incubated with protein variant at a molar ratio of more than 5 calculated as equivalents of activated PEG divided by moles of lysines in the protein of interest. For coupling to immobilized protein variant, the PEG:protein ratio should be optimized such that the PEG concentration is low enough for the buffer capacity to maintain alkaline pH throughout the reaction; while the PEG concentration is still high enough to ensure sufficient degree of modification of the protein. Further, it is important that the activated PEG is kept at conditions that prevent hydrolysis (i.e. dissolved in acid or solvents) and diluted directly into the alkaline reaction buffer. It is essential that primary amines are not present other than those occurring in the lysine residues of the protein. This can be secured by washing thoroughly in borate buffer. The reaction is stopped by separating the fluid phase containing unreacted PEG from the solid phase containing protein and derivatized protein. Optionally, the solid phase can then be washed with tris buffer, to block any unreacted sites on PEG chains that might still be present.

Introduction of Consensus Sequences for Post-Translational Modifications in the Epitope Areas

**[0193]** In another embodiment, the mutations are designed, such that recognition sites for post-translational modifications are introduced in the epitope areas, and the protein variant is expressed in a suitable host organism capable of the corresponding post-translational modification. These post-translational modifications may serve to shield the epitope and hence lower the immunogenicity of the protein variant relative to the protein backbone. Post-translational modifications include glycosylation, phosphorylation, N-terminal processing, acylation, ribosylation and sulfatation. A good example is N-glycosylation. N-glycosylation is found at sites of the sequence Asn-Xaa-Ser, Asn-Xaa-Thr, or Asn-Xaa-Cys, in which neither the Xaa residue nor the amino acid following the tri-peptide consensus sequence is a proline (T. E. Creighton, 'Proteins—Structures and Molecular Properties, 2nd edition, W.H. Freeman and Co., New York, 1993, pp. 91-93). It is thus desirable to introduce such recognition sites in the sequence of the backbone protein. The specific nature of the glycosyl chain of the glycosylated protein variant may be linear or branched depending on the protein and the host cells. Another example is phosphorylation: The protein sequence can be modified so as to introduce serine phosphorylation sites with the recognition sequence arg-arg-$(xaa)_n$-ser (where n=0, 1, or 2) (SEQ ID NOS: 38 and 39), which can be phosphorylated by the cAMP-dependent kinase or tyrosine phosphorylation sites with the recognition sequence -lys/arg-$(xaa)_3$-asp/glu-$(xaa)_3$-tyr (SEC) ID NO: 40), which can usually be phosphorylated by tyrosine-specific kinases (T. E. Creighton, "Proteins—Structures and molecular properties", 2nd ed., Freeman, N.Y., 1993).

Randomized Approaches to Introduce Modifications in Epitope Areas.

**[0194]** In order to generate protein variants, more than one amino acid residue may be substituted, added or deleted, these amino acids preferably being located in different epitope areas. In that case, it may be difficult to assess a priori how well the functionality of the protein is maintained while antigenicity is reduced, especially since the possible number of mutation-combinations becomes very large, even for a small number of mutations. In that case, it will be an advantage, to establish a library of diversified mutants each having one or more changed amino acids introduced and selecting those variants, which show good retention of function and at the same time a significant reduction in antigenicity.

**[0195]** A diversified library can be established by a range of techniques known to the person skilled in the art (Reetz M T; Jaeger K E, in 'Biocatalysis—from Discovery to Application' edited by Fessner W D, Vol. 200, pp. 31-57 (1999); Stemmer, Nature, vol. 370, p. 389-391, 1994; Zhao and Arnold, Proc. Natl. Acad. Sci., USA, vol. 94, pp. 7997-8000, 1997; or Yano et al., Proc. Natl. Acad. Sci., USA, vol. 95, pp 5511-5515, 1998). These include, but are not limited to, 'spiked mutagenesis', in which certain positions of the protein sequence are randomized by carring out PCR mutagenesis using one or more oligonucleotide primers which are synthesized using a mixture of nucleotides for certain positions (Lanio T, Jeltsch A, Biotechniques, Vol. 25(6), 958,962,964-965 (1998)). The mixtures of oligonucleotides used within each triplet can be designed such that the corresponding amino acid of the mutated gene product is randomized within some predetermined distribution function. Algorithms have been disclosed, which facilitate this design (Jensen L J et al., Nucleic Acids Research, Vol. 26(3), 697-702 (1998)).

**[0196]** In an embodiment substitutions are found by a method comprising the following steps: 1) a range of substitutions, additions, and/or deletions are listed encompassing several epitope areas (preferably in the corresponding epitope sequences, anchor amino aids, and/or hot spots), 2) a library is designed which introduces a randomized subset of these changes in the amino acid sequence into the target gene, e.g. by spiked mutagenesis, 3) the library is expressed, and preferred variants are selected. In another embodiment, this method is supplemented with additional rounds of screening and/or family shuffling of hits from the first round of screening (J. E. Ness, et al, Nature Biotechnology, vol. 17, pp. 893-896, 1999) and/or combination with other methods of reducing immunogenicity by genetic means (such as that disclosed in WO 92/10755).

**[0197]** The library may be designed, such that at least one amino acid of the epitope area is substituted. In a preferred embodiment at least one amino acid of the epitope sequence itself is changed, and in an even more preferred embodiment, one or more hot spot amino acids are changed. The library may be biased such that towards introducing an amino acid of different size, hydrophilicity, and/or polarity relative to the original one of the 'protein backbone'. For example changing a small amino acid to a large amino acid, a hydrophilic amino acid to a hydrophobic amino acid, a polar amino acid to a non-polar amino acid or a basic to an acidic amino acid. Other changes may be the addition or deletion of at least one amino acid of the epitope area, preferably deleting an anchor amino acid. Furthermore, substituting some amino acids and deleting or adding others may change an epitope.

**[0198]** Diversity in the protein variant library can be generated at the DNA triplet level, such that individual codons are variegated e.g. by using primers of partially randomized sequence for a PCR reaction. Further, several techniques have been described, by which one can create a library with such

diversity at several locations in the gene, which are too far apart to be covered by a single (spiked) oligonucleotide primer. These techniques include the use of in vivo recombination of the individually diversified gene segments as described in WO 97/07205 on page 3, line 8 to 29 or by using DNA shuffling techniques to create a library of full length genes that combine several gene segments each of which are diversified e.g. by spiked mutagenesis (Stemmer, Nature 370, pp. 389-391, 1994 and U.S. Pat. Nos. 5,605,793 and 5,830,721). In the latter case, one can use the gene encoding the "protein backbone" as a template double-stranded polynucleotide and combining this with one or more single or double-stranded oligonucleotides as described in claim 1 of U.S. Pat. No. 5,830,721. The single-stranded oligonucleotides could be partially randomized during synthesis. The double-stranded oligonucleotides could be PCR products incorporating diversity in a specific region. In both cases, one can dilute the diversity with corresponding segments containing the sequence of the backbone protein in order to limit the number of changes that are on average introduced. As mentioned above, methods have been established for designing the ratios of nucleotides (A; C; T; G) used at a particular codon during primer synthesis, so as to approximate a desired frequency distribution among a set of desired amino acids at that particular codon. This allows one to bias the partially randomized mutagenesis towards e.g. introduction of post-translational modification sites, chemical modification sites, or simply amino acids that are different from those that define the epitope or the epitope area. One could also approximate a sequence in a given location or epitope area to the corresponding location on a homologous, human protein.

**[0199]** Occasionally, one would be interested in testing a library that combines a number of known mutations in different locations in the primary sequence of the 'protein backbone'. These could be introduced post-translational or chemical modification sites, or they could be mutations, which by themselves had proven beneficial for one reason or another (e.g. decreasing antigenicity, or improving specific activity, performance, stability, or other characteristics). In such cases, it may be desirable to create a library of diverse combinations of known sequences. For example if 12 individual mutations are known, one could combine (at least) 12 segments of the 'protein backbone' gene in which each segment is present in two forms: one with and one without the desired mutation. By varying the relative amounts of those segments, one could design a library (of size $2^{12}$) for which the average number of mutations per gene can be predicted. This can be a useful way of combining elements that by themselves give some, but not sufficient effect, without resorting to very large libraries, as is often the case when using 'spiked mutagenesis'. Another way to combine these 'known mutations' could be by using family shuffling of oligomeric DNA encoding the known changes with fragments of the full length wild type sequence.

Assays for Reduced Allergenicity

**[0200]** When protein variants have been constructed based on the methods described in this invention, it is desirable to confirm their antibody binding capacity, functionality, immunogenicity and/or allergenicity using a purified preparation. For that use, the protein variant of interest can be expressed in larger scale, purified by conventional techniques, and the antibody binding and functionality should be examined in detail using dose-response curves and e.g. direct or competitive ELISA (C-ELISA).

**[0201]** The potentially reduced allergenicity (which is likely, but not necessarily true for a variant w. low antibody binding) should be tested in in vivo or in vitro model systems: e.g. an in vitro assays for immunogenicity such as assays based on cytokine expression profiles or other proliferation or differentiation responses of epithelial and other cells incl. B-cells and T-cells. Further, animal models for testing allergenicity should be set up to test a limited number of protein variants that show desired characteristics in vitro. Useful animal models include the guinea pig intratracheal model (GPIT) (Ritz, et al. Fund. Appl. Toxicol., 21, pp. 31-37, 1993), mouse subcutaneous (mouse-SC) (WO 98/30682, Novo Nordisk), the rat intratracheal (rat-IT) (WO 96/17929, Novo Nordisk), and the mouse intranasal (MINT) (Robinson et al., Fund. Appl. Toxicol. 34, pp. 15-24, 1996) models.

**[0202]** The immunogenicity of the protein variant is measured in animal tests, wherein the animals are immunised with the protein variant and the immune response is measured. Specifically, it is of interest to determine the allergenicity of the protein variants by repeatedly exposing the animals to the protein variant by the intratracheal route and following the specific IgG and IgE titers. Alternatively, the mouse intranasal (MINT) test can be used to assess the allergenicity of protein variants. By the present invention the allergenicity is reduced at least 3 times as compared to the allergenicity of the parent protein, preferably 10 times reduced, more preferably 50 times.

**[0203]** However, the present inventors have demonstrated that the performance in ELISA correlates closely to the immunogenic responses measured in animal tests. To obtain a useful reduction of the allergenicity of a protein, the IgE binding capacity of the protein variant must be reduced to at least below 75%, preferably below 50%, more preferably below 25% of the IgE binding capacity of the parent protein as measured by the performance in IgE ELISA, given the value for the IgE binding capacity of the parent protein is set to 100%.

**[0204]** Thus a first assessment of the immunogenicity and/or allergenicity of a protein can be made by measuring the antibody binding capacity or antigenicity of the protein variant using appropriate antibodies. This approach has also been used in the literature (WO 99/47680).

Assays for Altered Immunotherapeutic Effect

**[0205]** The immunotherapeutic effect of allergen vaccines can be assessed a number of different ways. One is to measure the specific IgE binding, the reduction of which indicates a better allergen vaccine potential (WO 99/47680, ALK-ABELLO). Also, several cellular assays could be employed to show the modified immuneresponse indicative of good allergen vaccine potential as shown in several publications, all of which are hereby incorporated by reference (van Neerven et al, "T lymphocyte responses to allergens: Epitope-specificity and clinical relevance", Immunol Today, 1996, vol. 17, pp. 526-532; Hoffmann et al., Allergy, 1999, vol. 54, pp. 446-454, WO 99/07880).

**[0206]** Eventually, clinical trials with allergic patients could be employed using cellular or clinical end-point measurements. (Ebner et al., Clin. Exp. All., 1997, vol. 27, pp. 107-1015; Int. Arch. Allergy Immunol., 1999, vol. 119, pp 1-5).

Determining Functionality

**[0207]** A wide variety of protein functionality assays are available in the literature. Especially, those suitable for auto-

mated analysis are useful for this invention. Several have been published in the literature such as protease assays (WO 99/34011, Genencor International; J. E. Ness, et al, Nature Biotechn., 17, pp. 893-896, 1999), oxidoreductase assays (Chemy et al., Nature Biotechn., 17, pp. 379-384, 1999, and assays for several other enzymes (WO 99/45143, Novo Nordisk). Those assays that employ soluble substrates can be employed for direct analysis of functionality of immobilized protein variants.

Cross-Reactivity

**[0208]** A related objective is to reduce cross-reactivity between 'commercial allergens' and 'environmental allergens'. Cross-reactivities between food allergens of different origin are well-known (Akkerdaas et al, Allergy 50, pp 215-220, 1995). Similarly, cross-reactivities between other environmental allergens (like pollen, dust mites etc.) and commercial allergens (like enzyme proteins) have been established in the literature (J. All. Clin. Immunol., 1998, vol. 102, pp. 679-686 and by the present inventors. The molecular reason for this cross-reactivity can be explored using epitope mapping. By finding epitope patterns using antibodies raised against environmental allergen (donor protein) and mapping this information on a commercial allergen (the acceptor protein), one may find the epitopes that are common to both proteins, and hence responsible for the cross-reactivity. Obviously, one can also use the commercial allergen as donor and the environmental allergen as acceptor. By modifying the commercial allergen using protein engineering in the epitope areas identified as described above, one can reduce the cross-reactivity of the commercial allergen variant towards the environmental allergens (and vice versa). Hence, the use of the modified commercial allergens would be safer than using the unmodified commercial allergen.

**[0209]** Testing of this approach would be done using an antibody-binding assay with the protein variant (and its parent protein as control) and antibodies raised against the protein that cross-reacts with the parent protein. The method is otherwise identical to those described in the Methods section for characterization of allergencitiy and antigenicity.

Wash Performance etc.

**[0210]** The modifications of the enzymes in the epitope areas as disclosed the present application may cause other effects to the enzyme than modified immunogenicity. A modification may also change the performance of the enzyme, such as the wash performance, thermo stability, storage stability and increased catalytical activity of the enzyme.

**[0211]** The ability of an enzyme to catalyze the degradation of various naturally occurring substrates present on the objects to be cleaned during e.g. wash is often referred to as its washing ability, wash-ability, detergency, or wash performance. Throughout this application the term wash performance will be used to encompass this property.

Commercial Enzyme Applications

Industrial Applications

**[0212]** Another aspect of the invention is a composition comprising at least one protein (polypeptide) or enzyme of the invention. The composition may comprise other polypeptides, proteins or enzymes and/or ingredients normally used in personal care products, such as shampoo, soap bars, skin lotion, skin creme, hair dye, toothpaste, household articles, agro chemicals, personal care products, such as cleaning preparations e.g. for contact lenses, cosmetics, toiletries, oral and dermal pharmaceuticals, compositions used for treating textiles, compositions used for manufacturing food, e.g. baking, and feed etc.

**[0213]** Examples of said proteins(polypeptides)/enzymes include enzymes exhibiting protease, lipolytic enzyme, oxidoreductase, carbohydrase, transferase, such as transglutaminase, phytase and/or anti-microbial polypeptide activity. These enzymes may be present as conjugates with reduced activity.

**[0214]** The protein of the invention may furthermore typically be used in detergent composition. It may be included in the detergent composition in the form of a non-dusting granulate, a stabilized liquid, or a protected enzyme. Non-dusting granulates may be produced, e.g., as disclosed in U.S. Pat. Nos. 4,106,991 and 4,661,452 (both to Novo Industri NS) and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethylene glycol, PEG) with mean molecular weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in patent GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Other enzyme stabilizers are well known in the art. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

**[0215]** The detergent composition may be in any convenient form, e.g. as powder, granules, paste or liquid. A liquid detergent may be aqueous, typically containing up to 70% water and 0-30% organic solvent, or non-aqueous.

**[0216]** The detergent composition comprises one or more surfactants, each of which may be anionic, nonionic, cationic, or zwitterionic. The detergent will usually contain 0-50% of anionic surfactant such as linear alkylbenzenesulfonate (LAS), alpha-olefinsulfonate (AOS), alkyl sulfate (fatty alcohol sulfate) (AS), alcohol ethoxysulfate (AEOS or AES), secondary alkanesulfonates (SAS), alpha-sulfo fatty acid methyl esters, alkyl- or alkenylsuccinic acid, or soap. It may also contain 0-40% of nonionic surfactant such as alcohol ethoxylate (AEO or AE), carboxylated alcohol ethoxylates, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamine oxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, or polyhydroxy alkyl fatty acid amide (e.g. as described in WO 92/06154).

**[0217]** The detergent composition may additionally comprise one or more other enzymes, such as e.g. proteases, amylases, lipolytic enzymes, cutinases, cellulases, peroxidases, oxidases, and further anti-microbial polypeptides.

**[0218]** The detergent may contain 1-65% of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTMPA), alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g. SKS-6 from Hoechst). The detergent may also be unbuilt, i.e. essentially free of detergent builder.

**[0219]** The detergent may comprise one or more polymers. Examples are carboxymethylcellulose (CMC), poly(vinylpyrrolidone) (PVP), polyethyleneglycol (PEG), poly(vinyl alcohol) (PVA), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

**[0220]** The detergent may contain a bleaching system which may comprise a $H_2O_2$ source such as perborate or percarbonate which may be combined with a peracid-forming bleach activator such as tetraacetylethylenediamine (TAED) or nonanoyloxybenzenesulfon-ate (NOBS). Alternatively, the bleaching system may comprise peroxyacids of, e.g., the amide, imide, or sulfone type.

**[0221]** The detergent composition of the invention comprising the polypeptide of the invention may be stabilized using conventional stabilizing agents, e.g. a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative such as, e.g., an aromatic borate ester, and the composition may be formulated as described in, e.g., WO 92/19709 and WO 92/19708.

**[0222]** The detergent may also contain other conventional detergent ingredients such as, e.g., fabric conditioners including clays, foam boosters, suds suppressors, anti-corrosion agents, soil-suspending agents, anti-soil-redeposition agents, dyes, bactericides, optical brighteners, or perfume.

**[0223]** The pH (measured in aqueous solution at use concentration) will usually be neutral or alkaline, e.g. in the range of 7-11.

Dishwashing Composition

**[0224]** Further, a modified enzyme according to the invention may also be used in dishwashing detergents.

**[0225]** Dishwashing detergent compositions comprise a surfactant which may be anionic, non-ionic, cationic, amphoteric or a mixture of these types. The detergent will contain 0-90% of non-ionic surfactant such as low- to non-foaming ethoxylated propoxylated straight-chain alcohols.

**[0226]** The detergent composition may contain detergent builder salts of inorganic and/or organic types. The detergent builders may be subdivided into phosphorus-containing and non-phosphorus-containing types. The detergent composition usually contains 1-90% of detergent builders.

**[0227]** Examples of phosphorus-containing inorganic alkaline detergent builders, when present, include the water-soluble salts especially alkali metal pyrophosphates, orthophosphates, and polyphosphates. An example of phosphorus-containing organic alkaline detergent builder, when present, includes the water-soluble salts of phosphonates. Examples of non-phosphorus-containing inorganic builders, when present, include water-soluble alkali metal carbonates, borates and silicates as well as the various types of water-insoluble crystalline or amorphous alumino silicates of which zeolites are the best-known representatives.

**[0228]** Examples of suitable organic builders include the alkali metal, ammonium and substituted ammonium, citrates, succinates, malonates, fatty acid sulphonates, carboxymetoxy succinates, ammonium polyacetates, carboxylates, polycarboxylates, aminopolycarboxylates, polyacetyl carboxylates and polyhydroxsulphonates.

**[0229]** Other suitable organic builders include the higher molecular weight polymers and co-polymers known to have builder properties, for example appropriate polyacrylic acid, polymaleic and polyacrylic/polymaleic acid copolymers and their salts.

**[0230]** The dishwashing detergent composition may contain bleaching agents of the chlorine/bromine-type or the oxygen-type. Examples of inorganic chlorine/bromine-type bleaches are lithium, sodium or calcium hypochlorite and hypobromite as well as chlorinated trisodium phosphate. Examples of organic chlorine/bromine-type bleaches are heterocyclic N-bromo and N-chloro imides such as trichloroisocyanuric, tribromoisocyanuric, dibromoisocyanuric and dichloroisocyanuric acids, and salts thereof with water-solubilizing cations such as potassium and sodium. Hydantoin compounds are also suitable.

**[0231]** The oxygen bleaches are preferred, for example in the form of an inorganic persalt, preferably with a bleach precursor or as a peroxy acid compound. Typical examples of suitable peroxy bleach compounds are alkali metal perborates, both tetrahydrates and monohydrates, alkali metal percarbonates, persilicates and perphosphates. Preferred activator materials are TAED and glycerol triacetate.

**[0232]** The dishwashing detergent composition of the invention may be stabilized using conventional stabilizing agents for the enzyme(s), e.g. a polyol such as e.g. propylene glycol, a sugar or a sugar alcohol, lactic acid, boric acid, or a boric acid derivative, e.g. an aromatic borate ester.

**[0233]** The dishwashing detergent composition of the invention may also contain other conventional detergent ingredients, e.g. deflocculant material, filler material, foam depressors, anti-corrosion agents, soil-suspending agents, sequestering agents, anti-soil redeposition agents, dehydrating agents, dyes, bactericides, fluorescers, thickeners and perfumes.

**[0234]** Finally, the enzyme of the invention may be used in conventional dishwashing-detergents, e.g. in any of the detergents described in any of the following patent publications: EP 518719, EP 518720, EP 518721, EP 516553, EP 516554, EP 516555, GB 2200132, DE 3741617, DE 3727911, DE 4212166, DE 4137470, DE 3833047, WO 93/17089, DE 4205071, WO 52/09680, WO 93/18129, WO 93/04153, WO 92/06157, WO 92/08777, EP 429124, WO 93/21299, U.S. Pat. No. 5,141,664, EP 561452, EP 561446, GB 2234980, WO 93/03129, EP 481547, EP 530870, EP 533239, EP 554943, EP 346137, U.S. Pat. No. 5,112,518, EP 318204, EP 318279, EP 271155, EP 271156, EP 346136, GB 2228945, CA 2006687, WO 93/25651, EP 530635, EP 414197, and U.S. Pat. No. 5,240,632.

Personal Care Applications

**[0235]** A particularly useful application area for low allergenic proteins or of proteins with low cross-reactivity to environmental allergens would be in personal care products where the end-user is in close contact with the protein, and where certain problems with allergenicity has been encountered in experimental set-ups (Kelling et al., J. All. Clin. 1 mm., 1998, Vol. 101, pp. 179-187 and Johnston et al., Hum. Exp. Toxicol., 1999, Vol. 18, p. 527).

**[0236]** First of all the conjugate or compositions of the invention can advantageously be used for personal care products, such as hair care and hair treatment products. This include products such as shampoo, balsam, hair conditioners, hair waving compositions, hair dyeing compositions, hair tonic, hair liquid, hair cream, shampoo, hair rinse, hair spray.

**[0237]** Further contemplated are oral care products such as dentifrice, oral washes, chewing gum.

**[0238]** Also contemplated are skin care products and cosmetics, such as skin cream, skin milk, cleansing cream,

cleansing lotion, cleansing milk, cold cream, cream soap, nourishing essence, skin lotion, milky lotion, calamine lotion, hand cream, powder soap, transparent soap, sun oil, sun screen, shaving foam, shaving cream, baby oil lipstick, lip cream, creamy foundation, face powder, powder eye-shadow, powder, foundation, make-up base, essence powder, whitening powder.

**[0239]** Also for contact lenses hygiene products the conjugate of the invention can be used advantageously. Such products include cleaning and disinfection products for contact lenses.

Proteases

**[0240]** Proteases are well-known active ingredients for cleaning of contact lenses. They hydrolyse the proteinaceous soil on the lens and thereby makes it soluble. Removal of the protein soil is essential for the wearing comfort.

**[0241]** Proteases are also effective ingredients in skin cleaning products, where they remove the upper layer of dead keratinaseous skin cells and thereby make the skin look brighter and fresher.

**[0242]** Proteases are also used in oral care products, especially for cleaning of dentures, but also in dentifrices.

**[0243]** Further, proteases are used in toiletries, bath and shower products, including shampoos, conditioners, lotions, creams, soap bars, toilet soaps, and liquid soaps.

Lipolytic Enzymes

**[0244]** Lipolytic enzymes can be applied for cosmetic use as active ingredients in skin cleaning products and anti-acne products for removal of excessive skin lipids, and in bath and shower products such as creams and lotions as active ingredients for skin care.

**[0245]** Lipolytic enzymes can also be used in hair cleaning products (e.g. shampoos) for effective removal of sebum and other fatty material from the surface of hair.

**[0246]** Lipolytic enzymes are also effective ingredients in products for cleaning of contact lenses, where they remove lipid deposits from the lens surface.

Oxidoreductases

**[0247]** The most common oxidoreductase for personal care purposes is an oxidase (usually glucose oxidase) with substrate (e.g. glucose) that ensures production of $H_2O_2$, which then will initiate the oxidation of for instance $SON^-$ or $I^-$ into antimicrobial reagents ($SCNO^-$ or $I_2$) by a peroxidase (usually lactoperoxidase). This enzymatic complex is known in nature from e.g. milk and saliva.

**[0248]** It is being utilised commercially as anti-microbial system in oral care products (mouth rinse, dentifrice, chewing gum) where it also can be combined with an amyloglucosidase to produce the glucose. These systems are also known in cosmetic products for preservation.

**[0249]** Anti-microbial systems comprising the combination of an oxidase and a peroxidase are know in the cleaning of contact lenses.

**[0250]** Another application of oxidoreductases is oxidative hair dyeing using oxidases, peroxidases and laccases.

**[0251]** Free radicals formed on the surface of the skin (and hair) known to be associated with the ageing process of the skin (spoilage of the hair). The free radicals activate chain reactions that lead to destruction of fatty membranes, collagen, and cells. The application of free radical scavengers such as Superoxide dismutase into cosmetics is well known (R. L. Goldemberg, DCI, Nov. 93, p. 48-52).

**[0252]** Protein disulfide isomerase (PDI) is also an oxidoreductase. It can be utilised for waving of hair (reduction and reoxidation of disulfide bonds in hair) and repair of spoiled hair (where the damage is mainly reduction of existing disulfide bonds).

Carbohydrases

**[0253]** Plaque formed on the surface of teeth is composed mainly of polysaccharides. They stick to the surface of the teeth and the microorganisms. The polysaccharides are mainly α-1,6 bound glucose (dextran) and α-1,3 bound glucose (mutan). The application of different types of glucanases such as mutanase and dextranase helps hydrolysing the sticky matrix of plaque, making it easier to remove by mechanical action.

**[0254]** Also other kinds of biofilm for instance the biofilm formed in lens cases can be removed by the action of glucanases.

Food and Feed

**[0255]** Further conjugated enzymes or polypeptides with reduced immunogenicity according to the invention may advantageously be used in the manufacturing of food and feed.

Proteases

**[0256]** The gluten in wheat flour is the essential ingredient responsible for the ability of flour to be used in baked foodstuffs. Proteolytic enzymes are sometimes needed to modify the gluten phase of the dough, e.g. a hard wheat flour can be softened with a protease.

**[0257]** Neutrase® is a commercially available neutral metallo protease that can be used to ensure a uniform dough quality and bread texture, and to improve flavour. The gluten proteins are degraded either moderately or more extensively to peptides, whereby close control is necessary in order to avoid excessive softening of the dough.

**[0258]** Proteases are also used for modifying milk protein.

**[0259]** To coagulate casein in milk when producing cheese proteases such as rennet or chymosin may be used.

**[0260]** In the brewery industry proteases are used for brewing with unmalted cereals and for controlling the nitrogen content.

**[0261]** In animal feed products proteases are used so to speak to expand the animals digestion system.

Lipolytic Enzymes

**[0262]** Addition of lipolytic enzyme results in improved dough properties and an improved breadmaking quality in terms of larger volume, improved crumb structure and whiter crumb colour. The observed effect can be explained by a mechanism where the lipolytic enzyme changes the interaction between gluten and some lipids fragment during dough mixing. This results in an improved gluten network.

**[0263]** The flavour development of blue roan cheese (e.g. Danablue), certain Italian type cheese, and other dairy products containing butter-fat, are dependent on the degradation of milk fat into free fatty acids. Lipolytic enzymes may be used for developing flavour in such products.

**[0264]** In the oil- and fat producing industry lipases are used e.g. to minimize the amount of undesirable side-products, to modify fats by interesterification, and to synthesis of esters.

Oxidoreductases

**[0265]** Further oxidoreductases with reduced immunogenicity according to the invention may advantageously be used in the manufacturing of food and feed.

**[0266]** Several oxidoreductases are used for baking, glucose oxidase, lipoxygenase, peroxidase, catalase and combinations hereof. Traditionally, bakers strengthen gluten by adding ascorbic acid and potassium bromate. Some oxidoreductases can be used to replace bromate in dough systems by oxidation of free sulfydryl units in gluten proteins. Hereby disulphide linkages are formed resulting in stronger, more elastic doughs with greater resistance.

**[0267]** Gluzyme™ (Novozymes A/S) is a glucose oxidase preparation with catalase activity that can be used to replace bromate. The dough strengthen is measured as greater resistance to mechanical shock, better oven spring and larger loaf volume.

Carbohydrases

**[0268]** Flour has varying content of amylases leading to differences in the baking quality. Addition of amylases can be necessary in order to standardize the flour. Amylases and pentosanases generally provide sugar for the yeast fermentation, improve the bread volume, retard retrogradation, and decrease the staling rate and stickiness that results from pentosan gums. Examples of carbohydrases are given below.

**[0269]** Certain maltogenic amylases can be used for prolonging the shelf life of bread for two or more days without causing gumminess in the product. Selectively modifies the gelatinized starch by cleaving from the non-reducing end of the starch molecules, low molecular wight sugars and dextrins. The starch is modified in such a way that retrogradation is less likely to occur. The produced low-molecular-weight sugars improve the baked goods water retention capacity without creating the intermediate-length dextrins that result in gumminess in the finished product. The enzyme is inactivated during bread baking, so it can be considered a processing aid that does not have to be declared on the label. Overdosing of Novamyl can almost be excluded.

**[0270]** The bread volume can be improved by fungal α-amylases which further provide good and uniform structure of the bread crumb. Said α-amylases are endoenzymes that produce maltose, dextrins and glucose. Cereal and some bacterial α-amylases are inactivated at temperatures above the gelatinization temperature of starch, therefore when added to wheat dough it results in a low bread volume and a sticky bread interior. Fungamyl has the advantage of being thermolabile and is inactivated just below the gelatinization temperature.

**[0271]** Enzyme preparations containing a number of pentosanase and hemi-cellulase activities can improve the handling and stability of the dough, and improves the freshness, the crumb structure and the volume of the bread.

**[0272]** By hydrolysing the pentosans fraction in flour, it will lose a great deal of its water-binding capacity, and the water will then be available for starch and gluten. The gluten becomes more pliable and extensible, and the starch gelatinizes more easily. Pentosanases can be used in combination with or as an alternative to emulsifiers.

**[0273]** Further carbohydrases are user for producing syrups from starch, which are widely used in soft drinks, sweets, meat products, dairy products, bread products, ice cream, baby food, jam etc.

**[0274]** The conversion of starch is normally carried out three steps. First the starch is liquefied, by the use of alpha-amylases. Maltodextrins, primary consisting of oligosaccharides and dextrins, are obtained.

**[0275]** The mixture is then treated with an amyloglucosidase for hydrolysing the oligosaccharides and dextrins into glucose. This way a sweeter product is obtained. If high maltose syrups are desired beta-amylases alone or in combination with a pullulanase (de-branching enzyme) may be used.

**[0276]** The glucose mixture can be made even sweeter by isomerization to fructose. For this an immobilized glucose isomerase can be used.

**[0277]** In the sugar industry, it is common practice to speed up the break down of present starch in cane juices. Thereby the starch content in the raw sugar is reduced and filtration at the refinery facilitated.

**[0278]** Furthermore dextranases are used to break down dextran in raw sugar juices and syrups.

**[0279]** In the alcohol industry alpha-amylases is advantageously being used for thinning of starch in distilling mashes.

**[0280]** In the brewing industry alpha-amylases is used for adjunct liquefaction.

**[0281]** In the dairy industry beta-galactosidases (lactase) is used when producing low lactose milk for persons suffering from lactose malabsorption.

**[0282]** When flavoured milk drinks are produced from lactase-treated milk, the addition of sugar can be reduced without reducing the sweetness of the product.

**[0283]** In the production of condensed milk, lactose crystallization can be avoided by lactase treatment, and the risk of thickening caused by casein coagulation in lactose crystals is thus reduced.

**[0284]** When producing ice cream made from lactase-treated milk (or whey) no lactose crystals will be formed and the defect, sandiness, will not occur.

**[0285]** Further, xylanases are known to be used within a number of food/feed industrial applications as described in WO 94/21785 (Novo Nordisk A/S).

**[0286]** Alpha-amylases are used in the animal feed industry to be added to cereal-containing feed to improve the digestibility of starch.

Anti-Microbial Polypeptides

**[0287]** Certain bacteriolytic enzymes may be used e.g. to wash carcasses in the meat packing industry (see U.S. Pat. No. 5,354,681 from Novo Industri A/S)

Transferases

**[0288]** Transglutaminases with reduced immunogenicity according to the invention may advantageously be used in the manufacturing of food and feed.

**[0289]** Transglutaminases has the ability to crosslinking protein.

**[0290]** This property can be used for gelling of aqueous phases containing proteins. This may be used for when producing of spreads (DK patent application no. 1071/84 from Novo Nordisk A/S).

**[0291]** Transglutaminases are being used for improvement of baking quality of flour e.g. by modifying wheat flour to be used in the preparation of cakes with improved properties, such as improved taste, dent, mouth-feel and a higher volume (see JP 1-110147).

**[0292]** Further producing paste type food material e.g. used as fat substitution in foods as ice cream, toppings, frozen desserts, mayonnaises and low fat spreads (see WO 93/22930 from Novo Nordisk A/S).

**[0293]** Furthermore for preparation of gels for yoghurt, mousses, cheese, puddings, orange juice, from milk and milk-like products, and binding of chopped meat product, improvement of taste and texture of food proteins (see WO 94/21120 and WO 94/21129 from Novo Nordisk A/S).

Phytases

**[0294]** Phytases of the invention may advantageously be used in the manufacturing of food, such as breakfast cereal, cake, sweets, drinks, bread or soup etc., and animal feed.

**[0295]** Phytases may be used either for exploiting the phosphorus bound in the phytate/phytic acid present in vegetable protein sources or for exploiting the nutritionally important minerals bound in phytic acid complexes.

**[0296]** Microbial phytase may be added to feedstuff of monogastric animals in order to avoid supplementing the feed with inorganic phosphorus (see U.S. Pat. No. 3,297,548).

**[0297]** Further phytases may be used in soy processing. Soyabean meal may contain high levels of the anti-nutritional factor phytate which renders this protein source unsuitable for application in baby food and feed for fish, calves and other non-ruminants, since the phytate chelates essential minerals present therein (see EP 0 420 358).

**[0298]** Also for baking purposes phytases may be used. Bread with better quality can be prepared by baking divided pieces of a dough containing wheat flour etc. and phytase (see JP-0-3076529-A).

**[0299]** A high phytase activity as in koji mold are known to be used for producing refined sake (see JP-0-6070749-A).

Textile Applications

Proteases

**[0300]** Proteases are used for degumming and sand washing of silk.

Lipolytic Enzymes

**[0301]** Lipolytic enzymes are used for removing fatty matter containing hydrophobic esters (e.g. triglycerides) during the finishing of textiles (see e.g. WO 93/13256 from Novo Nordisk A/S).

Oxidoreductases

**[0302]** In bleach clean up of textiles catalases may serve to remove excess hydrogen peroxide.

Carbohydrases

**[0303]** Cellulolytic enzymes are widely used in the finishing of denim garments in order to provide a localized variation in the colour density of the fabric (Enzyme facilitated "stone wash").

**[0304]** Also cellulolytic enzymes find use in the bio-polishing process. Bio-Polishing is a specific treatment of the yarn surface which improves fabric quality with respect to handle and appearance without loss of fabric wettability. Bio-polishing may be obtained by applying the method described e.g. in WO 93/20278.

**[0305]** During the weaving of textiles, the threads are exposed to considerable mechanical strain. In order to prevent breaking, the threads are usually reinforced by the coating (sizing) with a gelatinous substance (size). The most common sizing agent is starch in native or modified form. A uniform and durable finish can thus be obtained only after removal of the size from the fabric, the so-called desizing. Desizing of fabrics sized with a size containing starch or modified starch is preferably facilitated by use of amylolytic enzymes.

Oral and Dermal Pharmaceuticals

Proteases

**[0306]** Different combinations of highly purified proteases (e.g. Trypsin and Chymotrypsin) are used in pharmaceuticals to be taken orally, and dermal pharmaceuticals for combating e.g. inflammations, edemata and injuries.

Leather Production

Transferase

**[0307]** Transglutaminase is known to be used to casein-finishing leather by acting as a hardening agent (see WO 94/13839 from Novo Nordisk).

Hard Surface Cleaning

**[0308]** Cleaning of hard surfaces e.g. in the food industry is often difficult, as equipment used for producing dairies, meat, sea food products, beverages etc. often have a complicated shape. The use of surfactant compositions in the form gels and foams comprising enzymes have shown to facilitate and improve hard surface cleaning. Enzymes, which advantageously may be added in such surfactant compositions, are in particular proteases, lipolytic enzymes, amylases and cellulases.

**[0309]** Such hard surface cleaning compositions comprising enzymes may also advantageously be used in the transport sector, for instance for washing cars and for general vessel wash.

**[0310]** Furthermore this invention relates to the method by which the protein variants are being synthesised and expressed in host cells. This is achieved by culturing host cells capable of expressing a polypeptide in a suitable culture medium to obtain expression and secretion of the polypeptide into the medium, followed by isolation of the polypeptide from the culture medium. The host cell may be any cell suitable for the large-scale production of proteins, capable of expressing a protein and being transformed by an expression vector.

**[0311]** The host cell comprises a DNA construct as defined above, optionally the cells may be transformed with an expression vector comprising a DNA construct as defined above. The host cell is selected from any suitable cell, such as a bacterial cell, a fungal cell, an animal cell, such as an insect cell or a mammalian cell, or a plant cell.

Immunotherapy

**[0312]** A number of vaccination approaches have been described to for infective diseases as well as for non-infective

diseases (such as cancers). In a number of cases, the antigen provided is an isolated protein or protein-adjuvant mixture and more and more often, the protein is recombinant (e.g. the hepatitits B vaccine from Merck & Co). In these cases, it could be desirable to modify the immunogenicity of the antigen vaccine, such that it offers a stronger or more specific protection. This can be achieved by protein engineering of the amino acid sequence of the antigen, and would be greatly facilitated by the use of the methods of this invention for identification of epitopes on the antigen vaccine to be the favored sites for modification.

**[0313]** There are several examples of vaccine molecules that have been engineered to achieve a specific immune protection against virus, parasites or cancer (Ryu and Nam, Biotechnol. Prog., 2000, vol. 16 pp. 2-16; and references cited therein). "The goal is often to vaccinate with a minimal strucutre consisting of a well-defined antigen, to stimulate an effective specific immune response, while avoiding potentially hazardous risks" (Ryu and Nam, Biotechnol. Prog., 2000, vol. 16 pp. 2-16). Thus, the methods of this invention can be used to identify such minimal structures that define an antigen (or epitope thereof) whether in the form of the parent protein scaffold with a number of mutations introduced in it, or whether it is in the form of the antibody binding peptides themselves.

Allergen Vaccines

**[0314]** Today, a patient suffering allergic disease may be subjected to allergy vaccine therapy using allergens selected on the basis of testing the specificity of the patient's serum IgE against a bank of allergen extracts (or similar specificity tests of the patient's sensibilization such as skin prick test.

**[0315]** One could improve the quality of characterization by using antibody binding peptides corresponding to various epitope sequences on the protein allergens of interest. This would require a kit comprising reagents for such specificity characterization, e.g. the antibody binding peptides of desired specificity. It would be preferred to use antibody binding sequences in the kit, which correspond to defined epitope sequences known to be specific for the allergen under investigation (i.e. not identified on other allergens and/or not cross-reacting with sera raised against other allergens). This kit would be useful to specifying which allergy the patient is suffering from. This kit will lead to a more specific answer than those kits used today, and hence to a better selection of allergen vaccine therapy for the individual patient.

**[0316]** Further, the knowledge about cross-reacting epitopes may improve vaccine development.

**[0317]** In an extension of this approach, one could also characterize the patient's serum by identifying the corresponding antibody binding peptides among a random display library using the aforementioned methods. This again may lead to a better selection of allergen vaccine therapy.

**[0318]** Further, one could use the individual antibody binding sequences as allergen vaccines leading to more specific allergen vaccine. These antibody binding sequences could be administered in an isolated form or fused to a membrane protein of the phage display system, or to another protein, which may have beneficial effect for the immunoprotective effect of the antibody binding peptide (Dalum et al., Nature Biotechnology, 1999, Vol. 17, pp. 666-669).

D) Variations Possible

Parent Protein

**[0319]** The "parent protein" can in principle be any protein molecule of biological origin, non-limiting examples of which are peptides, polypeptides, proteins, enzymes, post-translationally modified polypeptides such as lipopeptides or glycosylated peptides, anti-microbial peptides or molecules, and proteins having pharmaceutical properties etc.

**[0320]** Accordingly the invention relates to a method, wherein the "parent protein" is chosen from the group consisting of polypeptides, small peptides, lipopeptides, antimicrobials, and pharmaceutical polypeptides.

**[0321]** The term "pharmaceutical polypeptides" is defined as polypeptides, including peptides, such as peptide hormones, proteins and/or enzymes, being physiologically active when introduced into the circulatory system of the body of humans and/or animals.

**[0322]** Pharmaceutical polypeptides are potentially immunogenic as they are introduced into the circulatory system.

**[0323]** Examples of "pharmaceutical polypeptides" contemplated according to the invention include insulin, ACTH, glucagon, somatostatin, somatotropin, thymosin, parathyroid hormone, pigmentary hormones, somatomedin, erythropoietin, luteinizing hormone, chorionic gonadotropin, hypothalmic releasing factors, antidiuretic hormones, thyroid stimulating hormone, relaxin, interferon, thrombopoietin (TPO) and prolactin.

**[0324]** However, the proteins are preferably to be used in industry, housekeeping and/or medicine, such as proteins used in personal care products (for example shampoo; soap; skin, hand and face lotions; skin, hand and face cremes; hair dyes; toothpaste), food (for example in the baking industry), detergents and pharmaceuticals.

Antimicrobial Peptides.

**[0325]** The antimicrobial peptide (AMP) may be, e.g., a membrane-active antimicrobial peptide, or an antimicrobial peptide affecting/interacting with intracellular targets, e.g. binding to cell DNA. The AMP is generally a relatively short peptide, consisting of less than 100 amino acid residues, typically 20-80 residues. The antimicrobial peptide has bactericidal and/or fungicidal effect, and it may also have antiviral or antitumour effects. It generally has low cytotoxicity against normal mammalian cells.

**[0326]** The antimicrobial peptide is generally highly cationic and hydrophobic. It typically contains several arginine and lysine residues, and it may not contain a single glutamate or asparatate. It usually contains a large proportion of hydrophobic residues. The peptide generally has an amphiphilic structure, with one surface being highly positive and the other hydrophobic.

**[0327]** The bioactive peptide and the encoding nucleotide sequence may be derived from plants, invertebrates, insects, amphibians and mammals, or from microorganisms such as bacteria and fungi.

**[0328]** The antimicrobial peptide may act on cell membranes of target microorganisms, e.g. through nonspecific binding to the membrane, usually in a membrane-parallel orientation, interacting only with one face of the bilayer.

**[0329]** The antimicrobial peptide typically has a structure belonging to one of five major classes: a helical, cystine-rich

(defensin-like), b-sheet, peptides with an unusual composition of regular amino acids, and peptides containing uncommon modified amino acids.

**[0330]** Examples of alpha-helical peptides are Magainin 1 and 2; Cecropin A, B and P1; CAP18; Andropin; Clavanin A or AK; Styelin D and C; and Buforin II. Examples of cystine-rich peptides are a-Defensin HNP-1 (human neutrophil peptide) HNP-2 and HNP-3; b-Defensin-12, Drosomycin, g1-purothionin, and Insect defensin A. Examples of b-sheet peptides are Lactoferricin B, Tachyplesin I, and Protegrin PG1-5. Examples of peptides with an unusual composition are Indolicidin; PR-39; Bactenicin Bac5 and Bac7; and Histatin 5. Examples of peptides with unusual amino acids are Nisin, Gramicidin A, and Alamethicin.

**[0331]** Another example is the antifungal peptide (AFP) from *Aspergillus* giganteus. As explained in detail in WO 94/01459, which is hereby incorporated by reference, the antifungal polypeptide having the amino acid sequence shown in FIG. **1** has been found in several strains of the fungal species *A. giganteus*, an example of which is the *A. giganteus* strain deposited with the Centraallbureau voor Schimmelcultures (CBS) under the deposition number CBS 526.65.

**[0332]** However, the antifungal polypeptide, or variants thereof, suitable for the use according to the invention are expected to be derivable from other fungal species, especially other *Aspergillus* species such as *A. pallidus, A. clavatus, A. longivesica, A. rhizopodus* and *A. clavatonanicus*, because of the close relationship which exists between these species and *A. giganteus.*

**[0333]** In one embodiment of the invention the protein is an enzyme, such as glycosyl hydrolases, carbohydrases, peroxidases, proteases, lipolytic enzymes, phytases, polysaccharide lyases, oxidoreductases, transglutaminases and glycoseisomerases, in particular the following.

Parent Proteases

**[0334]** Parent proteases (i.e. enzymes classified under the Enzyme Classification number E.C. 3.4 in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB)) include proteases within this group.

**[0335]** Examples include proteases selected from those classified under the Enzyme Classification (E.C.) numbers:

**[0336]** 3.4.11 (i.e. so-called aminopeptidases), including 3.4.11.5 (Prolyl aminopeptidase), 3.4.11.9 (X-pro aminopeptidase), 3.4.11.10 (Bacterial leucyl aminopeptidase), 3.4.11.12 (Thermophilic aminopeptidase), 3.4.11.15 (Lysyl aminopeptidase), 3.4.11.17 (Tryptophanyl aminopeptidase), 3.4.11.18 (Methionyl aminopeptidase).

**[0337]** 3.4.21 (i.e. so-called serine endopeptidases), including 3.4.21.1 (Chymotrypsin), 3.4.21.4 (Trypsin), 3.4.21.25 (Cucumisin), 3.4.21.32 (Brachyurin), 3.4.21.48 (Cerevisin) and 3.4.21.62 (Subtilisin);

**[0338]** 3.4.22 (i.e. so-called cysteine endopeptidases), including 3.4.22.2 (Papain), 3.4.22.3 (Ficain), 3.4.22.6 (Chymopapain), 3.4.22.7 (Asclepain), 3.4.22.14 (Actimidain), 3.4.22.30 (Caricain) and 3.4.22.31 (Ananain);

**[0339]** 3.4.23 (i.e. so-called aspartic endopeptidases), including 3.4.23.1 (Pepsin A), 3.4.23.18 (Aspergillopepsin I), 3.4.23.20 (Penicillopepsin) and 3.4.23.25 (Saccharopepsin); and

**[0340]** 3.4.24 (i.e. so-called metalloendopeptidases), including 3.4.24.28 (Bacillolysin).

Serine Proteases

**[0341]** A serine protease is an enzyme which catalyzes the hydrolysis of peptide bonds, and in which there is an essential serine residue at the active site (White, Handler and Smith, 1973 "*Principles of Biochemistry*," Fifth Edition, McGraw-Hill Book Company, NY, pp. 271-272).

**[0342]** The bacterial serine proteases have molecular weights in the 20,000 to 45,000 Dalton range. They are inhibited by diisopropylfluorophosphate. They hydrolyze simple terminal esters and are similar in activity to eukaryotic chymotrypsin, also a serine protease. A more narrow term, alkaline protease, covering a sub-group, reflects the high pH optimum of some of the serine proteases, from pH 9.0 to 11.0 (for review, see Priest (1977) *Bacteriological Rev.* 41 711-753).

Subtilases

**[0343]** A sub-group of the serine proteases tentatively designated subtilases has been proposed by Siezen et al., *Protein Engng.* 4 (1991) 719-737 and Siezen et al. *Protein Science* 6 (1997) 501-523. They are defined by homology analysis of more than 170 amino acid sequences of serine proteases previously referred to as subtilisin-like proteases. A subtilisin was previously often defined as a serine protease produced by Gram-positive bacteria or fungi, and according to Siezen et al. now is a subgroup of the subtilases. A wide variety of subtilases have been identified, and the amino acid sequence of a number of subtilases has been determined. For a more detailed description of such subtilases and their amino acid sequences reference is made to Siezen et al., (1997).

Savinase-Like Subtilisin

**[0344]** One subgroup of the subtilases may be classified as savinase-like subtilisins, having at least 81% homology to Savinase, preferably at least 85% homology, more preferably at least 90% homology, even more preferably at least 96% homology, most preferably at least 98% homology to Savinase.

Parent Subtilase

**[0345]** The term "parent subtilase" describes a subtilase defined according to Siezen et al. (1991 and 1997). For further details see description of "SUBTILASES" immediately above. A parent subtilase may also be a subtilase isolated from a natural source, wherein subsequent modifications have been made while retaining the characteristic of a subtilase. Furthermore, a parent subtilase may also be a subtilase which has been prepared by the DNA shuffling technique, such as described by J. E. Ness et al., Nature Biotechnology, 17, 893-896 (1999).

**[0346]** Alternatively the term "parent subtilase" may be termed "wild type subtilase".

Modification(s) of a Subtilase Variant

**[0347]** The term "modification(s)" used herein is defined to include chemical modification of a subtilase as well as genetic manipulation of the DNA encoding a subtilase. The

modification(s) can be replacement(s) of the amino acid side chain(s), substitution(s), deletion(s) and/or insertions in or at the amino acid(s) of interest.

Subtilase Variant

**[0348]** In the context of this invention, the term subtilase variant or mutated subtilase means a subtilase that has been produced by an organism which is expressing a mutant gene derived from a parent microorganism which possessed an original or parent gene and which produced a corresponding parent enzyme, the parent gene having been mutated in order to produce the mutant gene from which said mutated subtilase protease is produced when expressed in a suitable host.

**[0349]** Examples of relevant subtilisins comprise subtilisin BPN', subtilisin amylosacchariticus, subtilisin 168, subtilisin mesentericopeptidase, subtilisin Carlsberg, subtilisin DY, subtilisin 309, subtilisin 147, PD498 (WO 93/24623), thermitase, aqualysin, *Bacillus* PB92 protease, proteinase K, Protease TW7, and Protease TW3.

**[0350]** Preferred commercially available protease enzymes include Alcalase™, Savinase™ Primase™, Duralase™, Neutrase®, Dyrazym®, Esperase™, Pyrase®, Pancreatic Trypsin NOVO (PTN), Bio-Feed™ Pro, Clear-Lens Pro, and Relase® (Novozymes A/S), Maxatase™ Maxacal™, Maxapem™, Properase™, Purafect™, Purafect OxP™ (Genencor International Inc.).

**[0351]** It is to be understood that also protease variants are contemplated as the parent protease. Examples of such protease variants are disclosed in EP 130.756 (Genentech), EP 214.435 (Henkel), WO 87/04461 (Amgen), WO 87/05050 (Genex), EP 251.446 (Genencor), EP 260.105 (Genencor), Thomas et al., (1985), Nature. 318, p. 375-376, Thomas et al., (1987), J. Mol. Biol., 193, pp. 803-813, Russel et al., (1987), Nature, 328, p. 496-500, WO 88/08028 (Genex), WO 88/08033 (Amgen), WO 89/06279 (Novo Nordisk A/S), WO 91/00345 (Novo Nordisk A/S), EP 525 610 (Solvay) and WO 94/02618 (Gist-Brocades N.V.).

**[0352]** The activity of proteases can be determined as described in "Methods of Enzymatic Analysis", third edition, 1984, Verlag Chemie, Weinheim, vol. 5.

Parent Lipolytic Enzymes

**[0353]** Lipolytic enzymes are classified in EC 3.1.1 Carboxylic Ester Hydrolases according to Enzyme Nomenclature (available at http://www.chem.qmw.ac.uk/iubmb/enzyme). The lipolytic enzyme may have a substrate specificity with an activity such as EC 3.1.1.3 triacylglycerol lipase, EC 3.1.1.4 phospholipase A2, EC 3.1.1.5 lysophospholipase, EC 3.1.1.26 galactolipase, EC 3.1.1.32 phospholipase A1, EC 3.1.1.73 feruloyl esterase or EC 3.1.1.74 cutinase.

**[0354]** The parent lipolytic enzyme may be prokaryotic, particularly a bacterial enzyme, e.g. from *Pseudomonas*. Examples are *Pseudomonas* lipases, e.g. from *P. cepacia* (U.S. Pat. No. 5,290,694, pdb file 1OIL), *P. glumae* (N Frenken et al. (1992), Appl. Envir. Microbiol. 58 3787-3791, pdb files 1TAH and 1QGE), *P. pseudoalcaligenes* (EP 334 462) and *Pseudomonas* sp. strain SD 705 (FERM BP-4772) (WO 95/06720, EP 721 981, WO 96/27002, EP 812 910). The *P. glumae* lipase sequence is identical to the amino acid sequence of *Chromobacterium viscosum* (DE 3908131 A1). Other examples are bacterial cutinases, e.g. from *Pseudomonas* such as *P. mendocina* (U.S. Pat. No. 5,389,536) or *P. putida* (WO 88/09367).

**[0355]** Alternatively, the parent lipolytic enzyme may be eukaryotic, e.g. a fungal lipolytic enzyme such as lipolytic enzymes of the Humicola family and the Zygomycetes family and fungal cutinases.

**[0356]** Examples of fungal cutinases are the cutinases of *Fusarium solani pisi* (S. Longhi et al., Journal of Molecular Biology, 268 (4), 779-799 (1997)) and *Humicola insolens* (U.S. Pat. No. 5,827,719).

**[0357]** The parent lipolytic enzyme may be fungal and may have an amino acid sequence that can be aligned with SEQ ID NO: 1 which is the amino acid sequence shown in positions 1-269 of SEQ ID NO: 2 of U.S. Pat. No. 5,869,438 for the lipase from *Thermomyces lanuginosus* (synonym *Humicola lanuginosa*), described in EP 258 068 and EP 305 216 (trade name LIPOLASE). The parent lipolytic enzyme may particularly have an amino acid sequence with at least 50% homology with SEQ ID NO: 1. In addition to the lipase from *T. lanuginosus*, other examples are a lipase from *Penicillium camembertii* (P25234), a lipase from *Fusasrium*, lipase/phospholipase from *Fusarium oxysporum* (EP 130064, WO 98/26057), lipase from *F. heterosporum* (R87979), lysophospholipase from *Aspergillus foetidus* (W33009), phospholipase A1 from *A. oryzae* (JP-A 10-155493), lipase from *A. oryzae* (D85895), lipase/ferulic acid esterase from *A. niger* (Y09330), lipase/ferulic acid esterase from *A. tubingensis* (Y09331), lipase from *A. tubingensis* (WO 98/45453), lysophospholipase from *A. niger* (WO 98/31790), lipase from *F. solanii* having an isoelectric point of 6.9 and an apparent molecular weight of 30 kDa (WO 96/18729).

**[0358]** Other examples are the Zygomycetes family of lipases comprising lipases having at least 50% homology with the lipase of *Rhizomucor miehei* (P19515. This family also includes the lipases from *Absidia reflexa, A. sporophora, A. corymbifera, A. blakesleeana, A. griseola* (all described in WO 96/13578 and WO 97/27276) and *Rhizopus oryzae* (P21811). Numbers in parentheses indicate publication or accession to the EMBL, GenBank, GeneSeqp or Swiss-Prot databases.

**[0359]** Examples of lipases include lipases derived from the following microorganisms. The indicated patent publications are incorporated herein by reference:

**[0360]** *Humicola*, e.g. *H. brevispora, H. brevis* var. *thermoidea.*

**[0361]** *Pseudomonas*, e.g. *Ps. fragi, Ps. stutzeri, Ps. cepacia* and *Ps. fluorescens* (WO 89/04361), or *Ps. plantarii* or *Ps. gladioli* (U.S. Pat. No. 4,950,417 (Solvay enzymes)) or *Ps. alcaligenes* and *Ps. pseudoalcaligenes* (EP 218 272) or.

**[0362]** *Candida*, e.g. *C. cylindracea* (also called *C. rugosa*) or *C. antarctica* (WO 88/02775) or *C. antarctica* lipase A or B (WO 94/01541 and WO 89/02916).

**[0363]** *Geotricum*, e.g. *G. candidum* (Schimada et al., (1989), J. Biochem., 106, 383-388).

**[0364]** *Rhizopus*, e.g. *R. delemar* (Hass et al., (1991), Gene 109, 107-113) or *R. niveus* (Kugimiya et al., (1992) Biosci. Biotech. Biochem 56, 716-719) or *R. oryzae.*

**[0365]** *Bacillus*, e.g. *B. subtilis* (Dartois et al., (1993) Biochemica et Biophysica acta 1131, 253-260) or *B. stearothermophilus* (JP 64/7744992) or *B. pumilus* (WO 91/16422).

**[0366]** Specific examples of readily available commercial lipases include Lipolase® (WO 98/35026) Lipolase™ Ultra, Lipozyme®, Palatase®, Novozym® 435, Lecitase® (all available from Novozymes A/S).

**[0367]** Examples of other lipases are Lumafast™, *Ps. mendocian* lipase from Genencor Int. Inc.; Lipomax™, *Ps.*

*pseudoalcaligenes* lipase from Gist Brocades/Genencor Int. Inc.; *Fusarium solani* lipase (cutinase) from Unilever; *Bacillus* sp. lipase from Solvay enzymes. Other lipases are available from other companies.

**[0368]** It is to be understood that also lipase variants are contemplated as the parent enzyme. Examples of such are described in e.g. WO 93/01285 and WO 95/22615.

**[0369]** The activity of the lipase can be determined as described in "Methods of Enzymatic Analysis", Third Edition, 1984, Verlag Chemie, Weinhein, vol. 4, or as described in AF 95/5 GB (available on request from Novozymes A/S).

Parent Oxidoreductases

**[0370]** Parent oxidoreductases (i.e. enzymes classified under the Enzyme Classification number E.C. 1 (Oxidoreductases) in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB)) include oxidoreductases within this group.

**[0371]** Examples include oxidoreductases selected from those classified under the Enzyme Classification (E.C.) numbers:

**[0372]** Glycerol-3-phosphate dehydrogenase (NAD) (1.1.1.8), Glycerol-3-phosphate dehydrogenase [NAD(P)] (1.1.1.94), Glycerol-3-phosphate 1-dehydrogenase [NADP] (1.1.1.94), Glucose oxidase (1.1.3.4), Hexose oxidase (1.1.3.5), Catechol oxidase (1.1.3.14), Bilirubin oxidase (1.3.3.5), Alanine dehydrogenase (1.4.1.1), Glutamate dehydrogenase (1.4.1.2), Glutamate dehydrogenase [NAD(P)] (1.4.1.3), Glutamate dehydrogenase (NADP) (1.4.1.4), L-Amino acid dehydrogenase (1.4.1.5), Serine dehydrogenase (1.4.1.7), Valine dehydrogenase (NADP) (1.4.1.8), Leucine dehydrogenase (1.4.1.9), Glycine dehydrogenase (1.4.1.10), L-Amino-acid oxidase (1.4.3.2.), D-Amino-acid oxidase(1.4.3.3), L-Glutamate oxidase (1.4.3.11), Protein-lysine 6-oxidase (1.4.3.13), L-lysine oxidase (1.4.3.14), L-Aspartate oxidase (1.4.3.16), D-amino-acid dehydrogenase (1.4.99.1), Protein disulfide reductase (1.6.4.4), Thioredoxin reductase (1.6.4.5), Protein disulfide reductase (glutathione) (1.8.4.2), Laccase (1.10.3.2), Catalase (1.11.1.6), Peroxidase (1.11.1.7), Lipoxygenase (1.13.11.12), Superoxide dismutase (1.15.1.1).

**[0373]** Said glucose oxidases may be derived from *Aspergillus niger*.

**[0374]** Said laccases may be derived from *Polyporus pinsitus, Myceliophthora thermophila, Coprinus cinereus, Rhizoctonia solani, Rhizoctonia praticola, Scytalidium thermophilum* and *Rhus vernicifera*. Because of the homology found between the above mentioned laccases (see WO 98/38287), they are considered to belong to the same class of laccases, namely the class of "*Coprinus*-like laccases". Accordingly, in the present context, the term "*Coprinus*-like laccase" is intended to indicate a laccase which, on the amino acid level, displays a homology of at least 50% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO: 3, or at least 55% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO: 3, or at least 60% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO: 3, or at least 65% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO: 3, or at least 70% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO: 3, or at least 75% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO: 3, or at least 80% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO: 3, or at least 85% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO: 3, or at least 90% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO: 3, at least 95% and less than 100% or at least 98% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO: 3.

**[0375]** Bilirubin oxidases may be derived from Myrothecium verrucaria.

**[0376]** The peroxidase may be derived from e.g. Soy bean, Horseradish or *Coprinus cinereus*.

**[0377]** The protein disulfide reductase may be any of the mentioned in Danish application nos. 768/93, 265/94 and 264/94 (Novo Nordisk A/S), which are hereby incorporated as references, including Protein Disulfide reductases of bovine origin, Protein Disulfide reductases derived from *Aspergillus oryzae* or *Aspergillus niger*, and DsbA or DsbC derived from *Escherichia coli*.

**[0378]** Specific examples of readily available commercial oxidoreductases include Gluzyme™ (enzyme available from Novozymes A/S). However, other oxidoreductases are available from others.

**[0379]** It is to be understood that also variants of oxidoreductases are contemplated as the parent enzyme.

**[0380]** The activity of oxidoreductases can be determined as described in "Methods of Enzymatic Analysis", third edition, 1984, Verlag Chemie, Weinheim, vol. 3.

Parent Carbohydrases

**[0381]** Parent carbohydrases may be defined as all enzymes capable of breaking down carbohydrate chains (e.g. starches) of especially five and six member ring structures (i.e. enzymes classified under the Enzyme Classification number E.C. 3.2 (glycosidases) in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB)). Also included in the group of carbohydrases according to the invention are enzymes capable of isomerizing carbohydrates e.g. six member ring structures, such as D-glucose to e.g. five member ring structures like D-fructose.

**[0382]** Examples include carbohydrases selected from those classified under the Enzyme Classification (E.C.) numbers: alpha-amylase (3.2.1.1), beta-amylase (3.2.1.2), glucan 1,4-alpha-glucosidase (3.2.1.3), cellulase (3.2.1.4), endo-1,3(4)-beta-glucanase (3.2.1.6), endo-1,4-beta-xylanase (3.2.1.8), dextranase (3.2.1.11), chitinase (3.2.1.14), polygalacturonase (3.2.1.15), lysozyme (3.2.1.17), beta-glucosidase (3.2.1.21), alpha-galactosidase (3.2.1.22), beta-galactosidase (3.2.1.23), amylo-1,6-glucosidase (3.2.1.33), xylan 1,4-beta-xylosidase (3.2.1.37), glucan endo-1,3-beta-D-glucosidase (3.2.1.39), alpha-dextrin endo-1,6-glucosidase (3.2.1.41), sucrose alpha-glucosidase (3.2.1.48), glucan endo-1,3-alpha-glucosidase (3.2.1.59), glucan 1,4-beta-glucosidase (3.2.1.74), glucan endo-1,6-beta-glucosidase (3.2.1.75), arabinan endo-1,5-alpha-arabinosidase (3.2.1.99), lactase (3.2.1.108), chitonanase (3.2.1.132) and xylose isomerase (5.3.1.5).

**[0383]** Examples of relevant carbohydrases include alpha-1,3-glucanases derived from *Trichoderma harzianum*; alpha-1,6-glucanases derived from a strain of *Paecilomyces*; beta-glucanases derived from *Bacillus subtilis*; beta-glucanases derived from *Humicola insolens*; beta-glucanases derived from *Aspergillus niger*, beta-glucanases derived from a strain of *Trichoderma*; beta-glucanases derived from a strain of *Oerskovia xanthineolytica*; exo-1,4-alpha-D-glucosidases (glucoamylases) derived from *Aspergillus niger*; alpha-amylases derived from *Bacillus subtilis*; alpha-amylases derived from *Bacillus amyloliquefaciens*; alpha-amylases derived from *Bacillus stearothermophilus*; alpha-amylases derived

from *Aspergillus oryzae*; alpha-amylases derived from non-pathogenic microorganisms; alpha-galactosidases derived from *Aspergillus niger*, Pentosanases, xylanases, cellobiases, cellulases, hemi-cellulases derived from *Humicola insolens*; cellulases derived from *Trichoderma reesei*; cellulases derived from non-pathogenic mold; pectinases, cellulases, arabinases, hemi-celluloses derived from *Aspergillus niger*, dextranases derived from *Penicillium lilacinum*; endo-glucanase derived from non-pathogenic mold; pullulanases derived from *Bacillus acidopullyticus*; beta-galactosidases derived from *Kluyveromyces fragilis*; xylanases derived from *Trichoderma reesei.*

**[0384]** Specific examples of readily available commercial carbohydrases include Alpha-Gal™ Bio-Feed™ Alpha, Bio-Feed™ Beta, Bio-Feed™ Plus, Bio-Feed™ Plus, Novozyme® 188, Carezyme® (SEQ ID NO: 5), Celluclast®, Cellusoft®, Ceremyl®, Citrozym™, Denimax™ Dezyme™, Dextrozyme™, Finizym®, Fungamyl™, Gamanase™, Glucanex®, Lactozym®, Maltogenase™, Pentopan™, Pectinex™, Promozyme®, Pulpzyme™, Novamyl™, Termamyl®, AMG (Amyloglucosidase Novo), Maltogenase®, Sweetzyme®, Aquazym®, Natalase® (SEQ ID NO: 4), SP722, AA560 (all enzymes available from Novozymes A/S). Other carbohydrases are available from other companies.

**[0385]** The parent cellulase is preferably a microbial cellulase. As such, the cellulase may be selected from bacterial cellulases, e.g. *Pseudomonas* cellulases or *Bacillus*, such as the *Bacillus* strains described in U.S. Pat. No. 4,822,516, U.S. Pat. No. 5,045,464 or EP 468 464, or *B. lautus* (cf. WO 91/10732), cellulases. More preferably, the parent cellulases may be a fungal cellulase, in particular *Humicola, Trichoderma, Irpex, Aspergillus, Penicillium, Myceliophthora* or *Fusarium* cellulases. Examples of suitable parent cellulases are described in, e.g. WO 91/17244. Examples of suitable *Trichoderma* cellulases are those described in T. T. Teeri, *Gene* 51, 1987, pp. 43-52. Preferably, the parent cellulase is selected from the cellulases classified in family 45, e.g. the enzymes EG B (*Pseudomonas fluorescens*) and EG V (*Humicola insolens*), as described in Henrissat, B. et al.: *Biochem. J.* (1993), 293, p. 781-788.

The Termamyl-Like Alpha-Amylase

**[0386]** It is well known that a number of alpha-amylases produced by *Bacillus* spp. are highly homologous on the amino acid level. For instance, the *B. licheniformis* alpha-amylase comprising the amino acid sequence shown in SEQ ID NO: 4 of WO 00/29560 (commercially available as Termamyl®) has been found to be about 89% homologous with the *B. amyloliquefaciens* alpha-amylase comprising the amino acid sequence shown in SEQ ID NO: 5 of WO 00/29560 and about 79% homologous with the *B. stearothermophilus* alpha-amylase comprising the amino acid sequence shown in SEQ ID NO: 3 of WO 00/29560. Further homologous alpha-amylases include an alpha-amylase derived from a strain of the *Bacillus* sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, all of which are described in detail in WO 95/26397, and the alpha-amylase described by Tsukamoto et al., *Biochemical and Biophysical Research Communications,* 151 (1988), pp. 25-31.

**[0387]** Still further homologous alpha-amylases include the alpha-amylase produced by the *B. licheniformis* strain described in EP 0252666 (ATCC 27811), and the alpha-amylases identified in WO 91/00353 and WO 94/18314. Other commercial Termamyl-like *B. licheniformis* alpha-amylases are Optitherm® and Takatherm® (available from Solvay), Maxamyl® (available from Gist-brocades/Genencor), Spezym AA® and Spezyme Delta AA™ (available from Genencor), and Keistase® (available from Daiwa).

**[0388]** Because of the substantial homology found between these alpha-amylases, they are considered to belong to the same class of alpha-amylases, namely the class of "Termamyl-like alpha-amylases".

**[0389]** Accordingly, in the present context, the term "Termamyl-like alpha-amylase" is intended to indicate an alpha-amylase which, at the amino acid level, exhibits a substantial homology to Termamyl®, i.e., the *B. licheniformis* alpha-amylase having the amino acid sequence shown in SEQ ID NO: 4 (WO 00/29560). In other words, a Termamyl-like alpha-amylase is an alpha-amylase which has the amino acid sequence shown in SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7 or 8 of WO 00/29560, and the amino acid sequence shown in SEQ ID NO: 1 of WO 95/26397 (the same as the amino acid sequence shown as SEQ ID NO: 7 of WO 00/29560) or in SEQ ID NO: 2 of WO 95/26397 (the same as the amino acid sequence shown as SEQ ID NO: 8 of WO 00/29560) or in Tsukamoto et al., 1988, (which amino acid sequence is shown in SEQ ID NO: 6 of WO 00/29560) or i) which displays at least 60% homology (identity), preferred at least 70%, more preferred at least 75%, even more preferred at least 80%, especially at least 85%, especially preferred at least 90%, especially at least 95%, even especially more preferred at least 97%, especially at least 99% homology with at least one of said amino acid sequences shown in SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7 or 8 of WO 00/29560 and/or ii) displays immunological cross-reactivity with an antibody raised against one or more of said alpha-amylases, and/or iii) is encoded by a DNA sequence which hybridizes, under the low to very high stringency conditions (said conditions described below) to the DNA sequences encoding the above-specified alpha-amylases which are apparent from SEQ ID NOS: 9, 10, 11, 12, and 32, respectively, of the present application (which encodes the amino acid sequences shown in SEQ ID NOS: 1, 2, 3, 4, and 5 herein, respectively), from SEQ ID NO: 4 of WO 95/26397 (which DNA sequence, together with the stop codon TAA, is shown in SEQ ID NO: 13 herein and encodes the amino acid sequence shown in SEQ ID NO: 8 herein) and from SEQ ID NO: 5 of WO 95/26397 (shown in SEQ ID NO: 14 herein), respectively.

**[0390]** In connection with property i), the "homology" (identity) may be determined by use of any conventional algorithm, preferably by use of the gap progamme from the GCG package version 8 (August 1994) using default values for gap penalties, i.e., a gap creation penalty of 3.0 and gap extension penalty of 0.1 (Genetic Computer Group (1991) Programme Manual for the GCG Package, version 8, 575 Science Drive, Madison, Wis., USA 53711).

**[0391]** The parent Termamyl-like alpha-amylase backbone may in an embodiment have an amino acid sequence which has a degree of identity to SEQ ID NO: 4 (WO 00/29560) of at least 65%, preferably at least 70%, preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least about 90%, even more preferably at least 95%, even more preferably at least 97%, and even more preferably at least 99% identity determined as described above.

**[0392]** A structural alignment between Termamyl® (SEQ ID NO: 4) and a Termamyl-like alpha-amylase may be used to identify equivalent/corresponding positions in other Ter-

mamyl-like alpha-amylases. One method of obtaining said structural alignment is to use the Pile Up programme from the GCG package using default values of gap penelties, i.e., a gap creation penalty of 3.0 and gap extension penalty of 0.1. Other structural alignment methods include the hydrophobic cluster analysis (Gaboriaud et al., (1987), FEBS LETTERS 224, pp. 149-155) and reverse threading (Huber, T; Torda, A E, PROTEIN SCIENCE Vol. 7, No. 1 pp. 142-149 (1998).

Parent Glucoamylases

**[0393]** Parent glucoamylase contemplated according to the present invention include fungal glucoamylases, in particular fungal glucoamylases obtainable from an *Aspergillus* strain, such as an *Aspergillus niger* or *Aspergillus awamori* glucoamylases and variants or mutants thereof, homologous glucoamylases, and further glucoamylases being structurally and/or functionally similar to SEQ ID NO: 2 (WO 00/04136). Specifically contemplated are the *Aspergillus niger* glucoamylases G1 and G2 disclosed in Boel et al. (1984), "Glucoamylases G1 and G2 from *Aspergillus niger* are synthesized from two different but closely related mRNAs", EMBO J. 3 (5), p. 1097-1102. The G2 glucoamylase is disclosed in SEQ ID NO: 2 (WO 00/04136). The G1 glucoamylase is disclosed in SEQ ID NO: 13 (WO 00/04136). Another AMG backbone contemplated is *Talaromyces emersonii*, especially *Talaromyces emersonii* DSM disclosed in WO 99/28448 (Novo Nordisk).

**[0394]** The homology referred to above of the parent glucoamylase is determined as the degree of identity between two protein sequences indicating a derivation of the first sequence from the second. The homology may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wis., USA 53711) (Needleman, S. B. and Wunsch, C. D., (1970), Journal of Molecular Biology, 48, p. 443-453). Using Gap with the following settings for polypeptide sequence comparison: Gap creation penalty of 3.0 and Gap extension penalty of 0.1, the mature part of a polypeptide encoded by an analogous DNA sequence of the invention exhibits a degree of identity preferably of at least 60%, such as 70%, at least 80%, at least 90%, more preferably at least 95%, more preferably at least 97%, and most preferably at least 99% with the mature part of the amino acid sequence shown in SEQ ID NO: 2 (WO 00/04136).

**[0395]** Preferably, the parent glucoamylase comprise the amino acid sequences of SEQ ID NO: 2 (WO 00/04136); or allelic variants thereof; or fragments thereof that has glucoamylase activity.

**[0396]** A fragment of SEQ ID NO: 2 is a polypeptide which have one or more amino acids deleted from the amino and/or carboxyl terminus of this amino acid sequence. For instance, the AMG G2 (SEQ ID NO: 2) is a fragment of the *Aspergillus niger* G1 glucoamylase (Boel et al. (1984), EMBO J. 3 (5), p. 1097-1102) having glucoamylase activity. An allelic variant denotes any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequences. An allelic variant of a polypeptide is a polypeptide encoded by an allelic variant of a gene.

**[0397]** It is to be understood that also carbohydrase variants are contemplated as the parent enzyme.

**[0398]** The activity of carbohydrases can be determined as described in "Methods of Enzymatic Analysis", third edition, 1984, Verlag Chemie, Weinheim, vol. 4.

Parent Transferases

**[0399]** Parent transferases (i.e. enzymes classified under the Enzyme Classification number E.C. 2 in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB)) include transferases within this group.

**[0400]** The parent transferases may be any transferase in the subgroups of transferases: transferases transferring one-carbon groups (E.C. 2.1); transferases transferring aldehyde or residues (E.0 2.2); acyltransferases (E.C. 2.3); glucosyltransferases (E.C. 2.4); transferases transferring alkyl or aryl groups, other that methyl groups (E.C. 2.5); transferases transferring nitrogeneous groups (2.6).

**[0401]** In a preferred embodiment the parent transferase is a transglutaminase E.C 2.3.2.13 (Protein-glutamine μ-glutamyltransferase).

**[0402]** Transglutaminases are enzymes capable of catalyzing an acyl transfer reaction in which a gamma-carboxyamide group of a peptide-bound glutamine residue is the acyl donor. Primary amino groups in a variety of compounds may function as acyl acceptors with the subsequent formation of monosubstituted gamma-amides of peptide-bound glutamic acid. When the epsilon-amino group of a lysine residue in a peptide-chain serves as the acyl acceptor, the transferases form intramolecular or intermolecular gamma-glutamyl-epsilon-lysyl crosslinks.

**[0403]** Examples of transglutaminases are described in the pending DK patent application no. 990/94 (Novo Nordisk A/S).

**[0404]** The parent transglutaminase may be of human, animal (e.g. bovine) or microbial origin.

**[0405]** Examples of such parent transglutaminases are animal derived Transglutaminase, FXIIIa; microbial transglutaminases derived from *Physarum polycephalum* (Klein et al., Journal of Bacteriology, Vol. 174, p. 2599-2605); transglutaminases derived from *Streptomyces* sp., including *Streptomyces lavendulae, Streptomyces lydicus* (former *Streptomyces libani*) and *Streptoverticillium* sp., including *Streptoverticillium mobaraense, Streptoverticillium cinnamoneum*, and *Streptoverticillium griseocarneum* (Motoki et al., U.S. Pat. No. 5,156,956; Andou et al., U.S. Pat. No. 5,252,469; Kaempfer et al., Journal of General Microbiology, Vol. 137, p. 1831-1892; Ochi et al., International Journal of Sytematic Bacteriology, Vol. 44, p. 285-292; Andou et al., U.S. Pat. No. 5,252,469; Williams et al., Journal of General Microbiology, Vol. 129, p. 1743-1813).

**[0406]** It is to be understood that also transferase variants are contemplated as the parent enzyme.

**[0407]** The activity of transglutaminases can be determined as described in "Methods of Enzymatic Analysis", third edition, 1984, Verlag Chemie, Weinheim, vol. 1-10.

Parent Phytases

**[0408]** Parent phytases are included in the group of enzymes classified under the Enzyme Classification number E.C. 3.1.3 (Phosphoric Monoester Hydrolases) in accordance

with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB)).

**[0409]** Phytases are enzymes produced by microorganisms which catalyse the conversion of phytate to inositol and inorganic phosphorus

**[0410]** Phytase producing microorganisms comprise bacteria such as *Bacillus subtilis, Bacillus natto* and *Pseudomonas*; yeasts such as *Saccharomyces cerevisiae*; and fungi such as *Aspergillus niger, Aspergillus ficuum, Aspergillus awamori, Aspergillus oryzae, Aspergillus terreus* or *Aspergillus nidulans*, and various other *Aspergillus* species).

**[0411]** Examples of parent phytases include phytases selected from those classified under the Enzyme Classification (E.C.) numbers: 3-phytase (3.1.3.8) and 6-phytase (3.1.3.26).

**[0412]** The activity of phytases can be determined as described in "Methods of Enzymatic Analysis", third edition, 1984, Verlag Chemie, Weinheim, vol. 1-10, or may be measured according to the method described in EP-A1-0 420 358, Example 2A.

Lyases

**[0413]** Suitable lyases include Polysaccharide lyases: Pectate lyases (4.2.2.2) and pectin lyases (4.2.2.10), such as those from *Bacillus licheniformis* disclosed in WO 99/27083.

Isomerases

Protein Disulfide Isomerase

**[0414]** Without being limited thereto suitable protein disulfide isomerases include PDIs described in WO 95/01425 (Novo Nordisk A/S) and suitable glucose isomerases include those described in Biotechnology Letter, Vol. 20, No 6, June 1998, pp. 553-56.

**[0415]** Contemplated isomerases include xylose/glucose Isomerase (5.3.1.5) including Sweetzyme®.

Environmental Allergens

**[0416]** The environmental allergens that are of interest for epitope mapping include allergens from pollen, dust mites, mammals, venoms, fungi, food items, and other plants.

**[0417]** Pollen, allergens include but are not limited to those of the order Fagales, Oleales, Pinales, Poales, Asterales, and Urticales; including those from *Betula, Alnus, Corylus, Carpinus, Olea, Phleum pratense* and *Artemisia vulgaris*, such as Aln g1, Cor a1, Car b1, Cry j1, Amb a1 and a2, Art v1, Par j1, Ole e1, Ave v1, and Bet v1 (WO 99/47680).

**[0418]** Mite allergens include but are not limited to those from Derm. farinae and Derm. pteronys., such as Der f1 and f2, and Der p1 and p2.

**[0419]** From mammals, relevant environmental allergens include but are not limited to those from cat, dog, and horse as well as from dandruff from the hair of those animals, such as Fel d1; Can f1; Equ c1; Equ c2; Equ c3.

**[0420]** Venum allergens include but are not limited to PLA2 from bee venom as well as *Apis* m1 and m2, Ves g1, g2 and g5, Ves v5 and to Pol and Sol allergens.

**[0421]** Fungal allergens include those from *Alternaria* alt. and Cladospo. herb. such as Alt a1 and Cla h1.

**[0422]** Food allergens include but are not limited to those from milk (lactoglobulin), egg (ovalbumin), peanuts, hazelnuts, wheat (alpha-amylase inhibitor),

**[0423]** Other plant allergens include latex (hevea brasiliensis).

**[0424]** In addition, a number of proteins of interest for expression in transgenic plants could be useful objects for epitope engineering. If for instance a heterologous enzyme is introduced into a transgenic plant e.g. to increase the nutritional value of food or feed derived from that plant, that enzyme may lead to allergenicity problems in humans or animals ingesting the plant-derived material. Epitope mapping and engineering of such heterologous enzymes or other proteins of transgenic plants may lead to reduction or elimination of this problem. Hence, the methods of this patent are also useful for potentially modifying proteins for heterologous expression in plants and plant cells.

Materials and Methods

Materials

ELISA Reagents:

**[0425]** Horse Radish Peroxidase labelled pig anti-rabbit-Ig (Dako, DK, P217, dilution 1:1000)

Rat anti-mouse IgE (Serotec MCA419; dilution 1:100)

Mouse anti-rat IgE (Serotec MCA193; dilution 1:200)

Biotin-labelled mouse anti-rat IgG1 monoclonal antibody (Zymed 03-9140; dilution 1:1000)

Biotin-labelled rat anti-mouse IgG1 monoclonal antibody (Serotec MCA336B; dilution 1:2000)

Streptavidin-horse radish peroxidase (Kirkegård & Perry 14-30-00; dilution 1:1000).

Buffers and Solutions:

**[0426]** PBS (pH 7.2 (1 liter))

| | |
|---|---|
| NaCl | 8.00 g |
| KCl | 0.20 g |
| $K_2HPO_4$ | 1.04 g |
| $KH_2PO_4$ | 0.32 g |

**[0427]** Washing buffer PBS, 0.05% (v/v) Tween 20

**[0428]** Blocking buffer PBS, 2% (wt/v) Skim Milk powder

**[0429]** Dilution buffer PBS, 0.05% (v/v) Tween 20, 0.5% (wt/v) Skim Milk powder

**[0430]** Citrate buffer 0.1M, pH 5.0-5.2

**[0431]** Stop-solution (DMG-buffer)

**[0432]** Sodium Borate, borax (Sigma)

**[0433]** 3,3-Dimethyl glutaric acid (Sigma)

**[0434]** Tween 20: Poly oxyethylene sorbitan mono laurate (Merck cat no. 822184)

**[0435]** PMSF (phenyl methyl sulfonyl flouride) from Sigma

**[0436]** Succinyl-Alanine-Alanine-Proline-Phenylalanine-paranitro-anilide (Suc-AAPF-pNP) Sigma no. S-7388, Mw 624.6 g/mol.

**[0437]** mPEG (Fluka)

Coloring Substrate:

**[0438]** OPD: o-phenylene-diamine, (Kementec cat no. 4260)

Methods

Automatic Epitope Mapping

Implementation

**[0439]** The implementation consists of 3 pieces of code:
1. The core program (see above), written in C (see Appendix A).
2. A "wrapping" cgi-script run by the web server, written in Python (see Appendix B).
3. A HTML page defining the input/submission form (see Appendix C).
**[0440]** The wrapper receives the input and calls the core program and several other utilities.
Apart from the standard Unix utility programs (my, rm, awk, etc.) the following must be installed:
**[0441]** A web server capable of running cgi-scripts, e.g. Apache
**[0442]** Python 1.5 or later
**[0443]** Gnuplot 3.7 or later
**[0444]** DSSP, version July 1995

The Core Program

Inputs

**[0445]** 1. A Brookhaven PDB file with the structure of the protein
2. The output of DSSP called with the above PDB file.
3. Maximum distance between adjacent residues
4. Minimum solvent accessible surface area for each residue
5. Maximum epitope size (max distance between any two residues in epitope)
6. Maximum number of non-redundant epitopes to include (0=all)
7. The shortest acceptable epitope (as a fraction of the length of the epitope consensus sequence).
8. Epitope consensus sequence describing which residues are possible at the different positions. An example is shown below:
KR (Lys or Arg allowed)
AILV– (Ala, Ile, Leu, Val or missing residue allowed)
* (All Residues Allowed, but there Must be a Residue)

? (All or Missing Residue Allowed)

**[0446]** DE (Asp or Glu allowed)
(*, ? or – in first or last position is allowed but obsolete. (– in first position is ignored.))
**[0447]** Examples of matching epitopes:

KAAKD (SEQ ID NO: 41), KLASD (SEQ ID NO: 42), KLYSD (SEQ ID NO: 43), KLY-D (SEQ ID NO: 44), R-M-D.

The Epitope Searching Algorithm

**[0448]** The "core" of the program is the algorithm that scans the protein surface for the epitope patterns. The principle is that several "trees" are built, where each of their branches describes one epitope:
1. All residues in the protein are checked according to: a) Does the residue type match the first residue of the epitope consensus sequence. b) Is the surface accessibility greater than or equal to the given threshold. If both requirements are fulfilled, the protein residue is considered as one root in the epitope tree. Remark that there are usually many roots.
2. For each of the residues defined as roots, all residues within the given threshold distance between adjacent residues (e.g. 7 Angstroms) are checked for the same as above: a) Does the residue type match the second residue of the epitope consensus sequence. b) Is the surface accessibility greater than or equal to the given threshold. If yes, the protein residue is considered as a "child" of the root. The spatial position of a residue is defined as the coordinates of its C-alpha atom.
3. The procedure from step 2 is repeated for the next residue in the epitope consensus sequence, where each of the "childs" found in step 2 are now "roots" of new childs. If a gap is defined in the epitope consensus sequence, a "missing" residue is allowed, and the coordinates of the root (also called "parent") is used.
4. This procedure is repeated for all residues in the epitope consensus sequence.
5. In this way a number of trees (corresponding to the number of roots found in step 1) are found. Notice that the same protein residue can be present many places in the trees.
6. If no epitopes that matches the length of the epitope consensus sequence are found, the longest shorter epitopes that matches the first n residues of the epitope consensus sequence are used, where n is an integer smaller than the length of the epitope consensus sequence. If n is smaller than the length of the epitope consensus sequence multiplied by the fraction value defining the shortest acceptable epitope length, no epitopes are written to the output, and steps 7, 8 and 9 are skipped.
7. The epitopes are extracted from the trees by traversing down from each of the "childs" in the last level. The algorithm also finds epitopes which have the same protein residue present more than once. This is, of course, an artifact and such epitopes are discarded. Every epitope is then checked for its size, that is, the maximum distance between any two residues which are members of the epitope. If this exceeds the threshold, the epitope is discarded.
8. Redundant epitopes are removed. Epitopes containing one or more gaps are redundant if they are subsets of other epitopes without or with fewer gaps. For example: A82-gap-F45-G44-K43 is a subset of A82-L46-F45-G44-K43, and is therefore discarded.
9. For every epitope, the total solvent accessible surface area is calculated (by adding the contributions from each residue as found by the DSSP program). The epitopes are sorted according to this area in descending order. If a maximum number of n non-redundant epitopes has been specified, the n epitopes with largest solvent accessible surface area are selected.
10. The output consists of a list of the found epitopes, along with information of the epitope consensus sequence used and other internal parameters. A separate file containing the number of epitopes that each of the protein residues is a member of is also written.
The wrapper

Inputs

**[0449]** 1. One PDB file, describing one structure, or one ZIP file, containing a number of PDB files, each describing one structure. The ZIP file must not contain subfolders.
2. An epitope consensus sequence or which part of the current epitope library to use (full library or IgE part or IgG part).
3. Maximum distance between adjacent residues
4. Minimum solvent accessible surface area for each residue

5. Maximum epitope size (max distance between any two residues in epitope)
6. Maximum number of non-redundant epitopes to include (0=all)
7. Whether to use sequential numbering (1,2,3,4, etc) or PDB-file numbering.

DESCRIPTION

**[0450]** The core program accepts only one structure and one epitope consensus sequence. It is usually desirable to use a library of epitope consensus sequences and sometimes several protein structures. The wrapper reads the user input and calls the utility programs and the core program the necessary number of times. The output is collected and presented on the web page returned to the user.

**[0451]** Depending on the type of input, the wrapper works in different modes:

**[0452]** Epitope consensus can be given directly or taken from a library

**[0453]** Input type can be a single PDB file or a collection of PDB file given as a ZIP-file.

**[0454]** Any of the four possible combinations are allowed.

**[0455]** The epitope library consists of a number of text files, each containing one epitope consensus sequence as specified above.

**[0456]** The layout of the wrapper is like this:

1. Check if the program is already in use from somewhere else (this is done by checking for a lock file when the wrapper starts. If it does not exist, it is created and removed again when the program is finished).
2. If the epitope consensus sequences are to be read from the library, make an internal list of the desired library entries.
3. If the input type is a ZIP file, unzip the file and create one new directory for each of the contained PDB files. Move each PDB file to its corresponding directory.
4. Do a loop over the structures and/or epitope consensus sequences. For each structure/epitope consensus sequence pair, DSSP and the core program is called with the required parameters. If the input type is a ZIP file, the outputs are put in the appropriate directories.
5. If the epitope library is used, a sum file containing the total number of epitopes each residue is a member of. (Such a file is generated by the core program for each epitope consensus sequence—here a sum of these files is calculated). If input type is a ZIP file, a sum file is generated for each structure and put in the appropriate directory.
6. If the epitope library is used, a file containing the total number of epitopes found from each entry in the epitope library. If the input type is a PDB file, the file contains only one line (with a number of data corresponding to the library size). If the input type is a ZIP file, there is one line for each structure.
7. Depending on the combination of input type (ZIP or single PDB) and epitope consensus sequence source (typed-in or epitope library), different information is returned to the user:

Single PDB+typed in epitope: Graph of numbers of epitopes that each residue is a member of. List of found epitopes.

ZIP file+typed in epitope: Graphs (one for each structure) of numbers of epitopes that each residue is a member of. Lists (one for each structure) of found epitopes.

Single PDB+epitope library: Graph of numbers of epitopes that each residue is a member of (total for the complete library).

ZIP file+epitope library: Graphs (one for each structure) of numbers of epitopes that each residue is a member of (total for the complete library).

Data flow sheets for the four different are shown in the FIG. 8. For all modes except Single PDB+typed in epitope, a ZIP file containing all output files is created and returned to the user.

Immunisation of Brown Norway Rats:

**[0457]** Twenty intratracheal (IT) immunisations were performed weekly with 0,100 ml 0.9% (wt/vol) NaCl (control group), or 0,100 ml of a protein dilution (~0, 1-1 mg/ml). Each group contained 10 rats. Blood samples (2 ml) were collected from the eye one week after every second immunisation. Serum was obtained by blood clothing and centrifugation and analysed as indicated below.

Immunisation of Balb/C Mice:

**[0458]** Twenty subcutaneous (SC) immunisations were performed weekly with 0.05 ml 0.9% (wt/vol) NaCl (control group), or 0.050 ml of a protein dilution (~0.01-0.1 mg/ml). Each group contained 10 female Balb/C mice (about 20 grams) purchased from Bomholdtgaard, Ry, Denmark. Blood samples (0.100 ml) were collected from the eye one week after every second immunisation. Serum was obtained by blood clothing and centrifugation and analysed as indicated below.

ELISA Procedure for Detecting Serum Levels of IgE and IgG:

**[0459]** Specific IgG1 and IgE levels were determined using the ELISA specific for mouse or rat IgG1 or IgE. Differences between data sets were analysed by using appropriate statistical methods.

Activation of CovaLink Plates:

**[0460]** A fresh stock solution of cyanuric chloride in acetone (10 mg/ml) is diluted into PBS, while stirring, to a final concentration of 1 mg/ml and immediately aliquoted into CovaLink NH2 plates (100 microliter per well) and incubated for 5 minutes at room temperature. After three washes with PBS, the plates are dryed at 50° C. for 30 minutes, sealed with sealing tape, and stored in plastic bags at room temperature for up to 3 weeks.

**[0461]** Mouse anti-Rat IgE was diluted 200× in PBS (5 microgram/ml). 100 microliter was added to each well. The plates were coated overnight at 4° C.

**[0462]** Unspecific adsorption was blocked by incubating each well for 1 hour at room temperature with 200 microliter blocking buffer. The plates were washed 3× with 300 microliter washing buffer.

**[0463]** Unknown rat sera and a known rat IgE solution were diluted in dilution buffer: Typically 10×, 20× and 40× for the unknown sera, and 1/2 dilutions for the standard IgE starting from 1 μg/ml. 100 microliter was added to each well. Incubation was for 1 hour at room temperature.

**[0464]** Unbound material was removed by washing 3× with washing buffer. The anti-rat IgE (biotin) was diluted 2000× in dilution buffer. 100 microliter was added to each well. Incubation was for 1 hour at room temperature. Unbound material was removed by washing 3× with washing buffer.

**[0465]** Streptavidin was diluted 1000× in dilution buffer. 100 microliter was added to each well. Incubation was for 1

hour at room temperature. Unbound material was removed by washing 3× with 300 microliter washing buffer. OPD (0.6 mg/ml) and $H_2O_2$ (0.4 microliter/ml) were dissolved in citrate buffer. 100 microliter was added to each well. Incubation was for 30 minutes at room temperature. The reaction was stopped by addition of 100 microliter $H_2SO_4$. The plates were read at 492 nm with 620 nm as reference.

**[0466]** Similar determination of IgG can be performed using anti Rat-IgG and standard rat IgG reagents.

**[0467]** Similar determinations of IgG and IgE in mouse serum can be performed using the corresponding species-specific reagents.

Direct IgE Assay:

**[0468]** To determine the IgE binding capacity of protein variants one can use an assay, essentially as described above, but using sequential addition of the following reagents:

1) Mouse anti-rat IgE antibodies coated in wells;

2) Known amounts of rat antiserum containing IgE against the parent protein;

3) Dilution series of the protein variant in question (or parent protein as positive control);

4) Rabbit anti-parent antibodies

5) HRPO-labelled anti-rabbit Ig antibodies for detection using OPD as described.

**[0469]** The relative IgE binding capacity (end-point and/or affinity) of the protein variants relative to that of the parent protein are determined from the dilution-response curves. The IgE-positive serum can be of other animals (including humans that inadvertently have been sensitized to the parent protein) provided that the species-specific anti-IgE capture antibodies are changed accordingly.

Competitive ELISA (C-ELISA):

**[0470]** C-ELISA was performed according to established procedures. In short, a 96 well ELISA plate was coated with the parent protein. After proper blocking and washing, the coated antigen was incubated with rabbit anti-enzyme polyclonal antiserum in the presence of various amounts of modified protein (the competitior). The residual amount of rabbit antiserum was detected by horseraddish peroxidase-labelled pig anti-rabbit immunoglobulin.

Protein Sequences and Alignments:

**[0471]** For purposes of the present invention, the degree of homology may be suitably determined by means of computer programs known in the art, such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wis., USA 53711) (Needleman, S. B. and Wunsch, C. D., (1970), Journal of Molecular Biology, 48, 443-45).

Subtilisin Proteases:

**[0472]** In the present invention, corresponding (or homologous) positions in subtilisin protease sequences are defined by alignment with Subtilisin Novo (BPN') from *B. amyloliquefaciens*, as shown in Table 1A for Alcalase, Protease B, Esperase, Protease C, Protease D, Protease E, Protease A, PD498, Properase, Relase, Savinase.

TABLE 1A

Alignment of different proteases to the sequence of BPN'

```
                                  Alcalase:
  69.5% identity in 275 residues overlap; Score: 953.0; Gap frequency: 0.4%

Alcalase,     1AQTVPYGIPLIKADKVQAQGFKGANVKVAVLDTGIQASHPDLNVVGGASFVAGEAYN-TD
BPN',         1AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
               ** ****   ***     **  * ****** * **  ***** * **** *  *  *  *

Alcalase,    60GNGHGTHVAGTVAALDNTTGVLGVAPSVSLYAVKVLNSSGSGSYSGIVSGIEWATTNGMD
BPN',        61DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
                * ************ *  ********  *******   *** ** *  *****  * **

Alcalase,   120VINMSLGGASGSTAMKQAVDNAYARGVVVVAAAGNSGSSGNTNTIGYPAKYDSVIAVGAV
BPN',       121VINMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
               ******** *** * * *** * * ********** ** *   * *** ** ********

Alcalase,   180DSNSNRASFSSVGAELEVMAPGAGVYSTYPTNTYATLNGTSMASPHVAGAAALILSKHPN
BPN',       181DSSNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
               **   ******** ** *****    ** * * *   **********************

Alcalase,   240LSASQVRNRLSSTATYLGSSFYYGKGLINVEAAAQ (SEQ ID NO: 45)
BPN',       241WTNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO: 46)
                  ***  *  * * ** *********** ****

                                  Protease B:
  59.6% identity in 275 residues overlap; Score: 820.0; Gap frequency: 2.2%

PROTEASE B,   1AQTIPWGISRVQAPAAHNRGLTGSGVKVAVLDTGI-STHPDLNIRGGASFVPGE-PSTQD
BPN',         1AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
               **  * * *   *** *  * *** ***** * ** * ****   **** ** * *  **

PROTEASE B,  59GNGHGTHVAGTIAALNNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWAGNNGMH
BPN',        61DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
                * ******** ***************  ********  ***  * *  * ***  * *
```

TABLE 1A-continued

Alignment of different proteases to the sequence of BPN'

```
PROTEASE B, 119VANLSLGSPSPSATLEQAVNSATSRGVLVVAASGNSGA----GSISYPARYANAMAVGAT
BPN',       121VINMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
               * * *** ** ** *  **  *   ** **** ** *         **  *    ****

PROTEASE B, 175DQNNNRASFSQYGAGLDIMAPGVNIQSTYPGSTYASDNGTSMATPHVAGAAALVKQKNPS
BPN',       181DSSNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
               *  * *****  *  ** ***** **** **  *   ****** *********   * *

PROTEASE B, 235WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAATR (SEQ ID NO: 47)
BPN',       241WTNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO: 46)
               * * * *  * ** * **    ** ** *  **
                                 Esperase:
  54.7% identity in 274 residues overlap; Score: 745.0; Gap frequency: 2.2%

Esperase,     1QTVPWGISFINTQQAHNRGIFGNGARVAVLDTGI-ASHPDLRIAGGASFISSE-PSYHDN
BPN',         2QSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQDD
                * ** * * *     *  *  *    *** * **  *****  *****   ** *   *

Esperase,    59NGHGTHVAGTIAALNNSIGVLGVAPSADLYAVKVLDRNGSGSLASVAQGIEWAINNNMHI
BPN',        62NSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMDV
                * ******** ***************  *******   ***       ****** ***

Esperase,   119INMSLGSTSGSSTLELAVNRANNAGILLVGAAGNTGRQG----VNYPARYSGVMAVAAVD
BPN',       122INMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAVD
               ******  ***  *  **  *   *   * **** *  *    * **  *  * ** ***

Esperase,   175QNGQRASFSTYGPEIEISAPGVNVNSTYTGNRYVSLSGTSMATPHVAGVAALVKSRYPSY
BPN',       182SSNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPNW
                  ******  ***    ****   **  ** *    ***** ***** ***  *  *

Esperase,   235TNNQIRQRINQTATYLGSPSLYGNGLVHAGRATQ (SEQ ID NO: 48)
BPN',       242TNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO: 46)
               ** * *     * * **    ** **     * *

                                 Protease C:
  59.6% identity in 275 residues overlap; Score: 825.0; Gap frequency: 2.2%

ProteaseC,    1AQSVPWGISRVQAPAAHNRGLTGSGVRVAVLDTGI-STHPDLNIRGGASFVPGE-PSTQD
BPN',         1AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
                ***** * *   *** *  * *** * *** * ** * ****   **** ** * *  **

ProteaseC,   59GNGHGTHVAGTIAALNNSIGVLGVAPSAELYAVKVLGASGSGSYSSIAQGLEWAGNNGMH
BPN',        61DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
                * ******** ***************  ********  *** ** *  * ***  * *

ProteaseC,  119VASLSLGSPSPSATLEQAVNSATSRGVLVVAASGNSGA----GSISYPARYANAMAVGAT
BPN',       121VINMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
               *   *** ** ** *  **  *   ** **** ** *         **  *    ****

ProteaseC,  175DQNNNRASFSQYGAGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVKQKNPS
BPN',       181DSSNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
               *  * *****  *  **  ****  *** **  *   ****** *********   * *

ProteaseC,  235WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAAAR (SEQ ID NO: 49)
BPN',       241WTNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO: 46)
               * * * *  * ** * **    ** ** *  ***

                                 Protease D:
  59.3% identity in 275 residues overlap; Score: 815.0; Gap frequency: 2.2%

ProteaseD,    1AQSVPWGISRVQAPAAHNRGLTGSGVKVAVLDTGI-STHPDLNIRGGASFVPGE-PSTQD
BPN',         1AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
                ***** * *   *** *  * *** ***** * ** * ****   **** ** * *  **

ProteaseD,   59GNGHGTHVAGTIAALDNSIGVLGVAPSAELYAVKVLGASGSGAISSIAQGLEWAGNNGMH
BPN',        61DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
                * ******** *** ***********  ********  ***  * *  * ***  * *

ProteaseD,  119VANLSLGSPSPSATLEQAVNSATSRGVLVVAASGNSGA----GSISYPARYANAMAVGAT
BPN',       121VINMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
               * * *** ** ** *  **  *   ** **** ** *         **  *    ****
```

TABLE 1A-continued

Alignment of different proteases to the sequence of BPN'

```
ProteaseD,  175DQNNNRASFSQYGAGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVKQKNPS
BPN',       181DSSNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
               *  * *****  *  **  ****  *** **  *   ****** *********   * *

ProteaseD,  235WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAATR (SEQ ID NO: 50)
BPN',       241WTNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO: 46)
               * * * *  * ** * **    ** ** *  **
```

Protease E:

58.2% identity in 275 residues overlap; Score: 800.0; Gap frequency: 2.2%

```
ProteaseE,    1AQSVPWGISRVQAPAAHNRGLTGSGVKVAVLDTGI-STHPDLNIRGGASFVPGE-PSTQD
BPN',         1AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
               ***** * *   *** *  * *** ***** * ** * ****   **** ** * *  **

ProteaseE,   59GNGHGTHVAGTIAALNNSIGVLGVAPSAELYAVKVLGASGGGAISSIAQGLEWAGNNGMH
BPN',        61DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
               * ******** ***************  ********  * *  * *  * ***  * *

ProteaseE,  119VANLSLGSPSPSATLEQAVNSATSRGVLVVAASGNSGA----DSISYPARYANAMAVGAT
BPN',       121VINMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
               * * *** ** ** *  **  *   ** **** ** *         **  *    ****

ProteaseE,  175DQNNNRASFSQYGAGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAVLVKHKNPS
BPN',       181DSSNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
               *  * *****  *  **  ****  *** **  *   ****** ******* *   * *

ProteaseE,  235WSNVRIRDHLKKTATSLGSTNLYGSGLVNAEAATR (SEQ ID NO: 51)
BPN',       241WTNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO: 46)
               * *   *  *  * * **    ** ** *  **
```

Protease A:

58.9% identity in 275 residues overlap; Score: 812.0; Gap frequency: 2.2%

```
Protease A,   1AQSVPWGISRVQAPAAHNRGLTGSGVKVAVLDTGI-STHPDLNIRGGASFVPGE-PSTQD
BPN',         1AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
               ***** * *   *** *  * *** ***** * ** * ****   **** ** * *  **

Protease A,  59GNGHGTHVAGTIAALNNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWAGNNGMH
BPN',        61DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
               * ******** ***************  ********  ***  * *  * ***  * *

Protease A, 119VANLSLGSPSAGGTLEQAVNSATSRGVLVVAASGNSGA----GSISAPASYANAMAVGAT
BPN',       121VINMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
               * * *** **    *  **  *   ** **** ** *          *  *    ****

Protease A, 175DQNNNRASFSQYGPGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVKQKNPS
BPN',       181DSSNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
               *  * *****  ** **  ****  *** ** *   ****** *********   * *

Protease A, 235WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAATR (SEQ ID NO: 52)
BPN',       241WTNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO: 46)
               * * * *  * ** * **    ** ** *  **
```

PD498:

47.7% identity in 266 residues overlap; Score: 487.0; Gap frequency: 4.9%

```
PD498,       13YGPQNTSTPAAWDVTRGSSTQTVAVLDSGVDYNHPDLARKVIKGYDFIDRDN-NPMDLNG
BPN',         6YGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDL--KVAGGASMVPSETPNFQDDNS
               **      **         *   *** *** *  ****  **  *         *  * *

PD498,       72HGTHVAGTVAADTNNGIGVAGMAPDTKILAVRVLDANGSGSLDSIASGIRYAADQGAKVL
BPN',        64HGTHVAGTVAA-LNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMDVI
               ***********  ** *** * **     ** **   ***    *  **  *      *

PD498,      132NLSLGCECNSTTLKSAVDYAWNKGAVVVAAAGND----NVSRTFQPASYPNAIAVGAIDS
BPN',       123NMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAVDS
               * ***    *  ** *** *   * ********       *    *  **  ***** **


PD498,      188NDRKASFSNYGTWVDVTAPGVNIASTVPNNGYSYMSGTSMASPHVAGLAALLASQGKN--
BPN',       183SNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPNWT
              ****  *   ** **** * ** * * *    ********** ***  *   *
```

TABLE 1A-continued

Alignment of different proteases to the sequence of BPN'

```
PD498,      246NVQIRQAIEQTADKISGTGTNFKYGK (SEQ ID NO: 53)
BPN',       243NTQVRSSLQNTTTKL---GDSFYYGK (SEQ ID NO: 46)
               * * *     *  *    *  * ***
```

Properase:
58.9% identity in 275 residues overlap; Score: 813.0; Gap frequency: 2.2%

```
Properase,    1AQSVPWGISRVQAPAAHNRGLTGSGVKVAVLDTGI-STHPDLNIRGGASFVPGE-PSTQD
BPN',         1AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
               ***** * *   *** *  * *** ***** * ** * ****   **** ** * *  **

Properase,   59GNGHGTHVAGTIAALNNSIGVLGVAPNAELYAVKVLGASGGGSNSSIAQGLEWAGNNGMH
BPN',        61DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
                * ******** **************   ********  * *  * *  * ***  * *

Properase,  119VANLSLGSPSPSATLEQAVNSATSRGVLVVAASGNSGA----GSISYPARYANAMAVGAT
BPN',       121VINMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
               * * *** ** ** *  **  *   ** **** ** *         **  *    ****

Properase,  175DQNNNRASFSQYGAGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVKQKNPS
BPN',       181DSSNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
               *  * *****  *  **  ****  *** **  *   ****** *********   * *

Properase,  235WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAATR (SEQ ID NO: 54)
BPN',       241WTNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO: 46)
               * * * *  * ** * **    ** ** *  **
```

Relase:
60.7% identity in 275 residues overlap; Score: 858.0; Gap frequency: 1.8%

```
Relase,       1AQSVPWGISRVQAPAAHNRGLTGSGVKVAVLDTGIDSTHPDLNIRGGASFVPGE-PSTQD
BPN',         1AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
               ***** * *   *** *  * *** ***** * **** ****   **** ** * *  **

Relase,      60GNGHGTHVAGTIAALDNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWAGNNGMD
BPN',        61DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
                * ******** *** ***********  ********  ***  * *  * ***  * **
Relase,     120VANLSLGSPSPSATLEQAVNSATSRGVLVVAASGNSGA----GSISYPARYANAMAVGAT
BPN',       121VINMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
               * * *** ** ** *  **  *   ** **** ** *         **  *    ****

Relase,     176DQNNNRASFSQYGAELDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVLQKNPS
BPN',       181DSSNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
               *  * *****  * ***  ****  *** **  *   ****** ********* * * *

Relase,     236WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAATR (SEQ ID NO: 55)
BPN',       241WTNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO: 46)
               * * * *  * ** * **    ** ** *  **
```

Savinase:
59.6% identity in 275 residues overlap; Score: 821.0; Gap frequency: 2.2%

```
Savinase,     1AQSVPWGISRVQAPAAHNRGLTGSGVKVAVLDTGI-STHPDLNIRGGASFVPGE-PSTQD
BPN',         1AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
               ***** * *   *** *  * *** ***** * ** * ****   **** ** * *  **

Savinase,    59GNGHGTHVAGTIAALNNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWAGNNGMH
BPN',        61DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
                * ******** ***************  ********  ***  * *  * ***  * *

Savinase,   119VANLSLGSPSPSATLEQAVNSATSRGVLVVAASGNSGA----GSISYPARYANAMAVGAT
BPN',       121VINMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
               * * *** ** ** *  **  *   ** **** ** *         **  *    ****

Savinase,   175DQNNNRASFSQYGAGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVKQKNPS
BPN',       181DSSNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
               *  * *****  *  **  ****  *** **  *   ****** *********   * *

Savinase,   235WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAATR (SEQ ID NO: 56)
BPN',       241WTNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO: 46)
               * * * *  * ** * **    ** ** *  **
```

**[0473]** To find the homologous positions in subtilisin protease sequences not shown in the alignment of Table 1A, the sequence of interest is aligned to the sequence of BPN' as shown in Table 1B for YaB protease and Subtilisin sendai. The new sequence is aligned to the BPN' sequence by using the GAP alignment to the most homologous sequence found by the GAP program. GAP is provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wis., USA 53711) (Needleman, S. B. and Wunsch, C. D., (1970), Journal of Molecular Biology, 48, 443-45).

**[0474]** The sequence of the YaB protease is disclosed by Kaneko, R.; Koyama, N.; Tsai, Y.-C.; Juang, R.-Y.; Yoda, K.; Yamasaki, M.; Molecular cloning of the structural gene for alkaline elastase YaB, a new subtilisin produced by an alkalophilic *Bacillus* strain. J. Bacteriol. 171:5232 (1989), it has Swissprot number P20724, and is shown in SEQ ID NO: 35.

**[0475]** The sequence of the Subtilisin sendai is disclosed by Yamagata, Y.; Isshiki, K.; Ichishima, E.; Subtilisin Sendai from alkalophilic *Bacillus* sp.: molecular and enzymatic properties of the enzyme and molecular cloning and characterization of the gene, aprS. Enzyme Microb. Technol. 17:653 (1995), it has SPTREMBL accession number Q45522, and is shown in SEQ ID NO: 34.
Identity to savinase: 81.7%
identity to savinase: 82.09%

Swissprot: P20724

**[0476]**

TABLE 1B

Alignment of YAB protease to BPN': 55.3% identity
CLUSTAL W (1.7) multiple sequence alignment

```
YAB    -QTVPWGINRVQAPIAQSRGFTGTGVRVAVLDTGISN-HADLRIRGGASFVPGE-PNISD
BPN´   AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
        *:**:*:.:::**  :*:*:**:.*:***:*:**.. *.**:: ****:**.* **:.*

YAB    GNGHGTQVAGTIAALNNSIGVLGVAPNVDLYGVKVLGASGSGSISGIAQGLQWAANNGMH
BPN´   DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
       .*.***:****:**************.  **.***** :***. * * :*::**  *.*.

YAB    IANMSLGSSAGSATMEQAVNQATASGVLVVAASGNSG----AGNVGFPARYANAMAVGAT
BPN´   VINMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
       : *****..:***::: **::*.****:****:**.*    :..**:*.:*...:****.

YAB    DQNNNRATFSQYGAGLDIVAPGVGVQSTVPGNGYASFNGTSMATPHVAGVAALVKQKNPS
BPN´   DSSNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
       *..*:**:**. *. **::****.:***:*** *.::******:*****.***: .*:*.

YAB    WSNVQIRNHLKNTATNLGNTTQFGSGLVNAEAATR (SEQ ID NO: 57)
BPN´   WTNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO: 46)
       *:*.*:*. *:**:*:**::  :*.**:*.:**::

       Alignment of Subtilisin sendai to BPN': 55.6% identity.
            CLUSTAL W (1.7) multiple sequence alignment

sendai NQVTPWGITRVQAPTAWTRGYTGTGVRVAVLDTGIS-THPDLNIRGGVSFVPGE-PSYQD
BPN´   AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
        * .*:*:::::**:  ::****:.*:***:*:**. :****:: **.*:**.* *.:**

sendai GNGHGTHVAGTIAALNNSIGVVGVAPNAELYAVKVLGANGSGSVSSIAQGLQWTAQNNIH
BPN´   DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
       .*.********:*********:****.: ********  ***. * * :*::*:  **:.

sendai VANLSLGSPVGSQTLELAVNQATNAGVLVVAATGNNG----SGTVSYPARYANALAVGAT
BPN´   VINMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
       * *:***.* ** :*: **::*. :**:****:**:*    *.**.**.:*...:****.

sendai DQNNNRASFSQYGTGLNIVAPGVGIQSTYPGNRYASLSGTSMATPHVAGVAALVKQKNPS
BPN´   DSSNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
       *..*:*****. *. *:::****.**** ***:*.: .*****:*****.***: .*:*.

sendai WSNTQIRQHLTSTATSLGNSNQFGSGLVNAEAATR (SEQ ID NO: 58)
BPN´   WTNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO: 46)
       *:***:*. * .*:*.**:*  :*.**:*.:**::
```

**[0477]** These alignments reveal that that homology between various subtilisin proteases ranges between 100% and 40%.

**[0478]** Unless specified, subtilisin sequences and positions mentioned in the present invention, are given in the BPN' numeration, and can be converted by alignment as 50 described above (Tables 1A and 1B).

**[0479]** Sequence identities between different pairs of proteases are given below:

Sequence identity to BPN':

| | |
|---|---|
| Savinase | 60.4% |
| Alcalase | 69.5% |
| BLAPR | 60.4% |
| ProteaseC | 0.4% |
| ProteaseD | 0.0% |
| ProteaseE | 8.2% |
| Protease A | 0.0% |
| Properase | 9.6% |
| Relase | 61.5% |
| PD498 | 44.8% |
| sendai | 55.6% |
| YAB | 55.3% |

Sequence identity to Savinase:

| | |
|---|---|
| Alcalase | 60.9% |
| BLAPR | 98.1% |
| ProteaseC | 8.5% |
| ProteaseD | 8.9% |
| ProteaseE | 6.7% |
| Protease A | 7.8% |
| Properase | 8.9% |
| Relase | 98.1% |
| PD498 | 44.3% |
| sendai | 81.4% |
| YAB | 81.8% |

Structures

**[0480]** The protein structure of PD498 is disclosed in WO 98/35026 (Novo Nordisk). The structure of Savinase can be found in BETZEL et al, J.MOL.BIOL., Vol. 223, p. 427, 1992 (1 svn.pdb).

Homology Modelling

**[0481]** Three dimensional structural models of the subtilisins properase, release, ProteaseC, ProteaseD, ProteaseE, and PROTEASE B were constructed based on three dimensional structure of Savinase (Protein Data Bank entry 1SVN; Betzel, C., Klupsch, S., Papendorf, G., Hastrup, S., Branner, S., Wilson, K. S.: Crystal structure of the alkaline proteinase Savinase from *Bacillus lentus* at 1.4 Å resolution. *J Mol Biol* 223 pp. 427 (1992)) using the Modeller 5o (Šali, A.; T. L. Blundell, "Definition of general topological equivalence in protein structures: A procedure involving comparison of properties and relationships through simulated annealing and dynamic programming," *J. Mol. Biol.,* 212 403-428 (1990)) module of the Insight 2000 molecular modelling package (Biosym inc.). Default parameters were used with the alignments shown in FIG. **1**A as input, e.g. alignment between the columns labelled Savinase and PROTEASE B served as input alignment in construction of a PROTEASE B structural model. The Modeller module by default output ten structural models, of these the model with lowest 'modeller objective function' score was chosen as representing PROTEASE B structure.

Lipase:

**[0482]** The sequence of the *T. lanuginosus* lipase (trade name Lipolase) is provided in SEQ ID NO: 1 and the structure is disclosed in WO 98/35026 and as "1tib", available in Structural Classification of Proteins (SCOP) on the Internet.

Amylase:

**[0483]** The amylase used in the examples is the alpha-amylase of *Bacillus halmapalus* (WO 96/23873), which is called amylase SP722 (the wild-type). Its sequence is shown in SEQ ID NO: 2 and the corresponding protein structure was built from the BA2 structure, as described in WO 96/23874. The first four amino acids of the structural model are not defined, hence the sequence used for numeration of amino acid residues in the examples of this invention is four amino acids shorter than the one of the full length protein SP722.

**[0484]** Several variants of this amylase are available (WO 96/23873). One particularly useful variant has deleted two amino acid residues at D-G at positions 183 and 184 of the SEQ ID NO: 2 (corresponding to residues 179 and 180 of the modelled structure). This variant is called JE-1 or Natalase.

**[0485]** Another amylase that is particularly useful is the amylase AA560: This alkaline alpha-amylase may be derived from a strain of *Bacillus* sp. DSM 12649. The strain was deposited on 25 Jan. 1999 by the assignee under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure at Deutshe Sammmlung von Microorganismen and Zellkulturen GmbH (DSMZ), Mascheroder Weg 1b, D-38124 Braunschweig DE.

Laccase:

**[0486]** The laccase used in this invention is that from *Coprinus cinereus* (WO 98/38287), the sequence of which is shown as SEQ ID NO: 3. The structure of the *Myceliophthora thermophila* laccase can be built by homology modeling to the *Coprinus cinereus* laccase as shown in WO 98/38287.

Cellulase:

**[0487]** The cellulase sequence and structure used in the present invention is that of the core fragment of endoglucanase V from *Humicola insolens* (aka Cel45 or Carezyme). The core fragment structure is available as 3eng.pdb (G. J. DAVIES et al. ACTA CRYSTALLOGR., SECT.D, Vol. 52, p. 7 1996; G. J. DAVIES et al. BIOCHEMISTRY, V. 34, p. 16210, 1995); SwissProt accession number P43316, and the sequences shown in SEQ ID 4. The corresponding full-length sequence is disclosed in WO 91/17243 and shown here in SEQ ID NO: 5. The numeration of all description and claims of this invention pertain to the core fragment, however, it is contemplated that all claims are also valid for the corresponding positions in the full-length protein.

TABLE 1

Alignment and numeration scheme for subtilisins
(SEQ ID NOS: 46, 45, 47, 48, 49, 50, 51, 52, 54, 55, 56, 53, respectively)

| | BPN' | Alcalase | Pro-teaseB | Esperase | Pro-teaseC | Pro-teaseD | Pro-teaseE | Pro-teaseA | Properase | Relase | Savinase | PD498 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| -6 | | | | | | | | | | | | W |
| -5 | | | | | | | | | | | | S |
| -4 | | | | | | | | | | | | P |
| -3 | | | | | | | | | | | | N |
| -2 | | | | | | | | | | | | D |
| -1 | | | | | | | | | | | | P |
| 1 | A | A | A | | A | A | A | A | A | A | A | Y |
| 2 | Q | Q | Q | Q | Q | Q | Q | Q | Q | Q | Q | Y |
| 3 | S | T | T | T | S | S | S | S | S | S | S | S |
| 3a | | | | | | | | | | | | A |
| 4 | V | V | I | V | V | V | V | V | V | V | V | Y |
| 5 | P | P | P | P | P | P | P | P | P | P | P | Q |
| 6 | Y | Y | W | W | W | W | W | W | W | W | W | Y |
| 7 | G | G | G | G | G | G | G | G | G | G | G | G |
| 8 | V | I | I | I | I | I | I | I | I | I | I | P |
| 9 | S | P | S | S | S | S | S | S | S | S | S | Q |
| 10 | Q | L | R | F | R | R | R | R | R | R | R | N |
| 11 | I | I | V | I | V | V | V | V | V | V | V | T |
| 12 | K | K | Q | N | Q | Q | Q | Q | Q | Q | Q | S |
| 13 | A | A | A | T | A | A | A | A | A | A | A | T |
| 14 | P | D | P | Q | P | P | P | P | P | P | P | P |
| 15 | A | K | A | Q | A | A | A | A | A | A | A | A |
| 16 | L | V | A | A | A | A | A | A | A | A | A | A |
| 17 | H | Q | H | H | H | H | H | H | H | H | H | W |
| 18 | S | A | N | N | N | N | N | N | N | N | N | D |
| 19 | Q | Q | R | R | R | R | R | R | R | R | R | V |
| 20 | G | G | G | G | G | G | G | G | G | G | G | |
| 21 | Y | F | L | I | L | L | L | L | L | L | L | T |
| 22 | T | K | T | F | T | T | T | T | T | T | T | R |
| 23 | G | G | G | G | G | G | G | G | G | G | G | G |
| 24 | S | A | S | N | S | S | S | S | S | S | S | S |
| 25 | N | N | G | G | G | G | G | G | G | G | G | S |
| 26 | V | V | V | A | V | V | V | V | V | V | V | T |
| 27 | K | K | K | R | R | K | K | K | K | K | K | Q |
| 28 | V | V | V | V | V | V | V | V | V | V | V | T |

TABLE 1-continued

Alignment and numeration scheme for subtilisins
(SEQ ID NOS: 46, 45, 47, 48, 49, 50, 51, 52, 54, 55, 56, 53, respectively)

| | BPN' | Alcalase | Pro-teaseB | Esperase | Pro-teaseC | Pro-teaseD | Pro-teaseE | Pro-teaseA | Properase | Relase | Savinase | PD498 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 28a | | | | | | | | | | | | V |
| 29 | A | A | A | A | A | A | A | A | A | A | A | A |
| 30 | V | V | V | V | V | V | V | V | V | V | V | V |
| 31 | I | L | L | L | L | L | L | L | L | L | L | L |
| 32 | D | D | D | D | D | D | D | D | D | D | D | D |
| 33 | S | T | T | T | T | T | T | T | T | T | T | S |
| 34 | G | G | G | G | G | G | G | G | G | G | G | G |
| 35 | I | I | I | I | I | I | I | I | I | I | I | V |
| 36 | D | Q | | | | | | | | D | | D |
| 37 | S | A | S | A | S | S | S | S | S | S | S | Y |
| 38 | S | S | T | S | T | T | T | T | T | T | T | N |
| 39 | H | H | H | H | H | H | H | H | H | H | H | H |
| 40 | P | P | P | P | P | P | P | P | P | P | P | P |
| 41 | D | D | D | D | D | D | D | D | D | D | D | D |
| 42 | L | L | L | L | L | L | L | L | L | L | L | L |
| 43 | K | N | N | R | N | N | N | N | N | N | N | A |
| 44 | V | V | I | I | I | I | I | I | I | I | I | R |
| 44a | | | | | | | | | | | | K |
| 44b | | | | | | | | | | | | V |
| 45 | A | V | R | A | R | R | R | R | R | R | R | I |
| 46 | G | G | G | G | G | G | G | G | G | G | G | K |
| 47 | G | G | G | G | G | G | G | G | G | G | G | G |
| 48 | A | A | A | A | A | A | A | A | A | A | A | Y |
| 49 | S | S | S | S | S | S | S | S | S | S | S | D |
| 50 | M | F | F | F | F | F | F | F | F | F | F | F |
| 51 | V | V | V | I | V | V | V | V | V | V | V | I |
| 52 | P | A | P | S | P | P | P | P | P | P | P | D |
| 53 | S | G | G | S | G | G | G | G | G | G | G | R |
| 54 | E | E | E | E | E | E | E | E | E | E | E | D |
| 55 | T | A | P | | P | P | P | P | P | P | P | N |
| 56 | P | Y | | P | | | | | | | | N |
| 57 | N | N | S | S | S | S | S | S | S | S | S | P |
| 58 | F | | T | Y | T | T | T | T | T | T | T | M |
| 59 | Q | T | Q | H | Q | Q | Q | Q | Q | Q | Q | |
| 60 | D | D | D | D | D | D | D | D | D | D | D | D |

TABLE 1-continued

Alignment and numeration scheme for subtilisins
(SEQ ID NOS: 46, 45, 47, 48, 49, 50, 51, 52, 54, 55, 56, 53, respectively)

| | BPN' | Alcalase | Pro-teaseB | Esperase | Pro-teaseC | Pro-teaseD | Pro-teaseE | Pro-teaseA | Properase | Relase | Savinase | PD498 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 61 | D | G | G | N | G | G | G | G | G | G | G | L |
| 62 | N | N | N | N | N | N | N | N | N | N | N | N |
| 63 | S | G | G | G | G | G | G | G | G | G | G | G |
| 64 | H | H | H | H | H | H | H | H | H | H | H | H |
| 65 | G | G | G | G | G | G | G | G | G | G | G | G |
| 66 | T | T | T | T | T | T | T | T | T | T | T | T |
| 67 | H | H | H | H | H | H | H | H | H | H | H | H |
| 68 | V | V | V | V | V | V | V | V | V | V | V | V |
| 69 | A | A | A | A | A | A | A | A | A | A | A | A |
| 70 | G | G | G | G | G | G | G | G | G | G | G | G |
| 71 | T | T | T | T | T | T | T | T | T | T | T | T |
| 72 | V | V | I | I | I | I | I | I | I | I | I | V |
| 73 | A | A | A | A | A | A | A | A | A | A | A | A |
| 74 | A | A | A | A | A | A | A | A | A | A | A | A |
| 75 | L | L | L | L | L | L | L | L | L | L | L | D |
| 75a | | | | | | | | | | | | T |
| 76 | N | D | N | N | N | D | N | N | N | D | N | N |
| 77 | N | N | N | N | N | N | N | N | N | N | N | N |
| 78 | S | T | S | S | S | S | S | S | S | S | S | G |
| 79 | I | T | I | I | I | I | I | I | I | I | I | I |
| 80 | G | G | G | G | G | G | G | G | G | G | G | G |
| 81 | V | V | V | V | V | V | V | V | V | V | V | V |
| 82 | L | L | L | L | L | L | L | L | L | L | L | A |
| 83 | G | G | G | G | G | G | G | G | G | G | G | G |
| 84 | V | V | V | V | V | V | V | V | V | V | V | M |
| 85 | A | A | A | A | A | A | A | A | A | A | A | A |
| 86 | P | P | P | P | P | P | P | P | P | P | P | P |
| 87 | S | S | S | S | S | S | S | S | N | S | S | D |
| 88 | S | V | A | A | A | A | A | A | A | A | A | T |
| 89 | A | S | E | D | E | E | E | E | E | E | E | K |
| 90 | L | L | L | L | L | L | L | L | L | L | L | I |
| 91 | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | L |
| 92 | A | A | A | A | A | A | A | A | A | A | A | A |
| 93 | V | V | V | V | V | V | V | V | V | V | V | V |
| 94 | K | K | K | K | K | K | K | K | K | K | K | R |

TABLE 1-continued

Alignment and numeration scheme for subtilisins
(SEQ ID NOS: 46, 45, 47, 48, 49, 50, 51, 52, 54, 55, 56, 53, respectively)

| | BPN' | Alcalase | Pro-teaseB | Esperase | Pro-teaseC | Pro-teaseD | Pro-teaseE | Pro-teaseA | Properase | Relase | Savinase | PD498 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 95 | V | V | V | V | V | V | V | V | V | V | V | V |
| 96 | L | L | L | L | L | L | L | L | L | L | L | L |
| 97 | G | N | G | D | G | G | G | G | G | G | G | D |
| 98 | D | S | A | R | A | A | A | A | A | A | A | A |
| 99 | A | S | S | N | S | S | S | S | S | S | S | N |
| 100 | G | G | G | G | G | G | G | G | G | G | G | G |
| 101 | S | S | S | S | S | S | G | S | G | S | S | S |
| 102 | G | G | G | G | G | G | G | G | G | G | G | G |
| 103 | Q | S | S | S | S | A | A | S | S | S | S | S |
| 104 | Y | Y | V | L | Y | I | I | V | N | V | V | L |
| 105 | S | S | S | A | S | S | S | S | S | S | S | D |
| 106 | W | G | S | S | S | S | S | S | S | S | S | S |
| 107 | I | I | I | V | I | I | I | I | I | I | I | I |
| 108 | I | V | A | A | A | A | A | A | A | A | A | A |
| 109 | N | S | Q | Q | Q | Q | Q | Q | Q | Q | Q | S |
| 110 | G | G | G | G | G | G | G | G | G | G | G | G |
| 111 | I | I | L | I | L | L | L | L | L | L | L | I |
| 112 | E | E | E | E | E | E | E | E | E | E | E | R |
| 113 | W | W | W | W | W | W | W | W | W | W | W | Y |
| 114 | A | A | A | A | A | A | A | A | A | A | A | A |
| 115 | I | T | G | I | G | G | G | G | G | G | G | A |
| 116 | A | T | N | N | N | N | N | N | N | N | N | D |
| 117 | N | N | N | N | N | N | N | N | N | N | N | Q |
| 118 | N | G | G | N | G | G | G | G | G | G | G | G |
| 119 | M | M | M | M | M | M | M | M | M | M | M | A |
| 120 | D | D | H | H | H | H | H | H | H | D | H | K |
| 121 | V | V | V | I | V | V | V | V | V | V | V | V |
| 122 | I | I | A | I | A | A | A | A | A | A | A | L |
| 123 | N | N | N | N | S | N | N | N | N | N | N | N |
| 124 | M | M | L | M | L | L | L | L | L | L | L | L |
| 125 | S | S | S | S | S | S | S | S | S | S | S | S |
| 126 | L | L | L | L | L | L | L | L | L | L | L | L |
| 127 | G | G | G | G | G | G | G | G | G | G | G | G |
| 128 | G | G | S | S | S | S | S | S | S | S | S | C |
| 129 | P | A | P | T | P | P | P | P | P | P | P | E |

TABLE 1-continued

Alignment and numeration scheme for subtilisins
(SEQ ID NOS: 46, 45, 47, 48, 49, 50, 51, 52, 54, 55, 56, 53, respectively)

| | BPN' | Alcalase | Pro-teaseB | Esperase | Pro-teaseC | Pro-teaseD | Pro-teaseE | Pro-teaseA | Properase | Relase | Savinase | PD498 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 130 | S | S | S | S | S | S | S | S | S | S | S | C |
| 131 | G | G | P | G | P | P | P | A | P | P | P | N |
| 132 | S | S | S | S | S | S | S | G | S | S | S | S |
| 133 | A | T | A | S | A | A | A | G | A | A | A | T |
| 134 | A | A | T | T | T | T | T | T | T | T | T | T |
| 135 | L | M | L | L | L | L | L | L | L | L | L | L |
| 136 | K | K | E | E | E | E | E | E | E | E | E | K |
| 137 | A | Q | Q | L | Q | Q | Q | Q | Q | Q | Q | S |
| 138 | A | A | A | A | A | A | A | A | A | A | A | A |
| 139 | V | V | V | V | V | V | V | V | V | V | V | V |
| 140 | D | D | N | N | N | N | N | N | N | N | N | D |
| 141 | K | N | S | R | S | S | S | S | S | S | S | Y |
| 142 | A | A | A | A | A | A | A | A | A | A | A | A |
| 143 | V | Y | T | N | T | T | T | T | T | T | T | W |
| 144 | A | A | S | N | S | S | S | S | S | S | S | N |
| 145 | S | R | R | A | R | R | R | R | R | R | R | K |
| 146 | G | G | G | G | G | G | G | G | G | G | G | G |
| 147 | V | V | V | I | V | V | V | V | V | V | V | A |
| 148 | V | V | L | L | L | L | L | L | L | L | L | V |
| 149 | V | V | V | L | V | V | V | V | V | V | V | V |
| 150 | V | V | V | V | V | V | V | V | V | V | V | V |
| 151 | A | A | A | G | A | A | A | A | A | A | A | A |
| 152 | A | A | A | A | A | A | A | A | A | A | A | A |
| 153 | A | A | S | A | S | S | S | S | S | S | S | A |
| 154 | G | G | G | G | G | G | G | G | G | G | G | G |
| 155 | N | N | N | N | N | N | N | N | N | N | N | N |
| 156 | E | S | S | T | S | S | S | S | S | S | S | D |
| 157 | G | G | G | G | G | G | G | G | G | G | G | N |
| 158 | S | S | A | R | A | A | A | A | A | A | A | V |
| 159 | T | S | | Q | | | | | | | | |
| 160 | G | G | G | G | G | G | D | G | G | G | G | S |
| 161 | S | N | S | | S | S | S | S | S | S | S | R |
| 162 | S | T | I | | I | I | I | I | I | I | I | T |
| 163 | S | N | S | | S | S | S | S | S | S | S | F |
| 164 | T | T | | | | | | | | | | |

TABLE 1-continued

Alignment and numeration scheme for subtilisins
(SEQ ID NOS: 46, 45, 47, 48, 49, 50, 51, 52, 54, 55, 56, 53, respectively)

| | BPN' | Alcalase | Pro-teaseB | Esperase | Pro-teaseC | Pro-teaseD | Pro-teaseE | Pro-teaseA | Properase | Relase | Savinase | PD498 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 165 | V | I | | V | | | | | | | | |
| 166 | G | G | | N | | | | | | | | |
| 167 | Y | Y | Y | Y | Y | Y | Y | A | Y | Y | Y | Q |
| 168 | P | P | P | P | P | P | P | P | P | P | P | P |
| 169 | G | A | A | A | A | A | A | A | A | A | A | A |
| 170 | K | K | R | R | R | R | R | S | R | R | R | S |
| 171 | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| 172 | P | D | A | S | A | A | A | A | A | A | A | P |
| 173 | S | S | N | G | N | N | N | N | N | N | N | N |
| 174 | V | V | A | V | A | A | A | A | A | A | A | A |
| 175 | I | I | M | M | M | M | M | M | M | M | M | I |
| 176 | A | A | A | A | A | A | A | A | A | A | A | A |
| 177 | V | V | V | V | V | V | V | V | V | V | V | V |
| 178 | G | G | G | A | G | G | G | G | G | G | G | G |
| 179 | A | A | A | A | A | A | A | A | A | A | A | A |
| 180 | V | V | T | V | T | T | T | T | T | T | T | I |
| 181 | D | D | D | D | D | D | D | D | D | D | D | D |
| 182 | S | S | Q | Q | Q | Q | Q | Q | Q | Q | Q | S |
| 183 | S | N | N | N | N | N | N | N | N | N | N | N |
| 184 | N | S | N | G | N | N | N | N | N | N | N | D |
| 185 | Q | N | N | Q | N | N | N | N | N | N | N | R |
| 186 | R | R | R | R | R | R | R | R | R | R | R | K |
| 187 | A | A | A | A | A | A | A | A | A | A | A | A |
| 188 | S | S | S | S | S | S | S | S | S | S | S | S |
| 189 | F | F | F | F | F | F | F | F | F | F | F | F |
| 190 | S | S | S | S | S | S | S | S | S | S | S | S |
| 191 | S | S | Q | T | Q | Q | Q | Q | Q | Q | Q | N |
| 192 | V | V | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| 193 | G | G | G | G | G | G | G | G | G | G | G | G |
| 194 | P | A | A | P | A | A | A | P | A | A | A | T |
| 195 | E | E | G | E | G | G | G | G | G | E | G | W |
| 196 | L | L | L | I | L | L | L | L | L | L | L | V |
| 197 | D | E | D | E | D | D | D | D | D | D | D | D |
| 198 | V | V | I | I | I | I | I | I | I | I | I | V |
| 199 | M | M | M | S | V | V | V | V | V | V | V | T |

TABLE 1-continued

Alignment and numeration scheme for subtilisins
(SEQ ID NOS: 46, 45, 47, 48, 49, 50, 51, 52, 54, 55, 56, 53, respectively)

| | BPN' | Alcalase | Pro-teaseB | Esperase | Pro-teaseC | Pro-teaseD | Pro-teaseE | Pro-teaseA | Properase | Relase | Savinase | PD498 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 200 | A | A | A | A | A | A | A | A | A | A | A | A |
| 201 | P | P | P | P | P | P | P | P | P | P | P | P |
| 202 | G | G | G | G | G | G | G | G | G | G | G | G |
| 203 | V | A | V | V | V | V | V | V | V | V | V | V |
| 204 | S | G | N | N | N | N | N | N | N | N | N | N |
| 205 | I | V | I | V | V | V | V | V | V | V | V | I |
| 206 | Q | Y | Q | N | Q | Q | Q | Q | Q | Q | Q | A |
| 207 | S | S | S | S | S | S | S | S | S | S | S | S |
| 208 | T | T | T | T | T | T | T | T | T | T | T | T |
| 209 | L | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | V |
| 210 | P | P | P | T | P | P | P | P | P | P | P | P |
| 211 | G | T | G | G | G | G | G | G | G | G | G | N |
| 212 | N | N | S | N | S | S | S | S | S | S | S | N |
| 213 | K | T | T | R | T | T | T | T | T | T | T | G |
| 214 | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| 215 | G | A | A | V | A | A | A | A | A | A | A | S |
| 216 | A | T | S | S | S | S | S | S | S | S | S | Y |
| 217 | Y | L | D | L | L | L | L | L | L | L | L | M |
| 218 | N | N | N | S | N | N | N | N | N | N | N | S |
| 219 | G | G | G | G | G | G | G | G | G | G | G | G |
| 220 | T | T | T | T | T | T | T | T | T | T | T | T |
| 221 | S | S | S | S | S | S | S | S | S | S | S | S |
| 222 | M | M | M | M | M | M | M | M | M | M | M | M |
| 223 | A | A | A | A | A | A | A | A | A | A | A | A |
| 224 | S | S | T | T | T | T | T | T | T | T | T | S |
| 225 | P | P | P | P | P | P | P | P | P | P | P | P |
| 226 | H | H | H | H | H | H | H | H | H | H | H | H |
| 227 | V | V | V | V | V | V | V | V | V | V | V | V |
| 228 | A | A | A | A | A | A | A | A | A | A | A | A |
| 229 | G | G | G | G | G | G | G | G | G | G | G | G |
| 230 | A | A | A | V | A | A | A | A | A | A | A | L |
| 231 | A | A | A | A | A | A | A | A | A | A | A | A |
| 232 | A | A | A | A | A | A | V | A | A | A | A | A |
| 233 | L | L | L | L | L | L | L | L | L | L | L | L |
| 234 | I | I | V | V | V | V | V | V | V | V | V | L |

TABLE 1-continued

Alignment and numeration scheme for subtilisins
(SEQ ID NOS: 46, 45, 47, 48, 49, 50, 51, 52, 54, 55, 56, 53, respectively)

| | BPN' | Alcalase | Pro-teaseB | Esperase | Pro-teaseC | Pro-teaseD | Pro-teaseE | Pro-teaseA | Properase | Relase | Savinase | PD498 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 235 | L | L | K | K | K | K | K | K | K | L | K | A |
| 236 | S | S | Q | S | Q | Q | H | Q | Q | Q | Q | S |
| 237 | K | K | K | R | K | K | K | K | K | K | K | Q |
| 238 | H | H | N | Y | N | N | N | N | N | N | N | G |
| 239 | P | P | P | P | P | P | P | P | P | P | P | K |
| 240 | N | N | S | S | S | S | S | S | S | S | S | N |
| 241 | W | L | W | Y | W | W | W | W | W | W | W | |
| 242 | T | S | S | T | S | S | S | S | S | S | S | |
| 243 | N | A | N | N | N | N | N | N | N | N | N | N |
| 244 | T | S | V | N | V | V | V | V | V | V | V | V |
| 245 | Q | Q | Q | Q | Q | Q | R | Q | Q | Q | Q | Q |
| 246 | V | V | I | I | I | I | I | I | I | I | I | I |
| 247 | R | R | R | R | R | R | R | R | R | R | R | R |
| 248 | S | N | N | Q | N | N | D | N | N | N | N | Q |
| 249 | S | R | H | R | H | H | H | H | H | H | H | A |
| 250 | L | L | L | I | L | L | L | L | L | L | L | I |
| 251 | Q | S | K | N | K | K | K | K | K | K | K | E |
| 252 | N | S | N | Q | N | N | K | N | N | N | N | Q |
| 253 | T | T | T | T | T | T | T | T | T | T | T | T |
| 254 | T | A | A | A | A | A | A | A | A | A | A | A |
| 255 | T | T | T | T | T | T | T | T | T | T | T | D |
| 256 | K | Y | S | Y | S | S | S | S | S | S | S | K |
| 257 | L | L | L | L | L | L | L | L | L | L | L | I |
| 258 | G | G | G | G | G | G | G | G | G | G | G | S |
| 259 | D | S | S | S | S | S | S | S | S | S | S | G |
| 260 | S | S | T | P | T | T | T | T | T | T | T | T |
| 261 | F | F | N | S | N | N | N | N | N | N | N | G |
| 262 | Y | Y | L | L | L | L | L | L | L | L | L | T |
| 263 | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | N |
| 264 | G | G | G | G | G | G | G | G | G | G | G | F |
| 264a | | | | | | | | | | | | K |
| 265 | K | K | S | N | S | S | S | S | S | S | S | Y |
| 266 | G | G | G | G | G | G | G | G | G | G | G | G |
| 267 | L | L | L | L | L | L | L | L | L | L | L | K |
| 268 | I | I | V | V | V | V | V | V | V | V | V | I |

TABLE 1-continued

Alignment and numeration scheme for subtilisins
(SEQ ID NOS: 46, 45, 47, 48, 49, 50, 51, 52, 54, 55, 56, 53, respectively)

| | BPN' | Alcalase | Pro-teaseB | Esperase | Pro-teaseC | Pro-teaseD | Pro-teaseE | Pro-teaseA | Properase | Relase | Savinase | PD498 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 269 | N | N | N | H | N | N | N | N | N | N | N | N |
| 270 | V | V | A | A | A | A | A | A | A | A | A | S |
| 271 | Q | E | E | G | E | E | E | E | E | E | E | N |
| 272 | A | A | A | R | A | A | A | A | A | A | A | K |
| 273 | A | A | A | A | A | A | A | A | A | A | A | A |
| 274 | A | A | T | T | A | T | T | T | T | T | T | V |
| 275 | Q | Q | R | Q | R | R | R | R | R | R | R | R |
| 276 | | | | | | | | | | | | Y |

## EXAMPLES

### Example 1

### Identification of Epitope Sequences and Epitope Patterns

**[0488]** High diversity libraries ($10^{12}$) of phages expressing random hexa-, nona- or dodecapetides as part of their membrane proteins, were screened for their capacity to bind purified specific rabbit IgG, and purified rat and mouse IgG1 and IgE antibodies. The phage libraries were obtained according to prior art (se WO 9215679 hereby incorporated by reference).

**[0489]** The antibodies were raised in the respective animals by subcutaneous, intradermal, or intratracheal injection of relevant proteins (e.g. proteases, lipolytic enzymes, amylases, oxidoreductases) dissolved in phosphate buffered saline (PBS). The respective antibodies were purified from the serum of immunised animals by affinity chromatography using paramagnetic immunobeads (Dynal AS) loaded with pig anti-rabbit IgG, mouse anti-rat IgG1 or IgE, or rat anti-mouse IgG1 or IgE antibodies.

**[0490]** The respective phage libraries were incubated with the IgG, IgG1 and IgE antibody coated beads. Phages, which express oligopeptides with affinity for rabbit IgG, or rat or mouse IgG1 or IgE antibodies, were collected by exposing these paramagnetic beads to a magnetic field. The collected phages were eluted from the immobilised antibodies by mild acid treatment, or by elution with intact enzyme. The isolated phages were amplified as know to the specialist. Alternatively, immobilised phages were directly incubated with *E. coli* for infection. In short, F-factor positive *E. coli* (e.g. XL-1 Blue, JM101, TG1) were infected with M13-derived vector in the presence of a helper-phage (e.g. M13K07), and incubated, typically in 2xYT containing glucose or IPTG, and appropriate antibiotics for selection. Finally, cells were removed by centrifugation. This cycle of events was repeated 2-5 times on the respective cell supernatants. After selection round 2, 3, 4, and 5, a fraction of the infected *E. coli* was incubated on selective 2xYT agar plates, and the specificity of the emerging phages was assessed immunologically. Thus, phages were transferred to a nitrocellulase (NC) membrane. For each plate, 2 NC-replicas were made. One replica was incubated with the selection antibodies, the other replica was incubated with the selection antibodies and the immunogen used to obtain the antibodies as competitor. Those plaques that were absent in the presence of immunogen, were considered specific, and were amplified according to the procedure described above.

**[0491]** The specific phage-clones were isolated from the cell supernatant by centrifugation in the presence of polyethylenglycol. DNA was isolated, the DNA sequence coding for the oligopeptide was amplified by PCR, and the DNA sequence was determined, all according to standard procedures. The amino acid sequence of the corresponding oligopeptide was deduced from the DNA sequence.

**[0492]** Thus, a number of peptide sequences with specificity for the protein specific antibodies, described above, were obtained. These sequences were collected in a database, and analysed by sequence alignment to identify epitope patterns. For this sequence alignment, conservative substitutions (e.g. aspartate for glutamate, lysine for arginine, serine for threonine) were considered as one. This showed that most sequences were specific for the protein the antibodies were raised against. However, several cross-reacting sequences were obtained from phages that went through 2 selection rounds only. In the first round 22 epitope patterns were identified.

**[0493]** In further rounds of phage display, more antibody binding sequences were obtained leading to more epitope patterns. Further, the literature was searched for peptide sequences that have been found to bind environmental allergen-specific antibodies (J All Clin Immunol 93 (1994) pp. 34-43; Int Arch Appl Immunol 103 (1994) pp. 357-364; Clin Exp Allergy 24 (1994) pp. 250-256; Mol Immunol 29 (1992) pp. 1383-1389; J Immunol 121 (1989) pp. 275-280; J. Immunol 147 (1991) pp. 205-211; Mol Immunol 29 (1992) pp. 739-749; Mol Immunol 30 (1993) pp. 1511-1518; Mol Immunol 28 (1991) pp. 1225-1232; J. Immunol 151 (1993) pp. 7206-7213). These antibody binding peptide sequences were included in the database.

**[0494]** A first generation database of antibody binding peptides identified and their corresponding epitope patterns are shown in Table 2-7 below.

Tables 2-7: Overview of the antibody binding peptide sequences, epitope patterns and epitope sequences. The type of antibody used for identifying the antibody binding sequences is indicated as IgG or IgE and the species from which the antibodies were derived are indicated as mo (mouse), ra (rat) and hu (human).

TABLE 2

Savinase antibody binding peptide sequences, epitope patterns and epitope sequences.

| Antibody binding peptide | Method of identification | Epitope pattern | Donor | Acceptor | Epitope Sequence (BPN') |
|---|---|---|---|---|---|
| VQVYGDTSA (SEQ ID NO: 59) | Phage display | Q > Y > D > | Savinase | savinase | Q206 V81 Y214 G80 D41 T208 |
| LQCVGS (SEQ ID NO: 60) | Protein fragments | | a-amylase inhibitor | savinase | L21 Q236 V26 G25 S24 |
| KRFANTELA (SEQ ID NO: 61) | Phage display | R/K R F > N | Savinase | savinase | K251 R247 A174 N173 |
| LDQIFFTRW (SEQ ID NO: 62) | Phage display | D/E Q I F F T | Savinase | savinase | L42/L75 D41 Q2 I79 |
| FNDAFFVKM (SEQ ID NO: 63) | Phage display | | Savinase | savinase | N185 D181 A187 F189 V203 |
| ANIPIWSRSA (SEQ ID NO: 64) | Phage display | > R S A | Savinase | savinase | R145 S144 A142 |
| ANIPIWSRSA (SEQ ID NO: 64) | Phage display | > R S A | Savinase | savinase | S188 R186 S190 A179 |
| RQSTDFGTT (SEQ ID NO: 65) | Phage display | R Q > > D/E | Savinase | savinase | R186 Q191 S156 |
| VQVYGDTSA (SEQ ID NO: 66) | Phage display | Q > Y > D > | Savinase | savinase | Q191 Y192 G193/A194/G195 D197 S265 |
| RRFSNATRA (SEQ ID NO: 67) | Phage display | R/K R F > N | Savinase | savinase | K251 R247 A174 N173 |
| CTARLRAGNACG (SEQ ID NO: 68) | Phage display | A R > A | Savinase | savinase | A172/A169 R170 A194 G193 N261 |
| LDQIFFTRW (SEQ ID NO: 69) | Phage display | D/E Q I F F T | Savinase | savinase | D60 Q59 I44/I35 |
| LDQIFFTRW (SEQ ID NO: 69) | Phage display | D/E Q I F F T | Savinase | savinase | L42/L75 D41 Q2 I79 |
| EQIFFTSGL (SEQ ID NO: 70) | Phage display | D/E Q I F F T | Savinase | savinase | E112 Q109 I79 |
| GRFSNSKFK (SEQ ID NO: 71) | Phage display | L > G R S | Savinase | savinase | L196 G195 R170 S163 |
| AVLRDC (SEQ ID NO: 72) | Protein fragments | | a-amylase inhibitor | savinase | A254 V268 L267 R10 D181 |
| LQCVGS (SEQ ID NO: 73) | Protein fragments | | a-amylase inhibitor | savinase | L217 Q206 V81 G80 S3 |
| LRQCNERCV (SEQ ID NO: 74) | Phage display | R Q > > D/E | Savinase | savinase | L267 R10 Q12 N269 E271 R275 |
| SPVTKRASLKIDSKK (SEQ ID NO: 75) | Protein fragments | | Der p II | savinase | A88 S87/T22 L233 K235 I246 |
| RQSTDFGTT (SEQ ID NO: 76) | Phage display | R Q > > D/E | Savinase | savinase | R247 Q245 S240/S242 |
| FCTNNCELS (SEQ ID NO: 77) | Phage display | N > > E L | Savinase | savinase | T143 N173 N140 E136 L135 |
| FCTNNCELS (SEQ ID NO: 77) | Phage display | N > > E L | Savinase | savinase | N117 N116 E112 L111 |
| DFHVKYAAQ (SEQ ID NO: 78) | Phage display | | Savinase | savinase | |

TABLE 2-continued

Savinase antibody binding peptide sequences, epitope patterns and epitope sequences.

| | | | | | |
|---|---|---|---|---|---|
| VAQYKALPVVLENA (SEQ ID NO: 79) | Protein fragments | | Fel d I | savinase | L135 P168 V139 L111 E112 N116 |
| AAYPDV (SEQ ID NO: 80) | Protein fragments | A > > > > Y P > | a-amylase inhibitor | savinase | A215 Y214 P40 D41 V81 |
| EQIFFTSGL (SEQ ID NO: 81) | Phage display | D/E Q I F F T | Savinase | savinase | E271 Q12 I8 |
| VDAAF (SEQ ID NO: 82) | Protein fragments | | Poa p IX | savinase | V203 D181 A179 A187 F189 |
| AVLRDC (SEQ ID NO: 83) | Protein fragments | | a-amylase inhibitor | savinase | A232 V234 L250 R247 D197 |
| RAFRRNANW (SEQ ID NO: 84) | Phage display | A R > A | Savinase | savinase | A272/A273 R275 R19 N18 A15/A16 |
| CTARLRAGNACG (SEQ ID NO: 85) | Phage display | A R > A | Savinase | savinase | A15/A16 R19 L21 R275 A272 A273 N269 |
| TFHDAPALQ (SEQ ID NO: 86) | Phage display | | Savinase | savinase | H39 D41 A74/A73 P86 A88 L90 |
| CTARVVALGVCG (SEQ ID NO: 87) | Phage display | A R > A | Savinase | savinase | R145 V147 V149 A151 L124/L126 G127 |
| GRFSNSKFK (SEQ ID NO: 88) | Phage display | L > G R S | Savinase | savinase | L148 G146 R145 S144/S141 N140 |
| RRFANDHTR (SEQ ID NO: 89) | Phage display | R/K R F > N | savinase | savinase | K27 R45 N43 D41 H39 T38/T213 |
| KRFANTEPA (SEQ ID NO: 90) | Phage display | R/K R F > N | savinase | savinase | K251 R247 A174 N173 |
| YKVSAL (SEQ ID NO: 91) | Protein fragments | | a-amylase inhibitor | savinase | Y91 K27 V26 S24 G23 L21 |
| TGKYVS (SEQ ID NO: 92) | Protein fragments | | a-amylase inhibitor | savinase | S24 G25 K27 Y91 V93 |

| Antibody binding peptide | Epitope # | IgG | IgE |
|---|---|---|---|
| VQVYGDTSA (SEQ ID NO: 59) | sav1.1 | Ra | |
| LQCVGS (SEQ ID NO: 60) | sav19.1 | | Hu |
| KRFANTELA (SEQ ID NO: 61) | sav6.1 | Ra-Mo | Mo |
| LDQIFFTRW (SEQ ID NO: 62) | sav5.1 | Ra | |
| FNDAFFVKM (SEQ ID NO: 63) | sav11.0 | Ra | |
| ANIPIWSRSA (SEQ ID NO: 64) | sav3.2-lac1.0-lip4.0-pd5.0 | Ra | |
| ANIPIWSRSA (SEQ ID NO: 64) | sav3.1-lac1.0-lip4.0-pd5.0 | Ra | |
| RQSTDFGTT (SEQ ID NO: 65) | sav2.2 | Ra | |
| VQVYGDTSA (SEQ ID NO: 66) | sav1.2 | Ra | |

TABLE 2-continued

Savinase antibody binding peptide sequences, epitope patterns and epitope sequences.

| | | | |
|---|---|---|---|
| RRFSNATRA (SEQ ID NO: 67) | sav6.1 | Ra-Mo | Mo |
| CTARLRAGNACG (SEQ ID NO: 68) | sav10.4 | Ra | |
| LDQIFFTRW (SEQ ID NO: 69) | sav5.2 | Ra | |
| LDQIFFTRW (SEQ ID NO: 69) | sav5.1 | Ra | |
| EQIFFTSGL (SEQ ID NO: 70) | sav5.4 | Ra | |
| GRFSNSKFK (SEQ ID NO: 71) | sav9.2-je4.0-lip5.1-5.2 | Ra | |
| AVLRDC (SEQ ID NO: 72) | sav18.1-pd18.1-18.2 | | Hu |
| LQCVGS (SEQ ID NO: 73) | sav19.2 | | Hu |
| LRQCNERCV (SEQ ID NO: 74) | sav2.1 | Ra | |
| SPVTKRASLKIDSKK (SEQ ID NO: 75) | sav16.0-pd7.0 | | Hu |
| RQSTDFGTT (SEQ ID NO: 76) | sav2.3 | Ra | |
| FCTNNCELS (SEQ ID NO: 77) | sav7.2 | Ra | |
| FCTNNCELS (SEQ ID NO: 77) | sav7.1 | Ra | |
| DFHVKYAAQ (SEQ ID NO: 78) | sav8.0 | Ra | |
| VAQYKALPVVLENA (SEQ ID NO: 79) | sav12.0-pd8.0 | | Hu |
| AAYPDV (SEQ ID NO: 80) | sav13.0-pd13.1-13.2 | | Hu |
| EQIFFTSGL (SEQ ID NO: 81) | sav5.3 | Ra | |
| VDAAF (SEQ ID NO: 82) | sav15.0-pd12.0 | Hu | |
| AVLRDC (SEQ ID NO: 83) | sav18.2-pd18.1-18.2 | | Hu |
| RAFRRNANW (SEQ ID NO: 84) | sav10.1 | Ra | |
| CTARLRAGNACG (SEQ ID NO: 85) | sav10.2 | Ra | |
| TFHDAPALQ (SEQ ID NO: 86) | sav4.0 | Ra | |
| CTARVVALGVCG (SEQ ID NO: 87) | sav10.3 | Ra | |
| GRFSNSKFK (SEQ ID NO: 88) | sav9.1-je4.0-lip5.1-5.2 | Ra | |

TABLE 2-continued

Savinase antibody binding peptide sequences, epitope patterns and epitope sequences.

| | | |
|---|---|---|
| RRFANDHTR (SEQ ID NO: 89) | sav6.2 | Ra |
| KRFANTEPA (SEQ ID NO: 90) | sav6.1 | Ra-Mo Mo |
| YKVSAL (SEQ ID NO: 91) | sav14.0-pd14.0 | Hu |
| TGKYVS (SEQ ID NO: 92) | sav17.0-pd17.1-17.2 | Hu |

TABLE 3

PD498 antibody binding peptide sequences, epitope patterns and epitope sequences.

| Epitope pattern | Donor | Acceptor | Epitope Sequence (BPN') | Epitope # | IgG | IgE |
|---|---|---|---|---|---|---|
| | Fel d I | pd498 | V198 A254 Q252 Y276 K239 A235 L233 P86 | pd8.0 | | Hu |
| A > > > > Y P > | a-amylase inhibitor | pd498 | *3aA Y1/Y2 P-4/P-1 D-2 V81 | pd13.2 | | Hu |
| | Poa p IX | pd498 | S182 Y6 G7 P8 T13 P14 A15 A16 | pd11.0 | Hu | |
| > K L > > | Poa p IX | pd498 | Y171 K136 L135 A108 Y113 | pd4.4 | Hu | |
| | a-amylase inhibitor | pd498 | Y48/Y37 K46 *44aaV A43 L42 | pd14.0 | | Hu |
| | Poa p IX | pd498 | V196/V198 D197 A174/A176 A169 F163 | pd12.0 | Hu | |
| K Q S | Poa p IX | pd498 | A142 A147 V148 K120 Q27 S24/S25 | pd2.3 | Hu | |
| K Q S | pd498 | pd498 | R44 K89 Q27 S236 K120 G146 | pd2.2 | Ra | |
| | Der p II | pd498 | *28aV T88 *44a K R44 A43 L42 | pd7.0 | | Hu |
| > K L > > | pd498 | pd498 | N56/N55 K46 L91 A29/A119 T28 | pd4.3 | Ra | |
| > K L > > | pd498 | pd498 | N240/N243 K239 L233/L234 A16 T21 R22 | pd4.1 | Ra | |
| > K L > > | Poa p IX | pd498 | Y37 K46 L91 A114 Y113 | pd4.5 | Hu | |
| > K L > > | pd498 | pd498 | N240/N243 K239 L233/L234 A16 T21 R22 | pd4.1 | Ra | |
| Y I > K L | pd498 | pd498 | Y113 I111 A108/A138 K136 L135 | pd3.1 | Ra | |
| K Q S | pd498 | pd498 | A115 K145 N243 N240 K239 Q237 S236 | pd2.1 | Ra | |
| > R Y > K/R | pd498 | pd498 | R94 R53 Y48 Q117 R112 S109/S137 | pd1.5-lac2.0 | Ra | |
| | Phl p V | pd498 | A169 Q167 F163 T162 S160 G193 | pd10.0 | | Hu |
| Y I > K L | pd498 | pd498 | Y276 I246 K239 L234 S236 | pd3.2 | Ra | |
| > K L > > | pd498 | pd498 | N240/N243 K239 L233/L234 R22 P86 | pd4.2 | Ra | |
| A > > > > Y P > | a-amylase inhibitor | pd498 | *3aA Y2 P14 D18 V19 | pd13.1 | | Hu |
| K Q S | Poa p IX | pd498 | A15 A16 V274 K239 Q237 S236 | pd2.4 | Hu | |
| | a-amylase inhibitor | pd498 | G146 K145 Y141 V139 S137 | pd17.2 | | Hu |
| | a-amylase inhibitor | pd498 | A273 V274 L233 R22 D87 | pd18.1 | | Hu |

TABLE 3-continued

PD498 antibody binding peptide sequences, epitope patterns and epitope sequences.

| Epitope pattern | Donor | Acceptor | Epitope Sequence (BPN') | Epitope # | IgG | IgE |
|---|---|---|---|---|---|---|
| A R > A | Par j 1 + Par o 1 | pd498 | N10 S12 A15/A16 R275 A273/A249 R247 A174 D197 S170 | pd9.0 | Hu + Ra | Hu |
| | pd498 | pd498 | R22 G23 L233 S236 | pd6.2 | Ra | |
| > R Y > K/R | pd498 | pd498 | R94 R53 Y48 P57 K46 L91 | pd1.4-lac2.0 | Ra | |
| > R Y > K/R | pd498 | pd498 | R94 R53 Y48 P57 K46 L91 | pd1.4-lac2.0 | Ra | |
| | a-amylase inhibitor | pd498 | L96 R94 S33 V35 Y37 | pd15.0 | | Hu |
| > R Y > K/R | pd498 | pd498 | S109/S137 R112 Y141 N144 K145 | pd1.3-lac2.0 | Ra | |
| > R Y > K/R | pd498 | pd498 | T162 R161 Y192 N191 K186 | pd1.2-lac2.0 | Ra | |
| > R Y > K/R | pd498 | pd498 | T133/T134 R112 Y141 N144 K145 | pd1.1-lac2.0 | Ra | |
| | a-amylase inhibitor | pd498 | A92 *44aaV L42 R44 D75 | pd18.2 | | Hu |
| | a-amylase inhibitor | pd498 | S236 G238 K239 Y276 V274 S270 | pd17.1 | | Hu |
| | a-amylase inhibitor | pd498 | S12 P14 W17 S-5 W-6 | pd16.0 | | Hu |
| > R S A | pd498 | pd498 | S137 R112 S109 A108 | pd5.0-lac1.0-lip4.0-sav3.1-3.2 | Ra | |
| | pd498 | pd498 | S215 M217 I205 M222 G219 | pd6.1 | Ra | |

TABLE 4

Antibody binding peptide sequences, epitope patterns and epitope sequences for the *T. lanuginosus* lipase (Lipolase).

| Antibody binding peptide | Method of identification | Epitope pattern | Donor | Acceptor | Epitope Sequence | Epitope # | IgG | IgE |
|---|---|---|---|---|---|---|---|---|
| QRPPRYELE (SEQ ID NO: 93) | Phage display | R P P R | lipolase | lipolase | | lip1.0 | Ra | |
| ELEYRPPRQ (SEQ ID NO: 94) | Phage display | > E Y | lipolase | lipolase | L124 E129 Y164 | lip2.1 | Ra | |
| HEYDMRVAW (SEQ ID NO: 95) | Phage display | > E Y | lipolase | lipolase | H215 E219 Y220 | lip2.2 | Ra | |
| HEYPMDFHL (SEQ ID NO: 96) | Phage display | > E Y | lipolase | lipolase | H215 E219 Y220 | lip2.2 | Ra | |
| SEYSMSITP (SEQ ID NO: 97) | Phage display | > E Y | lipolase | lipolase | S217 E219 Y220 | lip2.3 | Ra | |
| CVWPAHAPLSCG (SEQ ID NO: 98) | Phage display | > P > > P A P > S | lipolase | lipolase | P253 P250 A243 P208/P207 S214/S216/S217 | lip3.0 | Ra | |
| CSWPSPAPLSCG (SEQ ID NO: 99) | Phage display | > P > > P A P > S | lipolase | lipolase | P253 P250 A243 P208/P207 S214/S216/S217 | lip3.0 | Ra | |
| CDFPLHAPLSCG (SEQ ID NO: 100) | Phage display | > P > > P A P > S | lipolase | lipolase | P253 P250 A243 P208/P207 S214/S216/S217 | lip3.0 | Ra | |

TABLE 4-continued

Antibody binding peptide sequences, epitope patterns and epitope sequences for the *T. lanuginosus* lipase (Lipolase).

| Antibody binding peptide | Method of identification | Epitope pattern | Donor | Acceptor | Epitope Sequence | Epitope # | IgG | IgE |
|---|---|---|---|---|---|---|---|---|
| CLFPSPAPRSCG (SEQ ID NO: 101) | Phage display | > P > > P A P > S | lipolase | lipolase | P253 P250 A243 P208/P207 S214/S216/S217 | lip3.0 | Ra | |
| CDGPAPAPWSCG (SEQ ID NO: 102) | Phage display | > P > > P A P > S | lipolase | lipolase | P253 P250 A243 P208/P207 S214/S216/S217 | lip3.0 | Ra | |
| CSFPLPAPRSCG (SEQ ID NO: 103) | Phage display | > P > > P A P > S | lipolase | lipolase | P253 P250 A243 P208/P207 S214/S216/S217 | lip3.0 | Ra | |
| CVYPSPAPWSCG (EQ ID NO: 104) | Phage display | > P > > P A P > S | lipolase | lipolase | P253 P250 A243 P208/P207 S214/S216/S217 | lip3.0 | Ra | |
| PEYTMNALS (SEQ ID NO: 105) | Phage display | > E Y | lipolase | lipolase | P218 E219 Y220 | lip2.4 | Ra | |
| CSRSAKGARLCG (EQ ID NO: 106) | Phage display | > R S A | lipolase | lipolase | R209 S214 A182 | lip4.0-lac1.0-pd5.0-sav3.1-3.2 | Ra | |
| LEYPMSASQ (SEQ ID NO: 107) | Phage display | > E Y | lipolase | lipolase | L124 E129 Y164 | lip2.1 | Ra | |
| RKLTLSGRS (SEQ ID NO: 108) | Phage display | L > G R S | lipolase | lipolase | L67 G65 R81 S83/S85 | lip5.1-je4.0-sav9.0 | Ra | |
| RKLTLSGRS (SEQ ID NO: 109) | Phage display | L > G R S | lipolase | lipolase | L96/L97 G212 R209/ R179 S214 | lip5.2-je4.0-sav9.0 | Ra | |
| SYGAPATPAA (SEQ ID NO: 110) | Protein fragments | | Poa p IX | lipolase | S170 Y171 G172 A173 P174 A150 T153 | lip6.0 | Hu | |
| PAAGYTPAAP (SEQ ID NO: 111) | Protein fragments | | Poa p IX | lipolase | A18/A19/A20 G65 Y53 T123 | lip7.0 | Hu | Hu |
| YKLAY (SEQ ID NO: 112) | Protein fragments | | Poa p IX | lipolase | Y138 K74 L75 A68 Y16 | lip8.1 | Hu | |
| YKLAY (SEQ ID NO: 112) | Protein fragments | | Poa p IX | lipolase | Y53 K127 L67 A68 Y16 | lip8.2 | Hu | |
| KYDDYVATLS (SEQ ID NO: 113) | Protein fragments | | Poa p IX | lipolase | Y194 D167 D165 Y164 V132 A131 L52 S54 | lip9.0 | Hu | |
| EVKATPAGEL (SEQ ID NO: 114) | Protein fragments | | Poa p IX | lipolase | E43 V44 K46 A47 T72 | lip10.0 | Hu | |
| CGYSNAQGVDYWI (SEQ ID NO: 115) | Protein fragments | | Der p I | lipolase | Y53 S54 N25/N26 A18/A19/A20 Q15 V44 | lip15.0 | Hu | Hu |
| VPGIDPNACHYMKC (SEQ ID NO: 116) | Protein fragments | | Der p II | lipolase | P256 I255 D254 P253 N200 H198 Y261 | lip16.0 | | Hu |
| SPVTKRASLKIDSKK (SEQ ID NO: 117) | Protein fragments | | Der p II | lipolase | R179 A182 S216/S217 I238 K237 I235 D234 S224 K223 | lip17.0 | | Hu |
| IMSALAMVYLGAK (SEQ ID NO: 118) | Protein fragments | | Ovalbumin | lipolase | V140 Y138 L69 A49 A47 K46 | lip18.0 | Hu | |
| ELGVRE (SEQ ID NO: 119) | Protein fragments | | a-amylase inhibitor | lipolase | E99 L97 G109/G177 V176 R175 D242 | lip11.0 | | Hu |
| GCRKEV (SEQ ID NO: 120) | Protein fragments | | a-amylase inhibitor | lipolase | G106 C107 R108 K98 E99 | lip12.0 | | Hu |

TABLE 4-continued

Antibody binding peptide sequences, epitope patterns and epitope sequences for the *T. lanuginosus* lipase (Lipolase).

| Antibody binding peptide | Method of identification | Epitope pattern | Donor | Acceptor | Epitope Sequence | Epitope # | IgG | IgE |
|---|---|---|---|---|---|---|---|---|
| LRSVYQ (SEQ ID NO: 121) | Protein fragments | | a-amylase inhibitor | lipolase | L147 R81 S79 V77 Y16 Q15 | lip13.0 | | Hu |
| SGPWSW (SEQ ID NO: 122) | Protein fragments | | a-amylase inhibitor | lipolase | S170 G172 P174 W89 S83 | lip14.0 | | Hu |

TABLE 5

Amylase (Natalase) antibody binding peptide sequences, epitope patterns and epitope sequences.

| Antibody binding peptide | Method of identification | Epitope pattern | Donor | Acceptor | Epitope Sequence |
|---|---|---|---|---|---|
| ARIDPRGPS (SEQ ID NO: 123) | Phage display | A > I D P R/K | amylase | amylase | A380 K381 I382 D383 P384 R389 |
| ARIDPRHGS (SEQ ID NO: 124) | Phage display | A > I D P R/K | amylase | amylase | A380 K381 I382 D383 P384 R389 |
| CSVAKIDPRTCG (SEQ ID NO: 125) | Phage display | A > I D P R/K | amylase | amylase | A109 K138 D140 P142 R144 |
| CSVAKIDPRTCG (SEQ ID NO: 125) | Phage display | A > 1 D P R/K | amylase | amylase | A380 K381 I382 D383 P384 R389 |
| AKIDPKPDT (SEQ ID NO: 126) | Phage display | A > I D P R/K | amylase | amylase | A109 K138 D140 P142 R144 |
| AKIDPKPDT (SEQ ID NO: 126) | Phage display | A > I D P R/K | amylase | amylase | A380 K381 I382 D383 P384 R389 |
| ARIDPRHGS (SEQ ID NO: 127) | Phage display | A > I D P R/K | amylase | amylase | A109 K138 D140 P142 R144 |
| QIYNDTGPT (SEQ ID NO: 128) | Phage display | Q > Y > D > | amylase | amylase | Q390 L386 Y368/Y367 D366 |
| QIYNDTGPT (SEQ ID NO: 128) | Phage display | Q > Y > D > | amylase | amylase | Q170 I173 Y196 D195 |
| QIYNDTGPT (SEQ ID NO: 128) | Phage display | Q > Y > D > | amylase | amylase | Q357 I352 Y349 D366 |
| QIYNDTGPT (SEQ ID NO: 128) | Phage display | Q > Y > D > | amylase | amylase | Q331 I370 Y368/Y367 D366 |
| CGSATIDPRQCG (SEQ ID NO: 129) | Phage display | A > I D P R/K | amylase | amylase | A109 K138 D140 P142 R144 |
| CNADNQMYPQCG (SEQ ID NO: 130) | Phage display | A > > > > Y P > | amylase | amylase | N29 A27 D26/D25 Y8 P41/P42 |
| ARIDPRGPS (SEQ ID NO: 131) | Phage display | A > I D P R/K | amylase | amylase | A109 K138 D140 P142 R144 |
| CGSATIDPRQCG (SEQ ID NO: 132) | Phage display | A > I D P R/K | amylase | amylase | A380 K381 I382 D383 P384 R389 |
| CDADSSGYPLCG (SEQ ID NO: 133) | Phage display | A > > > > Y P > | amylase | amylase | A107/A109 D108 Y57 P41/42 |
| QLYGDEQLP (SEQ ID NO: 134) | Phage display | Q > Y > D > | amylase | amylase | Q331 I370 Y368/Y367 D366 |
| QLYGDEQLP (SEQ ID NO: 134) | Phage display | Q > Y > D > | amylase | amylase | Q357 I352 Y349 D366 |

TABLE 5-continued

Amylase (Natalase) antibody binding peptide sequences, epitope patterns and epitope sequences.

| | | | | | |
|---|---|---|---|---|---|
| QLYGDEQLP (SEQ ID NO: 134) | Phage display | Q > Y > D > | amylase | amylase | Q170 I173 Y196 D195 |
| QLYGDEQLP (SEQ ID NO: 134) | Phage display | Q > Y > D > | amylase | amylase | Q390 L386 Y368/Y367 D366 |
| RYAQIDPRW (SEQ ID NO: 135) | Phage display | A > I D P R/K | amylase | amylase | A380 K381 I382 D383 P384 R389 |
| RYAQIDPRW (SEQ ID NO: 135) | Phage display | A > I D P R/K | amylase | amylase | A109 K138 D140 P142 R144 |
| GEFNLGRSS (SEQ ID NO: 136) | Phage display | L > G R S | amylase | amylase | L88 G92 R31 S28 |
| CNADSWGYPRCG (SEQ ID NO: 137) | Phage display | A > > > > Y P > | amylase | amylase | N29 A27 D26/D25 Y8 P41/P42 |
| CNADNQMYPQCG (SEQ ID NO: 138) | Phage display | A > > > > Y P > | amylase | amylase | N102 A233 D232 Y54 P41/P42 |
| CNADSWGYPRCG (SEQ ID NO: 137) | Phage display | A > > > > Y P > | amylase | amylase | N102 A233 D232 Y54 P41/P42 |
| GEFNLGRSS (SEQ ID NO: 139) | Phage display | L > G R S | amylase | amylase | L62 G63/G76 R78 S79 |

| Antibody binding peptide | Epitope # | IgG | IgE |
|---|---|---|---|
| ARIDPRGPS (SEQ ID NO: 123) | je1.1 | | Ra |
| ARIDPRHGS (SEQ ID NO: 124) | je1.1 | | Ra |
| CSVAKIDPRTCG (SEQ ID NO: 125) | je1.2 | | Ra |
| CSVAKIDPRTCG (SEQ ID NO: 125) | je1.1 | | Ra |
| AKIDPKPDT (SEQ ID NO: 126) | je1.2 | | Ra |
| AKIDPKPDT (SEQ ID NO: 126) | je1.1 | | Ra |
| ARIDPRHGS (SEQ ID NO: 127) | je1.2 | | Ra |
| QIYNDTGPT (SEQ ID NO: 128) | je2.4 | | Ra |
| QIYNDTGPT (SEQ ID NO: 128) | je2.3 | | Ra |
| QIYNDTGPT (SEQ ID NO: 128) | je2.2 | | Ra |
| QIYNDTGPT (SEQ ID NO: 128) | je2.1 | | Ra |
| CGSATIDPRQCG (SEQ ID NO: 129) | je1.2 | | Ra |
| CNADNQMYPQCG (SEQ ID NO: 130) | je3.1 | | Ra |
| ARIDPRGPS (SEQ ID NO: 131) | je1.2 | | Ra |

TABLE 5-continued

Amylase (Natalase) antibody binding peptide sequences, epitope patterns and epitope sequences.

| | | | |
|---|---|---|---|
| CGSATIDPRQCG (SEQ ID NO: 132) | je1.1 | | Ra |
| CDADSSGYPLCG (SEQ ID NO: 133) | e3.3 | | Ra |
| QLYGDEQLP (SEQ ID NO: 134) | je2.1 | | Ra |
| QLYGDEQLP (SEQ ID NO: 134) | je2.2 | | Ra |
| QLYGDEQLP (SEQ ID NO: 134) | je2.3 | | Ra |
| QLYGDEQLP (SEQ ID NO: 134) | je2.4 | | Ra |
| RYAQIDPRW (SEQ ID NO: 135) | je1.1 | | Ra |
| RYAQIDPRW (SEQ ID NO: 135) | je1.2 | | Ra |
| GEFNLGRSS (SEQ ID NO: 136) | je4.1-sav9.0-lip5.1-5.2 | | Ra |
| CNADSWGYPRCG (SEQ ID NO: 137) | je3.1 | | Ra |
| CNADNQMYPQCG (SEQ ID NO: 138) | je3.2 | | Ra |
| CNADSWGYPRCG (SEQ ID NO: 137) | je3.2 | | Ra |
| GEFNLGRSS (SEQ ID NO: 139) | je4.2-sav9.0-lip5.1-5.2 | | Ra |

TABLE 6

Cellulase (Carezyme; Cel45 from *Humicola insolens*) antibody binding peptide sequences, epitope patterns and epitope sequences.

| Antibody binding peptide | Method of identification | Epitope pattern | Donor | Acceptor | Epitope Sequence | Epitope # | IgG | IgE |
|---|---|---|---|---|---|---|---|---|
| CVHAGPRAGTCG (SEQ ID NO: 140) | Phage display | > G > > A G | carezyme | carezyme | P23 R201 A83 G84 | car1.1 | | Ra |
| CVHAGPRAGTCG (SEQ ID NO: 140) | Phage display | V H > G > | carezyme | carezyme | | car2.0 | | Ra |
| CLSGPLAGRVCG (SEQ ID NO: 141) | Phage display | > G > > A G | carezyme | carezyme | P23 R201 A83 G84 | car1.1 | | Ra |
| CRISPWYSVPCG (SEQ ID NO: 142) | Phage display | | carezyme | carezyme | | car3.0 | | Ra |
| CLSGPAAGQSCG (SEQ ID NO: 143) | Phage display | > G > > A G | carezyme | carezyme | P23 R201 A83 G84 | car1.1 | | Ra |
| | Phage display | A > I D P R/K | je-1 | carezyme | R146 I131 D133 P137 | car11.2 | Ra | |
| CITRGTRAGWCG (SEQ ID NO: 144) | Phage display | > G > > A G | carezyme | carezyme | P23 R201 A83 G84 | car1.1 | | Ra |
| | Phage display | A R > A | savinase | carezyme | A191 R200 R201 A83 N81 | car6.2 | | Ra |
| CLSGPAAGQSCG (SEQ ID NO: 143) | Phage display | > G > > A G | carezyme | carezyme | T95/S96 G27 P98 A100 G101 | car1.2 | | Ra |

TABLE 6-continued

Cellulase (Carezyme; Cel45 from *Humicola insolens*) antibody binding peptide sequences, epitope patterns and epitope sequences.

| Antibody binding peptide | Method of identification | Epitope pattern | Donor | Acceptor | Epitope Sequence | Epitope # | IgG | IgE |
|---|---|---|---|---|---|---|---|---|
| | Phage display | A > I D P R/K | je-1 | carezyme | A195 R37 I38 D40 L44 | car11.1 | Ra | |
| | Phage display | Q > Y > D > | savinase, je-1 | carezyme | Q59 Y54 G134 D133 T136 | car10.0 | | Ra |
| | Phage display | > P > > A P > S | lipoprime | carezyme | W62/W169 P61 P165 A162 P160 | car9.0 | | Ra |
| CITRGTRAGWCG (SEQ ID NO: 144) | Phage display | > G > > A G | carezyme | carezyme | T95/S96 G27 P98 A100 G101 | car1.2 | | Ra |
| | Phage display | R/K R F > N | savinase | carezyme | R7 R170 F174 A177 | car7.0 | | Ra |
| CLSGPLAGRVCG (SEQ ID NO: 145) | Phage display | > G > > A G | carezyme | carezyme | T95/S96 G27 P98 A100 G101 | car1.2 | | Ra |
| | Phage display | A R > A | savinase | carezyme | A1 R4 R7 A177 N176 | car6.1 | | Ra |
| | Phage display | > P > R D T G | laccase | carezyme | D178 P180 R4 D2 S183 | car5.0 | | Ra |
| | Phage display | > R Y > K/R | pd498 | carezyme | R170 R153 Y168 P165 K164 L163 | car4.0 | | Ra |
| | Phage display | D/E Q I F F T | savinase | carezyme | Q36 I38 F41 F29 T197 | car8.0 | | Ra |
| CLTAGPSAGYCG (SEQ ID NO: 146) | Phage display | > G > > A G | carezyme | carezyme | T95/S96 G27 P98 A100 G101 | car1.2 | | Ra |
| CYTTGRLAGLCG (SEQ ID NO: 147) | Phage display | > G > > A G | carezyme | carezyme | P23 R201 A83 G84 | car1.1 | | Ra |
| CYTTGRLAGLCG (SEQ ID NO: 147) | Phage display | > G > > A G | carezyme | carezyme | T95/S96 G27 P98 A100 G101 | car1.2 | | Ra |
| CVHSGPRAGYCG (SEQ ID NO: 148) | Phage display | > G > > A G | carezyme | carezyme | P23 R201 A83 G84 | car1.1 | | Ra |
| CVHSGPRAGYCG (SEQ ID NO: 148) | Phage display | V H > G > | carezyme | carezyme | | car2.0 | | Ra |
| CVHAGPRAGTCG (SEQ ID NO: 149) | Phage display | > G > > A G | carezyme | carezyme | T95/S96 G27 P98 A100 G101 | car1.2 | | Ra |
| CVHSGPRAGYCG (SEQ ID NO: 148) | Phage display | > G > > A G | carezyme | carezyme | T95/S96 G27 P98 A100 G101 | car1.2 | | Ra |
| CVHSGLSRRLLR (SEQ ID NO: 150) | Phage display | V H > G > | carezyme | carezyme | | car2.0 | | Ra |
| CVTRGPNAGSCG (SEQ ID NO: 151) | Phage display | > G > > A G | carezyme | carezyme | T95/S96 G27 P98 A100 G101 | car1.2 | | Ra |
| CLTAGPSAGYCG (SEQ ID NO: 152) | Phage display | > G > > A G | carezyme | carezyme | P23 R201 A83 G84 | car1.1 | | Ra |
| CVTRGPNAGSCG (SEQ ID NO: 151) | Phage display | > G > > A G | carezyme | carezyme | P23 R201 A83 G84 | car1.1 | | Ra |
| CITSGPRAGNCG (SEQ ID NO: 153) | Phage display | > G > > A G | carezyme | carezyme | T95/S96 G27 P98 A100 G101 | car1.2 | | Ra |
| CITSGPRAGNCG (SEQ ID NO: 153) | Phage display | > G > > A G | carezyme | carezyme | P23 R201 A83 G84 | car1.1 | | Ra |

TABLE 7

Laccase (*Myceliophthora thermopila* laccase) antibody binding peptide sequences, epitope patterns and epitope sequences.

| Antibody binding peptide | Method of identification | Epitope pattern | Donor | Acceptor | Epitope Sequence | Epitope # | IgG | IgE |
|---|---|---|---|---|---|---|---|---|
| PQSD5PGESQ (SEQ ID NO: 154) | Phage display | P > S/T D P G | laccase | laccase | P180 R175 T168 D166 P165 G265 | lac3.2 | Ra | |
| WPKSDAGDS (SEQ ID NO: 155) | Phage display | P > > D A G | laccase | laccase | P241 R409 S410/S416 D434 A389 G390 | lac4.1 | Ra | |
| PQSDAGVVM (SEQ ID NO: 156) | Phage display | P > > D A G | laccase | laccase | P241 R409 S410/S416 D434 A389 G390 | lac4.1 | Ra | |
| DPVRDTGAG (SEQ ID NO: 157) | Phage display | > P > R D T G | laccase | laccase | P241 R409 D434 T432 G430/G390 | lac5.1 | Ra | |
| GPSRDAGLL (SEQ ID NO: 158) | Phage display | P > > D A G | laccase | laccase | P241 R409 S410/S416 D434 A389 G390 | lac4.1 | Ra | |
| PASDAGRGP (SEQ ID NO: 159) | Phage display | P > > D A G | laccase | laccase | P241 R409 S410/S416 D434 A389 G390 | lac4.1 | Ra | |
| PRDSTGLAL (SEQ ID NO: 160) | Phage display | P > S/T D P G | laccase | laccase | P378 R379 T442 D443 P445 G446 | lac3.1 | Ra | |
| PQSDPGESQ (SEQ ID NO: 161) | Phage display | P > S/T D P G | laccase | laccase | P378 R379 T442 D443 P445 G446 | lac3.1 | Ra | |
| RYPFLRATN (SEQ ID NO: 162) | Phage display | > R Y > K/R | laccase | laccase | | lac2.0-pd1.1-1.4 | Ra | |
| GAARDARSA (SEQ ID NO: 163) | Phage display | > R S A | laccase | laccase | | lac1.0-lip4.0-pd5.0-sav3.1-3.2 | Ra | |
| PRSDTGFGS (SEQ ID NO: 164) | Phage display | > P > R D T G | laccase | laccase | P241 R409 D434 T432 G430/G390 | lac5.1 | Ra | |
| LPRSDPGGR (SEQ ID NO: 165) | Phage display | P > S/T D P G | laccase | laccase | P180 R175 T168 D166 P165 G265 | lac3.2 | Ra | |
| DPARDTGDV (SEQ ID NO: 166) | Phage display | > P > R D T G | laccase | laccase | P241 R409 D434 T432 G430/G390 | lac5.1 | Ra | |
| APKSDNGIT (SEQ ID NO: 167) | Phage display | P > > D A G | laccase | laccase | P241 R409 S410/S416 D434 A389 G390 | lac4.1 | Ra | |
| PKSDPGTNW (SEQ ID NO: 168) | Phage display | P > S/T D P G | laccase | laccase | P378 R379 T442 D443 P445 G446 | lac3.1 | Ra | |
| PRTDPGWLA (SEQ ID NO: 169) | Phage display | P > S/T D P G | laccase | laccase | P378 R379 T442 D443 P445 G446 | lac3.1 | Ra | |
| LPRSDPGGR (SEQ ID NO: 170) | Phage display | P > S/T D P G | laccase | laccase | P378 R379 T442 D443 P445 G446 | lac3.1 | Ra | |
| PSSDPGARS (SEQ ID NO: 171) | Phage display | P > S/T D P G | laccase | laccase | P180 R175 T168 D166 P165 G265 | lac3.2 | Ra | |
| HVFDKNVTR (SEQ ID NO: 172) | Phage display | | laccase | laccase | | lac6.0 | | |
| PRSDPGTPT (SEQ ID NO: 173) | Phage display | P > S/T D P G | laccase | laccase | P378 R379 T442 D443 P445 G446 | lac3.1 | Ra | |
| PRSDPGTPT (SEQ ID NO: 173) | Phage display | P > S/T D P G | laccase | laccase | P180 R175 T168 D166 P165 G265 | lac3.2 | Ra | |
| PRDSTGLAL (SEQ ID NO: 174) | Phage display | P > S/T D P G | laccase | laccase | P180 R175 T168 D166 P165 G265 | lac3.2 | Ra | |

TABLE 7-continued

Laccase (*Myceliophthora thermopila* laccase) antibody binding peptide sequences, epitope patterns and epitope sequences.

| Antibody binding peptide | Method of identification | Epitope pattern | Donor | Acceptor | Epitope Sequence | Epitope # | IgG | IgE |
|---|---|---|---|---|---|---|---|---|
| PRTDPGWLA (SEQ ID NO: 175) | Phage display | P > S/T D P G | laccase | laccase | P180 R175 T168 D166 P165 G265 | lac3.2 | | Ra |
| PSSDPGARS (SEQ ID NO: 176) | Phage display | P > S/T D P G | laccase | laccase | P378 R379 T442 D443 P445 G446 | lac3.1 | | Ra |
| PKSDPGTNW (SEQ ID NO: 177) | Phage display | P > S/T D P G | laccase | laccase | P180 R175 T168 D166 P165 G265 | lac3.2 | | Ra |
| WPKSDAGDS (SEQ ID NO: 178) | Phage display | P > > D A G | laccase | laccase | P350 S349 D80 A79 G78 | lac4.2 | | Ra |
| PQSDAGVVM (SEQ ID NO: 179) | Phage display | P > > D A G | laccase | laccase | P350 S349 D80 A79 G78 | lac4.2 | | Ra |
| GPSRDAGLL (SEQ ID NO: 180) | Phage display | P > > D A G | laccase | laccase | P350 S349 D80 A79 G78 | lac4.2 | | Ra |
| PASDAGRGP (SEQ ID NO: 181) | Phage display | P > > D A G | laccase | laccase | P350 S349 D80 A79 G78 | lac4.2 | | Ra |
| APKSDNGIT (SEQ ID NO: 182) | Phage display | P > > D A G | laccase | laccase | P350 S349 D80 A79 G78 | lac4.2 | | Ra |
| WPKSDAGDS (SEQ ID NO: 183) | Phage display | P > > D A G | laccase | laccase | P300 R234 S211 D213 A296 | lac4.3 | | Ra |
| PQSDAGVVM (SEQ ID NO: 184) | Phage display | P > > D A G | laccase | laccase | P300 R234 S211 D213 A296 | lac4.3 | | Ra |
| GPSRDAGLL (SEQ ID NO: 185) | Phage display | P > > D A G | laccase | laccase | P300 R234 S211 D213 A296 | lac4.3 | | Ra |
| PASDAGRGP (SEQ ID NO: 186) | Phage display | P > > D A G | laccase | laccase | P300 R234 S211 D213 A296 | lac4.3 | | Ra |
| APKSDNGIT (SEQ ID NO: 187) | Phage display | P > > D A G | laccase | laccase | P300 R234 S211 D213 A296 | lac4.3 | | Ra |
| DPVRDTGAG (SEQ ID NO: 188) | Phage display | > P > R D T G | laccase | laccase | P378 R379 D469 T473 G446 | lac5.2 | | Ra |
| PRSDTGFGS (SEQ ID NO: 189) | Phage display | > P > R D T G | laccase | laccase | P378 R379 D469 T473 G446 | lac5.2 | | Ra |
| DPARDTGDV (SEQ ID NO: 190) | Phage display | > P > R D T G | laccase | laccase | P378 R379 D469 T473 G446 | lac5.2 | | Ra |
| DPVRDTGAG (SEQ ID NO: 191) | Phage display | > P > R D T G | laccase | laccase | P60 R59 D51/D53 T10/T12 G30 | lac5.3 | | Ra |
| PRSDTGFGS (SEQ ID NO: 192) | Phage display | > P > R D T G | laccase | laccase | P60 R59 D51/D53 T10/T12 G30 | lac5.3 | | Ra |
| DPARDTGDV (SEQ ID NO: 193) | Phage display | > P > R D T G | laccase | laccase | P60 R59 D51/D53 T10/T12 G30 | lac5.3 | | Ra |
| DPVRDTGAG (SEQ ID NO: 194) | Phage display | > P > R D T G | laccase | laccase | P157/P155 R23 D118 T114 G113 | lac5.4 | | Ra |
| PRSDTGFGS (SEQ ID NO: 195) | Phage display | > P > R D T G | laccase | laccase | P157/P155 R23 D118 T114 G113 | lac5.4 | | Ra |
| DPARDTGDV (SEQ ID NO: 196) | Phage display | > P > R D T G | laccase | laccase | P157/P155 R23 D118 T114 G113 | lac5.4 | | Ra |

Example 2

Localisation of Epitope Sequences and Epitope Areas on the 3D-Structure of Acceptor Proteins

**[0495]** Epitope sequences were assessed manually on the screen on the 3D-structure of the protein of interest, using appropriate software (e.g. SwissProt Pdb Viewer, WebLite Viewer).

**[0496]** In a first step, the identified epitope patterns were fitted with the 3D-structure of the enzymes. A sequence of at least 3 amino acids, defining a specific epitope pattern, was localised on the 3D-structure of the acceptor protein. Conservative mutations (e.g. aspartate for glutamate, lysine for arginine, serine for threonine) were considered as one for those patterns for which phage display had evidenced such exchanges to occur. Among the possible sequences provided by the protein structure, only those were retained where the sequence matched a primary sequence, or where it matched a structural sequence of amino acids, where each amino acid was situated within a distance of 5 Å from the next one. Occasionally, the mobility of the amino acid side chains, as provided by the software programme, had to be taken in to consideration for this criterium to be fulfilled.

**[0497]** Secondly, the remaining anchor amino acids as well as the variable amino acids, i.e. amino acids that were not defining a pattern but were present in the individual sequences identified by phage library screening, were assessed in the area around the various amino acid sequences localised in step 1. Only amino acids situated within a distance of 5 Å from the next one were included.

**[0498]** Finally, an accessibility criterium was introduced. The criterium was that at least half of the anchor amino acids had a surface that was >30% accessible. Typically, 0-2 epitopes were retained for each epitope pattern. In some cases, two different amino acids could with equal probability be part of the epitope (e.g. two leucines located close to each other in the protein 3D-structure). For example, in Savinase two epitopes actually fit to the antibody binding peptide LDQIFFTRW (SEQ ID NO:62): L75 D41 Q2 179 and L42 D41 Q2 I79. A shorthand notation for such a situation is: L42/L75 D41 Q2 I79.

**[0499]** Thus, a number of epitope sequences were identified and localised on the surface of various proteins. As suggested by sequence alignment of the antibody binding peptides, structural analysis confirmed most of the epitopes to be enzyme specific, with only few exceptions. Overall, most of the identified epitopes were at least partially structural. However, some proteins (e.g. amylase) expressed predominantly primary sequence epitopes. Typically, the epitopes were localised in very discrete areas of the enzymes, and different epitope sequences often shared some amino acids (hot-spots).

**[0500]** The identified epitope sequences are shown in Tables 2-7.

Birch Allergen:

**[0501]** Bet v1 (WO 99/47680) was used as the parent protein for identification of epitope sequences that may cross react with enzyme epitopes. The structural coordinates from 1BV1.pdb (Gajhede et al., NAT.STRUCT.BIOL., Vol. 3, p. 1040, 1996) were used as well the corresponding sequence (Swissprot accession number P15494). The epitope pattern P>PAP>S (which had been identified from antibody binding peptides specific for anti-Lipolase antibodies) was found to match three (overlapping) epitope sequences on the surface of Bet v1:

Bet v1 1.1: P31 A34 P35 A37 P59 S39/S40;

Bet v1 1.2: P63 L62 P59 A37 P35 S39/S40; and

Bet v1 1.3: P59 S39/S40 P31 A34 P35 S39/S40.

Example 3

Epitope Areas

**[0502]** It is common knowledge that amino acids that surround binding sequences can affect binding of a ligand without participating actively in the binding process. Based on this knowledge, areas covered by amino acids with potential steric effects on the epitope-antibody interaction, were defined around the identified epitopes. Practically, all amino acids situated within 5 Å from the amino acids defining the epitope were included. The accessibility criterium was not included for defining epitope areas, as hidden amino acids can have an effect on the surrounding structures.

**[0503]** For Savinase, the following amino acid residues belong to the epitope area that correspond to each epitope sequence indicated in Table 2:

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sav1.1 | A1 | Q2 | S3 | P5 | H39 | P40 | D41 | L42 | N43 | G63 | T66 | |
| | H67 | A69 | G70 | T71 | A73 | A74 | L75 | N77 | S78 | I79 | G80 | |
| | V81 | L82 | G83 | N204 | V205 | Q206 | S207 | T208 | Y209 | P210 | S212 | |
| | T213 | Y214 | A215 | S216 | L217 | | | | | | | |
| sav1.2 | S153 | G154 | N155 | S156 | G157 | A158 | G160 | S161 | I162 | S163 | A169 | |
| | R170 | A174 | M175 | A176 | V177 | G178 | R186 | F189 | S190 | Q191 | Y192 | |
| | G193 | A194 | G195 | L196 | D197 | I198 | V199 | T220 | R247 | K251 | A254 | |
| | T255 | S256 | T260 | N261 | L262 | Y263 | G264 | S265 | G266 | L267 | | |
| sav2.1 | W6 | G7 | I8 | R10 | V11 | Q12 | A13 | P14 | A15 | A16 | R19 | L21 |
| | V84 | T180 | D181 | Q182 | N183 | N184 | I198 | V199 | A200 | P201 | H226 | |
| | V227 | A230 | L233 | V234 | K237 | N238 | H249 | L250 | T253 | A254 | T255 | |
| | S256 | L257 | S265 | G266 | L267 | V268 | N269 | A270 | E271 | A272 | A273 | |
| | T274 | R275 | | | | | | | | | | |
| sav2.2 | S153 | G154 | N155 | S156 | G157 | A158 | S161 | I162 | S163 | G178 | A179 | |
| | T180 | D181 | N184 | N185 | R186 | A187 | S188 | F189 | S190 | Q191 | Y192 | |
| | G193 | L196 | T220 | L262 | Y263 | | | | | | | |
| sav2.3 | A142 | T143 | G146 | V147 | L148 | Y171 | A172 | N173 | A174 | M175 | D197 | |
| | A231 | V234 | K235 | N238 | P239 | S240 | W241 | S242 | N243 | V244 | Q245 | |
| | I246 | R247 | N248 | H249 | L250 | K251 | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sav3.1 | S153 | G154 | N155 | S156 | G157 | A158 | V177 | G178 | A179 | T180 | D181 | |
| | N184 | N185 | R186 | A187 | S188 | F189 | S190 | Q191 | Y192 | V199 | A200 | |
| | P201 | G202 | V203 | N218 | G219 | T220 | A223 | L262 | Y263 | | | |
| sav3.2 | L111 | E112 | G115 | N116 | M119 | A138 | V139 | N140 | S141 | A142 | S144 | |
| | R145 | G146 | V147 | V149 | N173 | N243 | | | | | | |
| sav4.0 | Q2 | H17 | T22 | G23 | S24 | G25 | V26 | K27 | V28 | V30 | I35 | |
| | S37 | T38 | H39 | P40 | D41 | L42 | N43 | I44 | R45 | G46 | T66 | |
| | A69 | G70 | T71 | I72 | A73 | A74 | L75 | N76 | N77 | I79 | G80 | |
| | V81 | L82 | G83 | V84 | A85 | P86 | S87 | A88 | E89 | L90 | Y91 | |
| | A92 | T208 | Y209 | P210 | S212 | T213 | Y214 | | | | | |
| sav5.1 | A1 | Q2 | S3 | V4 | I35 | S37 | H39 | P40 | D41 | L42 | N43 | I44 |
| | T66 | A69 | G70 | A73 | A74 | L75 | N76 | N77 | S78 | I79 | G80 | |
| | V81 | L82 | G83 | P86 | L90 | T208 | Y214 | | | | | |
| sav5.2 | V30 | T33 | G34 | I35 | S37 | T38 | L42 | N43 | I44 | R45 | G46 | |
| | E54 | S57 | T58 | Q59 | D60 | G61 | N62 | G63 | H64 | G65 | T66 | |
| | H67 | A69 | L90 | Y91 | A92 | K94 | P210 | | | | | |
| sav5.3 | V4 | P5 | W6 | G7 | I8 | S9 | R10 | V11 | Q12 | A13 | P14 | |
| | A15 | A16 | R19 | N269 | A270 | E271 | A272 | A273 | T274 | R275 | | |
| sav5.4 | A1 | Q2 | P40 | D41 | F50 | L75 | N77 | S78 | I79 | G80 | V81 | |
| | V104 | S105 | S106 | I107 | A108 | Q109 | G110 | L111 | E112 | W113 | A114 | |
| | G115 | N116 | Q137 | A138 | S141 | A142 | Y214 | | | | | |
| sav6.1 | V139 | N140 | T143 | L148 | V149 | A151 | P168 | A169 | Y171 | A172 | N173 | |
| | A174 | M175 | A176 | D197 | I198 | N243 | V244 | Q245 | I246 | R247 | N248 | |
| | H249 | L250 | K251 | N252 | T253 | A254 | S265 | | | | | |
| sav6.2 | Q2 | G25 | V26 | K27 | V28 | A29 | I35 | S37 | T38 | H39 | P40 | |
| | D41 | L42 | N43 | I44 | R45 | G46 | G47 | Q59 | T66 | A69 | G70 | |
| | A73 | A74 | L75 | N77 | I79 | G80 | V81 | L82 | A88 | E89 | L90 | |
| | Y91 | N117 | G118 | M119 | H120 | V121 | S207 | T208 | Y209 | P210 | G211 | |
| | S212 | T213 | Y214 | A215 | | | | | | | | |
| sav7.1 | K27 | L31 | I107 | A108 | Q109 | G110 | L111 | E112 | W113 | A114 | G115 | |
| | N116 | N117 | G118 | M119 | A122 | L124 | L135 | Q137 | A138 | V139 | S141 | |
| | A142 | R145 | V149 | | | | | | | | | |
| sav7.2 | V104 | I107 | A108 | L111 | S132 | A133 | T134 | L135 | E136 | Q137 | A138 | |
| | V139 | N140 | S141 | A142 | T143 | S144 | R145 | G146 | V147 | V149 | Y167 | |
| | P168 | Y171 | A172 | N173 | A174 | M175 | N243 | R247 | | | | |
| sav9.1 | L111 | E112 | A114 | G115 | N116 | M119 | H120 | V121 | A122 | E136 | Q137 | |
| | A138 | V139 | N140 | S141 | A142 | T143 | S144 | R145 | G146 | V147 | L148 | |
| | V149 | V150 | N173 | M175 | N243 | I246 | R247 | L250 | | | | |
| sav9.2 | L126 | G127 | S128 | P129 | A152 | S153 | G154 | S161 | I162 | S163 | Y167 | |
| | P168 | A169 | R170 | Y171 | A172 | A176 | V177 | G178 | Q191 | Y192 | G193 | |
| | A194 | G195 | L196 | D197 | I198 | V199 | T260 | N261 | L262 | Y263 | G264 | |
| sav10.1 | Q12 | A13 | P14 | A15 | A16 | H17 | N18 | R19 | G20 | L21 | T22 | |
| | N76 | L82 | G83 | V84 | A85 | P86 | L233 | V234 | K237 | N238 | H249 | |
| | L250 | T253 | N269 | A270 | E271 | A272 | A273 | T274 | R275 | | | |
| sav10.2 | V11 | Q12 | A13 | P14 | A15 | A16 | H17 | N18 | R19 | G20 | L21 | |
| | T22 | G23 | L233 | V234 | Q236 | K237 | N238 | H249 | L250 | T253 | A254 | |
| | T255 | L267 | V268 | N269 | A270 | E271 | A272 | A273 | T274 | R275 | | |
| sav10.3 | L31 | D32 | H64 | V68 | V95 | L96 | I107 | L111 | A114 | G115 | | |
| | N116 | M119 | V121 | A122 | N123 | L124 | S125 | L126 | G127 | S128 | P129 | |
| | V139 | S141 | A142 | T143 | S144 | R145 | G146 | V147 | L148 | V149 | V150 | |
| | A151 | A152 | S153 | S163 | Y167 | P168 | A169 | N173 | A174 | M175 | A176 | |
| | V177 | T220 | S221 | M222 | T224 | P225 | V227 | A228 | A231 | N243 | I246 | |
| | R247 | L250 | | | | | | | | | | |
| sav10.4 | P131 | S132 | A133 | L135 | E136 | V139 | A151 | A152 | S153 | G160 | | |
| | S161 | I162 | S163 | Y167 | P168 | A169 | R170 | Y171 | A172 | N173 | A174 | |
| | A176 | Q191 | Y192 | G193 | A194 | G195 | L196 | R247 | S259 | T260 | N261 | |
| | L262 | Y263 | G264 | | | | | | | | | |
| sav11.0 | W6 | G154 | N155 | S156 | G157 | A179 | T180 | D181 | Q182 | N183 | | |
| | N184 | N185 | R186 | A187 | S188 | F189 | S190 | Q191 | Y192 | P201 | G202 | |
| | V203 | N204 | V205 | L217 | N218 | G219 | T220 | L262 | Y263 | | | |
| sav12.0 | L31 | I107 | A108 | Q109 | G110 | L111 | E112 | W113 | A114 | G115 | | |
| | N116 | N117 | G118 | A122 | L124 | S132 | A133 | T134 | L135 | Q137 | A138 | |
| | V139 | N140 | S141 | T143 | R145 | V149 | A151 | S163 | Y167 | P168 | A169 | |
| | R170 | Y171 | N173 | A174 | | | | | | | | |
| sav13.0 | Q2 | S3 | P5 | T38 | H39 | P40 | D41 | L42 | N43 | H67 | | |
| | G70 | A73 | A74 | L75 | N77 | I79 | G80 | V81 | L82 | G83 | V205 | |
| | Q206 | S207 | T208 | Y209 | S212 | T213 | Y214 | A215 | S216 | L217 | | |
| sav14.0 | A16 | H17 | R19 | G20 | L21 | T22 | G23 | S24 | G25 | V26 | | |
| | K27 | V28 | A29 | V30 | I35 | I44 | R45 | G46 | G47 | V84 | A85 | |
| | P86 | S87 | A88 | E89 | L90 | Y91 | A92 | V93 | W113 | N117 | G118 | |
| | M119 | H120 | V121 | A232 | L233 | K235 | Q236 | K237 | T274 | | | |
| sav15.0 | W6 | R10 | G154 | N155 | S156 | G157 | V177 | G178 | A179 | T180 | | |
| | D181 | Q182 | N183 | N184 | N185 | R186 | A187 | S188 | F189 | S190 | Q191 | |
| | V199 | A200 | P201 | G202 | V203 | N218 | G219 | T220 | A223 | L257 | Y263 | |
| | L267 | | | | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sav16.0 | A13 | A16 | H17 | G20 | L21 | T22 | G23 | S24 | G25 | V26 | | |
| | V28 | I72 | A73 | V84 | A85 | P86 | S87 | A88 | E89 | L90 | H120 | |
| | G229 | A230 | A231 | A232 | L233 | V234 | K235 | Q236 | K237 | N238 | P239 | |
| | S240 | W241 | I246 | H249 | L250 | A270 | A273 | T274 | | | | |
| sav17.0 | T22 | G23 | S24 | G25 | V26 | K27 | V28 | A29 | V30 | L31 | | |
| | D32 | I35 | I44 | R45 | G46 | G47 | A48 | F50 | S87 | A88 | E89 | L90 |
| | Y91 | A92 | V93 | K94 | V95 | G110 | W113 | N117 | G118 | M119 | H120 | |
| | V121 | A232 | K235 | Q236 | | | | | | | | |
| sav18.1 | W6 | G7 | I8 | S9 | R10 | V11 | Q12 | A179 | T180 | D181 | | |
| | Q182 | N183 | N184 | N185 | R186 | A187 | I198 | V199 | A200 | P201 | V203 | |
| | H226 | V227 | A230 | H249 | L250 | K251 | N252 | T253 | A254 | T255 | S256 | |
| | L257 | S265 | G266 | L267 | V268 | N269 | A270 | | | | | |
| sav18.2 | A13 | A16 | H17 | L21 | T22 | G23 | V26 | V28 | V84 | A85 | | |
| | A88 | V121 | L148 | Y171 | A172 | N173 | V174 | M175 | A176 | G195 | L196 | |
| | D197 | I198 | V199 | V227 | A228 | G229 | A230 | A231 | A232 | L233 | V234 | |
| | K235 | Q236 | K237 | N238 | W241 | N243 | V244 | Q245 | I246 | R247 | N248 | |
| | H249 | L250 | K251 | N252 | T253 | A254 | Y263 | G264 | S265 | G266 | V268 | |
| | A270 | A273 | T274 | | | | | | | | | |
| sav19.1 | A16 | H17 | R19 | G20 | L21 | T22 | G23 | S24 | G25 | V26 | | |
| | K27 | V28 | S87 | A88 | E89 | H120 | V121 | A232 | L233 | V234 | K235 | |
| | Q236 | K237 | N238 | P239 | T274 | | | | | | | |
| sav19.2 | A1 | Q2 | S3 | V4 | P5 | D41 | H64 | H67 | G70 | T71 | | |
| | A74 | L75 | N77 | S78 | I79 | G80 | V81 | L82 | G83 | G202 | V203 | |
| | N204 | V205 | Q206 | S207 | T208 | Y209 | Y214 | A215 | S216 | L217 | N218 | |
| | G219 | M222 | | | | | | | | | | |

**[0504]** For PD498, the following amino acid residues belong to the epitope area that correspond to each epitope sequence indicated in Table 3:

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pd1.1 | D105 | A108 | S109 | G110 | I111 | R112 | Y113 | A114 | A115 | D116 | Q117 |
| | N131 | S132 | T133 | T134 | L135 | K136 | S137 | A138 | V139 | D140 | Y141 |
| | A142 | W143 | N144 | K145 | G146 | A147 | | | | | |
| pd1.2 | C128 | E129 | A153 | G154 | N155 | D156 | N157 | V158 | S160 | R161 | T162 |
| | F163 | Q167 | S170 | G178 | A179 | I180 | D181 | D184 | R185 | K186 | A187 |
| | S188 | F189 | S190 | N191 | Y192 | G193 | T194 | W195 | V196 | T220 | T262 |
| | N263 | | | | | | | | | | |
| pd1.3 | F50 | L104 | D105 | S106 | I107 | A108 | S109 | G110 | I111 | R112 | Y113 |
| | A114 | A115 | D116 | Q117 | T133 | T134 | L135 | K136 | S137 | A138 | V139 |
| | D140 | Y141 | A142 | W143 | N144 | K145 | G146 | A147 | | | |
| pd1.4 | T28 | *28aV | A29 | V30 | D32 | S33 | G34 | V35 | Y37 | *44aaV | I45 |
| | K46 | G47 | Y48 | D49 | F50 | I51 | R53 | D54 | N55 | N56 | P57 |
| | M58 | D60 | L61 | K89 | I90 | L91 | A92 | V93 | R94 | V95 | L96 |
| | D97 | A98 | Y113 | A114 | Q117 | A119 | | | | | |
| pd1.5 | D32 | S33 | G34 | K46 | G47 | Y48 | D49 | F50 | I51 | D52 | R53 |
| | D54 | N55 | P57 | M58 | L61 | L91 | A92 | V93 | R94 | V95 | L96 |
| | D97 | A98 | L104 | D105 | S106 | I107 | A108 | S109 | G110 | I111 | R112 |
| | Y113 | A114 | A115 | D116 | Q117 | G118 | A119 | T133 | T134 | L135 | K136 |
| | S137 | A138 | V139 | D140 | Y141 | A142 | | | | | |
| pd2.1 | V19 | T21 | I111 | R112 | Y113 | A114 | A1 | D116 | Q117 | G118 | A119 |
| | L122 | D140 | Y141 | A142 | W143 | N144 | K145 | G146 | A147 | V148 | L233 |
| | L234 | A235 | S236 | Q237 | G238 | K239 | N240 | N243 | V244 | Q245 | I246 |
| | R247 | Q248 | A249 | A273 | V274 | R275 | Y276 | | | | |
| pd2.2 | S24 | S25 | T26 | Q27 | T28 | *28aV | L42 | A43 | R44 | *44aK | *44aaV |
| | I45 | D75 | N77 | D87 | T88 | K89 | I90 | L91 | G118 | A119 | K120 |
| | V121 | L122 | G146 | A147 | V148 | A232 | A235 | S236 | | | |
| pd2.3 | R22 | G23 | S24 | S25 | T26 | Q27 | T28 | *28aV | D87 | T88 | K89 |
| | I111 | A115 | G118 | A119 | K120 | V121 | L122 | S137 | A138 | V139 | D140 |
| | Y141 | A142 | W143 | N144 | K145 | G146 | A147 | V148 | V149 | V150 | I175 |
| | A231 | A232 | A235 | S236 | N243 | I246 | R247 | | | | |
| pd2.4 | W-6 | S12 | T13 | P14 | A15 | A16 | V19 | T21 | R22 | G23 | S24 |
| | Q27 | L230 | A231 | L233 | L234 | A235 | S236 | Q237 | G238 | K239 | N240 |
| | N243 | Q245 | I246 | S270 | N271 | K272 | A273 | V274 | R275 | Y276 | |
| pd3.1 | L31 | K46 | G47 | Y48 | F50 | L91 | V93 | S103 | L104 | D105 | S106 |
| | I107 | A108 | S109 | G110 | I111 | R112 | Y113 | A114 | A115 | D116 | Q117 |
| | G118 | L122 | L124 | C130 | S132 | T133 | T134 | L135 | K136 | S137 | A138 |
| | V139 | D140 | Y141 | A142 | Q167 | P168 | Y171 | P172 | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pd3.2 | V19 | T21 | R22 | G23 | S24 | Q27 | K120 | V121 | V148 | L230 | A231 | |
| | A232 | L233 | L234 | A235 | S236 | Q237 | G238 | K239 | N240 | N243 | Q245 | |
| | I246 | R247 | Q248 | A249 | I250 | Q252 | T253 | K272 | A273 | V274 | R275 | |
| | Y276 | | | | | | | | | | | |
| pd4.1 | W-6 | S12 | T13 | P14 | A15 | A16 | W17 | D18 | V19 | T21 | R22 | |
| | G23 | S24 | M84 | A85 | P86 | D87 | T88 | A142 | W143 | G146 | A147 | |
| | V148 | G229 | L230 | A231 | A232 | L233 | L234 | A235 | S236 | Q237 | G238 | |
| | K239 | N240 | N243 | V244 | Q245 | I246 | R247 | Q248 | A249 | I250 | S270 | |
| | N271 | A273 | V274 | R275 | Y276 | | | | | | | |
| pd4.2 | W-6 | T13 | A16 | W17 | V19 | T21 | R22 | G23 | S24 | *44aK | A73 | |
| | A74 | *75aT | G83 | M84 | A85 | P86 | D87 | T88 | A142 | G146 | G146 | |
| | A147 | V148 | G229 | L230 | A231 | A232 | L233 | L234 | A235 | S236 | Q237 | |
| | G232 | K239 | N240 | N243 | V244 | Q245 | I246 | R247 | Q248 | A249 | I250 | |
| | S270 | A273 | V274 | R275 | Y276 | | | | | | | |
| pd4.3 | T26 | Q27 | T28 | *28aV | A29 | V30 | L31 | Y37 | *44aaV | I45 | | |
| | K46 | G47 | Y48 | D49 | D52 | R53 | D54 | N55 | N56 | P57 | M58 | |
| | V72 | T88 | K89 | I90 | L91 | A92 | V93 | Y113 | A114 | A115 | Q117 | |
| | G118 | A119 | K120 | V121 | L122 | N123 | A147 | A228 | A232 | | | |
| pd4.4 | K46 | G47 | F50 | L91 | V93 | S103 | L104 | D105 | S106 | I107 | A108 | |
| | S109 | G110 | I111 | R112 | Y113 | A114 | A115 | D116 | Q117 | G118 | C130 | |
| | S132 | T133 | T134 | L135 | K136 | S137 | A138 | V139 | D140 | Y141 | Q167 | |
| | P168 | A169 | S170 | Y171 | P172 | N173 | A174 | | | | | |
| pd4.5 | T28 | *28aV | A29 | V30 | L31 | V35 | D36 | Y37 | N38 | H39 | L42 | |
| | A43 | *44aaV | I45 | K46 | G47 | Y48 | F50 | N55 | N56 | P57 | | |
| | M58 | K89 | I90 | L91 | A92 | V93 | A108 | S109 | G110 | I111 | R112 | |
| | Y113 | A114 | A115 | D116 | Q117 | G118 | A119 | L122 | | | | |
| pd5.0 | F50 | S103 | L104 | D105 | S106 | I107 | A108 | S109 | G110 | I111 | R112 | |
| | Y113 | A114 | A115 | D116 | Q117 | T133 | T134 | L135 | K136 | S137 | A138 | |
| | V139 | D140 | Y141 | A142 | | | | | | | | |
| pd6.1 | Y4 | Y6 | G7 | G63 | H64 | H67 | V68 | T71 | N155 | A179 | F189 | |
| | P201 | G202 | V203 | N204 | I205 | A206 | S207 | V209 | G213 | Y214 | S215 | |
| | Y216 | M217 | S218 | G219 | T220 | S221 | M222 | A223 | S224 | P225 | H226 | |
| pd6.2 | W-6 | T13 | A16 | W17 | V19 | T21 | R22 | G23 | S24 | S25 | Q27 | |
| | M84 | A85 | P86 | D87 | T88 | G229 | L230 | A231 | A232 | L233 | L234 | |
| | A235 | S236 | Q237 | G238 | S270 | V274 | | | | | | |
| pd7.0 | R22 | G23 | S24 | S25 | Q27 | T28 | *28aV | A29 | V30 | V35 | D36 | |
| | Y37 | N38 | H39 | P40 | D41 | L42 | A43 | R44 | *44aK | *44aaV | T66 | |
| | A69 | G70 | V72 | A73 | A74 | D75 | N77 | A85 | P86 | D87 | T88 | |
| | K89 | I90 | L91 | A119 | V121 | L122 | N123 | T208 | A228 | A231 | | |
| pd8.0 | W-6 | T13 | A16 | W17 | T21 | R22 | G23 | Q27 | *44aK | A73 | A74 | |
| | *75aT | G83 | M84 | A85 | P86 | D87 | T88 | K120 | V121 | I175 | A176 | |
| | V177 | G178 | V196 | D197 | V198 | T199 | A200 | V227 | G229 | L230 | A231 | |
| | A232 | L233 | L234 | A235 | S236 | Q237 | G238 | K239 | N240 | N243 | Q245 | |
| | I246 | Q248 | A249 | I250 | Q252 | T253 | A254 | F264 | Y265 | G266 | I268 | |
| pd9.0 | W-6 | Y6 | G7 | P8 | Q9 | N10 | T11 | S12 | T13 | P14 | A15 | |
| | A16 | W17 | D18 | V19 | T21 | M84 | V139 | W143 | V148 | V149 | A151 | |
| | P168 | A169 | Y171 | P172 | N173 | A174 | I175 | A176 | D181 | S182 | N183 | |
| | D184 | D197 | P201 | L230 | L233 | L234 | K239 | N240 | N243 | V244 | Q245 | |
| | I246 | R247 | Q248 | A249 | I250 | E251 | Q252 | T253 | A254 | K267 | I268 | |
| | N269 | S270 | N271 | K272 | A273 | V274 | R275 | Y276 | | | | |
| pd10.0 | L124 | L126 | G127 | C128 | E129 | C130 | N131 | L135 | V139 | A151 | A152 | |
| | A153 | G154 | N155 | D156 | N157 | V158 | S160 | R161 | T162 | F163 | Q167 | |
| | P168 | A169 | S170 | Y171 | A174 | I175 | A176 | N191 | Y192 | G193 | T194 | |
| | W195 | V196 | T262 | N263 | F264 | *264aK | | | | | | |
| pd11.0 | W-6 | S-5 | Y2 | Y4 | Q5 | Y6 | G7 | P8 | Q9 | N10 | T11 | |
| | S12 | T13 | P14 | W17 | D18 | V19 | T21 | A82 | M84 | I180 | D181 | |
| | S182 | N183 | D184 | P201 | G202 | V203 | N204 | I205 | H226 | L233 | S270 | |
| | N271 | V274 | R275 | | | | | | | | | |
| pd12.0 | G127 | C128 | E129 | V139 | V148 | V149 | V150 | A151 | A152 | A153 | G154 | |
| | N155 | D156 | V158 | R161 | T162 | F163 | Q167 | P168 | A169 | S170 | Y171 | |
| | P172 | N173 | A174 | I175 | A176 | V177 | G178 | N191 | Y192 | G193 | T194 | |
| | W195 | V196 | D197 | V198 | T199 | A200 | V227 | R247 | I250 | E251 | A254 | |
| | N263 | F264 | *264aK | Y265 | G266 | I268 | | | | | | |
| pd13.1 | W-6 | S-5 | P-4 | D-2 | P-1 | Y1 | Y2 | S3 | *3aA | Y4 | Q5 | P8 |
| | Q9 | S12 | T13 | P14 | A15 | A16 | W17 | D18 | V19 | T21 | R22 | |
| | G80 | V81 | A82 | N271 | V274 | R275 | | | | | | |
| pd13.2 | W-6 | S-5 | P-4 | N-3 | D-2 | P-1 | Y1 | Y2 | S3 | *3aA | Y4 | Q5 |
| | P8 | Q9 | P14 | W17 | D41 | G70 | A74 | D75 | *75aT | N76 | N77 | |
| | G78 | I79 | G80 | V81 | A82 | G83 | A206 | S207 | T208 | Y214 | | |
| pd14.0 | T28 | V35 | D36 | Y37 | N38 | H39 | P40 | D41 | L42 | A43 | R44 | |
| | *44aK | *44aaV | I45 | K46 | G47 | Y48 | D49 | F50 | R53 | D54 | | |
| | N55 | N56 | P57 | M58 | T66 | A69 | G70 | A73 | A74 | D75 | K89 | I90 |
| | L91 | A92 | V93 | R94 | Y113 | T208 | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pd15.0 | V30 | L31 | D32 | S33 | G34 | V35 | D36 | Y37 | N38 | H39 | L42 | |
| | A43 | *44aaV | K46 | Y48 | D49 | F50 | I51 | N56 | P57 | M58 | | |
| | D60 | L61 | N62 | G63 | H64 | G65 | T66 | A69 | I90 | A92 | V93 | |
| | R94 | V95 | L96 | D97 | A98 | G100 | S101 | G102 | S103 | S106 | I107 | |
| | G110 | S125 | L126 | V209 | P210 | N211 | N212 | | | | | |
| pd16.0 | W-6 | S-5 | P-4 | N-3 | Y2 | G7 | P8 | Q9 | N10 | T11 | S12 | T13 |
| | P14 | A15 | A16 | W17 | D18 | V19 | T21 | R22 | *75aT | N76 | A82 | |
| | G83 | M84 | A85 | P86 | L233 | N269 | S270 | N271 | | | | |
| pd17.1 | T11 | S12 | A15 | A16 | D18 | V19 | T21 | R22 | G23 | S24 | Q27 | |
| | L230 | A232 | L233 | L234 | A235 | S236 | Q237 | G238 | K239 | N240 | N243 | |
| | Q245 | I246 | Q248 | A249 | Q252 | T253 | N269 | S270 | N271 | K272 | A273 | |
| | V274 | R275 | Y276 | | | | | | | | | |
| pd17.2 | A108 | I111 | R112 | A115 | D116 | K120 | L124 | T133 | T134 | L135 | K136 | |
| | S137 | A138 | V139 | D140 | Y141 | A142 | W143 | N144 | K145 | G146 | A147 | |
| | V148 | V149 | P168 | Y171 | N173 | A174 | N243 | | | | | |
| pd18.1 | W-6 | T13 | A16 | W17 | V19 | T21 | R22 | G23 | S24 | S25 | *44aK | |
| | M84 | A85 | P86 | D87 | T88 | K89 | G229 | L230 | A231 | A232 | L233 | |
| | L234 | A235 | S236 | Q237 | K239 | A249 | I250 | T253 | N269 | S270 | N271 | |
| | K272 | A273 | V274 | R275 | Y276 | | | | | | | |
| pd18.2 | D-2 | V30 | V35 | D36 | Y37 | N38 | H39 | P40 | D41 | L42 | A43 | |
| | R44 | *44aK | *44aaV | I45 | K46 | G47 | Y48 | P57 | T66 | A69 | | |
| | G70 | A73 | A74 | D75 | *75aT | N76 | N77 | I79 | V81 | A82 | A85 | |
| | P86 | D87 | T88 | K89 | I90 | L91 | A92 | V93 | R94 | T208 | | |

**[0505]** For Lipolase, the following amino acid residues belong to the epitope area that correspond to each epitope sequence indicated in Table 4:

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| lip2.1 | Y53 | F55 | V63 | L78 | F80 | W117 | V120 | A121 | D122 | T123 | L124 | |
| | R125 | Q126 | K127 | V128 | E129 | D130 | A131 | V132 | R133 | V140 | L159 | |
| | R160 | G161 | N162 | G163 | Y164 | D165 | I166 | G190 | | | | |
| lip2.2 | V2 | L6 | F10 | A173 | P174 | R175 | A182 | L193 | Y194 | R195 | I196 | |
| | T197 | P204 | R205 | Y213 | S214 | H215 | S216 | S217 | P218 | E219 | Y220 | |
| | W221 | I222 | I235 | V236 | K237 | I238 | E239 | I241 | D242 | A243 | G246 | |
| | N247 | N248 | | | | | | | | | | |
| lip2.3 | V2 | L6 | F10 | A182 | L185 | T186 | L193 | Y194 | R195 | I196 | T197 | |
| | H215 | S216 | S217 | P218 | E219 | Y220 | W221 | I222 | I235 | V236 | K237 | |
| | I238 | E239 | G240 | I241 | A243 | G246 | N247 | N248 | | | | |
| lip2.4 | V2 | L6 | F10 | L193 | Y194 | R195 | I196 | T197 | S216 | S217 | P218 | |
| | E219 | Y220 | W221 | I222 | I235 | V236 | K237 | I238 | E239 | G240 | A243 | |
| | G246 | N247 | N248 | | | | | | | | | |
| lip3.0 | L93 | K94 | F95 | H110 | A173 | P174 | R175 | V176 | G177 | N178 | R179 | |
| | A182 | L185 | T186 | L193 | R195 | N200 | D201 | I202 | P204 | R205 | L206 | |
| | P207 | P208 | R209 | E210 | F211 | G212 | Y213 | S214 | H215 | S216 | S217 | |
| | P218 | E219 | I238 | E239 | G240 | I241 | D242 | A243 | T244 | G245 | N248 | |
| | ?R259? | P250 | N251 | I252 | P253 | D254 | I255 | | | | | |
| lip4.0 | R175 | V176 | G177 | N178 | R179 | A180 | F181 | A182 | E183 | F184 | L185 | |
| | T186 | R205 | P207 | P208 | R209 | E210 | F211 | G212 | Y213 | S214 | H215 | |
| | S216 | S217 | I241 | D242 | N248 | | | | | | | |
| lip5.1 | A20 | Y21 | N25 | N26 | T50 | F51 | L52 | Y53 | S54 | F55 | E56 | |
| | V63 | T64 | G65 | F66 | L67 | A68 | L69 | I76 | V77 | L78 | S79 | F80 |
| | R81 | G82 | S83 | R84 | S85 | I86 | E87 | N88 | W89 | K127 | V128 | |
| | A131 | H145 | S146 | L147 | G148 | L151 | G266 | | | | | |
| lip5.2 | K94 | F95 | L96 | L97 | K98 | E99 | R108 | G109 | H110 | D111 | G112 | |
| | R175 | V176 | G177 | N178 | R179 | A180 | F181 | A182 | E183 | F184 | R205 | |
| | P207 | P208 | R209 | E210 | F211 | G212 | Y213 | S214 | H215 | S216 | I241 | |
| | D242 | N248 | | | | | | | | | | |
| lip6.0 | Q9 | F10 | N11 | F13 | A14 | S17 | V63 | F80 | R81 | W89 | L93 | |
| | F113 | S116 | W117 | F142 | T143 | G144 | H145 | S146 | L147 | G148 | G149 | |
| | A150 | L151 | A152 | T153 | V154 | A155 | G156 | A157 | V168 | F169 | S170 | |
| | Y171 | G172 | A173 | P174 | R175 | V176 | F181 | L185 | L193 | Y194 | R195 | |
| | I196 | T197 | D201 | V203 | P204 | L206 | P207 | H215 | H258 | Y261 | F262 | |
| | I265 | | | | | | | | | | | |
| lip7.0 | F13 | A14 | Q15 | Y16 | S17 | A180 | A19 | A20 | Y21 | C22 | G23 | |
| | N25 | N26 | I34 | C36 | A40 | C41 | F51 | L52 | Y53 | S54 | F55 | |
| | E56 | V63 | T64 | G65 | F66 | L67 | S79 | F80 | R81 | V120 | A121 | |
| | D122 | T123 | L124 | R125 | Q126 | K127 | V128 | L264 | I265 | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| lip8.1 | L12 | F13 | A14 | Q15 | Y16 | S17 | A18 | A19 | A20 | I34 | V44 | |
| | A49 | T50 | F51 | L52 | F66 | L67 | A68 | L69 | D70 | N71 | T72 | |
| | N73 | K74 | L75 | I76 | V77 | S79 | H135 | P136 | D137 | Y138 | R139 | |
| | V140 | V141 | T143 | | | | | | | | | |
| lip8.2 | L12 | F13 | A14 | Q15 | Y16 | S17 | A18 | A19 | A20 | I34 | V44 | |
| | A49 | T50 | F51 | L52 | Y53 | S54 | F55 | G65 | F66 | L67 | A68 | L69 |
| | D70 | N73 | L75 | I76 | V77 | L78 | S79 | T123 | L124 | R125 | Q126 | |
| | K127 | V128 | E129 | D130 | A131 | T143 | | | | | | |
| lip9.0 | L6 | F10 | N25 | N26 | D27 | A28 | A30 | G31 | T50 | F51 | L52 | |
| | Y53 | S54 | F55 | E56 | G65 | F66 | L67 | A68 | L69 | I76 | T123 | |
| | L124 | R125 | Q126 | K127 | V128 | E129 | D130 | A131 | V132 | R1333 | E134 | |
| | H135 | P136 | R139 | V140 | V141 | F142 | G156 | L159 | R160 | G161 | N162 | |
| | G163 | Y164 | D165 | I166 | D167 | V168 | F169 | S170 | G190 | G191 | T192 | |
| | L193 | Y194 | R195 | I196 | Y220 | | | | | | | |
| lip10.0 | N11 | L12 | Q15 | Y16 | I34 | T35 | C36 | C41 | P42 | E43 | V44 | |
| | E45 | K46 | A47 | D48 | A49 | D70 | N71 | T72 | N73 | K74 | | |
| lip11.0 | F95 | L96 | L97 | K98 | E99 | I100 | N101 | D102 | C107 | R108 | G109 | |
| | H110 | D111 | F113 | T114 | S115 | A150 | T153 | V154 | A173 | P174 | R175 | |
| | V176 | G177 | N178 | R179 | F181 | V203 | P204 | R205 | L206 | P207 | P208 | |
| | R209 | F211 | G212 | Y213 | S214 | H215 | G240 | I241 | D242 | A243 | T244 | |
| | N248 | | | | | | | | | | | |
| lip12.0 | L96 | L97 | K98 | E99 | I100 | N101 | D102 | C104 | S105 | G106 | C107 | |
| | R108 | G109 | H110 | T114 | S115 | V176 | G177 | N178 | A180 | F181 | F184 | |
| lip13.0 | N11 | L12 | F13 | A14 | Q15 | Y16 | S17 | A182 | A19 | A20 | Y21 | |
| | N26 | I34 | C36 | A40 | C41 | P42 | E43 | V44 | A49 | F55 | E56 | |
| | V63 | T64 | G65 | F66 | L67 | A68 | D70 | N73 | L75 | I76 | V77 | L78 |
| | S79 | F80 | R81 | G82 | S83 | R84 | W89 | W117 | L124 | V128 | V141 | |
| | F142 | T143 | G144 | H145 | S146 | L147 | G148 | G149 | A150 | L151 | A152 | |
| | A155 | | | | | | | | | | | |
| lip14.0 | Q9 | F10 | N11 | F13 | A14 | S17 | Y21 | R81 | G82 | S83 | R84 | |
| | S85 | I86 | E87 | N88 | W89 | I90 | G91 | N92 | L93 | F113 | T143 | |
| | G144 | H145 | S146 | L147 | G149 | A150 | T153 | V168 | F169 | S170 | Y171 | |
| | A173 | P174 | R175 | V176 | L193 | Y194 | R195 | I196 | T197 | D201 | V203 | |
| | P204 | L206 | P207 | H215 | H258 | Y261 | F262 | I265 | G266 | | | |
| lip15.0 | N11 | L12 | F13 | A14 | Q15 | Y16 | S17 | A18 | A19 | A20 | Y21 | |
| | C22 | G23 | K24 | N25 | N26 | D27 | A28 | I34 | T35 | C36 | A40 | |
| | C41 | P42 | E43 | V44 | E45 | K46 | A47 | A49 | F51 | L52 | Y53 | |
| | S54 | F55 | E56 | T64 | G65 | F66 | L67 | S79 | F80 | R81 | T123 | |
| | L124 | K127 | L264 | I265 | | | | | | | | |
| lip16.0 | A14 | E87 | I90 | H145 | G172 | I196 | T197 | H198 | T199 | N200 | D201 | |
| | I202 | P204 | R205 | W221 | I222 | K223 | S224 | G225 | T226 | G246 | N247 | |
| | N254 | I252 | P253 | D254 | I255 | P256 | A257 | H258 | L259 | W260 | Y261 | |
| | F262 | G263 | I265 | | | | | | | | | |
| lip17.0 | E1 | V2 | F7 | F10 | G177 | N178 | R179 | A180 | F181 | A182 | E183 | |
| | F184 | L185 | T186 | L193 | R195 | H198 | T199 | G212 | S214 | H215 | S216 | |
| | S217 | P218 | E219 | Y220 | W221 | I222 | K223 | S224 | G225 | T226 | V228 | |
| | P229 | V230 | T231 | R232 | N233 | D234 | I235 | V236 | K237 | I238 | E239 | |
| | G240 | I241 | D242 | A243 | T244 | G245 | G246 | I262 | | | | |
| lip18.0 | Q9 | F13 | Y16 | T32 | N33 | I34 | C41 | P42 | E43 | V44 | E45 | |
| | K46 | A47 | D48 | A49 | T50 | F51 | L52 | L67 | A68 | L69 | D70 | |
| | N71 | T72 | N73 | L75 | I76 | V128 | V132 | H135 | P136 | D137 | Y138 | |
| | R139 | V140 | V141 | F142 | Y164 | D165 | I166 | D167 | F169 | Y194 | | |

**[0506]** For Amylase, the following amino acid residues belong to the epitope area that correspond to each epitope sequence indicated in Table 5:

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| je1.1 | N2 | G3 | T4 | R33 | P346 | Y349 | I352 | L353 | T354 | R355 | P360 |
| | V362 | D366 | Y367 | M378 | K379 | A380 | K381 | I382 | D383 | P384 | I385 |
| | L386 | E387 | A388 | R389 | Q390 | N391 | F392 | A393 | Y394 | I450 | T451 |
| je1.2 | Y57 | D58 | Y60 | D61 | F65 | N66 | Q67 | L104 | G105 | G106 | A107 |
| | D108 | A109 | T110 | E111 | A135 | W136 | T137 | K138 | F139 | D140 | F141 |
| | P142 | G143 | R144 | G145 | N146 | T147 | Y148 | S149 | F151 | K152 | W153 |
| | R154 | F158 | | | | | | | | | |
| je2.1 | M6 | Y8 | E10 | W11 | H12 | D26 | L30 | R33 | V325 | D326 | N327 |
| | H328 | D329 | S330 | Q331 | P332 | G333 | E334 | E337 | F339 | K345 | Y349 |
| | V362 | F363 | Y364 | G365 | D366 | Y367 | Y368 | G369 | I370 | P371 | T372 |
| | H373 | S374 | V375 | P376 | A377 | M378 | K379 | I382 | D383 | L386 | |

-continued

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| je2.2 | L289 | L293 | V314 | P318 | T323 | F324 | V325 | D326 | F339 | K345 | P346 | |
| | L347 | A348 | Y349 | A350 | L351 | I352 | L353 | T354 | R355 | F356 | Q357 | |
| | G358 | Y359 | P360 | S361 | V362 | F363 | Y364 | G365 | D366 | Y367 | Y368 | |
| | G369 | P376 | A377 | M378 | K379 | I382 | I385 | R389 | Q397 | | | |
| je2.3 | N102 | V116 | E117 | V118 | P120 | R123 | D159 | G160 | V161 | D162 | W163 | |
| | Q168 | F169 | Q170 | N171 | R172 | I173 | Y174 | K175 | A182 | W183 | D184 | |
| | V187 | D188 | N193 | Y194 | D195 | Y196 | L197 | M198 | Y199 | A200 | D201 | |
| | V202 | H236 | | | | | | | | | | |
| je2.4 | T1 | N2 | T4 | M6 | Y8 | D26 | L30 | R31 | N32 | R33 | G34 | I35 |
| | V325 | D326 | F339 | K345 | Y349 | L353 | V362 | F363 | Y364 | G365 | D366 | |
| | Y367 | Y368 | G369 | I370 | P376 | A377 | M378 | K379 | I382 | D383 | P384 | |
| | I385 | L386 | E387 | A388 | R389 | Q390 | N391 | F392 | Y394 | H417 | | |
| je3.1 | M6 | Q7 | Y8 | F9 | E10 | L13 | H19 | W20 | N21 | R22 | L23 | |
| | R24 | D25 | D26 | A27 | S28 | N29 | L30 | R31 | N32 | R33 | I385 | |
| | W39 | I40 | P41 | P42 | A43 | W44 | V52 | G53 | Y54 | Y75 | A87 | L88 |
| | N91 | V93 | D98 | V100 | Y364 | Y368 | | | | | | |
| je3.2 | Y8 | F9 | W11 | H19 | W20 | W39 | I40 | P41 | P42 | A43 | W44 | |
| | D51 | V52 | G53 | Y54 | G55 | A56 | Y75 | D98 | V99 | V100 | M101 | |
| | N102 | H103 | L104 | D195 | L197 | M198 | A200 | D201 | V202 | R230 | I231 | |
| | D232 | A233 | V234 | K235 | H236 | I237 | E262 | H328 | | | | |
| je3.3 | Y8 | F9 | H19 | W20 | W39 | I40 | P41 | P42 | A43 | W44 | K45 | |
| | G46 | T47 | V52 | G53 | Y54 | G55 | A56 | Y57 | D58 | L59 | Q67 | |
| | K68 | Y75 | D98 | V100 | L104 | G105 | G106 | A107 | D108 | A109 | T110 | |
| | E111 | A135 | W136 | T137 | K138 | F139 | D140 | F141 | P142 | | | |
| je4.1 | L23 | D25 | D26 | A27 | S28 | N29 | L30 | R31 | N32 | R33 | G34 | I35 |
| | T36 | I38 | A84 | I85 | H86 | A87 | L88 | K89 | N90 | N91 | G92 | |
| | V93 | Q94 | V95 | Q390 | | | | | | | | |
| je4.2 | A43 | W44 | K45 | L59 | Y60 | D61 | L62 | G63 | E64 | F65 | V71 | |
| | R72 | T73 | K74 | Y75 | G76 | T77 | R78 | S79 | Q80 | L81 | E82 | |
| | S83 | Y148 | W219 | Y220 | T223 | L224 | | | | | | |

Example 4

**[0507]** Having identified 'antibody binding peptide' sequences and by consensus analysis also "epitope patterns" (e.g. >DF>>K>), one can identify potential epitope sequences on the 3-dimensional surface of a parent protein (=acceptor protein) in a semi-automated manner using the following method:

**[0508]** The anchor amino acid residues are transferred to a three dimensional structure of the protein of interest, by colouring D red, F white and K blue. Any surface area having all three residues within a distance of 18 Å, preferably 15 Å, more preferably 12 Å, is then claimed to be an epitope. The relevant distance can easily be measured using e.g. molecular graphics programs like Insight!! from Molecular Simulations Inc.

**[0509]** The residues in question should be surface exposed, meaning that the residue should be more than 20% surface exposed, preferably more than 50% surface exposed, more preferably 70% surface exposed. The percentage "surface accessible area" of an amino acid residue of the parent protein is defined as the Connolly surface (ACC value) measured using the DSSP program to the relevant protein part of the structure, divided by the residue total surface area and multiplied by 100. The DSSP program is disclosed in W. Kabsch and C. Sander, BIOPOLYMERS 22 (1983) pp. 2577-2637. The residue total surface areas of the 20 natural amino acids are tabulated in Thomas E. Creighton, PROTEINS; Structure and Molecular Principles, W.H. Freeman and Company, NY, ISBN: 0-7167-1566-X (1984).

**[0510]** Substitutions of one or more residue (s) within 18 Å, preferably 15 Å, more preferably 12 Å, around the geometrical center of the residues involved in the epitope, for a bigger or smaller residues, may destroy the epitope, and make the protein less antigenic.

**[0511]** Residues involved in epitope is 2, preferably 3 and more preferably 4

Example 5

Production, Selection, and Evaluation of Enzyme Variants with Reduced Antiqenicity or Immunogenicity

**[0512]** Epitope sequences and hot-spots amino acids were mutated using standard techniques know to the person skilled in the field (e.g. site-directed mutagenesis, error-prone PCR—see for example Sambrook et al. (1989), Molecular Cloning. A Laboratory Manual, Cold Spring Harbour, N Y).

**[0513]** In the examples shown below, variants were made by site-directed mutagenesis. Amino acid exchanges giving new epitopes or duplicating existing epitopes, according to the information collected in the epitope-database (See Example 1), were avoided in the mutagenesis process.

**[0514]** Enzyme variants were screened for reduced binding of antibodies raised against the backbone enzyme. Antibody binding was assessed by competitive ELISA as described in the Methods section.

**[0515]** Variants with reduced antibody binding capacity were further evaluated in the mouse SC animal model (See methods section).

**[0516]** The following variants showed reduced IgE and/or reduced IgG levels in the mouse model:

| Parent protein | Mutations | Target epitope sequences | % IgG response | % IgE response |
|---|---|---|---|---|
| Savinase | D181N | Sav11.0; Sav15.0 and Sav18.1. Hot spot amino acid. | 50 | 19 |
| Savinase | R170L; Q206E | Sav9.4; Sav10.4; Sav1.1; and Sav19.2 | 5 | 34 |
| Savinase | R170L, S57P | Sav9.4; Sav10.4 | 45 | 12 |
| Savinase | R247E | Sav2.3, Sav6.1, Sav18.2 Hot spot amino acid. | 75 | 30 |
| Savinase | R247Q | Sav2.3, Sav6.1, Sav18.2 Hot spot amino acid. | 17 | 20 |
| Savinase | R247H | Sav2.3, Sav6.1, Sav18.2 Hot spot amino acid. | 40 | 27 |
| Savinase | R247K | Sav2.3, Sav6.1, Sav18.2 Hot spot amino acid. | 74 | 34 |

## Example 6

### Production, Selection, and Evaluation of Enzyme Variants with Reduced Antigenicity or Immunogenicity

**[0517]** Hot-spots or epitopes were mutated using techniques known to the expert in the field (e.g. site-directed mutagenesis, error-prone PCR).

**[0518]** In the examples showed below, variants were made by site-directed mutagenesis. Amino acid exchanges giving new epitopes or duplicating existing epitopes according to the information collected in the epitope-database, were avoided in the mutagenesis process.

**[0519]** Enzyme variants were screened for reduced binding of antibodies raised against the backbone enzyme. This antibody binding was assessed by established assays (e.g. competitive ELISA, agglutination assay).

**[0520]** Variants with reduced antibody binding capacity were further evaluated in animal studies.

**[0521]** Mice were immunised subcutanuous weekly, for a period of 20 weeks, with 50 microliters 0.9% (wt/vol) NaCl (control group), or 50 microliters 0.9% (wt/vol) NaCl containing 10 micrograms of protein. Blood samples (100 microliters) were collected from the eye one week after every second immunization. Serum was obtained by blood clothing, and centrifugation.

**[0522]** Specific IgG1 and IgE levels were determined using the ELISA specific for mouse or rat IgG1 or IgE. Differences between data sets were analysed by using appropriate statistical methods.

A. Site-Directed Mutagenesis of Amino Acids Defining Epitopes, with an Effect on IgG1 and/or IgE Responses in Mice.

Epitope: A172/A169 R170A194 G193 N261

Pattern: AR>R>A>N

Antibody: IgG1+IgE

Backbone: Savinase

**[0523]** The variant carried the mutation R170F.

**[0524]** In a competitive IgE ELISA, this variant was less effective in competing for anti-savinase antibodies, giving a 15% lower endpoint inhibition as compared to the savinase backbone.

**[0525]** Mouse studies revealed an 80% reduction of the specific IgE levels, as compared to savinase backbone ($p<0.01$). The IgG1 levels were not significantly affected.

Epitope: S216 E219 Y220

Pattern: E Y>M

Antibody: IgG1

Backbone: Lipoprime

**[0526]** The variant carried the mutation S216W.

**[0527]** In a competitive IgG ELISA, the variant was less effective in competing for Lipolase antibodies, giving a 38% decrease in endpoint inhibition as compared to the enzyme backbone.

**[0528]** Mouse studies revealed a 69% decrease in specific IgG1 levels, compared to the lipolase backbone ($p<0.05$). The IgE levels were not significantly affected.

B. Site-Directed Mutagenesis of Epitopes, with Examples of Epitope Duplication, and New Epitope Formation, Respectively, Predicted by the Epitope-Database.

Epitope: T143 N173 N140 E136 L135

Pattern: S/T NN>EL

Antibody: IgG1

Backbone: Savinase

**[0529]** The variant carried the mutation E136R.

**[0530]** In a competitive IgG ELISA, the variants were less effective in competing for savinase antibodies, giving a 38% decrease in endpoint inhibition as compared to the savinase backbone.

**[0531]** Mouse studies revealed a dramatic increase in specific IgG1 levels, compared to savinase backbone ($p<0.01$). The IgE levels were not significantly affected.

**[0532]** Mutation E136R establishes an IgG1 epitope of the R Y P R/K pattern, previously identified on PD498. Apparently, this new epitope was more antigenic in mice than the existing epitope. The introduction of a savinase unrelated epitope on the savinase backbone could explain the observed discrepancy between competitive ELISA and animal studies.

**[0533]** In this example, it was found that using information derived exclusively from screening phage libraries with anti-

PD498 antibodies (to identify the R Y P R/K epitope pattern of Table 2) one could predict the outcome of a genetic engineering experiment for Savinase in which the E136R mutation created the PD498-epitope on the Savinase surface, leading to increased immunogenicity of this Savinase variant. This demonstrates that the epitope patterns identified may be used to predict the effect on immunogenicity of substitutions in proteins that are different from the parent protein(s) used to identify the epitope pattern.

C. Site-Directed Mutagenesis of Amino Acids Defining Epitope Areas, with a Differential Effect on IgG1 and IgE Antibody Levels in Mice, and an Inhibiting Effect on IgG Binding, Respectively.

Epitope: A172/A169 R170A194 G193 N261

Pattern: AR>R>A>N

Antibody: IgG1+IgE

Backbone: Savinase

**[0534]** Epitope area: P131, S132, A133, L135, E136, V139, A151, A152, S153, G161, S162, I165, S166, Y167, P168, Y171, N173, A174, A176, Q191, Y192, G195, L196, R247, S259, T260, L262, Y263, G264.

**[0535]** The variant was different at position Y167 by the mutation Y167I.

**[0536]** In a competitive IgE ELISA, the variant was less effective in competing for anti-savinase antibodies, giving a 8% lower endpoint inhibition as compared to the its backbone.

**[0537]** Mouse studies revealed a 75% reduction of the specific IgE levels, as compared to the backbone ($p<0.01$). In contrast, the IgG1 levels were dramatically increased ($p<0.01$).

Epitope: T143 N173 N140 E136 L135

Pattern: S/T NN>EL

Antibody: IgG1

Backbone: Savinase

**[0538]** Epitope area: V10A, 1107, A108, L111, E112, G115, S132, A133, T134, Q137, A138, V139, S141, A142, S144, R145, G146, V147, V149, Y167, P168, Y171, A172, A174, M175, N243, R247.

**[0539]** While variant no. 1 was mutated at the epitope position (N140D), variant no. 2 was mutated at N140 (N140D), but also at the epitope area position (A172D).

**[0540]** In a competitive IgG ELISA, variant no. 1 was less effective in competing for anti-savinase antibodies, as compared to savinase. This variant revealed a 21% lower endpoint inhibition as compared to the its backbone.

**[0541]** Variant no. 2 resulted in an endpoint inhibition that was 60% lower as compared to savinase, and 40% as compared to variant no. 1.

## Example 7

### Conjugation of Savinase Variant E136K with Activated Bis-PEG-1000

**[0542]** 4.9 mg of the Savinase variant was incubated in 50 mM Sodium Borate pH 9.5 with 12 mg of N-succinimidyl carbonate activated bis-PEG 1000 in a reaction volume of approximately 2 ml. The reaction was carried out at ambient temperature using magnetic stirring while keeping the pH within the interval 9.0-9.5 by addition of 0.5 M NaOH. The reaction time was 2 hours.

**[0543]** The derivatives was purified and reagent excess removed by size exclusion chromatography on a Superdex-75 column (Pharmacia) equilibrated in 50 mM Sodium Borate, 5 mM Succinic Acid, 150 mM NaCl, 1 mM $CaCl_2$ pH 6.0.

**[0544]** The conjugate was stored at −20° C., in the above described buffer.

**[0545]** Compared to the parent enzyme variant, the protease activity of the conjugate was retained (97% using Dimethyl-casein as substrate at pH 9).

## Example 8

**[0546]** Competitive ELISA was performed according to established procedures. In short, a 96 well ELISA plate was coated with the parent protein. After proper blocking and washing, the coated antigen was incubated with rabbit anti-enzyme polyclonal antiserum in the presence of various amounts of modified protein (the competitior).

**[0547]** The amount of residual rabbit antiserum was detected by pig anti-rabbit immunoglobulin, horseraddish peroxidase labelled.

| | |
|---|---|
| Epitope: | T143 N173 N140 E136 L135 |
| Pattern: | S/T N N > E L |
| Antibody: | IgG1 |
| Backbone: | Savinase |
| Mutation: | E136K |
| Modification: | bis-NHS-PEG1000 |

**[0548]** The data show that the derivative (60% endpoint inhibition) has reduced capacity to bind enzyme specific immunoglobulines, as compared to the parent protein (100% endpoint inhibition).

## Example 9

**[0549]** For this example the epitope sequences were determined in four environmental allergens (Bet v1; Der f2; Der p2 and PhI p2), based on their structures (1btv.pdb; 1ahm.pdb; a19v.pdb; and 1whp.pdb, respectively), sequences (SEQ ID NOS: 6, 7, 8 and 9, respectively) and computer modelling of the epitope patterns that had been assembled in our database (shown in Table 8). The allergens arise from common sources of allergy: Birch (Bet v1 from *Betula pendula*), House dust mites (Der f2 from *Dermatophagoides farinae* and Der p2 from *Dermatophagoides pteronyssinus*), and Timothy grass (PhI p2 from *Phleum pratense*).

**[0550]** The protein surface is scanned for epitope patterns matching the given "consensus" sequence of about 6-12 residues. First, residues on the protein surface that match the first residue of the consensus sequence are identified. Within a specified distance from each of these, residues on the protein surface that match the next residue of the consensus sequence are identified. This procedure is repeated for the remaining residues of the consensus sequence. The method is further described under the paragraph "Methods" above and the computer program can be found in the Appendixes.

**[0551]** The critical parameters used in this screening included:

**[0552]** i) a maximal distance between the alpha-carbon atoms of subsequent amino acids,

**[0553]** ii) a minimal accessibility of the amino acid of 20 Å2,

**[0554]** iii) the largest maximal distance between the most distinct amino acids should be less than 25 Å,

**[0555]** iv) the 5 best epitopes were taken,

**[0556]** v) the minimal homology with the epitope pattern of interest was 80%

**[0557]** In this way a number of potential epitopes are identified. The epitopes are sorted according to total surface accessible area, and certain entries removed:

**[0558]** 1) Epitopes that contain the same protein surface residue more than once. These are artefacts generated by the described algorithm.

**[0559]** 2) Epitopes which are "too big", i.e. where a distance between any two residues in the epitope exceeds a given threshold.

**[0560]** The epitope sequences found by this second generation mapping procedure were:

Bet v1:

Epi#02

A146, K32, Q36, F30, T142, R145, V12

A34, K32, Q36, F30, T142, R145, V12

Epi#03

L62, K65, - - - , 156, Y66

L24, K20, H76, I23, Y81

L24, K20, H76, 1104, Y81

Epi#04

K134, S136, Q132, K129, A130, A135

K134, S136, Q132, K129, V128, G1

Epi#05

G140, A146, R145, T10, G111, A106, T107, V12

G26, A146, R145, T10, G110, A106, T107, V12

G140, A146, R145, T10, G110, S11, S149, L152

G110, A106, S11, T9, G140, R145, T10, V12

G140, A146, R145, T10, G111, S11, S149, V12

Epi#06

G110, P108, D109, T107, A106, P14

G111, P108, D109, T107, A106, P14

A34, N28, D27, S40, K32, P35

G26, N28, D27, S39, K32, P35

A106, N78, D75, T77, A16, P14

G26, N28, D27, S39, Q36, P35

Epi#07

G46, T52, D69, S99, R70, V71, P50, D72

G49, T52, D69, S99, R70, V71, P50, D72

G48, T52, D69, S99, R70, V71, P50, D72

Epi#08

K123, E127, G1, V2, H121, F3

K65, E60, F64, V67, F58

K65, E60, F58, V67, F64

K129, E127, G1, V2, H121, F3

Epi#09

S149, L152, D156, N159, R17, L24, D75, K103, N78, A106, V12

L152, S149, D156, N159, R17, L24, D75, H76, N78, A106, V12

L152, D156, N159, R17, L24, D75, K80, N78, A106, V12

Epi#10

D109, A106, N78, T77, F79, R17, K20

E141, T10, R145, T142, F30, G26, K32

E8, T10, R145, T142, F30, G26, K32

Epi#11

F30, K32, I38, Q36, V33, E148

F22, F30, I38, Q36, V33, E148

F30, L143, 138, Q36, V33, E148

Epi#12

Y5, E6

Y83, E73

Y120, E127

Y5, E8

Y66, E87

Y81, E73

Epi#13

H76, A16, P14, T107, A106, P108, G110, G111

A16, R17, P14, T107, A106, P108, G110, G111

A157, R17, P14, T107, A106, P108, G111, G110

Epi#15

K65, P90, D93, I91, K97, G92

K32, P31, D27, I56, K65, G61

Epi#17

A153, S149, R145, S11

A106, S11, R145, S149

Epi#18

R145, S149, L152, A153, Y150, L151, H154, S155

R145, S149, L152, A153, S155, L151, A157, N159

Epi#22

D125, D93, P90, K65

D93, P90, P63, E60

Epi#23
K55, N43, E42, S57, L62, P63
K68, N43, E42, S40, F30, P35
K54, N43, E42, S57, F64, P63
K55, N43, E42, S40, F58, P35
Epi#24
E96, K97, E87, P90, F64, E60, K65
E127, K123, E96, P90, F64, P63, K65
E42, K68, E87, P90, F64, E60, K65
E42, K55, E87, P90, F64, E60, K65
D93, G92, E87, P90, F64, E60, K65
D125, K123, E96, P90, F64, P63, K65
Epi#25
R70, K55, I44, E45, E42
R70, K54, I44, E45, N47
R70, K68, I53, E45, N47
Epi#27
D93, E127, D125, K123
Epi#28
A146, Q36, F58, E60, L62, F64, P63, K65
I38, Q36, F58, E60, L62, F64, P63, K65
A34, Q36, F58, E60, L62, F64, P63, K65
L143, Q36, F58, E60, L62, F64, P63, K65
V33, Q36, F58, E60, L62, F64, G61, K65
Epi#29
G61, K65, L62, F58, E60
I56, K65, L62, F64, E60
G89, K65, L62, F64, E60
V67, K65, L62, F64, E60
Epi#30
G1, N4, S99, H121, K97, I91, P90
I113, I13, S149, H154, S155, L152, L151
I13, L152, A153, H154, S155, L151, V33
G110, I13, S149, H154, S155, L152, L151
G1, N4, S99, H121, K97, I98, V2
G1, N4, S99, H121, K97, I91, V85
Epi#33
K32, F30, P35, S39, S57, K65
Q36, F30, P35, S39, S40, K32
K32, F30, P35, S40, S57, K65
K65, F58, P35, S39, A34, R145
Epi#34
V105, P14, T107, V12, R145, Y150, S155
I113, P14, T107, V12, R145, Y150, S155
Epi#37
P50, V74, L24, R17, N159
P50, V74, L24, K20, N159
P14, R17, L24, K20, N159
Epi#38
L143, G140, E141, R145, V33, N28, P31, S39
L143, G140, E141, R145, V33, N28, P31, S40
L143, G140, E141, R145, V33, N28, P31, S57
Epi#39
A130, E127, H126, T94, P90, G89, L62
A130, E127, H121, T94, P90, G89, L62
Epi#40
A157, L152, A153, Y150, K32, S39
A153, L152, A157, Y150, K32, S40
R17, L151, A153, Y150, K32, S40
R145, L143, A34, Y150, A153, S155
R145, L143, G140, T9, K115, T10
Epi#41
P63, Y66, L62, S57
Epi#44
I23, R17, D156, Y150, S149, V12, T10
L24, R17, D156, Y150, S149, V12, P14
L24, R17, D156, Y158, A16, A106, P108
I13, R17, D156, Y150, S149, V12, T10
L151, R17, D156, Y150, S149, V12, T10
L24, R17, D156, Y150, S149, V12, T107
Epi#45
K32, P35, F30, Y150, R145, M139, G140
K32, P35, F30, Y150, R145, M139, L143
K32, P31, F30, Y150, R145, M139, G140
Epi#47
L152, S149, R145, L143, A34, F30, N28, P31, P35
A153, S149, R145, A146, A34, F30, N28, P31, P35
Epi#48
E60, K65, P90, P63, G61
E60, K65, P63, P90, G92
Epi#51
T94, H126, E127, D125, G124, K123, H121
D125, H126, E127, T94, K123, T122, H121

Der f2:

Epi#02

A98, K100, S101, P99, R128, R31

A98, K100, R128, P99, R31, V94

T91, N93, P95, P34, R31, R128

L61, N93, P95, P34, R31, R128

Epi#03

L40, K15, A39, I13, Y86

L40, K14, A39, I88, Y90

Epi#05

G32, A98, R31, P34, G20, T36, T91, Y90

G32, A98, R31, P34, G20, T36, T91, V94

G32, A98, R31, P34, G20, T36, T91, L37

G32, A98, R31, P34, G20, T36, T91, V18

Epi#06

A98, P99, D129, R31, K96, P95

G32, P99, D129, R128, R31, P95

A98, P99, D129, R31, K33, P95

A98, P99, D129, R31, K96, P34

A98, P99, D129, R128, K126, P26

Epi#07

T107, S57, D59, S101, R128, A98, P99, D129

T107, S57, D59, S101, R31, A98, P99, D129

Epi#08

K15, D87, V76, H74, F75

K14, D87, V76, H74, F75

K77, D87, V76, H74, F75

Epi#09

L61, D64, I68, H74, F75, T70, N71

N114, N46, D113, K48, N71, T70, T49

G83, N46, D113, K48, N71, T70, T49

Epi#10

L40, I13, D42, N44, V81, K48, N46, N114, G115

L40, I13, D42, N44, V81, K82, N46, N114, G115

L37, D19, G20, V18, V3, D4, K6, A120, T107, V105

Epi#11

F75, K51, I111, Q45, V116, D113

F75, K51, I111, Q45, V81, D113

Epi#12

Y90, E38

Epi#13

H30, R31, P95, A98, P99, S101, G60, L61

Epi#15

**[0561]** K96, P99, D129, 128, R128, A98

K96, P99, D129, I127, R128, A98

K96, P99, D129, I29, R128, A98

K55, P66, D64, I68, T70, G67

Epi#18

R31, R128, I28, G125, T123, H124, V105

R31, R128, I127, G125, T123, H124, V105

Epi#22

D1, M17, D4, V3, K6

D1, M17, D19, P34, K96

D1, M17, D4, V5, K6

Epi#23

K14, N11, E12, N44, Q85, P79

K14, N11, E12, N10, Q45, P79

K14, N11, E12, N44, Q84, P79

K14, N11, E12, L40, Q85, P79

Epi#24

D129, K100, E102, P99, R128, R31, K96

E62, G60, E102, P99, R128, R31, K96

D129, K126, E102, P99, R128, R31, K33

D129, K126, E102, P99, R31, P95, K96

Epi#25

R31, K96, I97, D59, E62

R128, R31, I97, D59, E102

R128, K126, I127, E102, N103

Epi#27

D64, E62, D59, K100

D59, E62, D64, K55

D87, E38, D19, K33

D19, E38, D87, K15

D19, E38, D87, K14

D19, E38, D87, K77

Epi#28

V16, D87, Q85, K14, E12, K15, Q2, D1

I13, D87, Q85, K14, E12, K15, Q2, D1

V3, D1, Q2, K15, E12, K14, Q85, D87

L40, D87, Q85, K14, E12, K15, Q2, D1

I88, D87, Q85, K14, E12, K15, Q2, D1

V76, D87, Q85, K14, E12, K15, Q2, D1

V18, D1, Q2, K15, E12, K14, Q85, D87

Epi#29
G32, N93, L61, E62
V94, N93, L61, E62
Epi#30
G60, I97, A98, H30, K96, P34, P95
I68, N71, H74, K77, P79, V81
G32, I97, A98, H30, K96, P95, P34
Epi#34
V105, P26, S24, G125, R128, S101, P99
W92, P34, T91, V94, R31, S101, P99
I28, P26, T123, G125, R128, S101, P99
Epi#37
A120, V16, L40, K14, N11
A39, V16, L40, K14, N11
Y90, A39, L40, K14, N11
Y86, A39, L40, K14, N11
Epi#39
A120, E38, T91, P34, G20, L37
A39, E38, T91, P34, G20, L37
Epi#40
G20, L37, A120, T123, K6, S24
A39, L37, A120, T123, K6, S24
G20, L37, A120, T107, K6, T123
Epi#41
P34, L37, V106, S57
Epi#42
P26, S24, G125, R128, R31
P99, S101, G125, R128, R31
Epi#44
V16, Q2, D19, P34, W92, Y90, A39, V18, T91
V16, Q2, D19, P34, W92, Y90, A39, V5, T123
V3, Q2, D19, P34, W92, Y90, A39, V18, T91
Epi#45
K77, H74, F75, N71, D69, G67
**[0562]** K77, H74, F75, N71, D69, V76
K77, H74, F75, N71, D69, V65
Epi#46
A98, R128, R31, P95, N93, G32
A98, R128, R31, P34, G20, Q2
Epi#48
Q2, D19, P34, P95, G32
H30, K96, P95, P34, G20
Epi#49
D87, D42, L40, Q85, Q84, C78, T47, Q45, K48
D87, D42, L40, Q85, Q84, C78, T47, Q45, K82
Epi#50
D19, W92, P34, T91
D19, W92, P34, P95
D19, W92, T91, T36
Epi#51
D129, H30, K33, R31, R128, K126, H124
R31, H30, D129, R128, K100, K126, H124
T123, H124, K126, R128, R31, K33, H30
Der p2:
Epi#03
L17, K89, A39, I13, Y86
L17, K89, A72, I88, Y90
L17, K89, A72, I52, Y90
Epi#04
K15, S1, Q2, K14, V16, L17
**[0563]** K15, S1, Q2, K14, A39, L17
K15, S1, Q2, K14, V40, I13
Epi#05
G60, A56, L61, P99, G32, R31, H30, I97
G60, A56, L61, P99, G32, R31, H30, I28
Epi#06
G60, A56, D64, S57, K55, P66
G83, N46, D114, T49, K48, P79
G60, N103, D59, S101, R31, P95
Epi#08
K55, D64, S57, V106, F35
K55, E62, S57, V106, F35
Epi#09
L61, G60, E102, R128, I28, K126, N103, T123, V105
L61, G60, E102, R128, I127, K100, N103, T123, V105
L61, G60, E102, R128, I127, H124, N103, T123, V105
Epi#10
SAS: 435, Size 24.47: D69, T91, N93, F35, G32, R31
SAS: 422, Size 20.74: E38, T91, N93, F35, G32, K96
Epi#11
K14, I13, Q85, V81, E42
K15, I13, Q85, V81, E42
K14, I13, Q85, V40, D87

Epi#12
Y86, E42
Y90, E53
Y90, E38
Epi#13
H30, A125, P26, T123, A122, P19, L37, P34, W92
H30, A125, P26, T123, A122, H124, S24, G23, G20
H30, A125, P26, T123, A122, P19, L17, G20, F35
Epi#15
K55, P66, D69, I68, K89, A72
K55, P66, D69, I68, K89, A39
K55, P66, D64, I54, K109, G115
K55, P66, D64, I54, K109, A9
Epi#18
R31, I29, A125, S101, E102, N103
R31, I29, A125, S101, E102, V104
R31, I29, A125, T123, A122, V105
Epi#22
D69, P66, D64, V65, K55
D64, P66, D69, T91, K89
D59, L61, D64, P66, W92
D59, L61, D64, V65, E62
D69, P66, D64, V65, E53
Epi#24
D64, K55, E62, P99, R31, P34, K96
E53, K55, E62, P99, R31, P95, K96
D64, K55, E62, P99, R31, A98, K96
Epi#25
R31, H30, I28, E102, N103
R128, K126, I127, E102, N103
R128, K126, I28, E102, V105
Epi#27
D64, E53, D69, K89
D69, E53, D64, K55
D59, E62, D64, K55
Epi#28
V40, D87, Q85, E42, Q84, G83, K82
G20, H22, Q2, L17, E38, L37, Q36, P34, K33
G20, H22, Q2, L17, E38, L37, F35, P34, K33
Epi#29
I97, K100, L61, E62
G60, N103, L61, E62
I127, N103, L61, E62
Epi#30
G60, N103, S101, H30, K96, I97, P95
G60, N103, A125, H30, K96, I97, P95
I28, I127, A125, H30, K96, I97, P95
Epi#33
Q36, F35, V106, S57, A56, K55
K33, F35, V106, S57, A56, K55
Epi#34
I28, P26, S24, G23, G20, T123, S57
I28, P26, S24, V3, G20, T123, T107
W92, P34, T91, V18, G20, T123, P26
Epi#37
P66, V63, L61, K100, N103
P95, A98, L61, K100, N103
P19, V18, L17, K89, D87
P19, V3, L17, K89, D87
T123, V104, L61, K100, N103
Epi#38
L61, G60, E102, A125, V105, N103, P99, S57
L61, G60, E62, A56, V105, N103, P99, S57
Epi#39
A125, E102, H124, T123, P26, G20, L17
Epi#40
G60, L61, A56, T107, K6, T123
A39, L17, G20, T123, P26, S24
G60, L61, A56, T107, K55, S57
G60, L61, A56, T123, K126, S101
Epi#41
P19, L17, V3, S1
P19, L17, V5, S24
Epi#44
V65, D64, P66, W92, Y90, A39, V18, P19
L61, D64, P66, W92, Y90, A39, V18, T91
Epi#45
R31, P34, F35, N93, V94
K96, P34, F35, N93, G32
Epi#47
I127, S101, R31, I97, A98, L61, N103, P99, P95
I28, S101, R31, I97, A98, L61, N103, P99, S57
Epi#48
H30, K96, P95, P99, G60
H30, K96, P34, P19, G20
H30, K96, P34, P19, V18

H30, K96, P34, P95, V94
H30, K96, P34, P19, V3
E38, K89, P70, P66, V65
H30, K96, P95, P34, G32
Q36, K89, P70, P66, V65
Epi#50
D69, Y90, W92, P66, P70
D69, Y90, W92, P34, P95
D69, Y90, W92, T91, P34
D69, Y90, W92, V94, P95
D69, Y90, W92, L37, P19
Epi#51
K126, H124, E102, R128, I28, R31, H30
T123, H124, K126, R128, I28, R31, H30
D4, H124, K126, R128, I28, R31, H30
PhI p2:
Epi#02
T87, K85, Q61, S38, R34, R67
T87, K85, Q61, P63, R34, V42
Epi#03
K10, A90, I88, Y86
K10, A18, I88, Y86
Epi#04
R34, S38, Q61, K85, T87, I88
R34, S38, Q61, K85, T87, A90
Epi#05
G47, A18, S12, T87, G89, T91, T5, V1
G73, A29, L69, T27, G50, T53, T45, V42
G11, A18, L20, T91, G89, A90, T87, I88
Epi#06
A93, P94, D79, R34, Q61, P59
A93, P94, D79, R34, Q61, P83
A93, P94, D80, R34, Q61, P59
A93, P94, D79, R34, Q61, P63
Epi#08
K10, E9, G11, A18, H16, F54
K46, E48, G47, A18, H16, F54
K10, E9, S12, A18, H16, F54
Epi#09
L69, T27, G73, N76, R67, V77, D79, R34, A43, T45, V42
L69, T27, A29, E30, R67, V77, D80, R34, A43, T45, V42
Epi#10
D55, A18, N13, S12, F54, G47, K46
T45, A18, N13, S56, F54, G47, K46
Epi#09
L60, S56, E57, D55, K15, N13, S12, G11
L60, S56, E57, D55, H16, F54, T45, T53
L60, S56, E57, D55, H16, F54, T45, G47
Epi#12
Y86, E84
Y23, E24
Epi#18
N76, R67, F78, V81, A93, Y92, T91, T5, P2, V1
Epi#19
D39, W41, S38, Q61, R34, G37
E40, W41, S38, Q61, R34, A43
Epi#22
D79, P94, D80, P83, K85
D79, P94, D80, P63, K85
Epi#23
K10, N13, E14, L60, Q61, P59
K10, N13, E14, L60, Q61, P83
K10, N13, E14, L60, Q61, P63
Epi#24
E58, K15, E57, P59, S56, E14, Q61
D55, K15, E57, P59, S56, E58, Q61
Epi#25
R34, R67, W41, D39, E40
Epi#26
S38, E40, W41, V42, E32, E30
S38, E40, W41, V42, A43, E32
Epi#27
E14, E57, E58, K15
D55, E14, E84, K85
Epi#28
G37, H36, Q61, K85, E84, L60, F54, A43, K46
G37, H36, Q61, K85, E84, L60, F54, S12, D55
G37, H36, Q61, K85, E84, L60, F54, S56, D55
G37, H36, Q61, K85, E84, L60, F54, A43, R67
G37, H36, Q61, K15, E57, L60, F54, A43, K46
G37, H36, Q61, K85, E84, L60, F54, S12, K15
G37, H36, Q61, K85, E84, L60, F54, S56, K15
G37, H36, Q61, K85, E84, L60, F54, A43, R34
G37, H36, Q61, K85, E84, L60, F54, A18, D55

Epi#29
G73, K72, L69, R67, E30
I88, N13, L60, F54, E57
G25, K72, L69, R67, E32
V77, K75, L69, R67, E30
G37, H36, L60, F54, E57
G37, Q61, L60, F54, E57
Epi#30
I88, N13, S12, H16, K15, P59, L60
I88, N13, S56, H16, K15, L60, P59
I88, N13, A18, H16, K15, P59, L60
Epi#33
K46, F54, V42, S56, K15
H16, F54, V42, S56, K15
Epi#34
V1, P2, T5, V4, P94, Y92, T87
V1, P2, T5, L20, G89, T91, T87
V81, P94, T5, V1, P2, Y92, T91
Epi#37
T27, A29, L69, K72, D26
A43, R67, L69, K75, N76
Epi#38
L20, G89, E9, A18, N13, P59, S56
Epi#40
G49, L20, G89, Y86, K85, T87
G49, L20, G89, T87, K10, S12
G49, L20, G89, T87, K10, T7
Epi#44
V77, R67, D79, P94, Y92, A93, V1, P2
L69, R67, D79, P94, Y92, A93, V1, T5
Epi#45
D79, P94, F78, N76, M74, L69
D80, P94, F78, R67, D79, V77
K3, P94, F78, N76, M74, G73
Epi#46
A43, R67, R34, P63, H36, Q61
V77, R67, R34, P63, H36, G37
L69, R67, R34, P63, G37, Q61
Epi#47
G37, E35, E40, A43, R34, L60, N13, P59, S56
V77, E32, E40, A43, R34, L60, N13, P59, S56
S38, G37, E40, A43, R34, L60, N13, P59, S56
Epi#48
E24, K3, P94, P2, V1
E84, D80, P94, P2, V1
Epi#50
D39, W41, A43, T45
D39, W41, V42, T45
Epi#51
D79, H36, E84, T87, K10, G11, H16
D39, H36, Q61, K85, P63, R34, W41
D79, H36, E40, D39, G37, R34, W41
Q61, H36, E84, T87, K10, G11, H16

**[0564]**

TABLE 8

Each row indicates an epitope pattern. At each position (from 1 to maximum of 12) the cells indicate which amino acids (single letter coding) are allowed at that position. The last column indicates the patterns identified using IgE antibody binding.

| Epitope Pattern Number | Position 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | TS | RQ | YS | NHC | KR | KR | P | HNP | L | | | | IgE |
| 2 | RV | R | Y- | PST | FR- | ALPQS- (SEQ ID NO: 197) | RKN | ALT | | | | | IgE |
| 3 | Y | I | AH- | K | L | | | | | | | | |
| 4 | AGIL (SEQ ID NO: 198) | ANRTV- (SEQ ID NO: 199) | KRY | Q | S | Y- | KR | | | | | | |
| 5 | GILVY (SEQ ID NO: 200) | STH | ASTR- (SEQ ID NO: 201) | G | PT- | RNAFLS (SEQ ID NO: 202) | A | G | | | | | IgE |

TABLE 8-continued

Each row indicates an epitope pattern. At each position (from 1 to maximum of 12) the cells indicate which amino acids (single letter coding) are allowed at that position. The last column indicates the patterns identified using IgE antibody binding.

| Epitope Pattern Number | Position 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | P | KRQSA (SEQ ID NO: 203) | STRC (SEQ ID NO: 204) | D | PAN | GA | | | | | | | IgE |
| 7 | D | P | AV- | R | S- | D | S- | T | G | | | | |
| 8 | F | HI- | VA- | FSG- | DE- | KA | | | | | | | IgE |
| 9 | NRGLTV- (SEQ ID NO: 205) | STAN (SEQ ID NO: 206) | ANF | RKH | D- | AILV- (SEQ ID NO: 207) | R- | (ENRSV- SEQ ID NO: 208) | AGI- | DGNT- (SEQ ID NO: 209) | LIS- | | IgE |
| 10 | KR | RG | F | C- | AST- | RN | NTA | DECT (SEQ ID NO: 210) | | | | | IgE |
| 11 | DE | V- | Q | I | FLK | F | | | | | | | |
| 12 | E | Y | | | | | | | | | | | IgE |
| 13 | FWYGL (SEQ ID NO: 211) | PG | ALS- | PH | A | T- | P | LRWA (SEQ ID NO: 212) | SAH | | | | IgE |
| 14 | GV | Q | ILV | I- | Y | GNR | DN | TEH | | | | | |
| 15 | AG | RKQT- (SEQ ID NO: 213) | I | D | P | RKN | | | | | | | IgE |
| 16 | DN | A | DA | SDN | QRSW (SEQ ID NO: 214) | GMR | Y | P | RQL | | | | |
| 17 | S- | R | S | A | | | | | | | | | |
| 18 | VLSFN (SEQ ID NO: 215) | AEHNPT- (SEQ ID NO: 216) | T- | L- | ST- | Y- | GAL | LIV- | CSF- | R | FRN- | SD | IgE |
| 19 | AGLKM (SEQ ID NO: 217) | R | Q | QSC | NTW | DEI | | | | | | | IgE |
| 20 | D | G | D | KN | L | LF- | P | K | V | A | | | IgE |
| 21 | P | S | I- | I | LR- | CI | | | | | | | IgE |
| 22 | EDKW (SEQ ID NO: 218) | ACLPTV WY- (SEQ ID NO: 219) | D- | ASLPM- (SEQ ID NO: 220) | D- | | | | | | | | IgE |
| 23 | AP | LQF | SYLN- (SEQ ID NO: 221) | E | N | RK | | | | | | | IgE |
| 24 | KQ | AELFPR- (SEQ ID NO: 222) | TSFR (SEQ ID NO: 223) | P | EA | GK | DE | | | | | | IgE |
| 25 | ENV | DE | IW- | RKH | R | | | | | | | | IgE |
| 26 | DE | AGE | PHV | W | E- | S | W | | | | | | IgE |
| 27 | K | DE | E | DE | | | | | | | | | IgE |

TABLE 8-continued

Each row indicates an epitope pattern. At each position (from 1 to maximum of 12) the cells indicate which amino acids (single letter coding) are allowed at that position. The last column indicates the patterns identified using IgE antibody binding.

| Epitope Pattern Number | Position 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 28 | DKR | APSG- (SEQ ID NO: 224) | QF- | CFIKLW- (SEQ ID NO: 225) | E | FIKLW- (SEQ ID NO: 226) | Q | DH- | AGILV (SEQ ID NO: 227) | | | | IgE |
| 29 | E | RF- | L | KRQHNG P (SEQ ID NO: 228) | GILV (SEQ ID NO: 229) | | | | | | | | IgE |
| 30 | LVP | LIP | IKLPQS- (SEQ ID NO: 230) | H | AS- | LIMN (SEQ ID NO: 231) | GI | | | | | | |
| 31 | D | FI- | MV- | FW | R | N | QR | L | | | | | |
| 32 | V | f- | DE | A | A | F | | | | | | | |
| 33 | KR | SA- | S | VP | YF | KQH | | | | | | | IgE |
| 34 | STP | STY | GPR- | GLV | STM | WP | IVW | | | | | | |
| 35 | I | M | S | A- | L | AG | | | | | | | |
| 36 | AW | A | PV- | K- | Q- | ST | Y- | G- | V- | A | A | TP | IgE |
| 37 | NYD | KR | L | ARV | TYAP (SEQ ID NO: 232) | | | | | | | | IgE |
| 38 | S | P | N | LR- | RV- | AR- | E | G | L | | | | |
| 39 | L | G | P | RT- | HL- | E | A | | | | | | |
| 40 | ST- | APK- | YT | AG | L- | AGR | | | | | | | IgE |
| 41 | St | V- | L | Yh- | P- | | | | | | | | |
| 42 | RQ | R | P- | H- | NQG | S | P | L | | | | | |
| 43 | T- | RI | ML | S | HQ | GL | YA | WC | I | | | | |
| 44 | PT | AGV | SA | Y | W- | P- | D- | RQ- | ILVS (SEQ ID NO: 233) | | | | IgE |
| 45 | LVG | MD- | RN | Y- | F | PH | KRD | | | | | | IgE |
| 46 | AGQ | HNQGC (SEQ ID NO: 234) | P | R | R | AVLCY (SEQ ID NO: 235) | | | | | | | IgE |
| 47 | PS | RP | N | LFQA- (SEQ ID NO: 236) | AR | AILMNV (SEQ ID NO: 237) | RE | AGSYLE (SEQ ID NO: 238) | LIAGVS (SEQ ID NO: 239) | | | | |
| 48 | GV | P | P | KHQD (SEQ ID NO: 240) | SHQE (SEQ ID NO: 241) | | | | | | | | |
| 49 | KN | Q- | TMC | WYC- | Q | Q | FP- | VP- | L | W- | D | | |
| 50 | PST | STAPLW V (SEQ ID NO: 242) | W | WY- | RHD | | | | | | | | |

TABLE 8-continued

Each row indicates an epitope pattern. At each position (from 1 to maximum of 12) the cells indicate which amino acids (single letter coding) are allowed at that position. The last column indicates the patterns identified using IgE antibody binding.

| Epitope Pattern Number | Position 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 51 | WH | TSKHRQG (SEQ ID NO: 243) | LIRKGP (SEQ ID NO: 244) | DSRTQG KH- (SEQ ID NO: 245) | DEKQHT (SEQ ID NO: 246) | H | RKQDT (SEQ ID NO: 247) | | | | | |
| 52 | Q | DNT- | W | R | STRE- (SEQ ID NO: 248) | A | FW | | | | | |

Example 10

**[0565]** For this example the third-generation epitope sequences were determined in further 11 environmental allergens (Bosd2, Equc1, Gald4-mutant (with alanine substituted for glycine in position 102), Hevb8, Profillin1-AC, Profillin1-AT, Profillin2-AC, Profillin-birch pollen, Rag weed pollen5 and Vesv5), based on their structures sequences (SEQ ID NOS: 12, 13, 15, 16, 17, 18, 19, 20, 21 and 22, respectively), their structures (1bj7.pdb, 1ew3.pdb, 1flu.pdb, 1g5u.pdb, 1prq.pdb, 1a0k.pdb, 1f2k.pdb, 1cqa.pdb, 1bbg.pdb, and 1qnx.pdb, respectively), and computer modelling of the epitope patterns that had been assembled in our database (shown in Table 8). Further, the epitope sequences of the four environmental allergens of example 9, Bet v1, Der f2, Der p2, and PhI p2, were redetermined.

**[0566]** The additional allergens arise from common sources of allergy: cows (Bos d2 which is a bovine member of the lipocalin family of allergens), horses (Equ C1, a major horse allergen also of the lipocalin family), Hen egg white (Lysozyme Gal D 4), Latex (Hey b8, a profilin from *Hevea brasiliensis*), *Acanthamoeba castellani* (Profilin1-AC, a profilin isoform IA and Profilin2-AC, a profilin isoform II), *Arabidosis thaliana* (Profillin1-AT a cytoskeleton profilin), Birch (Profilin-birch pollen (Birch pollen profilin), Rag weed pollen5 (Ragweed pollen allergen V from *Ambrosia trifida*) and whasp venom (Ves v5 allergen from Vespula vulgaris venom).

**[0567]** The protein surface is scanned for epitope patterns matching the given "consensus" sequence of about 6-12 residues. First, residues on the protein surface that match the first residue of the consensus sequence are identified. Within a specified distance from each of these, residues on the protein surface that match the next residue of the consensus sequence are identified. This procedure is repeated for the remaining residues of the consensus sequence. The method is further described under the paragraph "Methods" above and the program can be found in Appendixes.

**[0568]** The critical parameters used in this screening included:

**[0569]** i) a maximal distance between the alpha-carbon atoms of subsequent amino acids,

**[0570]** ii) a minimal accessability of the amino acid of 20 Å2,

**[0571]** iii) the largest maximal distance between the most distinct amino acids should be less than 25 Å,

**[0572]** iv) the best epitope were taken,

**[0573]** v) the homology with the epitope pattern of interest was 100%

**[0574]** In this way a number of potential epitopes are identified. The epitopes are sorted according to total surface accessible area, and certain entries removed:

**[0575]** a. Epitopes that contain the same protein surface residue more than once. These are artefacts generated by the described algorithm.

**[0576]** b. Epitopes which are "too big", i.e. where a distance between any two residues in the epitope exceeds a given threshold.

**[0577]** The epitope sequences found were:

Bosd2:

Epi#01

L65, P155, P156, R17, R40, N37, Y39, R41, T67

L65, P155, P156, R17, R40, N37, Y39, R41, S52

L64, P155, P156, R17, R40, N37, Y39, R41, T54

Epi#02

T121, K150, S122, R17, P156, Y39, R41, R40

T121, K150, S122, R17, P156, Y56, R36, V30

Epi#03

L128, K130, H92,17, Y76

L134, K130, H92,17, Y76

L128, K130, H92,191, Y76

Epi#04

R72, Y76, S50, Q73, K71, V69, I45

K71, Y76, S50, Q73, R72, V69, L80

K71, Y76, S50, Q73, R72, V69, I42

Epi#06
G14, P13, D47, S10, K11, P9
G14, P13, D47, S10, S94, P9
G14, P13, D47, C44, S10, P9
Epi#08
K71, E49, S50, V69, F82
K71, E49, S50, V79, F82
Epi#09
I7, S10, D8, E95, K119, N96, S122, T121
S10, I7, D8, E95, K11, N96, S122, T124
Epi#10
E15, T54, R41, T67, F55, R17, K119
E43, T54, R41, T67, F55, R17, K119
E31, T151, N153, C63, F55, R40, R41
E31, T151, N153, C154, F55, R41, R17
Epi#11
K26, I145, Q132, E143
K26, I145, Q132, E137
K26, I145, Q132, E129
Epi#12
Y105, E108
Y83, E81
Epi#15
N153, P156, D152, I149, T121, G120
R17, P156, D152, I149, T121, G120
N153, P156, D152, I149, R17, G14
Epi#18
R109, 1110, G107, Y83, T85, E81, V69
R109, 1110, G107, Y105, T85, E81, V69
Epi#19
E43, N46, S50, Q73, R72, K71
D47, N46, S50, Q73, R72, G75
E49, N46, S50, Q73, R72, K71
I45, N46, S50, Q73, R72, K71
Epi#20
V30, K28, P34, L57, L65, K58, D59, G32, D27
V30, K28, P34, L57, L64, K58, D59, G33, D27
Epi#22
D8, S10, D47, P13, E15
D8, S10, D47, P13, E43
D47, S10, D8, V93, E95
D8, S10, D47, C48, K71
Epi#23
K119, N96, E127, S122, L128, P125
K150, N147, E146, Y20, F123, P125
K11, N96, E127, S122, L128, P125
Epi#24
E129, K130, E126, P125, S122, L128, Q133
E126, K130, E129, P125, S122, R17, K119
E126, K130, E129, P125, T124, L128, Q133
Epi#25
R72, K71, I45, D47, N46
R72, K71, I45, E43, N46
Epi#27
D47, E49, E74, K71
D24, E143, E146, K150
D47, E43, E15, K119
Epi#28
L134, Q133, L128, E126, K130, F123, S122, K150
Q132, K130, E126, L128, F123, S122, K150
L65, D59, Q60, K58, E31, L57, G32, D27
G61, D59, Q60, K58, E31, K28, G32, D27
Epi#29
V69, K71, L80, R72, E74
I45, K71, L80, R72, E74
G61, Q60, L64, F55, E68
Epi#30
G120, N96, S94, H92, K130, L128, P125
I91, I7, S94, H92, K130, L128, P125
Epi#33
K130, F123, P125, S122, K150
K71, Y76, P9, S10, S94, K119
Epi#34
I7, P9, S10, G14, R17, T121, S122
I45, P13, S10, G14, R41, Y39, P156
Epi#37
T67, V69, L80, K71, Y76
P156, R40, L65, K58, D59
P155, R40, L65, K58, N153
Epi#38
L80, G84, E108, R109, N25, P141, S136
Epi#39
E137, R138, P141, G139, L134
E31, L57, R36, P34, G84, L80

Epi#40
R17, G120, T121, K150, S122
R17, G120, T121, K150, T151
Epi#41
P34, Y83, L80, V69, S52
P34, Y83, L80, V79, S50
Epi#42
L128, P125, S122, G120, R17, R41
L128, P125, S122, G120, R17, R40
Epi#44
S10, D47, P9, Y76, S50, V69, T67
I45, D47, P9, Y76, S50, V69, T67
Epi#45
D27, P34, F82, Y105, R109, D106, G107
D59, P34, F82, Y105, R109, D106, G107
K58, P34, F82, Y105, R109, D106, G107
D27, P34, F82, Y105, R109, D106, G84
Epi#46
Y39, R41, R40, P155, C63, Q60
Y20, R17, R40, P155, C63, Q60
Epi#47
L128, E126, E129, L134, R138, Q133, N142, P141, S136
V69, E81, E68, I42, R41, F55, N37, R40, P156
V69, E43, E15, I42, R41, F55, N37, R40, P156
S122, E127, E129, L134, R138, Q133, N142, P141, S136
Epi#48
E43, D47, P13, P9, V93
S10, D47, P9, P13, G14
E43, D47, P13, P9, V90
E49, D47, P13, P9, V93
Equc1:
Epi#02
L66, N68, A65, F90, S69, Y72, R64, V89
A65, R64, S31, F28, S112, Y123, R110, V108
L179, R180, Q178, F177, P143, Y38, R141, V145
L66, R64, S31, F28, S112, Y123, R110, V125
L66, N68, A65, F90, S69, Y72, R64, V62
Epi#03
K32, A65, I63, Y72
Epi#05
G35, A65, S69, T93, G97, R26, S112, Y123
G35, A65, S69, T93, G97, R26, S112, I25
Epi#07
G97, T93, S70, D91, S100, R110, V125, P132, D128
Epi#08
K129, D130, F127, V108, F90
K129, D130, F127, V108, F109
K129, D130, F127, V125, F136
K129, D130, F127, V125, F133
Epi#10
E48, N53, N80, T77, C83, F177, R175, K172
E82, N80, N53, T77, C83, F177, G181, R180
E52, N53, N80, T77, C83, F177, R175, K172
Epi#11
F133, K47, I167, Q158, V163, E165
Epi#12
Y38, E142
Y38, E36
Y139, E142
Epi#13
K129, P132, D45, 1167, Q158, G161
R131, P132, D45, 1167, K164, G161
Epi#16
P87, Y72, R64, S70, S69, D67, A65, N68
Epi#17
A65, S31, R64, S34
Epi#18
R64, S31, I30, A65, S34, L66, N68, S69
Epi#19
E82, N80, C83, Q178, R175, K172
Epi#22
D130, P132, D128, Y106, K129
Epi#23
D144, K150, E148, P147, S146, E151, K155
Epi#25
R160, K159, I156, E151, E148
Epi#27
E118, E142, D144, K172
E36, E142, D144, K172
Epi#28
I173, D174, Q178, L179, E85, C83, F177, G181, R180
I173, D174, Q178, L179, E85, C83, F177, P143, D144
Epi#29
G181, Q178, L179, R180, E36
G181, Q178, L179, R180, E85

Epi#30
I30, N27, S112, H119, I121, I25, V23
Epi#31
L122, R110, N27, R26, F28, I30, D96
L124, R110, N27, R26, F28, I30, D96
Epi#33
H119, Y38, V62, S34, S31, R64
Epi#34
V62, P87, M88, V89, R64, S31, S34
Epi#37
P87, V89, L66, R64, D67
Epi#40
R64, L66, A65, Y72, S34
R64, L66, A65, Y72, S69
Epi#41
P132, Y106, L101, V89, S100
P132, Y106, L101, V89, S70
Epi#44
V46, R131, D128, P132, Y106, S100, V89, P87
Epi#45
K129, P132, F127, Y106, N102, D91, V89
K129, P132, F127, Y106, N102, D104, G105
Epi#47
S146, E148, E152, V23, R26, A24, N27, R110, S112
V23, E115, E118, N116, R26, F28, N27, R110, S112
Gald4:
Epi#01
L75, N65, P70, R73, R61, N59, Y53, R45, T47
L75, N65, P70, R68, R61, N59, Y53, R45, T47
Epi#02
A90, N77, L75, R73, P70, R61, R68
A122, R125, Q121, T118, R114, R112
Epi#04
R21, Y20, S24, Q121, R125, R128, L129
R21, Y20, S24, Q121, R125, R128, G126
Epi#05
G16, A10, R128, G126, A122, T118, G117
G4, A10, R128, G126, A122, T118, G117
Epi#06
G67, P79, D66, R61, R73, P70
G67, N65, D66, S72, R73, P70
G49, N46, D48, R61, R73, P70
Epi#07
G71, T69, D66, S72, R73, P70, D48
G67, T69, D66, S72, R73, P70, D48
Epi#08
K1, D87, S86, V2, F38
K1, D87, S86, V2, F3
Epi#09
Epi#10
E7, A11, R14, A10, C6, F3, R5, R125
D87, A11, R14, A10, C6, F3, R5, R125
T47, N46, N44, S36, F34, R114, R112
D18, A10, R14, A11, C6, F3, R5, R125
T118, N113, R112, A110, F34, R114, K116
Epi#11
L129, I124, Q121, V120, D119
Epi#12
Y53, E35
Epi#15
R73, P70, D66, I78, A82
R73, P70, D66, I78, A90
Epi#17
A102, S100, R21, S24
Epi#18
R112, N113, R114, F34, V109, A107, A102, N103
N113, R112, R114, F34, V109, A107, N103, S100
Epi#19
D18, N19, S24, Q121, R125, L129
D18, N19, S24, Q121, R125, G126
Epi#22
D48, P70, D66, W63, W62
D66, P70, D48, T69, W62
D48, P70, D66, W63, K97
Epi#23
R45, N44, E35, N39, Q41, A42
R45, N44, E35, Y53, Q41, A42
Epi#25
R128, R125, W123, D119, N27
R128, R125, W123, D119, V120
Epi#26
W62, S72, W63, P79, A82, D87
W62, S72, W63, P79, G67, D66

Epi#28
G117, D119, Q121, I124, E7, C6, F3, A11, R14
A122, D119, Q121, I124, E7, C6, F3, A11, R14
Epi#29
G126, R125, L129, R128, E7
G16, R14, L129, R128, E7
Epi#30
I124, L129, A10, H15, I88, L84
I124, L129, A11, H15,188, L84
Epi#31
L75, R73, N65, R61, W62, I98, D101
L75, R73, N74, R61, W62, I98, D101
Epi#33
Q41, F38, V2, S86, S85, K1
Q41, F38, V2, S36, A110, R114
Epi#34
W63, W62, T69, G71, R73, S72, P70
W62, W63, S72, L75, R73, T69, P70
Epi#36
A110, A107, A102, S100, K96, A90, A82
Epi#37
A10, R128, L129, R14, D18
A10, R128, L129, K13, N19
Epi#40
R128, L129, A11, T89, A90, S85
R14, L129, A11, T89, A90, S85
Epi#41
Y53, L84, S81
Y53, L84, S86
Epi#42
P79, S81, N65, P70, R61, R73
P79, S81, N65, P70, R61, R68
Epi#44
L129, R14, D18, Y20, S24, V120, T118
L129, R14, D18, Y23, S24, V120, T118
Epi#46
L75, R61, R73, P70, N65, G67
L75, R73, R61, P70, N65, A82
L75, R61, R68, P70, N65, G67
Epi#47
S72, G71, R68, N65, R61, L75, N77, R73, P70
G67, S72, R68, N65, R61, L75, N77, R73, P70
Epi#49
D87, L84, Q41, Q57, Y53, T43, N44
D87, L84, Q57, Q41, Y53, T43, N46
D87, L84, Q41, Q57, Y53, T43, N39
Epi#50
R73, W62, W63, P79, S81
R73, W63, W62, S72, P70
Epi#51
D18, H15, K13, R14, L129, R125, W123
Epi#52
F34, A110, R112, R114, W111, N27, Q121
F3, A11, E7, R5, W123, D119, Q121
W123, A122, T118, R114, W111, N27, Q121
Hevb8:
Epi#01
L20, P109, P112, K86, R84, N116, Y125, Q129, T111
L110, P109, P112, K86, R84, N116, Y125, Q129, T111
Epi#02
A48, K43, Q41, F42, T70, Y72, R84, V74
T21, R19, P109, P112, R84, V74
A49, K43, Q41, F42, T70, Y72, R84, V74
Epi#03
L65, K86, I75, Y72
Epi#05
G30, A48, L60, P62, G58, T63, H66, G69
G58, A61, R84, P112, G113, T111, S89, G88
G80, A81, F54, P79, G58, T63, H66, G69
G77, A81, F54, P79, G58, T63, H66, G69
Epi#06
G58, P79, D55, S59, K52, P57
G80, P79, D55, S59, K52, P57
G77, P79, D55, S59, K52, P57
Epi#07
G17, T5, S2, D16, R19, P109, D107
Epi#08
K52, D45, S44, A49, H66, F42
Epi#10
E78, A81, R96, F54, G58, K52
D55, A81, R96, F54, G80, K52
Epi#11
F54, L60, I83, Q76, V82, E78
Epi#12
Y106, E108

Epi#13
H66, L65, P62, T63, A61, P57, A81, P79, G58
H66, L65, P62, T63, A61, P57, A81, P79, G80
H66, L60, P62, T63, A61, P57, A81, P79, G77
Epi#15
R19, P109, D107, I105, K86, G88
Epi#18
R19, G17, P109, S89
Epi#22
D29, S44, D45, A48, K52
D29, M51, D55, P79, E56
D45, M51, D55, P79, E78
D29, S44, D45, A49, K52
D45, M51, D55, P79, E56
D29, M51, D55, P57, E78
D29, M51, D55, P57, E56
D45, M51, D55, P57, K52
D45, M51, D55, P57, E78
Epi#24
D55, K52, E56, P79, F54, E78, Q76
D45, K52, E56, P57, F54, E78, Q76
Epi#25
R84, K86, I105, D107, E108
R96, H28, I26, D29, V3
Epi#26
W33, S2, W3, V32, G30, D29
Epi#27
D53, E56, D55, K52
Epi#28
V32, Q41, K43, E46, K52, F54, P57, D55
G69, Q41, K43, E46, K52, F54, P57, D55
Epi#29
G130, Q99, L127, R96, E78
L127, Q99, L131, R96, E78
G98, Q99, L127, R96, E78
Epi#30
G69, L67, A49, H66, K71, L65, P62
G80, M51, A48, H28, Q99, L127, L131
Epi#33
Q41, F42, V32, S31, S44, K43
Q41, F42, V47, S44, A48, K52
Q41, F42, V47, S44, A49, K52
Epi#34
I105, P112, S89, L110, R19, T21, S37
I105, P112, T111, L20, R19, T21, S37
Epi#37
T63, A49, L60, K52, D55
P62, V74, L60, K52, D45
P62, A61, L60, K52, D55
Epi#38
G77, E78, R96, V82, R84, N116, P112, S89
Epi#39
A48, E46, H66, T63, P62, G58, L60
A49, E46, H66, T63, P62, G58, L60
Epi#40
R19, L110, G113, T111, P109, S89
R19, L110, G113, T111, P112, S89
Epi#41
P62, L65, V47, S44
P109, Y106, L110, S89
P112, Y106, L110, S8
Epi#44
L20, R19, D16, W3, Y6, S2, G17, P109
L110, R19, D16, W3, Y6, S2, G17, P109
Epi#45
K52, P57, F54, R96, D124, L127
D55, P79, F54, R96, D124, L131
Epi#47
I75, G77, E78, V82, R84, N116, P112, S89
I75, G77, E78, I83, R84, N116, P112, P109
Epi#48
E78, Q76, P79, P57, G58
E78, Q76, P79, P57, G80
E78, Q76, P79, P57, G77
Epi#50
D9, W3, W33, S2, T5
D16, W3, W33, S2, T5
Epi#51
R19, H18, E108, S89, K87, K71, H66
R19, H18, E108, D107, K87, K71, H66
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Epi#01
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L116, N111, P106, K80, K81, N101, Y100, Q105, S83

Epi#02
T44, N51, P54, R56, T69, Y78, R71, V68
L24, K93, S92, R75, S76, Y78, R71, R56
Epi#03
L24, K93, I121, Y119
L24, K90, I121, Y119
Epi#04
K80, Y100, S83, Q105, K103, N101, G82
K80, Y100, S83, Q105, K103, T17, G12
K80, Y100, S83, Q105, K103, T17, G14
Epi#05
G34, A33, A36, T38, G64, A63, H66, V68
G34, A33, S32, T17, G12, T4, S1, Y5
Epi#06
A46, N50, D53, R56, A57, P54
A52, N50, D53, R56, A57, P54
A72, N50, D53, R56, A57, P54
A57, P54, D53, S47, Q43, P39
Epi#07
G64, T38, D61, S58, R56, A57, P54, D53
G64, T38, D61, S58, R56, A52, P54, D53
Epi#08
K103, E102, G82, V68, H66, F60
K81, E102, G82, V68, H66, F60
Epi#09
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L24, S47, D53, A57, V68, R71, L70, R56, N51, T44, T38
Epi#10
D74, N50, N51, T44, F60, R56, R71
D53, N50, N51, T44, F60, R56, R71
Epi#11
F125, K93, I121, Q123, D118
F125, K90, I121, Q123, D118
F49, K90, I121, Q123, D118
Epi#12
Y119, E114
Y100, E102
Epi#13
A57, R56, P54, T44, A40, P39, A36, G64, Y67
S58, A57, P54, T44, A40, P39, A36, G64, Y67
Epi#15
N51, P54, D53, I55, R56, A57
R56, P54, D53, I55, T69, A57
R56, P54, D53, I55, T44, A40
Epi#16
Q105, P106, Y100, G14, Q18, S32, A36, A33, D7
Q105, P106, Y100, G14, Q18, S32, A36, A63, D61
Epi#17
A110, S76, R75, S92
A72, S76, R75, S92
Epi#18
N51, N50, R75, S92, L24, S47, T44, P39, N27
N51, N50, R75, S92, L24, T28, T38, P39, N27
Epi#22
D53, S47, D25, L24, K93
D53, S58, D61, V68, K81
Epi#23
K103, N101, E102, S83, Q105, P106
K103, N101, E102, S83, Q105, A84
Epi#24
E114, K115, A110, P106, S83, E102, K103
D53, G59, A57, P54, R56, L70, K80
E102, K103, A15, P106, S83, A84, Q105
Epi#25
R71, R56, 155, D53, N50
R71, R56, 155, D53, N51
Epi#28
I104, Q105, K103, E102, K81, S83, K80
G107, Q105, K103, E102, K81, G82, K80
A84, Q105, K103, E102, K81, S83, K80
A110, Q105, K103, E102, K81, S83, K80
Epi#29
I121, K115, L116, E114
V112, K115, L116, E114
Epi#30
G59, I55, S58, H66, K80, L70, V68
G59, I55, S58, H66, K80, P106, V99
Epi#33
K80, Y78, V68, S58, A57, R56
K81, Y67, V68, S58, A57, R56
Epi#34
I55, P54, S58, V68, R71, Y78, P106
W29, W2, T4, V11, G12, Y5, S1

Epi#36

A63, A36, A33, V11, G14, Y100, S83, Q105, K103, P106, A110, A15

A63, A36, A33, V11, G14, Y100, T108, Q105, K103, P106, A15, A110

Epi#37

A57, R56, L70, R71, Y78

A57, V68, L70, R56, D53

Y78, R71, L70, R56, N51

P54, R56, L70, R71, D73

T69, R71, L70, R56, D53

Epi#38

G82, E102, A84, V99, N101, P106, S83

Epi#40

R71, L70, A72, Y78, K80, S83

R71, L70, G59, T69, K81, S83

R56, L70, A72, T69, K81, S83

Epi#41

P106, Y78, L70, V68, S58

Epi#42

P54, S47, N51, R56, R71

P54, S58, G59, R56, R71

Epi#44

S83, Q105, P106, Y78, A110, G107, T108

V68, R71, D73, Y78, A110, G107, T108

L70, R71, D73, Y78, A110, V112, T108

L70, R71, D73, Y78, A110, G107, P106

Epi#45

K81, H66, F60, R56, D53, G59

K80, H66, F60, R56, D53, G59

D61, H66, F60, R56, D53, G59

Epi#46

L70, R71, R56, P54, N51, A52

L70, R71, R56, P54, N51, A72

V68, R71, R56, P54, N51, A46

Y78, R71, R56, P54, G59, A57

Epi#47

V68, A57, R56, L70, R71, A52, N51, P54, S58

S58, A57, R56, L70, R71, A72, N51, P54, S47

Epi#49

D25, L24, Q43, Q41, T44, N51

D25, L24, Q43, Q41, T38, N27

Epi#50

D7, W2, W29, S1, T4

D7, Y5, W2, W29, S1

Epi#51

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K80, H66, D61, T38, P39, T28, W29

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Epi#01

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Epi#02

L42, K43, Q45, F66, T63, Y72, R84, V74

L42, K43, Q45, F66, T63, Y72, R84, V82

Epi#03

K96, I127, Y125

K86, I75, Y72

Epi#05

G77, A81, F54, P57, G58, A61, T63, V74

G58, A61, F59, P57, G77, A81, T97, G80

G80, A81, F54, P57, G58, A61, T63, Y72

Epi#06

G17, P109, D107, T21, K38, P40

G112, P109, D107, T21, K38, P40

G88, P89, D107, T21, K38, P40

Epi#08

K52, E55, G58, V74, F66

K51, E55, G58, A61, F59

Epi#09

D29, D48, K52, F59, A61, T63

D29, D48, K51, F59, A61, T63

Epi#10

E108, T111, N18, T21, F39, G68, K71

E108, T111, N18, T21, F105, G112, K86

Epi#11

F105, K86, I75, Q76, V82, E78

F66, K43, I47, Q28, V32, D29

F59, K52, I47, Q28, V32, D29

Epi#12

Y125, E130

Y125, E128

Epi#15

K43, P44, D29, I47, K52, G58

K43, P44, D48, I47, Q45, G49

K43, P44, D29, I47, K51, G80

Epi#20
K38, P40, F39, L42, K43, D48, G30, D29
K51, P57, F59, L60, K52, D48, G30, D29
Epi#22
D48, P44, D29, V32, W33
D48, P44, D29, V32, W3
Epi#24
D29, K51, E56, P57, F59, E55, Q79
D48, K52, E55, P57, F59, E56, Q79
Epi#25
R121, K95, I83, D53, E55
R121, K95, I83, E78, V82
Epi#26
W33, S2, W3, V32, G30, D29
Epi#27
E128, E130, D124, K96
E130, E128, D124, K95
Epi#28
I75, Q76, E78, Q79, P57, K51
A61, Q76, E78, Q79, P57, K52
V32, D29, Q99, E130, I127, S129, D124
V32, D29, Q99, 1127, E128, S129, D124
Epi#29
V32, Q41, L42, F66, E70
G69, Q41, L42, F66, E70
G68, Q41, L42, F66, E70
Epi#30
G17, N18, H19, Q114, L117, V15
G17, M110, H19, Q114, L117, V15
G113, M110, H19, Q114, L117, V15
Epi#33
Q41, F39, P40, S36, A37, K38
Epi#34
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Epi#37
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T111, V15, L117, R121, D124
Epi#39
A81, E55, P57, G58, L60
A81, E78, P57, G58, L60
Epi#40
R121, L117, G112, Y106, P109, T111
R121, L117, G112, Y106, P89, T111
Epi#41
Y125, L131, S129
Epi#44
I75, R84, Y72, A61, G58, P62
I75, R84, Y72, A61, V74, T63
Epi#45
K38, P40, F105, Y106, N18, D14, G17
K38, P40, F105, Y106, N18, D107, G88
K38, P40, F105, Y106, N18, D14, V15
Epi#48
E16, H19, P109, P89, G88
E16, H19, P109, P89, G112
Epi#49
D124, L131, Q99, Q28, T97, N98
D124, L131, Q99, Q28, T97, K96
Epi#50
D9, Y6, W3, W33, S2
D9, W3, W33, S2, S5
D9, W3, W33, V32, S31
Epi#51
D14, H19, E108, T111, L117, R121, H10
D107, H19, E16, Q114, L117, R121, H10
D14, H19, D107, T21, K38, Q35, W33
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Epi#01
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Epi#02
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T53, K50, A52, R56, T69, Y67, R66, V68
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Epi#03
L116, K115, I121, Y119
Epi#04
K81, Y100, S83, Q105, K103, T17, G12
K80, Y100, S83, Q105, K103, A84, G82
K81, Y100, S83, Q105, K103, T17, G14
K80, Y100, S83, Q105, K103, N101, I104
K81, Y100, S83, Q105, K103, A15, G107
Epi#06
A54, N47, D25, T28, A36, P39
A40, N27, D25, T28, A36, P39
A44, N47, D25, T28, A36, P39

G34, A33, D7, T31, A36, P39
A43, N47, D25, T28, A36, P39
Epi#08
K103, E102, G82, V68, F60
K103, E102, G82, V68, F60
K81, E102, G82, V68, F60
Epi#10
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E61, N58, R56, S57, F60, R66, K80
Epi#11
F125, K93, I121, Q105, E102
F125, K93, I121, Q123, D118
Epi#12
Y100, E102
Y119, E114
Epi#13
A52, A44, P39, A43, H24, S92, G124, Y119
A46, A44, P39, A43, H24, S92, G124, Y119
Epi#15
K103, P106, D118, I121, K93, G124
K103, P106, D118, I121, Q105, G107
K103, P106, D118, I121, Q123, G122
Epi#16
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Q105, P106, Y78, R71, R56, D51, D74, A52, N47
Epi#18
R66, N58, R56, S57, V68, G82, S83, E102, N101
R66, N58, R56, S57, V68, G82, S83, P106, N101
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D74, A52, D51, T53, K50
D25, A44, D51, T53, K50
D74, A46, D51, T53, K50
D74, A72, D51, T53, K50
Epi#23
K103, N101, E102, S83, Q105, P106
K103, N101, E102, S83, Q105, A84
Epi#24
D74, K81, A84, P106, S83, E102, K103
D74, K81, E102, P106, T108, A15, K103
Epi#25
R66, K81, E102, N101
Epi#28
I121, D118, Q105, K103, E102, K81, G82, D74
G107, D118, Q105, K103, E102, K81, G82, D74
G122, D118, Q105, K103, E102, K81, G82, D74
Epi#29
I121, K115, L116, E114
V112, K115, L116, E114
Epi#30
I55, N47, A44, H24, K93, I121, L116
I55, N47, A43, H24, K93, I121, L116
Epi#31
R56, N58, R66, F60, V68, I55, D51
R66, N58, R56, F60, V68, I55, D51
Epi#33
K115, Y119, P106, S83, A84, K103
Q123, Y119, P106, S83, A84, K103
K81, Y67, V68, S57, A54, R56
K80, Y78, V68, S57, A54, R56
Epi#34
W29, W2, T8, V11, G12, T4, S1
W29, W2, T4, G12, G14, T13, T8
Epi#37
T108, V112, L116, K115, Y119
T108, A110, L116, K115, N111
T13, V112, L116, K115, D118
P106, A110, L116, K115, N111
Epi#38
G64, E61, A40, V37, N27, P39, S38
G82, E102, A84, V99, N101, P106, S83
Epi#39
A110, E114, T108, P106, G122, L116
Epi#40
G14, G12, T17, K103, S83
R56, A52, T53, A54, S57
R66, A63, T65, K81, S83
R56, A72, T53, A54, S57
R56, G59, T53, A54, S57
R66, G64, Y67, K81, S83
Epi#42
P106, S83, G82, R75, R71
Epi#44
S1, Q3, D7, W2, Y5, S32, G12, T8
S1, Q3, D7, W2, Y5, A30, A36, P39

S1, Q3, D7, W2, Y5, S32, V11, T8
S1, Q3, D7, W2, Y5, S32, G12, T4
S1, Q3, D7, W2, Y5, A30, A33, T31
S1, Q3, D7, W2, Y5, A30, A36, T28
S1, Q3, D7, W2, Y5, S32, G12, T13
S1, Q3, D7, W2, Y5, S32, G34, T31
Epi#45
K93, H24, F49, R75, D74, G82
D25, H24, F49, R75, D74, G82
Epi#47
A36, G64, E61, A40, A44, A54, N58, R56, S57
Epi#50
D7, Y5, W2, T8, S1
D7, W2, W29, T28, P39
Epi#51
K90, H24, K93, D25, P39, T28, W29
T91, H24, K93, D25, P39, T28, W29
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Epi#01
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Epi#02
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T50, K54, L62, T65, Y74, R86, V84
Epi#03
L133, K98, I129, Y127
Epi#04
S40, Q43, K45, T50, G32
S40, Q43, K45, T50, G51
S40, Q43, K45, T50, I49
Epi#05
G82, A81, A83, P59, G60, A63, T65, V76
G82, A83, A81, P59, G60, A63, H61, V76
G79, A81, A83, P59, G60, A63, T65, V76
Epi#06
G70, P46, D31, T50, K54, P59
A81, P59, D55, T50, Q47, P46
G32, P46, D31, T50, K45, P42
G51, P46, D31, T50, K54, P59
Epi#08
A81, E57, G60, A63, H61, F56
A81, E57, G60, V76, H68, F44
K54, E57, G60, A63, H61, F56
Epi#11
F56, K98, I85, Q78, V84, E122
F56, K98, I27, Q37, V34, D31
F56, K97, I85, Q78, V84, E80
Epi#12
Y6, E9
Y127, E122
Epi#13
H68, L62, P64, T65, A63, P59, A81, G82, G79
H61, L62, P64, T65, A63, P59, A81, G79, F56
H68, L62, P64, T65, A63, P59, A83, G79, G60
Epi#15
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K45, P46, D31, I49, K54, G60
K45, P46, D31, I49, K54, G82
K45, P46, D31, I49, T50, G51
Epi#16
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Q116, P114, Y108, M12, Q37, S40, A23, A24, D8
R86, P114, Y108, M12, S39, S40, A23, A24, D8
Epi#22
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D130, L124, D126, Y127, E122
D130, L128, D126, Y127, E122
Epi#23
R123, N118, E122, L124, L11, A23
R123, N118, E122, L124, L11, A36
R123, N118, E122, L124, L11, A24
Epi#24
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E57, K54, E58, P59, F56, A81, Q78
E58, G60, E57, P59, F56, E80, Q78
Epi#25
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R86, K88, I77, E80, V84
R86, K88, I107, E109, V112
Epi#27
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D55, E57, E58, K54
Epi#28
V34, D31, Q101, K98, E122, L128, Q131, G132, D130
I129, D126, Q131, L128, E122, K98, Q101, G100, D130

I72, H68, Q47, F44, E48, K45, Q43, G70, K73
I72, H68, Q47, I49, E48, K45, Q43, G71, K73
Epi#29
I129, Q101, L128, R123, E122
G132, Q131, L128, R123, E122
Epi#30
I77, M75, A63, H61, P59, L62, P64
G90, M75, A63, H61, K54, L62, P64
Epi#33
Q116, Y108, P111, S91, K89
K88, Y108, P111, S91, K8
Epi#34
V76, P64, M75, L62, G51, T50, P46
I27, W35, S33, V34, G32, T50, P46
V76, P64, T65, L62, G51, T50, P46
Epi#35
A24, L22, A23, S39, M12, 1107
A23, L11, A36, S39, M12, I10
Epi#37
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Y108, A23, L11, R123, Y127
A23, A24, L11, R123, Y127
Epi#39
A81, E57, H61, T65, P64, G60, L62
A81, E58, H61, T65, P64, G60, L62
Epi#40
R123, L11, A23, Y108, P111, S91
R123, L11, A24, Y108, P111, T113
Epi#41
P111, Y108, L22, V112, S91
P114, Y108, L22, V112, S91
Epi#43
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Epi#44
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L133, Y127, E122, M119, R123, L124, N118, R86, P114
Epi#48
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S91, K88, P114, P111, V112
Epi#50
H10, Y6, W3, S2, T5
H10, Y6, W3, T5, S39
Epi#51
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Q101, H30, D31, T50, K45, Q47, H68
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Epi#03
L4, K37, A33, I34, Y17
L4, K37, A33, I34, Y29
Epi#05
A33, N36, T40, G3, S20, L4
A33, N38, T40, G3, S20, Y25
A33, N36, G3, T40, S20, I22
Epi#06
A33, N36, D2, C19, K24, P21
A33, N38, D2, S20, K24, P21
Epi#09
I22, L4, D2, N38, D1, K37, A33, N36, T40
T9, G15, E7, V14, D30, K32, N36, T40, L4
T9, G15, E7, V14, D30, K32, N38, N36, L4
Epi#12
Y17, E7
Y6, E7
Epi#20
V27, K24, P21, L4, K37, D2, G3, D1
V27, K24, P21, L4, N36, D2, G3, D1
Epi#22
D1, D2, L4, K37
D1, D2, P21, K24
D2, L4, T40, D1
Epi#23
N10, E7, Y6, L4, P21
Epi#25
K32, I34, D30, V14
K37, I34, D30, V14
K16, I34, D30, V14
Epi#33
K32, Y17, V27, S20, K24
K16, Y6, P21, S20, K24

Epi#34
I22, P21, S20, V27, G12, Y17, T9
I22, P21, S20, V27, G12, Y29, S31
Epi#40
G12, G15, Y29, K37, T40
G15, G12, Y17, K16, T9
G12, G15, Y29, K32, S31
Epi#41
P21, Y6, L4, S20
Epi#44
L4, D2, P21, Y25, S20, V27, T40
L4, D2, P21, Y25, S20, G3, T40
Vesv5:
Epi#01
L59, P67, P65, K143, K144, N64, Y140, R62, T61
L59, P67, P70, R57, K204, N73, Y201, Q202, T203
L59, P67, P69, R57, K72, N73, Y201, Q202, T203
L152, N149, P142, K145, K143, N64, Y140, R62, T61
Epi#02
L9, K7, Q108, P191, Y107, R102, V13
L9, K7, Q108, S192, Y107, R102, V13
Epi#03
L9, K7, A105, I6, Y3
Epi#04
K106, Y107, S192, Q108, K7, A105, I6
K106, Y107, S192, Q108, K7, V13, G12
Epi#05
G58, A56, R57, P69, G66, R62, T61, L59
G58, A56, R57, P69, G63, R62, T61, L59
Epi#06
G66, N64, D139, R62, K138, P67
G66, N64, D139, R62, K138, P65
G63, N64, D139, R62, K138, P67
Epi#08
K145, E199, S147, F151
K196, E198, S147, F151
K144, E199, S147, F151
Epi#09
L152, D150, S147, K144, N64, T61, L59
L152, D150, D139, K153, F151, S147, N197
D139, N64, R62, D135, K153, F151, S147, N197
Epi#10
E199, N197, N194, S147, F151, G148, K143
E199, N197, N194, S147, F151, G148, K196
E199, N197, N194, S147, F151, G148, K145
Epi#11
K179, I176, Q177, V30, E178
K29, I176, Q177, V30, E178
Epi#12
Y201, E199
Epi#13
S147, L200, P142, T203, A56, P70, L59, P67, G66
S147, L200, P142, T203, A56, P69, L59, P67, G58
S147, L200, P142, T203, A56, P70, L59, P67, G63
S147, L200, P142, T203, A56, P69, L59, P67, Y140
Epi#15
K106, P191, D103, I6, K5, A105
K106, P191, D103, I6, K7, G12
Epi#16
R57, P70, Y201, M74, Q53, N76, D50, A56, N73
R57, P69, Y201, M74, Q53, N76, D50, A56, N73
Q108, P191, Y107, R102, Q111, S192, D103, A105, N2
Epi#18
R57, L59, T61, P67, N64
R57, L59, T61, P65, N64
Epi#19
E167, N164, S192, Q108, R102, K7
E198, N194, S192, Q108, R102, K7
D103, T100, C8, Q108, R102, K7
Epi#22
L9, D103, T100, K10
A105, D103, L9, K7
D50, L45, D43, T37, K38
S147, D150, L152, K153
Epi#23
K196, N197, E199, N164, Q202, P70
K145, N197, E199, N164, Q202, P69
Epi#24
E198, K196, E199, P142, T203, P69, K143
E198, K145, E199, P142, T203, P70, K204
E198, K196, E199, P142, T203, P70, K72
E198, K145, E199, P142, F146, F151, K196

Epi#25
R57, K54, D50, N76
R57, K54, D50, E47
Epi#27
D43, E40, D125, K122
D50, E47, D43, K38
Epi#28
Q202, E199, K196, F151, S147, K144
Q202, E199, K196, F195, S147, K145
Epi#29
G58, R57, L59, R62, E136
G148, K145, L200, F195, E199
G148, K145, L200, F195, E198
Epi#33
K23, Y19, P24, S21, A16, K18
K23, Y34, P24, S21, A16, R102
Epi#34
I176, W180, T116, L115, G117, T119, S118
V31, P24, S21, L22, G35, Y34, T37
Epi#37
P69, R57, L59, K54, D50
P70, R57, L59, R62, D135
A56, R57, L59, R62, N64
P69, R57, L59, R62, D139
Epi#39
E199, L200, T203, P70, G58, L59
E198, L200, T203, P69, G58, L59
Epi#40
R57, L59, G58, T203, P69, T61
R57, L59, A56, Y201, K204, T203
R57, L59, A56, Y201, K72, T203
Epi#41
P24, Y19, L22, S21
P24, Y34, L36, S33
Epi#42
P191, S192, Q111, H98, R102, Q108
Epi#44
L59, R57, P70, Y201, A56, G58, T61
L59, R57, P69, Y201, A56, G58, T203
L59, R57, P70, Y201, A56, G58, P67
Epi#45
K153, H156, F151, Y140, N149, D150, L152
D135, H156, F151, Y140, N141, D150, L152
K143, P142, F146, Y140, N149, D150, L152
Epi#47
G58, L59, R57, M74, A56, Q202, N73, P70, P69
G148, Y140, R62, L59, R57, A56, N73, P70, P67
G66, G63, R62, L59, R57, A56, N73, P70, P67
G155, E136, R62, L59, R57, A56, N73, P70, P67
Epi#48
Q202, K204, P69, P67, G58
Q202, K204, P70, P67, G63
Q202, K72, P70, P67, G66
Epi#49
D125, D43, L45, V78, Q42, Q39, T37, K38
D125, D43, L45, V78, Q42, Q39, T37, K41
Epi#50
H98, Y96, W90, L22, S21
H98, Y96, W90, P24, S33
Epi#52
F0, A16, R102, W90, N25, Q95
F0, A16, R102, W90, N25, Q93
Betv1:
Epi#03
SAS: 270, Size 11.07: L24, K20, H76, I23, Y81
SAS: 204, Size 11.96: L24, K20, A16, I23, Y81
Epi#05
SAS: 298, Size 14.01: G110, A106, A16, P14, G111, T10
SAS: 242, Size 14.01: G110, A106, A16, P14, G111, T107
Epi#08
SAS: 464, Size 11.12: K123, E127, G1, H121, F3
SAS: 455, Size 12.95: K129, E127, G1, H121, F3
SAS: 438, Size 13.31: K123, D125, G1, H121, F3
SAS: 428, Size 11.12: K123, E127, V2, H121, F3
SAS: 425, Size 11.65: K123, E127, G124, H121, F3
Epi#09
SAS: 466, Size 20.55: D109, A106, V105, K80, A16, T77
SAS: 444, Size 20.55: D109, G110, V105, K80, A16, T77
SAS: 427, Size 20.55: D109, G111, V105, K80, A16, T77
SAS: 398, Size 19.17: T10, G110, V105, K80, A16, T77
SAS: 381, Size 19.17: T10, G111, V105, K80, A16, T77
Epi#10
SAS: 558, Size 15.18: D75, T77, N78, A106, F79, R17, K20
SAS: 549, Size 21.96: E6, T7, N4, F3, G1, K123
SAS: 517, Size 13.31: D75, T77, N78, A16, F79, R17, K20
SAS: 497, Size 15.13: D75, T77, N78, A16, F22, R17, K20

Epi#12

SAS: 335, Size 9.08: T7, Y5, E6, N4

SAS: 331, Size 11.28: R145, Y150, E148, L152

SAS: 326, Size 10.37: R70, Y83, E73, P50

SAS: 311, Size 10.32: 1116, Y5, E6, N4

SAS: 308, Size 8.33: R145, Y150, E148, S149

Epi#18

SAS: 328, Size 24.67: S117, K103, F79, V105, A16, Y158, L24

Epi#22

SAS: 533, Size 9.96: D125, D93, K123, E127

SAS: 533, Size 9.96: D93, D125, K123, E127

SAS: 476, Size 11.40: D125, D93, K123, E96

SAS: 476, Size 11.40: D93, D125, K123, E96

SAS: 400, Size 17.99: D125, D93, P90, E87

Epi#23

SAS: 451, Size 22.02: K68, N43, E42, S57, F64, P63

SAS: 450, Size 22.02: K55, N43, E42, S57, F64, P63

SAS: 428, Size 22.02: K68, N43, E42, S57, L62, P63

SAS: 427, Size 22.02: K55, N43, E42, S57, L62, P63

SAS: 412, Size 18.85: K68, N43, E42, S40, F30, P35

Epi#24

SAS: 734, Size 18.92: E127, K123, E96, P90, S136, E131, K129

SAS: 729, Size 18.92: D93, K123, E96, P90, S136, E131, K129

SAS: 716, Size 19.57: E127, K123, E96, P90, S136, E131, K134

SAS: 711, Size 19.57: D93, K123, E96, P90, S136, E131, K134

SAS: 708, Size 20.49: D125, K123, E96, P90, S136, E131, K129

Epi#25

SAS: 467, Size 12.68: R70, K55, I44, E42, E45

SAS: 425, Size 12.68: R70, K54, I44, E42, E45

SAS: 420, Size 14.01: R70, K55, I44, D27, E42

Epi#27

SAS: 613, Size 14.25: D93, E127, A130, E131, K129

SAS: 595, Size 16.54: D93, E127, A130, E131, K134

SAS: 592, Size 16.70: D125, E127, A130, E131, K129

SAS: 574, Size 19.79: D125, E127, A130, E131, K134

SAS: 524, Size 18.78: D93, E127, A130, E131, K137

Epi#28

SAS: 869, Size 21.93: V33, Q36, F58, E60, L62, F64, P63, K65

SAS: 837, Size 21.83: V33, Q36, F58, E60, L62, F64, G61, K65

SAS: 808, Size 24.56: V33, Q36, F58, E60, L62, F64, P90, K65

SAS: 783, Size 21.83: V33, Q36, F58, E60, K65, F64, S57, K68

SAS: 782, Size 21.83: V33, Q36, F58, E60, L62, F64, S57, K65

Epi#29

SAS: 516, Size 9.52: G61, K65, L62, E60

SAS: 440, Size 8.70: G61, P63, L62, E60

SAS: 371, Size 6.78: G61, P59, L62, E60

Epi#32

SAS: 374, Size 17.88: F79, A16, A106, D109, V12

SAS: 354, Size 20.42: F22, A16, A106, D109, V12

Epi#33

SAS: 541, Size 18.79: K65, F64, P90, S136, A135, K134

SAS: 498, Size 9.15: Q36, F30, P35, S39, K32

SAS: 496, Size 11.27: Q36, F30, P35, S40, K32

SAS: 494, Size 12.19: Q36, F58, P35, S39, K32

SAS: 493, Size 18.79: K65, Y66, P90, S136, A135, K134

Epi#36

SAS: 447, Size 19.17: T77, A16, A106, V12, G110, T10

SAS: 430, Size 19.17: T77, A16, A106, V12, G111, T10

SAS: 392, Size 19.17: T77, A16, A106, V105, G110, T10

SAS: 391, Size 19.17: T77, A16, A106, V12, G110, T107

SAS: 375, Size 19.17: T77, A16, A106, V105, G111, T10

Epi#40

SAS: 246, Size 21.55: A106, A16, Y158, S155

SAS: 223, Size 13.25: A135, A130, Y5, T7

SAS: 196, Size 14.88: A135, A130, Y5, S117

SAS: 178, Size 10.62: A135, G140, T142, S136

Epi#44

SAS: 530, Size 19.04: L24, R17, D156, Y150, S149, V12, T10

SAS: 492, Size 19.04: 123, R17, D156, Y150, S149, V12, T10

SAS: 490, Size 17.39: L24, R17, D156, Y150, S149, V12, P14

SAS: 483, Size 23.09: L24, R17, D156, Y158, A16, A106, P108

SAS: 474, Size 20.83: L24, R17, D156, Y150, S149, V12, T107

Epi#45

SAS: 606, Size 21.41: K32, P35, F30, Y150, R145, V12

SAS: 546, Size 20.89: K32, P31, F30, Y150, R145, V12

SAS: 533, Size 15.19: K32, P35, F30, Y150, R145, G140

SAS: 533, Size 12.63: K32, P35, F30, Y150, R145, V33

SAS: 532, Size 19.60: K32, P35, F30, N28, D27, I44

Epi#47

SAS: 333, Size 21.03: R17, L24, N28, P31, P35

SAS: 300, Size 22.72: R17, L24, N28, P31, S39

SAS: 298, Size 21.80: R17, L24, N28, P31, S40

SAS: 269, Size 24.87: R17, L24, N28, P31, S57

Epi#48

SAS: 436, Size 14.26: S57, K65, P90, P63, G61

SAS: 414, Size 17.96: S39, K32, P35, P59, G61

SAS: 412, Size 17.96: S40, K32, P35, P59, G61

SAS: 389, Size 18.32: S57, K65, P63, P90, G92

SAS: 365, Size 21.15: S57, K65, P59, P35, V33

**[0578]** "SAS" is solvent accessible surface. "Size" is the total surface area of the epitope in A2.

Derf2:

Epi#02

A98, K100, S101, P99, R128, R31

A98, K100, R128, P99, R31, V94

T91, N93, P95, P34, R31, R128

L61, N93, P95, P34, R31, R128

Epi#03

L40, K15, A39, I13, Y86

L40, K14, A39, I88, Y90

Epi#05

G32, A98, R31, P34, G20, T36, T91, Y90

G32, A98, R31, P34, G20, T36, T91, V94

G32, A98, R31, P34, G20, T36, T91, L37

G32, A98, R31, P34, G20, T36, T91, V18

Epi#06

A98, P99, D129, R31, K96, P95

G32, P99, D129, R128, R31, P95

A98, P99, D129, R31, K33, P95

A98, P99, D129, R31, K96, P34

A98, P99, D129, R128, K126, P26

Epi#07

T107, S57, D59, S101, R128, A98, P99, D129

T107, S57, D59, S101, R31, A98, P99, D129

Epi#08

K15, D87, V76, H74, F75

K14, D87, V76, H74, F75

K77, D87, V76, H74, F75

Epi#09

L61, D64, I68, H74, F75, T70, N71

N114, N46, D113, K48, N71, T70, T49

G83, N46, D113, K48, N71, T70, T49

Epi#10

L40, I13, D42, N44, V81, K48, N46, N114, G115

L40, I13, D42, N44, V81, K82, N46, N114, G115

L37, D19, G20, V18, V3, D4, K6, A120, T107, V105

Epi#11

F75, K51, I111, Q45, V116, D113

F75, K51, I111, Q45, V81, D113

Epi#12

Y90, E38

Epi#13

H30, R31, P95, A98, P99, S101, G60, L61

Epi#15

K96, P99, D129, 128, R128, A98

K96, P99, D129, I127, R128, A98

**[0579]** K96, P99, D129, 129, R128, A98

K55, P66, D64, I68, T70, G67

Epi#18

R31, R128, I28, G125, T123, H124, V105

R31, R128, I127, G125, T123, H124, V105

Epi#22

D1, M17, D4, V3, K6

D1, M17, D19, P34, K96

D1, M17, D4, V5, K6

Epi#23

K14, N11, E12, N44, Q85, P79

K14, N11, E12, N10, Q45, P79

K14, N11, E12, N44, Q84, P79

K14, N11, E12, L40, Q85, P79

Epi#24

D129, K100, E102, P99, R128, R31, K96

E62, G60, E102, P99, R128, R31, K96

D129, K126, E102, P99, R128, R31, K33

D129, K126, E102, P99, R31, P95, K96

Epi#25

R31, K96, I97, D59, E62

R128, R31, 197, D59, E102

R128, K126, I127, E102, N103

Epi#27
D64, E62, D59, K100
D59, E62, D64, K55
D87, E38, D19, K33
D19, E38, D87, K15
D19, E38, D87, K14
D19, E38, D87, K77
Epi#28
V16, D87, Q85, K14, E12, K15, Q2, D1
I13, D87, Q85, K14, E12, K15, Q2, D1
V3, D1, Q2, K15, E12, K14, Q85, D87
L40, D87, Q85, K14, E12, K15, Q2, D1
I88, D87, Q85, K14, E12, K15, Q2, D1
V76, D87, Q85, K14, E12, K15, Q2,D1
V18, D1, Q2, K15, E12, K14, Q85, D87
Epi#29
G32, N93, L61, E62
V94, N93, L61, E62
Epi#30
G60, I97, A98, H30, K96, P34, P95
I68, N71, H74, K77, P79, V81
G32, I97, A98, H30, K96, P95, P34
Epi#34
V105, P26, S24, G125, R128, S101, P99
W92, P34, T91, V94, R31, S101, P99
I28, P26, T123, G125, R128, S101, P99
Epi#37
A120, V16, L40, K14, N11
A39, V16, L40, K14, N11
Y90, A39, L40, K14, N11
Y86, A39, L40, K14, N11
Epi#39
A120, E38, T91, P34, G20, L37
A39, E38, T91, P34, G20, L37
Epi#40
G20, L37, A120, T123, K6, S24
A39, L37, A120, T123, K6, S24
G20, L37, A120, T107, K6, T123
Epi#41
P34, L37, V106, S57
Epi#42
P26, S24, G125, R128, R31
P99, S101, G125, R128, R31
Epi#44
V16, Q2, D19, P34, W92, Y90, A39, V18, T91
V16, Q2, D19, P34, W92, Y90, A39, V5, T123
V3, Q2, D19, P34, W92, Y90, A39, V18, T91
Epi#45
K77, H74, F75, N71, D69, G67
K77, H74, F75, N71, D69, V76
K77, H74, F75, N71, D69, V65
Epi#46
A98, R128, R31, P95, N93, G32
A98, R128, R31, P34, G20, Q2
Epi#48
Q2, D19, P34, P95, G32
H30, K96, P95, P34, G20
Epi#49
D87, D42, L40, Q85, Q84, C78, T47, Q45, K48
D87, D42, L40, Q85, Q84, C78, T47, Q45, K82
Epi#50
D19, W92, P34, T91
D19, W92, P34, P95
D19, W92, T91, T36
Epi#51
D129, H30, K33, R31, R128, K126, H124
R31, H30, D129, R128, K100, K126, H124
T123, H124, K126, R128, R31, K33, H30
Derp2:
Epi#03
L17, K89, A39, I13, Y86
L17, K89, A72, I88, Y90
L17, K89, A72, I52, Y90
Epi#04
K15, S1, Q2, K14, V16, L17
K15, S1, Q2, K14, A39, L17
K15, S1, Q2, K14, V40, I13
Epi#05
G60, A56, L61, P99, G32, R31, H30, I97
G60, A56, L61, P99, G32, R31, H30, I28
Epi#06
G60, A56, D64, S57, K55, P66
G83, N46, D114, T49, K48, P79
G60, N103, D59, S101, R31, P95

Epi#08
K55, D64, S57, V106, F35
K55, E62, S57, V106, F35
Epi#09
L61, G60, E102, R128, 128, K126, N103, T123, V105
L61, G60, E102, R128, 1127, K100, N103, T123, V105
L61, G60, E102, R128, 1127, H124, N103, T123, V105
Epi#10
SAS: 435, Size 24.47: D69, T91, N93, F35, G32, R31
SAS: 422, Size 20.74: E38, T91, N93, F35, G32, K96
Epi#11
K14, I13, Q85, V81, E42
K15, I13, Q85, V81, E42
K14, I13, Q85, V40, D87
Epi#12
Y86, E42
Y90, E53
Y90, E38
Epi#13
H30, A125, P26, T123, A122, P19, L37, P34, W92
H30, A125, P26, T123, A122, H124, S24, G23, G20
H30, A125, P26, T123, A122, P19, L17, G20, F35
Epi#15
K55, P66, D69, I68, K89, A72
K55, P66, D69, I68, K89, A39
K55, P66, D64, I54, K109, G115
K55, P66, D64, I54, K109, A9
Epi#18
R31, 129, A125, S101, E102, N103
R31, 129, A125, S101, E102, V104
R31, 129, A125, T123, A122, V105
Epi#22
D69, P66, D64, V65, K55
D64, P66, D69, T91, K89
D59, L61, D64, P66, W92
D59, L61, D64, V65, E62
D69, P66, D64, V65, E53
Epi#24
D64, K55, E62, P99, R31, P34, K96
E53, K55, E62, P99, R31, P95, K96
D64, K55, E62, P99, R31, A98, K96
Epi#25
R31, H30, I28, E102, N103
R128, K126, I127, E102, N103
R128, K126, I28, E102, V105
Epi#27
D64, E53, D69, K89
D69, E53, D64, K55
D59, E62, D64, K55
Epi#28
V40, D87, Q85, E42, Q84, G83, K82
G20, H22, Q2, L17, E38, L37, Q36, P34, K33
G20, H22, Q2, L17, E38, L37, F35, P34, K33
Epi#29
I97, K100, L61, E62
G60, N103, L61, E62
I127, N103, L61, E62
Epi#30
G60, N103, S101, H30, K96, I97, P95
G60, N103, A125, H30, K96, I97, P95
I28, I127, A125, H30, K96, I97, P95
Epi#33
Q36, F35, V106, S57, A56, K55
K33, F35, V106, S57, A56, K55
Epi#34
I28, P26, S24, G23, G20, T123, S57
I28, P26, S24, V3, G20, T123, T107
W92, P34, T91, V18, G20, T123, P26
Epi#37
P66, V63, L61, K100, N103
P95, A98, L61, K100, N103
P19, V18, L17, K89, D87
P19, V3, L17, K89, D87
T123, V104, L61, K100, N103
Epi#38
L61, G60, E102, A125, V105, N103, P99, S57
L61, G60, E62, A56, V105, N103, P99, S57
Epi#39
A125, E102, H124, T123, P26, G20, L17
Epi#40
G60, L61, A56, T107, K6, T123
A39, L17, G20, T123, P26, S24
G60, L61, A56, T107, K55, S57
G60, L61, A56, T123, K126, S101

Epi#41

P19, L17, V3, S1

P19, L17, V5, S24

Epi#44

V65, D64, P66, W92, Y90, A39, V18, P19

L61, D64, P66, W92, Y90, A39, V18, T91

Epi#45

R31, P34, F35, N93, V94

K96, P34, F35, N93, G32

Epi#47

I127, S101, R31, 197, A98, L61, N103, P99, P95

I28, S101, R31, 197, A98, L61, N103, P99, S57

Epi#48

H30, K96, P95, P99, G60

H30, K96, P34, P19, G20

H30, K96, P34, P19, V18

H30, K96, P34, P95, V94

H30, K96, P34, P19, V3

E38, K89, P70, P66, V65

H30, K96, P95, P34, G32

Q36, K89, P70, P66, V65

Epi#50

D69, Y90, W92, P66, P70

D69, Y90, W92, P34, P95

D69, Y90, W92, T91, P34

D69, Y90, W92, V94, P95

D69, Y90, W92, L37, P19

Epi#51

K126, H124, E102, R128, 128, R31, H30

T123, H124, K126, R128, 128, R31, H30

D4, H124, K126, R128, 128, R31, H30

**[0580]** PhIp2:

Epi#02

T87, K85, Q61, S38, R34, R67

T87, K85, Q61, P63, R34, V42

Epi#03

K10, A90, I88, Y86

K10, A18, I88, Y86

Epi#04

R34, S38, Q61, K85, T87, I88

R34, S38, Q61, K85, T87, A90

Epi#05

G47, A18, S12, T87, G89, T91, T5, V1

G73, A29, L69, T27, G50, T53, T45, V42

G11, A18, L20, T91, G89, A90, T87, I88

Epi#06

A93, P94, D79, R34, Q61, P59

A93, P94, D79, R34, Q61, P83

A93, P94, D80, R34, Q61, P59

A93, P94, D79, R34, Q61, P63

Epi#08

K10, E9, G11, A18, H16, F54

K46, E48, G47, A18, H16, F54

K10, E9, S12, A18, H16, F54

Epi#09

L69, T27, G73, N76, R67, V77, D79, R34, A43, T45, V42

L69, T27, A29, E30, R67, V77, D80, R34, A43, T45, V42

Epi#10

D55, A18, N13, S12, F54, G47, K46

T45, A18, N13, S56, F54, G47, K46

Epi#09

L60, S56, E57, D55, K15, N13, S12, G11

L60, S56, E57, D55, H16, F54, T45, T53

L60, S56, E57, D55, H16, F54, T45, G47

Epi#12

Y86, E84

Y23, E24

Epi#18

N76, R67, F78, V81, A93, Y92, T91, T5, P2, V1

Epi#19

D39, W41, S38, Q61, R34, G37

E40, W41, S38, Q61, R34, A43

Epi#22

D79, P94, D80, P83, K85

D79, P94, D80, P63, K85

Epi#23

K10, N13, E14, L60, Q61, P59

K10, N13, E14, L60, Q61, P83

K10, N13, E14, L60, Q61, P63

Epi#24

E58, K15, E57, P59, S56, E14, Q61

D55, K15, E57, P59, S56, E58, Q61

Epi#25

R34, R67, W41, D39, E40

Epi#26
S38, E40, W41, V42, E32, E30
S38, E40, W41, V42, A43, E32
Epi#27
E14, E57, E58, K15
D55, E14, E84, K85
Epi#28
G37, H36, Q61, K85, E84, L60, F54, A43, K46
G37, H36, Q61, K85, E84, L60, F54, S12, D55
G37, H36, Q61, K85, E84, L60, F54, S56, D55
G37, H36, Q61, K85, E84, L60, F54, A43, R67
G37, H36, Q61, K15, E57, L60, F54, A43, K46
G37, H36, Q61, K85, E84, L60, F54, S12, K15
G37, H36, Q61, K85, E84, L60, F54, S56, K15
G37, H36, Q61, K85, E84, L60, F54, A43, R34
G37, H36, Q61, K85, E84, L60, F54, A18, D55
Epi#29
G73, K72, L69, R67, E30
I88, N13, L60, F54, E57
G25, K72, L69, R67, E32
V77, K75, L69, R67, E30
G37, H36, L60, F54, E57
G37, Q61, L60, F54, E57
Epi#30
I88, N13, S12, H16, K15, P59, L60
I88, N13, S56, H16, K15, L60, P59
I88, N13, A18, H16, K15, P59, L60
Epi#33
K46, F54, V42, S56, K15
H16, F54, V42, S56, K15
Epi#34
V1, P2, T5, V4, P94, Y92, T87
V1, P2, T5, L20, G89, T91, T87
V81, P94, T5, V1, P2, Y92, T91
Epi#37
T27, A29, L69, K72, D26
A43, R67, L69, K75, N76
Epi#38
L20, G89, E9, A18, N13, P59, S56
Epi#40
G49, L20, G89, Y86, K85, T87
G49, L20, G89, T87, K10, S12
G49, L20, G89, T87, K10, T7
Epi#44
V77, R67, D79, P94, Y92, A93, V1, P2
L69, R67, D79, P94, Y92, A93, V1, T5
Epi#45
D79, P94, F78, N76, M74, L69
D80, P94, F78, R67, D79, V77
K3, P94, F78, N76, M74, G73
Epi#46
A43, R67, R34, P63, H36, Q61
V77, R67, R34, P63, H36, G37
L69, R67, R34, P63, G37, Q61
Epi#47
G37, E35, E40, A43, R34, L60, N13, P59, S56
V77, E32, E40, A43, R34, L60, N13, P59, S56
S38, G37, E40, A43, R34, L60, N13, P59, S56
Epi#48
E24, K3, P94, P2, V1
E84, D80, P94, P2, V1
Epi#50
D39, W41, A43, T45
D39, W41, V42, T45
Epi#51
D79, H36, E84, T87, K10, G11, H16
D39, H36, Q61, K85, P63, R34, W41
D79, H36, E40, D39, G37, R34, W41
Q61, H36, E84, T87, K10, G11, H16

Example 11

**[0581]** For this example a third-generation epitope sequences were determined for some additional enzymes and redetermined for all of the enzymes in example 1-3. New enzymes are AMG (AMG pdb), BPN" (1sup.pdb), Esperase (structure see Appendix D), Natalase (structure modelling based on SP722), Amylase-AA560 (Structure modelling based on SP722), Protease A, Alcalase, Protease B, ProteaseC, ProteaseD, ProteaseE, Properase and Relase based on their sequences and structures. The structures of Protease B, Properase, Relase, Protease A, Alcalase, ProteaseC, ProteaseD and ProteaseE can be found by "Homology modelling" (see above) and computer modelling of the epiope patterns that had been assembled in our database (shown in Table 8). Furthermore, the epitope sequences were redetermined for CAREZYME, Laccase, PD498, Savinase, Amylase SP722, and Cellulase, according to the method.

**[0582]** The protein surface is scanned for epitope patterns matching the given "consensus" sequence of about 6-12 residues. First, residues on the protein surface that match the first residue of the consensus sequence are identified. Within a specified distance from each of these, residues on the protein surface that match the next residue of the consensus sequence are identified. This procedure is repeated for the remaining residues of the consensus sequence. The method is further

described under the paragraph "Methods" above and the program can be found in Appendixes.

**[0583]** The critical parameters used in this screening included:

**[0584]** i) a maximal distance between the alpha-carbon atoms of subsequent amino acids,

**[0585]** ii) a minimal accessability of the amino acid of 20 Å2,

**[0586]** iii) the largest maximal distance between the most distinct amino acids should be less than 25 Å

**[0587]** iv) the best epitope were taken,

**[0588]** v) the homology with the epitope pattern of interest was 100%

**[0589]** In this way a number of potential epitopes are identified. The epitopes are sorted according to total surface accessible area, and certain entries removed:

**[0590]** 1) Epitopes that contain the same protein surface residue more than once. These are artefacts generated by the described algorithm.

**[0591]** 2) Epitopes which are "too big", i.e. where a distance between any two residues in the epitope exceeds a given threshold.

**[0592]** The subtilisin sequences and positions mentioned in the following are not given in the BPN' numeration but in the subtilisins own numeration (see the alignment as described above in Tables 1A and 1B).

**[0593]** The epitope sequences found were:

AMG:

Epi#01

L104, P123, P107, R125, R122, N182, S184, Q172, T173

L104, P107, P123, R125, R122, N182, S184, Q172, S453

L104, P107, P123, R125, R122, N182, S184, Q172, T452

Epi#02

L234, R241, S240, F237, T173, Y175, R122, R125

L234, R241, S240, F237, T173, Y169, R125, R122

L234, R241, S240, F237, T173, Y175, R125, R54

Epi#03

L291, K404, I288, Y289

L66, K61, H254, I253, Y329

Epi#04

R122, Y175, S184, Q172, Y169, A454, I455

R122, Y175, S184, Q172, Y169, N171, A451

R125, Y175, S184, Q172, Y169, T452, A451

Epi#06

G31, A24, D25, S30, A27, P41

G146, N145, D144, T148, S149, P467

A471, N145, D144, T148, S149, P467

Epi#07

G294, T290, S405, D293, S287, R286, P307, D283

G294, T290, S287, D293, S296, R286, P307, D283

G207, T204, S200, D214, S209, R160, P157, D153

G294, T290, S405, D293, S287, R286, P307, D309

Epi#08

A27, D25, S30, V111, F49

A24, D25, S30, V111, F49

Epi#09

S149, T148, G146, N145, A471, R68, N69, T72, V470

S73, S76, T72, N69, R68, A471, N145, T148

Epi#10

D238, N182, N236, S240, F237, R241, K244

D238, T173, N182, S239, F237, R241, K244

Epi#11

F49, F109,191, Q85, E113

Epi#12

Y363, E342

Y311, E308

Y175, E180

Epi#13

S119, W120, P123, A102, P94, S92, G90, L98

S119, W120, P123, A102, P94, S92, G96, G90

Epi#15

K244, P307, D283, I288, T290, G294

R160, P157, D153, I154, T462, G90

R286, P307, D283, I288, T290, G294

Epi#16

L410, P46, Y48, R413, S397, S394, A392, A393, N395

R160, P157, Y458, G456, S211, S209, A205, A201, D214

Epi#17

A201, S209, R160, S459

A205, S209, R160, S459

Epi#19

D44, N45, S411, Q409, R413, L410

D47, N45, S411, Q409, R413, L410

Epi#20

K61, P434, L66, L423, N427, D65, G70, D71

Epi#22

D357, S356, D349, V346, D345

D349, S356, D357, A359, D345

D357, S356, D349, L348, D345

Epi#23

K404, N292, E299, S298, L295, A300

K404, N292, E299, S296, L295, A300

Epi#24

D336, K337, E259, P258, S431, L332, K378

D336, K337, E259, P258, S431, R429, K378

D336, K337, A261, P258, S436, E259, Q338

Epi#25

R125, R122, W120, E180, N182

R241, K244, E308, N313

Epi#26

W212, S200, E198, W437, V197, G438, E259

W212, S200, E198, W437, V197, A201, D214

Epi#27

D283, E280, D349, K352

D403, E408, D406, K404

D349, E280, D283, K244

D349, E280, D283, K279

Epi#28

L332, D336, Q338, K337, E259, C262, P272, D345

V374, D336, Q338, K337, E259, C262, P272, D345

G339, D336, Q338, K337, E259, C262, P272, D345

Epi#29

L295, G294, L291, R286, E299

I288, K404, L291, R286, E299

L348, K352, L354, F380, E299

Epi#33

K352, Y355, V374, S371, S365, K337

K352, Y355, V374, S365, S340, K337

Epi#34

V463, W466, S468, V470, P467, T464, T462

I469, W466, S468, V470, P467, T464, T462

I154, W466, S468, V470, P467, T464, T462

V463, W466, S468, V470, P467, S465, T464

Epi#37

T362, A359, L348, K352, D357

T360, V346, L348, K352, D357

T362, A359, L348, K352, D349

Epi#38

G438, E259, A435, R68, L66, N69, P434, S431

Epi#39

A353, E299, R286, P307, G243, L234

A300, E299, R286, P307, G243, L234

Epi#40

A205, L143, G146, Y147, P467, T464

G146, L143, A205, T204, A201, S209

A451, A450, T448, P446, S444

Epi#41

P467, Y147, L143, V206, S149

Epi#42

L66, P434, S431, N430, R429, R428

L104, P123, S95, G101, P94, R122, R125

L104, P107, S95, G96, P123, R125, Q172

Epi#44

L143, Q140, D144, W141, Y147, S468, V470, T72

V206, Q140, D144, W141, Y147, S468, V470, P467

S211, Q216, D214, P218, Y223, A451, A450, T448

S211, Q216, D214, P218, Y223, A450, G447, T448

Epi#45

R413, P46, F49, Y50, N110, D112, G31

R413, P41, F49, Y50, N110, D33, G31

D44, P46, F49, Y50, N110, D112, G31

Epi#46

Y175, R125, R122, P123, G174, Q172

Y169, R125, R122, P123, G174, Q172

V432, R429, R428, P434, N69, G70

Y175, R125, R122, P94, N93, G90

Y175, R122, R125, P123, N182, G121

Y175, R125, R122, P94, G101, A102

Y175, R125, R122, P94, G118, A115

Y175, R125, R122, P94, G101, G96

Y175, R122, R125, P123, N182, G183

Epi#48

S211, D214, P218, P446, G447

E259, K337, P258, P434, V432

S215, D214, P218, P446, G447

S209, D214, P218, P446, V445

E259, K337, P258, P434, V433

Epi#50

R122, Y175, W120, T117, S119

R125, Y175, W120, S119, T117

Epi#51

T390, H391, E408, Q409, R413, S411, W317

T390, H391, E408, S405, I288, K404, W317

D406, H391, E408, Q409, R413, S411, W317

T390, H391, E408, D406, K404, Q409, W317

Epi#52
W437, A260, T266, R273, W228, D264, Q225
BPN':
Epi#02
T255, K256, S260, F261, P194, Y262, R186, V203
L257, K256, S260, F261, P194, Y262, R186, V203
T253, K256, S260, F261, P194, Y262, R186, V203
Epi#03
K141, A137, I108, Y104
K136, A137, I108, Y104
K136, A134, I108, Y104
Epi#04
K265, Y262, S188, Q185, R186, N184, L257
K265, Y262, S188, Q185, Y263, R186, L257
K265, Y262, S188, Q185, R186, N184, G258
K265, Y262, S188, Q185, Y263, R186, G258
Epi#05
G80, A1, N77, P40, G211, S38, S37, V44
G80, A1, N77, P40, G211, S38, S37, L42
G127, A152, N155, T164, G160, S158, S188, Y262
Epi#06
G211, N212, D36, S37, K43, P40
G80, N212, D36, S38, K43, P40
G211, N212, D36, S38, K43, P86
Epi#08
K256, D259, S260, F261
K43, D36, S38, V44, F58
Epi#09
S105, S132, A133, A137, D140, K141, A144, S145, N118
S248, T244, A144, S145, D120, K27, N118, A116, N117
Epi#10
E54, T55, N57, S37, F58, G46, K43
T55, A48, N57, S37, F58, G46, K43
E54, T55, N57, S49, F58, G46, K43
Epi#11
K136, I108, Q103, V51, D98
Epi#12
Y171, E195
Epi#13
S101, W106, P52, T55, A48, P56, S49, G47, F58
S105, W106, P52, T55, A48, P56, S49, G47, W113
Epi#15
N25, P239, D120, I115, K141, A144
N240, P239, D120, I115, K141, A144
Epi#16
Q271, P14, Y21, G20, Q19, S18, A15, A272, N252
Q59, P210, Y214, G211, S38, D36, D61, A99, D98
Epi#17
A187, S188, R186, S183
A187, S188, R186, S182
Epi#18
N184, R186, S188, G157, S158, T159, S161
N184, R186, S188, G157, S158, T159, S162
N184, R186, S188, G157, S158, E156, N155
N184, R186, S188, G157, S158, E156, F189
Epi19
E156, N155, S188, Q185, R186, L257
E156, N155, S188, Q185, R186, G258
E156, N155, S188, Q185, R186, A187
Epi#22
D197, S260, D259, L257, K256
D197, S260, D259, Y263, K256
Epi#23
N155, E156, S188, Q185, A187
Epi#24
E156, G166, E195, P194, S260, L257, K256
D259, G264, E195, P194, S260, L257, K256
D197, K170, E195, P194, S260, L257, K256
Epi#25
K141, I115, D120, N25
K141, I115, D120, N118
K141, I115, E112, N118
Epi#26
W113, S49, W106, P52, E54, D98
W113, S49, W106, P52, E54, D60
W113, S49, W106, V51, E54, D98
Epi#28
A99, D61, Q59, F58, E54, L96, Q103, G102, D98
A99, D98, Q59, F58, E54, L96, Q103, G100, D61
A99, D61, Q59, F58, E54, L96, Q103, S101, D98
Epi#29
G102, Q103, L96, E54
G100, Q103, L96, E54

Epi#30

I79, N76, S87, H17, S18, P14, V4

I79, N76, S87, H17, Q19, P14, V4

Epi#31

L257, Q185, N184, R186, F189, V203, I205, D181

L267, Q10, N184, R186, F189, V203, I205, D181

Epi#33

K213, Y214, P210, S38, S37, K43

Q59, F58, V44, S38, S37, K43

Epi#34

W106, P52, M50, G47, P56, T55, S53

W106, P52, S49, G47, P56, T55, S53

I115, W113, M50, V51, P52, T55, S53

I108, W106, S105, V51, P52, T55, S53

Epi#35

A99, L96, S49, M50, 1108

A99, L96, S49, M50, 1107

Epi#36

A137, A134, A133, G131, Y104, S105, Q103, V51, A48, W113

A134, A137, A133, G131, Y104, S101, Q103, V51, A48, W113

Epi#37

Y262, R186, L257, K256, D259

Y263, R186, L257, K256, N252

Epi#39

E156, T164, P129, G127, L126

E156, T164, P129, G128, L126

E156, T164, P129, G154, L126

E156, T164, P129, G166, L126

Epi#40

R247, L250, A272, T255, K256, S260

R186, L257, G258, Y263, K256, S260

G264, L257, G258, T255, K256, S260

Epi#41

P194, Y262, L257, S260

P194, Y263, L257, S260

Epi#42

P194, S260, G258, R186, Q185

Epi#44

S182, Q185, D181, Y6, S9, V4, P14

S183, Q185, D181, Y6, S3, V4, P5

S248, R247, D197, P194, Y262, S260, G258, T255

S53, P52, W106, Y104, S105, V51, T55

Epi#45

K170, P194, F261, Y262, R186, D181, V203

D197, P194, F261, Y262, R186, D181, V203

Epi#46

S162, S158, E156, N155, A187, Q185, N184, R186, S188

S188, S158, E156, N155, A187, Q185, N184, R186, S183

S158, S188, E156, N155, A187, Q185, N184, R186, S182

S161, S158, E156, N155, A187, Q185, N184, R186, S183

G160, S158, E156, N155, A187, Q185, N184, R186, S188

Epi#48

S38, K43, P40, P210, G211

S37, K43, P86, P14, V4

S38, K43, P40, P210, G215

Epi#50

H238, W241, T242, P239

H238, W241, T244, T242

H238, W241, T242, T244

Epi#51

T242, H238, Q275, Q271, P14, S18, H17

Q245, H238, Q275, K237, P239, T242, W241

Q275, H238, Q245, T242, R247, T244, W241

Q245, H238, Q275, Q271, P14, Q19, H17

CAREZYME Core:

Epi#01

P61, P165, K164, R158, N154, Y168, R153, S151

P137, P49, K44, K13, N32, Y54, Q36, T39

P61, P165, K164, R158, N154, S152, R153, S151

Epi#02

L115, N118, S117, R4, T6, Y147, R146, V129

L115, N118, S5, R4, T6, Y147, R146, V129

Epi#03

K44, A43, I38, Y54

K13, A43, I38, Y54

Epi#04

R153, S151, Q145, Y147, R146, 1131

R153, S151, Q145, Y147, R146, G144

R153, S151, Q145, Y147, R146, L142

Epi#05

G3, A1, S183, T95, G101, A100, S96, G97

G3, A1, F184, T93, G101, T95, S96, G97

G97, A100, S96, T95, G101, T93, S183, G3

Epi#06
G140, P160, D161, R158, K164, P165
G50, P137, D133, R146, Q145, P143
A162, P165, D161, R158, K164, P160
Epi#07
G148, T6, S181, D178, R170, P165, D58
G128, T6, S181, D178, R170, P165, D58
Epi#08
K44, D42, S45, A43, F41
Epi#09
A191, E192, R196, A195, R200, N25, N202, $N_2O_6$
D161, R158, D157, R153, N176, S151, N154
Epi#10
D161, A57, N34, A162, F159, R158, K164
D2, A1, R185, S183, F184, G3, R4
Epi#11
F41, F29, I38, Q36, D58
Epi#12
Y168, E155
Y90, E91
Epi#13
A63, W62, P165, T60, A162, P160, L142, G149, Y147
A63, W62, P165, T60, A162, P160, L142, G128, Y147
A63, W169, P165, T60, A162, P160, L142, G144, Y147
Epi#15
P137, D133, I131, R146, G144
P137, D133, I131, R146, G148
P137, D133, I131, R146, G130
P137, D133, I131, R146, G128
P137, D133, I131, R146, G149
Epi#16
Q138, P137, Y54, R37, Q36, N34, A162, A57, D161
R170, P165, Y168, R153, S151, N176, D172, A63, D67
R170, P165, Y168, R153, S151, N176, D172, A63, D66
Epi#17
A1, S183, R4, S117
A100, S181, R4, S183
A1, S183, R4, S5
Epi#18
N118, R4, S181, - - - , G3, - - - , S117, L115, - - - , A78, S80
N34, N32, R37, F35, - - - , A33, Y54, S45, - - - , - - - , A43, V52
Epi#19
D157, N154, S151, Q145, R146, L142
D178, N176, S151, Q145, R146, G144
Epi#22
D40, A43, D42, W18, K20
D40, A43, D42, A19, K20
Epi#23
R158, N154, E155, L142, Q145, P143
R153, N154, E155, S151, Q145, P143
Epi#24
D42, K44, E48, P137, F139, A33, Q36
D40, K44, E48, P137, F139, A33, Q36
D161, K164, A162, P160, R158, L142, Q145
D161, K164, E155, P143, R158, L142, Q145
Epi#25
R158, K164, W169, D172, N176
R4, H119, 177, E82, N81
Epi#26
W18, S15, E82, W85, P23, A19, D42
W18, S15, E82, W85, P23, G84, D203
Epi#28
I31, D133, Q138, L142, E155, K164, F159, P165, D161
I131, D133, Q138, L142, E155, K164, F159, P143, R158
I131, D133, Q138, L142, E155, K164, F159, P160, R158
Epi#29
I131, R146, L142, R158, E155
G144, Q145, L142, R158, E155
Epi#30
G79, N81, A78, H119, S117, I77, L115
G79, N81, A78, H119, S76, I77, L115
Epi#31
L142, R158, N154, R153, W169, F171, D172
Epi#33
Q36, F29, P27, S15, A19, K20
K44, F41, P27, S15, A19, K20
Epi#34
V129, P143, S151, G144, R146, Y147, T6
V129, P143, S151, G148, R146, Y147, T6
V129, P143, S151, G149, R146, Y147, T6
Epi#36
A83, A22, A19, S15, K13, V52, A43, W18

Epi#37
Y147, R146, L142, R158, D161
Y147, R146, L142, R158, N154
Y147, R146, L142, R158, D157
Epi#38
E155, R158, P160, G140, L142
E155, R158, P143, G144, L142
Epi#40
G79, L115, G113, T111, A74, T6
G79, L115, G113, T111, A74, S15
G79, L115, G113, T111, A74, S110
G116, L115, G113, T111, A74, T6
G79, L115, G113, T111, A74, S76
Epi#42
L142, P143, S151, G144, R146, Q145
L142, P143, S151, G148, R146, Q145
L142, P143, S151, G149, R146, Q145
Epi#44
L142, R158, D161, P165, W62, Y168, S152, G144, P143
I131, R146, D133, P137, Y54, A33, V52, P49
L142, R158, D161, P165, W62, Y168, S152, G149, P143
Epi#45
R185, P208, F207, N206, D203, V24
D67, P213, F68, N65, D66, V64
R185, P208, F207, N206, D204, G205
Epi#46
A195, R200, R201, P23, N202, G205
A191, R200, R201, P23, N202, G205
V24, R201, R200, P190, Q211, A209
Epi#47
A191, A195, E192, V194, R200, N202, R201, P23
A195, A191, E192, V194, R200, N25, R201, P23
A191, A195, R196, V194, R200, N202, R201, P23
Epi#48
E48, K44, P49, P137, V52
E48, K44, P49, P137, G50
E48, K44, P49, P137, G140
Epi#50
D172, Y168, W62, V64, P213
D42, W18, A43, T39
D67, W173, W62, V64, P213
D66, W173, W62, V64, P213
D42, W18, S45, P49
D172, W169, W62, V64, P213
Epi#51
R4, H119, D2, T95, P98, K175, W169
R4, H119, D2, R185, P208, Q186, W85
R4, H119, D2, T95, G97, K175, W173
Epi#52
W18, A22, R200, R201, W85, Q186
Esperase:
Epi#01
N24, P239, R237, K235, N243, S240, Q245, T242
N24, P239, K235, R27, N117, Y91, R43, S87
N24, P239, R237, K235, N243, Y241, Q245, S240
Epi#02
T3, N76, L75, R43, S38, Y209, R213, V215
T3, N76, S87, R43, S38, Y209, R213, V215
T129, N166, Q161, R160, T156, Y192, R186, V203
Epi#03
R186, Y192, S261, Q161, R160, N155, G127
R186, Y192, S261, Q161, R160, N155, G157
R186, Y192, S261, Q161, R160, N155, L126
R186, Y192, S261, Q161, R160, T156, G162
R186, Y192, S261, Q161, R160, N155, A187
Epi#05
G102, A105, S133, T134, G131, R170, T129, Y167
G102, A105, S133, T134, G131, R170, T129, G127
G211, A37, R43, P40, G80, T3, S78, I79
Epi#06
G211, N61, D97, R98, S53, P55
G102, N99, D97, R98, S53, P55
G100, N99, D97, R98, S53, P55
Epi#07
211, T210, D60, S38, R43, P86, D89
Epi#08
A108, E136, S133, A105, F50
A108, E136, S132, A105, F50
A187, D181, S188, V203, F189
Epi#09
N212, G211, S38, H59, N61, N99, R98
S52, S53, R98, N99, N61, G211
Epi#10
T129, T156, N155, S188, F189, G157, R160
D181, N183, R186, S188, F189, G157, R160

T129, N166, N155, S188, F189, G157, R160
T129, T156, N155, S218, F189, G157, R160
D97, N99, N61, S57, F50, G102, R98
Epi#12
Y167, E136
Y192, E195
Y171, E136
Epi#13
S38, R43, P40, A37, H59, S57, P55, Y58
S38, R43, P40, A37, H59, S57, P55, F50
S38, R43, P40, A37, H59, S49, P55, Y58
Epi#15
N24, P86, D89, I44, R43, A45
N24, P86, D89, I44, R43, G46
N76, P86, D89, I44, R43, A45
N24, P86, D89, I44, R43, A37
Epi#16
Q161, P194, Y192, G157, R160, S188, D181, A187, N183
Q161, P194, Y192, R186, Q185, S188, D181, A187, N183
Q161, P194, Y192, G162, R160, S188, D181, A187, N155
Epi#17
A37, S38, R43, S87
Epi#18
N144, N140, R141, L137, S133, T134, E136, S132
N140, N144, R141, L137, S133, T134, A105, S103
N143, N144, R141, L137, S133, T134, E136, N140
Epi#19
I21, N18, Q15, Q275, R19, G20
I21, N18, Q15, Q275, R237, G20
E197, N265, S261, Q161, R160, G162
E197, N265, S261, Q161, R160, G157
I21, N18, Q15, Q275, R237, G25
Epi#23
R98, N61, E54, S53, F50, P55
R98, N61, E54, Y58, F50, P55
R98, N61, E54, S57, F50, P55
R98, N61, E54, S52, F50, A105
Epi#24
E195, G264, E197, P260, S261, P194, Q161
D89, G46, A48, P55, S52, F50, Q109
E197, G264, E195, P194, S261, L262, Q161
Epi#25
R98, H59, E54, N61
R98, H59, D60, N61
R43, H39, I44, D89, N24
R27, H120,I115, E112, N116
Epi#28
L104, Q109, I115, E112, W113, F50, S53, R98
A105, Q109, I115, E112, W113, F50, G102, R98
A108, Q109, I115, E112, W113, F50, S53, R98
V107, Q109, I115, E112, W113, F50, S53, R98
Epi#29
I147, N140, L137, R141, E136
G146, N140, L137, R141, E112
I115, N143, L137, R141, E136
G102, N99, L96, R98, E54
Epi#30
G211, N212, S38, H59, S57, I51, P55
G211, N61, S57, H59, S38, P40, L75
G211, N212, S38, H59, S49, I51, P55
G211, N212, S38, H59, P55, I51, L96
Epi#31
L257, Q185, N183, R186, F189, V203, D181
L262, Q185, N183, R186, F189, V203, D181
Epi#33
H59, Y58, P55, S52, S53, R98
Q109, F50, P55, S57, S53, R98
Q109, F50, P55, S49, S53, R98
Epi#34
I79, P40, S38, G211, R213, Y209, S216
I79, P40, S38, G211, R213, Y214, T210
I51, P55, S49, L96, R98, S53, S52
Epi#37
T134, A108, L137, R141, N144
Y256, A254, L257, R186, N183
A105, A108, L137, R141, N144
Epi#38
L257, G264, E195, L262, N265, P260, S259
L257, G264, E195, L262, N265, P260, S261
Epi#39
E195, R170, P194, G264, L257
E195, R170, P194, G264, L262

Epi#40
R141, L137, A108, T134, A105, S133
R43, L42, A37, Y58, P55, S52
R186, L257, A254, Y256, P260, S259
R186, L262, G258, Y256, P260, S259
R186, L257, G184, Y256, P260, S259
R141, L137, A108, T134, A105, S103
R186, L262, G264, Y256, P260, S259
R186, L257, A254, Y256, P260, S261
R186, L262, G258, Y256, P260, S261
R186, L257, G264, Y256, P260, S261
Epi#41
P260, Y256, L257, S259
Epi#42
L75, P86, S87, N24, P239, R237, Q275
L75, P86, S87, N24, P239, R237, R19
Epi#44
S53, R98, D97, Y58, S57, A48, P55
S53, R98, D97, Y58, S38, G211, T210
Epi#45
R19, H17, F22, N24, D89, G25
R43, P86, F22, N24, D89, G25
R272, H269, F10, N183, D181, V203
R272, H269, F10, N183, D181, G184
R43, P86, F22, N24, D89, G46
Epi#46
R19, R237, P239, N24, G20
R19, R237, P239, N24, G25
Epi#47
G162, Y192, R160, N155, A187, Q185, N183, R186, S188
G157, Y192, R160, N155, A187, Q185, N183, R186, S188
S261, Y192, R160, N155, A187, Q182, N183, R186, S188
L262, Y192, R160, N155, A187, Q182, N183, R186, S188
Epi#48
S261, Q161, P194, P260, G258
S261, Q161, P194, P260, G264
Epi#50
D181, W6, V4, T3
D181, W6, V203, S188
D181, W6, V4, S9
D181, W6, T3, P5
Epi#51
R98, H64, T210, R213, P40, S38, H59
R98, H64, T210, R213, G211, S38, H59
R19, H17, Q15, Q275, R272, Q252, H269
Laccase:
Epi#02
A14, N15, S17, F21, P180, Y176, R266, V177
T22, N15, P18, F21, P180, Y176, R266, V177
A274, N275, A181, R175, P180, Y176, R266, V177
A24, N15, S17, F21, P180, Y176, R266, V177
T272, N275, A181, R175, P180, Y176, R266, V177
Epi#03
L184, K173, I186, Y256
Epi#04
R234, S211, Q261, K264, N267, G271
R234, S211, Q261, K264, R266, G268,
R259, S211, Q302, R234, N299, A301
R259, S211, Q236, R234, N299, A301
Epi#05
G372, A371, L369, P350, G81, S349, S351, V352
G372, A371, L369, P350, G81, S351, S349, Y347
Epi#06
G286, N289, D291, T293, S295, P292
G214, P252, D254, T293, S295, P298
A288, N289, D291, T293, S295, P292
Epi#07
G214, T294, D291, R283, V253, P252, D254
G30, T12, D53, R59, A497, P89, D51
G30, T10, D51, R59, A497, P55, D53
Epi#08
A371, E348, S349, A346, F335
A14, D53, G90, A92, H91, F93
A181, E183, G20, V16, F21
A181, E183, G20, A182, F21
Epi#09
N41, A100, N43, V6, D42, R37, N4, T8, L94
N41, A100, N43, V6, D42, R37, N4, T8, N47
L369, N366, E376, R379, N472, A471, V474
Epi#10
E183, A181, N275, T272, F273, G268, R266
D129, N41, N43, A100, F69, G72, R71
E183, A181, N275, A274, F273, G271, K264

Epi#11
F93, L486, I489, Q485, V481, E482
Epi#12
Y490, E488
Y375, E376
Epi#13
N366, P370, D367, I358, Q363, A471
N366, P370, D367, I358, Q363, G361
R379, P378, D326, I319, T321, G323
R379, P378, D326, I319, T321, G318
R379, P378, D326, I319, T321, A324
Epi#15
N366, P370, D367, I358, Q363, A471
N366, P370, D367, I358, Q363, G361
R379, P378, D326, I319, T321, G323
R379, P378, D326, I319, T321, G318
R379, P378, D326, I319, T321, A324
Epi#16
R175, P180, Y176, R266, Q164, N267, D166, A163, D205
R283, P292, Y256, G214, Q251, D254, A285, A288, N289
R283, P292, Y256, G214, Q251, D254, D291, A290, N289
Epi#17
A306, S413, R409, S414
A411, S413, R409, S414
A306, S410, R409, S414
A411, S414, R409, S410
Epi#19
E216, N250, Q251, Q191, R283, G286
E190, N250, Q251, Q191, R283, A288
E216, N250, Q251, Q191, R283, A290
E190, N250, Q251, Q191, R283, A285
Epi#22
D491, P494, D492, P495, E496
D492, P494, D491, L493, E496
Epi#23
R339, N460, E348, S349, L369, A371
R339, N460, E348, S351, L369, P370
R339, N460, E348, S351, L369, A365
R339, N460, E348, S351, L369, P350
R283, N188, E190, N250, Q191, P252
Epi#24
D475, G72, A476, P445, R379, A471, Q363
D53, G90, A497, P495, T498, P55, Q501
D53, G90, A497, P495, S499, L58, Q501
Epi#25
R37, K40, D129, N130
R37, K40, D129, N41
Epi#27
E142, E139, D138, K194,
E142, E139, D138, K193
Epi#28
L58, Q501, I500, E496, L493, P495, D492
G286, D254, Q191, K194, E190, K193, G192, D138
A288, D254, Q191, K193, E190, K194, G192, D138
G192, D248, Q191, K194, E139, L136, A135, D138
V253, D254, Q191, K193, E190, K194, G192, D138
A285, D254, Q191, K193, E190, K194, G192, D138
Epi#29
G390, Q332, L329, R330, E435
V374, N366, L369, E348
I500, P495, L493, E496
G344, Q332, L333, R330, E435
Epi#30
G412, N304, A306, H309, I312, P314, V419
I312, L311, A315, H309, P229, L136, P132
Epi#31
L329, Q332, N343, R330, F331, V386, D434
L333, Q332, N343, R330, F331, V386, D434
L58, Q501, N54, R59, F112, M459, F456, D205
L58, Q501, N54, R59, F112, M459, I454, D205
Epi#33
Q485, Y490, P494, S499, A497, R59
Q251, Y256, P292, S295, A296, R234
H153, F21, V16, S17, A182, K173
H153, F21, P18, S17, A182, K173
Epi#34
V431, P395, T432, G433, G412, T415, S414
V431, P388, T432, G412, G433, S414, T415
V419, P320, T321, G323, P322, Y416, S414
V431, P395, T432, G390, G433, S414, T415
Epi#35
A371, L369, A362, S360, M359, I358
G372, L369, A362, S360, M359, I358
A365, L369, A362, S360, M359, I358

Epi#36

A362, A471, A476, V474, G361, S360, Q357, P350, A371, A365

A290, A288, A285, V253, Y256, S295, A296, W257

A288, A285, A287, V253, Y256, S295, A296, W257

Epi#37

P132, A135, L136, K194, N250

A135, A134, L136, K194, D138

P298, A301, L303, R234, N299

Epi#38

L356, G81, E348, A371, V374, L369, N366, P370, S351

L356, G81, E348, A371, V374, L369, N366, P370, S349

Epi#39

A411, E435, T432, P395, G393, L392

A1, E142, L35, R37, P34, G30, L27

A389, E435, T432, P395, G394, L392

Epi#40

R330, L333, G390, T432, A411, S414

G393, L392, G394, T432, A411, S414

R330, L333, G390, T432, A411, T415

Epi#41

P370, L369, V352, S351

P350, L369, V352, S351

Epi#42

L392, P395, S428, G430, P388, R330, Q332

Epi#44

S360, Q363, D367, P370, Y347, A371, G372, T345

V253, Q191, D254, P292, W257, Y256, S295, A296, P298

S360, Q363, D367, P370, Y347, S349, V352, P350

V253, Q191, D254, P292, W257, Y256, S295, G214, P252

Epi#45

R409, P322, F418, Y416, N420, D313, V419

K423, P314, F418, Y416, N420, D313, V419

R175, P180, F21, Y176, R266, D166, G268

Epi#46

A296, R259, R234, P300, N299, A301

Y256, R259, R234, P300, N299, Q302

Epi#47

I212, S211, R234, L303, A301, N299, P300, P298

I212, S211, R234, V232, A301, N299, P300, P298

Epi#48

S158, Q160, P157, P155, V504

S499, Q501, P55, P155, V504

E488, Q485, P480, P479, V481

Epi#49

D367, L369, V352, P350, Q357, Q363, M359, N478

D367, L369, P370, P350, Q357, Q363, M359, N478

Epi#50

D291, Y256, W257, S295, P298

D254, Y256, W257, T293, S295

Epi#51

D307, H309, E228, T218, P229, T231, H230

R234, H215, E216, T231, P229, H230, H309

D248, H215, E216, T231, P229, H230, H309

Epi#52

F69, A100, T98, R71, W75, T73, Q70

F97, A100, T98, R71, W75, T73, Q70

Natalase:

Epi#01

P344, P382, R387, R33, N32, S28, R31, T36

P344, P382, R387, R33, N29, S28, R31, T36

Epi#02

A87, N21, Q18, R24, S28, R31, R33

A87, K89, S83, R24, S28, R31, R33

Epi#03

L307, K305, H402, I404, Y398

L307, K305, H401, I404, Y398

L307, K305, A304, I404, Y398

Epi#04

R167, S166, Q168, R172, N171, I173

R177, Y131, S128, Q125, R123, N124, I127

Epi#05

G178, A180, N124, P120, G190, S187, H234, L195

G178, A180, N124, P120, G190, R123, S187, Y192

G178, A180, N124, P120, G190, S187, H234, Y192

Epi#06

A87, N21, D25, R24, Q18, P14

G145, N146, D150, T147, R144, P142

G143, N146, D150, T147, R144, P142

G450, N451, D447, T455, K452, P453

A87, N21, D25, R22, Q18, P14

G454, N451, D447, T455, K452, P453

A378, P382, D447, T455, K452, P453

Epi#07
G145, T147, D150, S149, R213, V208, P205, D201
Epi#08
K305, D400, A304, H402, F399
K305, D400, A304, H401, F399
Epi#09
S79, S83, D25, R22, R24, H86, N90, S28, R31
N439, A460, N459, V444, K478, N417, T413, T414
Epi#10
E254, N249, R248, T245, F239, R212, R213
E254, N249, R248, T245, F239, R241, K275
Epi#11
F169, I173, Q170, D162
L195, I173, Q170, D162
Epi#12
Y192, E188
Y357, E354
Epi#13
H12, L13, P369, A375, P374, S372, P330, W11
H12, L13, P369, A375, P374, S372, P330, L334
H12, L13, P369, A375, P374, S372, P330, G331
Epi#15
N451, P453, D447, I448, T449, A378
N451, P453, D447, I448, K452, G450
Epi#16
Q313, P316, Y357, R353, Q395, D397, D400, A304, N308
Q355, P316, Y357, G356, R353, D397, D400, A304, D302
Epi#17
A87, S83, R24, S28
A87, S28, R24, S83
Epi#18
R33, N32, R31, S28, G92, N90
Epi#19
D16, N50, S48, Q49, R72, G69
D25, N21, Q80, Q18, R24, A87
E82, T77, Q18, Q80, R72, G69
Epi#22
D461, A460, W463, W433
Epi#23
K478, N417, E410, N439, Q438, A460
K478, N417, E410, N439, Q438, A441
Epi#24
E332, G331, E335, P330, S372, A375, K379
D381, K379, A375, P369, S372, P374, K377
Epi#25
R154, K138, W136, D162, N171
R213, R212, W217, E216, N249
R154, K138, W136, E134, N112
R241, K236, W183, D203, E206
Epi#26
W163, S166, E134, W136, V161, E117, E126
W163, S166, E134, W136, V161, E117, D130
W163, S166, E134, W136, V161, E117, D162
Epi#27
D203, E206, D201, K236
E117, E126, D130, K175
D201, E206, D203, K179
E126, E117, D162, K175
Epi#28
L195, D162, Q168, W163, E134, W136, Q165, S166, R167
I173, D162, Q170, W163, E134, W136, Q165, S166, R167
V161, D162, Q170, W163, E134, W136, Q165, S166, R167
Epi#29
G331, P330, L334, F337, E335
G178, K175, L114, R177, E117
Epi#30
G450, N451, H446, K478, I448, P453
G454, N451, H446, K478, I448, P453
Epi#31
Q168, N171, R172, W163, M196, I173, D162
Q170, N171, R172, W163, V161, I173, D162
Epi#33
K377, Y366, P369, S372, A375, K379
K377, Y366, P374, S372, A375, K379
Epi#34
W433, W463, T457, V444, G454, T455, P453
W433, W463, T457, V456, G454, T455, P453
Epi#37
Y156, R177, L114, K175, D130
T132, R177, L114, K175, N124
Epi#38
G429, E431, N469, P428, S472
G430, E431, N469, P428, S472

Epi#39
E10, H12, T370, P330, G331, L334
E10, L13, T370, P330, G331, L334
Epi#40
A378, A375, Y366, P369, S372
R177, L114, G178, Y156, K138, T110
A375, A378, Y366, P369, T370
Epi#41
P369, L13, V52, S48
Epi#42
P316, S281, G356, R353, Q355
P316, S281, G356, R353, Q395
Epi#44
V208, R213, W217, Y148, S149, G145, P142
S28, R33, D381, Y365, A378, A375, P369
L13, D16, P14, W11, Y362, A375, V373, T370
S333, D327, P330, W11, Y362, A375, V373, P369
Epi#45
D108, P142, F65, Y60, N146, D150, G145
D140, P142, F65, Y60, N146, D150, G145
Epi#46
Y392, R387, R33, P382, G450, G454
Y392, R387, R33, P382, Q388, G3
Epi#47
S83, S79, E82, I85, R24, A87, N90, R31, S28
A250, G252, E254, N249, R248, F256, N279, R241, S238
Epi#48
S372, H371, P374, P369, V373
Epi#49
D51, W11, L13, V52, P14, Q18, Q80, T77, N21
D51, W11, L13, V52, P14, Q18, Q80, T77, K74
Epi#50
D461, Y435, W433, W463, T457
D400, Y398, W433, W463, T457
D397, Y435, W433, W463, T457
Epi#51
T394, H396, D397, D400, K305, H402, H401
T455, H446, K478, T457, G442, Q438, W463
Epi#52
W136, A109, E134, R167, W163, N171, Q170
W136, A109, E134, R167, W163, N171, Q168

PD498:
Epi#02
T262, K258, S260, F266, T198, Y196, R168, V166
T262, K258, S260, F266, T264, Y196, R168, V166
T141, N139, Q171, F170, S167, Y196, R168, V166
Epi#03
L99, K51, A49, I53, Y56
L99, K51, A49, I53, Y43
Epi#04
R28, S331, Q333, K97, R50, 153
R28, S331, Q333, K97, R50, A49
Epi#05
G108, A106, N107, G110, S109, S111, 159
G110, A106, N107, G108, S109, S111, L112
G108, A106, N107, G110, S111, S117, Y121
G108, A106, N107, G110, S111, S109, G135
G110, A106, L68, P214, G217, S219, Y220
G108, A106, N107, G110, S111, S109, L134
Epi#06
G135, N163, D164, R168, S174, P176
G162, N165, D164, R168, S174, P176
A22, N274, D25, S2, S9, P6
G154, N152, D148, T142, K144, P176
A22, P21, D25, S2, S9, P6
G154, N152, D148, S145, K144, P176
Epi#07
29, T332, S331, D95, S240, R28, V26, P21, D25
G29, T332, S330, D95, S331, R28, V26, P21, D25
Epi#08
K258, D257, S260, F266
K190, D185, S192, V207, F193
Epi#09
N215, N44, R50, 153, K54, N64, N63, R61
N44, A49, R50, 153, K54, N63, N64, R61
Epi#10
D188, N187, R189, S260, F266, G263, K258
D185, N187, R189, S260, F266, G263, K258
Epi#12
Y268, E253
Epi#15
R50, P46, D82, I87, T83, G86
N215, P46, D82, I87, T83, G86

Epi#18
N216, N44, R50, 153, A49, P46, N215
N215, N44, R50, 153, A49, P46, N216
Epi#19
D95, T332, S240, Q241, R28, G29
D95, T332, S330, Q241, R28, G29
Epi#22
D185, S192, D164, Y196, K267
D105, S111, D113, T141, K144
Epi#24
D95, K51, A49, P46, R50, K97
Epi#25
R120, K153, W151, D148, N152
R189, K190, D188, N187
R189, K190, D185, N208
Epi#27
D201, E253, D257, K258
D257, E253, D201, K267
Epi#28
I259, D257, Q254, E253, K267, F266, S260, R189
I259, D257, Q254, E253, K267, F266, S260, K258
Epi#29
L68, G108, L134, F170, E137
G135, N163, L134, F170, E137
Epi#30
G110, N107, A106, H71, L68, L104, L112
G108, N107, A106, H71, L68, P214, V213
G110, N107, A106, H71, P214, L68, L104
G110, N107, A106, H71, L68, L104, L134
Epi#33
Q12, Y220, V207, S222, S192, R189
190, F193, V207, S222, S192, R189
Q16, Y13, V207, S222, S192, R189
Epi#34
V26, W1, T27, G29, R28, S331, T332
W1, P21, T27, V26, R278, Y279, T255
Epi#35
G135, L134, S225, M221, I209
G110, L134, S225, M221, I209
G108, L134, S225, M221, I209
G162, L134, S225, M221, I209
Epi#37
A49, V52, L99, K54, N63
SAS: 309, Size 17.16: Y121, A127, L99, K54, N63
SAS: 307, Size 13.09: Y43, V52, L99, K54, N63
Epi#40
R189, G261, Y268, K258, S260
R189, G261, Y268, K258, T262
Epi#42
P3, S2, Q16, P21, R28, Q241
Epi#43
W199, Y196, G162, Q171, S140, L112, I115, T142
Epi#44
S145, D148, P176, W199, Y196, S167, G162, T169
S174, D201, P176, W199, Y196, S167, G197, T198
Epi#47
S330, S331, R28, V26, A22, Q16, N17, P21, S2
G242, S240, R28, V26, A22, Q16, N17, P21, S2
G29, S331, R28, V26, A22, Q16, N17, P21, S2
Epi#48
S2, D25, P21, P3, G86
S9, Q16, P21, P3, G86
Epi#50
R168, Y196, W199, T264, T198
D164, Y196, W199, T264, S260
Savinase:
L21, N18, P14, R19, K231, N232, S236, Q239, S234
L21, N18, P14, R19, K231, N232, S234, Q230, S24
L21, N18, P14, R19, K231, N232, S234, Q230, T22
Epi#02
T254, N255, A188, R164, S158, Y186, R180, V197
T249, N263, Q12, R10, P14, R19, R269
T249, N263, S9, R10, P14, R19, R269

**[0594]** Epi#03

K27, A86, I43, Y89
Epi#04
K229, S234, Q230, K231, R269, A266
K27, S24, Q230, K231, R269, A15
K231, S234, Q239, R241, N246, A248
Epi#05
G187, A188, N255, T254, G252, S250, T249, L251
G189, A188, N255, T254, G252, S250, T249, L261

Epi#06
G252, N179, D175, S182, S154, P127
A188, N255, D191, R164, S158, P127
A188, N255, D191, R164, S128, P127
Epi#08
A131, E134, S139, A106, F49
A166, E134, S139, A106, F49
Epi#09
S103, T132, A131, E134, A166, R164, N167, S142, R143
Epi#10
D175, N177, N179, S182, F183, G155, R180
D175, N212, N153, S182, F183, G155, R180
Epi#11
F49, L94, 1105, Q107, V102, E134
F49, K92, I105, Q107, V102, E134
Epi#12
Y161, E134
Y165, E134
Epi#13
S76, L73, P39, T207, A209, P204, S206, G205, Y208
S85, L73, P39, T207, A209, P204, S206, G205, Y203
Epi#16
R164, P127, Y161, G152, S158, N255, D191, A166, N167
R164, P129, Y161, G152, S158, N255, D191, A166, N138
Epi#17
A156, S158, R164, S128
A188, S158, R164, S126
Epi#18
N177, N179, R180, S182, G155, S154, A156, S158
N177, N178, R180, S182, G155, S154, N153, F183
Epi#19
D175, N179, S182, Q185, R180, L256
D175, N179, S182, Q185, R180, L251
I240, W235, S234, Q239, R241, K245
D175, N179, S182, Q185, R180, G252
Epi#23
R143, N114, E110, S139, Q135, A131
R143, N115, E110, N138, Q135, A131
Epi#24
D58, G59, E53, P51, F49, P54, Q57
D58, G59, E53, P51, S48, P54, Q57
D58, G59, E53, P54, S55, F49, Q107
Epi#25
R19, R269, E265, N18
R269, R19, E265, N18
Epi#28
V102, Q107, F49, E53, K92, Q57, G46, R44
A47, Q107, F49, E53, K92, Q57, G46, R44
V50, Q107, F49, E53, K92, Q57, G46, R44
Epi#29
I77, N74, L41, R44, E87
V4, N74, L41, R44, E87
G20, N18, L21, R19, E265
Epi#30
G59, N60, S97, H62, L94, P51, P54
G98, N60, S97, H62, L94, P51, P54
Epi#31
L256, R180, N178, R10, W6, V197, D175
L251, R180, N178, R10, W6, V197, D175
Epi#33
Q107, F49, P51, S48, S55, K92
Q107, F49, P54, S55, A47, K92
Epi#34
V102, P129, S128, G125, R164, Y161, P127
V102, P129, S126, G125, R164, S158, P127

**[0595]** Epi#37

T254, A188, L256, R180, N177
T254, A188, L256, R180, N179
Epi#38
L94, G59, E53, A96, N60, P204, S206
L94, G59, E53, A96, N60, P204, S36
Epi#39
A131, E134, L133, T132, P129, G125, L124
A166, E134, L133, T132, P129, G125, L124
Epi#40
R44, L41, G78, T207, P39, T37
R19, L21, G20, T22, K231, S234
R180, L256, G252, T254, A188, S158
Epi#41
P127, Y161, L133, V102, S99
P127, Y161, L133, V102, S103
P127, Y161, L133, V102, S101
P127, Y161, L133, V102, S126

Epi#42
L73, P84, S85, N74, H17, P14, R19, R269
L80, P5, S3, N74, H17, P14, R19, R269
L21, P84, S85, N74, H17, P14, R19, R269
Epi#43
105, W111, A47, G46, Q57, S36, L41, I43, T37
Epi#44
S126, R164, P127, Y161, S158, A188, T254
S128, R164, P129, Y161, S158, A188, T254
Epi#46
A15, R269, R19, P14, N18, G20
A266, R269, R19, P14, N18, A15
Epi#48
S55, Q57, P54, P51, G52
E53, Q57, P54, P51, G52
Epi#50
R10, W6, S3, S76
R241, W235, S234, P233
R10, W6, V4, S9
**[0596]** Epi#51
Q239, H243, T247, R269, R19, K231, W235
R19, H17, E265, R269, K231, S234, W235
Epi#52
A15, S9, R10, W6, N198, Q176
A15, S9, R10, W6, N198, Q200
Amylase SP722:
Epi#02
T419, N423, P422, F396, T5, Y398, R393, R37
T419, N418, P422, F396, T5, Y398, R393, R37
Epi#03
L313, K311, H408, I410, Y404
L313, K311, H407, I410, Y404
Epi#04
R171, S170, Q172, R176, N175, I177
R181, Y135, S132, Q129, R127, N128, I131
Epi#05
G184, A186, N128, P124, G196, S193, H240, L201
G184, A186, N128, P124, G196, R127, S193, Y198
Epi#06
G147, N150, D154, T151, R148, P146
G149, N150, D154, T151, R148, P146
Epi#07
G149, T151, D154, S153, R219, V214, P211, D207
Epi#08
K311, D406, A310, H407, F405
K311, D308, A310, H408, F405
Epi#09
T461, R485, K484, N423, T419, N418
R485, K484, N423, T420, T419
Epi#10
E260, N255, R254, T251, F245, R218, R219
T419, N423, N395, T5, F396, R393, R37
E260, T257, N255, T251, F245, R218, R219
Epi#11
F173, I177, Q174, D166
L201, I177, Q174, D166
Epi#12
Y363, E360
Y398, E360
Y198, E194
Epi#13
H16, L17, P375, A381, P380, S378, P336, W15
H16, L17, P375, A381, P380, S378, P336, G337
H16, L17, P375, A381, P380, S378, P336, L340
Epi#15
N457, P459, D453, I454, K458, G456
K458, P459, D453, I454, T455, A384
N457, P459, D453, I454, K458, G460
Epi#16
Q319, P322, Y363, R359, Q401, D403, D406, A310, N314
Q319, P322, Y363, G362, R359, D403, D406, A310, N314
Q319, P322, Y363, R359, R415, D403, D406, A310, N314
Epi#17
A91, S32, R28, S87
A91, S87, R82, S83
Epi#18
R485, V450, G448, T463, T461, H452, V462
N126, N128, R127, G196, Y198, S193, N195, N125
N25, R26, R28, S87, I89, A91, H90, N94
Epi#19
D20, N54, S52, Q53, R76, G73
D20, N19, Q22, Q84, R76, G73
D29, N25, Q22, Q84, R28, A91
Epi#20
K385, P350, L355, L313, K311, D308, G305, D432

Epi#22
D183, A186, D209, W189, K242
D183, A186, D209, W189, E190
D183, A186, D209, P211, E212
D209, A186, D183, Y160, W159
D183, A186, D209, W187, W189
Epi#23
R415, N418, E416, N445, Q444, A466
K446, N445, E416, Y441, Q444, A466
Epi#24
D387, K385, A381, P375, S378, P380, K383
E341, G337, E338, P336, S378, A381, K385
D333, G337, E341, P336, S378, A381, K385
Epi#25
R485, H452, I454, E391, N36
R485, K484, I454, E391, N395
Epi#26
W167, S170, E138, W140, V117, G182, D183
W167, S170, E138, W140, V165, E121, D134
W167, S170, E138, W140, V165, E121, E130
Epi#27
E212, E216, D154, K156
E216, E212, D209, K242
Epi#28
L201, D166, Q172, W167, E138, W140, Q169, S170, R171
L201, D166, Q169, W140, E138, W167, F173, S170, R171
L201, D166, Q174, W167, E138, W140, Q169, S170, R171
Epi#29
V214, N215, L217, R219, E222
G96, H90, L228, R82, E86
V214, R219, L217, R218, E212
Epi#30
G456, N457, H452, K484, I454, P459
G362, M323, S287, H324, K320, P322, V318
G362, M323, S287, H321, K320, P322, V318
G460, N457, H452, K484, I454, P459
Epi#31
L217, R219, N215, R218, F245, V214, D248
L217, R219, N215, R218, F245, M208, D209
Epi#33
K383, Y372, P375, S378, A381, K385
K383, Y372, P380, S378, A381, K38
Epi#34
W439, W469, T463, V450, R485, T461, P459
W439, W469, T463, V462, R485, T461, P459
Epi#37
T251, R218, L217, R219, N215
P211, V214, L217, R219, N215
A256, R218, L217, R219, N215
Epi#38
G435, E437, N475, P434, S478
G436, E437, N475, P434, S478
Epi#39
E338, H16, T376, P336, G337, L340
E14, H16, T376, P336, G337, L340
Epi#40
A384, A381, Y372, P375, S378
A384, A381, Y372, P375, T376
Epi#41
P375, L17, V56, S52
Epi#42
S378, P380, Y372, A381, A384, P375
S378, P375, Y372, A381, A384, P388
S378, P375, Y372, A381, A384, T455
Epi#45
K72, P146, F69, Y64, R148, D154, G149
K311, H408, F405, N409, D432, G304
D406, H408, F405, N409, D432, G304
Epi#46
Y398, R393, R37, P388, Q394, G7
Y398, R359, R393, P388, G456, G460
Y398, R393, R37, P388, Q394, G38
Epi#47
A256, G258, E260, N255, R254, F262, N285, R247, S244
S193, Y198, E194, N125, R127, Q129, N123, R176, P124
Epi#48
S378, H377, P380, P375, V379
H16, H377, P375, P380, V379
Epi#49
D55, W15, L17, P18, Q22, Q84, T81, N25
D55, W15, L17, P18, Q22, Q84, T81, K78
Epi#50
D467, Y441, W439, W469, T463
D406, Y404, W439, W469, T463
D183, Y160, W159, W140, T114
D403, Y441, W439, W469, T463

Epi#51
D406, H408, D308, K311, L313, Q319, H321
Epi#52
W140, A113, E138, R171, W167, N175, Q174
W140, A113, E138, R171, W167, D166, Q172
Amylase AA560:
Epi#01
L390, P388, P350, K383, K385, N457, S478, R458, T461
L390, P388, P350, K383, K385, N457, S478, R458, T452
L390, P388, P350, K383, K385, N457, S478, R458, T455
Epi#02
L390, K395, Q394, R393, T5, Y398, R359, R400
L173, K172, S170, T136, Y135, R118, R181
L173, R171, S170, T136, Y135, R118, R181
L390, K395, Q394, R393, T5, Y398, R400, R415
Epi#03
K438, H407, I410, Y404
Epi#04
K172, S170, Q169, R171, N174, L173
R171, S170, Q169, K172, N175, I177
Epi#05
G456, A459, R458, T461, G460, T452, T463, V450
G456, A459, R458, T452, G460, T461, T463, G448
Epi#06
A51, N54, D20, R76, Q71, P146
G73, A51, D55, S52, K72, P146
Epi#07
G456, T455, S384, D387, R393, P388, D453
Epi#08
K259, S255, V222, H252, F245
K259, G258, A256, H252, F245
Epi#09
N128, V131, R176, D166, K172, N175, N174, R171
Epi#10
467, N445, R444, F441, R415, R400
D467, A466, R444, F441, R415, R400
Epi#11
F69, K72, I75, Q53, V56, D55
Epi#12
Y16, E337
Y363, E360
Y198, E194
Epi#15
K385, P388, D453, I454, R458, A459
K385, P388, D387, I454, T452, A459
K385, P388, D387, I454, R458, G456
Epi#17
A87, S29, R28, S32
A91, S29, R28, S32
Epi#18
N445, R444, A466, T463, T461, N471, N437
N445, R444, A466, T463, T461, T452, V450
Epi#19
166, W167, S170, Q169, R171, K172
E138, W167, S170, Q169, R171, K172
E134, T136, S170, Q169, R171, K172
Epi#22
D209, P211, D207, Y160, D183
Epi#23
R400, N418, E416, N445, Q449, A466
R82, N83, E68, N70, F69, P146
Epi#24
E134, G133, E130, P124, R176, L173, K172
E134, K179, E130, P124, R176, L173, K172
Epi#25
R444, K446, W469, D467, N445
R171, K172, W167, D166, N175
R171, K172, W167, D166, N174
Epi#26
W167, S170, E138, W140, V165, E121, E130
W167, S170, E138, W140, V165, E121, E134
W167, S170, E138, W140, V165, E121, D166
Epi#27
E130, E121, D166, K172
D36, E391, D387, K385
E134, E121, D166, K172
Epi#28
L201, D166, Q169, W140, E138, K172, S170, R171
L173, D166, Q169, K172, E138, W167, S170, R171
Epi#29
V131, R176, L173, R171, E138
I177, N175, L173, R171, E138
I177, N174, L173, R171, E138
Epi#30
I39, N33, S29, H23, P18, L17, P375
G38, N33, S29, H23, L17, P375, P380

G362, M323, S287, H321, Q319, P322, V318
G417, N423, A420, H421, K395, L390, P388
G21, N25, S29, H23, P18, L17, P375
G399, N418, A420, H421, K395, L390, P388
Epi#31
L173, R171, N174, R176, W167, M202, I177, D166
L173, R171, N174, R176, W167, V165, I177, D166
Epi#33
K108, Y58, V56, S52, A51, K72
Epi#34
W439, W469, T463, V450, G460, T452, T461
W15, P18, T376, G378, P375, Y372, S384
W469, W439, S473, G460, R458, T461, T463
Epi#37
P124, R176, L173, K172, N175
P124, R176, L173, R171, N174
Epi#40
R400, G399, Y396, P422, T419
R400, G417, Y396, P422, T419
**[0597]** Epi#41
P375, Y16, L17, V56, S52
P18, Y16, L17, V56, S52
Epi#42
P350, S478, G433, H408, R310, Q311
P322, S287, N285, H324, R320, Q319
P322, S287, G362, H321, R320, Q319
Epi#44
L17, D20, P18, W15, Y368, A381, G378, T376
L340, D333, P336, W15, Y368, A381, G378, P375
Epi#45
K72, P146, F69, Y64, N150, D144, G147
D112, P146, F69, Y64, N150, D144, G149
Epi#46
Y398, R359, R393, P388, G456, A459
Y363, R359, R393, P388, Q394, G7
Y363, R359, R393, P388, Q394, G38
Epi#47
I75, E68, R76, N83, R82, Q84, N90, R28, S29
G133, E134, E130, V131, R176, L173, N174, R171, S170
Epi#48
S384, K383, P380, P375, G378
E337, H377, P380, P375, V379
Epi#50
R444, W469, W439, S473, T461
D183, Y160, W159, W140, T114
Epi#51
R320, H321, Q319, P322, H324, H286
Epi#52
W140, A113, E138, R171, W167, D166, Q169
W140, A113, E115, R118, W159, T114, Q169
Protease A:
Epi#01
L21, N18, P14, R19, K237, N238, S242, Q245, S240
L21, N18, P14, R19, K237, N238, S240, Q236, S24
Epi#02
T255, N269, Q12, R10, P14, R19, R275
T255, N269, S9, R10, P14, R19, R275
Epi#03
K27, A88, I44, Y91
Epi#04
K235, S240, Q236, K237, R275, A15
K27, S24, Q236, K237, R275, A15
K237, S240, Q245, R247, N252, A254
R145, S141, Q137, Y171, N173, A172
Epi#06
G61, N62, D60, T38, Q59, P55
G211, P210, D60, T38, Q59, P55
A98, N62, D60, T38, Q59, P55
G100, N62, D60, T38, Q59, P55
Epi#08
A131, E136, S141, A108, F50
A172, E136, S141, A108, F50
A98, E54, G53, V51, F50
Epi#09
S162, S170, A172, N173, V244, H249, N252, S256, T260
S259, S256, T260, N261, L262, R186, N185, S188, N155
S162, S170, A172, N173, V244, H249, N248, N252, T255
S156, S162, N261, S259, L262, R186, N185, S188, N155
Epi#10
D181, N183, N185, S188, F189, G157, R186
D181, N218, N155, S156, F189, G157, R186
Epi#12
Y171, E136
Y91, E89

Epi#13
S78, L75, P40, T213, A215, P210, S212, G211, Y209
S87, L75, P40, T213, A215, P210, S212, G211, Y214
Epi#16
L262, P194, Y192, G195, S162, N261, D197, A172, N140
L262, P194, Y192, G157, S162, N261, D197, A172, N173
L262, P194, Y192, G161, S162, S170, D197, A172, N173
Epi#17
A138, S141, R145, S144
A108, S141, R145, S144
Epi#18
N185, N183, R186, L262, S259, T260, P194, N261
N185, N183, R186, L262, Y192, T260, P194, S162
Epi#19
I246, W241, S240, Q245, R247, K251
D181, N185, S188, Q191, R186, L262
Epi#23
R145, N116, E112, S141, Q137, A138
R145, N117, E112, S141, Q137, A108
Epi#24
E136, G133, A131, P129, S103, F50, Q109
E136, G132, A131, P129, S103, A108, Q137
D60, G61, E54, P52, F50, P55, Q59
Epi#25
R275, R19, E271, N18
Epi#28
G20, H17, Q12, E271, L21, Q236, S240, K237
A15, H17, Q12, E271, L21, Q236, S240, K237
Epi#29
V244, Q245, L148, R145, E112
V244, N173, L148, R145, E112
Epi#30
G61, N62, A98, H64, L96, P52, P55
G20, N18, A15, H17, S87, L75, P40
I79, N76, S87, H17, Q12, P14, V4
G100, N62, A98, H64, L96, P52, P55
Epi#31
L262, R186, N184, R10, W6, V203, D181
L257, R186, N184, R10, W6, V203, D181
Epi#33
Q109, F50, P52, S49, S56, K94
Q109, F50, P55, S56, A48, K94
Epi#34
W241, P239, S242, G146, R145, S141, S144
I165, P194, T260, G258, R186, S188, S156
V104, P129, S130, G127, G102, S101, S99
V244, W241, S242, G146, R145, S141, S144
I165, P194, S170, G127, P129, S130, S103
Epi#37
P14, A15, L21, R19, N18
T143, R145, L148, R247, N252
T143, V244, L148, R145, N116
Epi#38
L96, G97, E54, A98, N62, P210, S212
L96, G97, E54, A98, N62, P210, S37
Epi#39
A15, E271, H17, R19, P14, G20, L21
A254, E271, H17, R19, P14, G20, L21
A272, E271, H17, R19, P14, G20, L21
Epi#40
R186, L257, G258, T260, P194, S162
R186, L262, G161, Y192, P194, T260
Epi#41
P194, Y192, L262, S259
P194, Y192, L196, S162
Epi#42
L82, P5, S3, N76, H17, P14, R19, R275
L82, P5, S9, Q12, H17, P14, R19, R275
Epi#43
W113, A48, G47, Q59, S37, L42, I44, T38
Epi#44
V244, R247, D197, P194, Y192, S162, G195, T260
V244, R247, D197, P194, Y192, S170, G195, T260
S56, Q59, D60, P210, Y214, S212, G211, T38
S56, Q59, D60, P210, Y209, S212, G211, T38
Epi#46
A15, R275, R19, P14, N18, G20
A272, R275, R19, P14, N18, G20
A272, R275, R19, P14, N18, A15
Epi#47
S130, A131, E136, N173, A172, N140, R145, S144
S105, A131, E136, N173, A172, N140, R145, S144
Epi#48
E54, Q59, P55, P52, G53
S56, Q59, P55, P52, G53
S49, Q59, P55, P52, G53

Epi#50
R10, W6, S3, S78
R10, W6, V4, S9
R10, W6, V203, S188
Epi#51
Q245, H249, T253, R275, K237, S240, W241
R19, H17, E271, R275, K237, S240, W241
R145, H120, K27, S24, K237, S240, W241
R145, H120, K235, K237, P239, S240, W241
Epi#52
A15, S9, R10, W6, N204, Q206
A15, S9, R10, W6, N204, Q182
Alcalase:
Epi#01
L10, P5, P9, K15, K12, N269, S251, R249, T253
L82, P5, P9, K15, K12, N269, S251, R249, T253
Epi#02
T115, N141, A144, R145, S242, R247, R249
A138, N141, A144, R145, S242, R247, R249
Epi#03
L196, K170, A129, 1165, Y167
L196, K170, A194, I165, Y171
Epi#04
R145, Y143, S173, Q137, K136, T133, A134
K170, Y167, S132, Q137, K136, N141, A144
Epi#05
G53, A52, F50, G102, S105, S103, Y104
G53, A52, F50, G102, S101, S103, Y104
Epi#06
A24, N25, D120, R145, S242, P239
A144, N141, D140, R145, S242, P239
Epi#08
K265, E197, S260, A194, F261
A56, E54, G53, A52, F50
Epi#10
T162, N161, N163, A194, F261, G264, K265
E195, N161, N163, S158, F261, G258, K265
Epi#12
Y57, E54
Y262, E197
Epi#13
S38, A37, P40, T213, A215, H64, L217, G204, Y206
S38, A37, P40, T213, A215, H64, S98, G100, G61
S87, L75, P40, T213, A215, H64, L217, G204, Y6
Epi#16
L10, P9, Y6, G204, S182, N183, D181, A187, N185
Q2, P5, Y206, G204, S182, N183, D181, A203, N218
L10, P9, Y6, G204, S182, N183, D181, A187, N155
Epi#17
A144, S244, R247, S252
A272, S252, R249, S244
A144, S244, R249, S251
A254, S252, R249, S244
Epi#18
N141, R145, A144, Y143, S244, N248, S252
Epi#19
N248, S244, Q245, R249, A272
N240, S242, Q245, R249, A254
N240, S242, Q245, R249, L241
Epi#22
D76, L82, D14, A18, K15
D181, L10, D14, A18, K15
Epi#23
K27, N117, E112, N141, Q137, A134
K27, N117, E112, N141, Q137, A138
K27, N117, E112, S109, F50, A52
Epi#24
D120, K27, A24, P86, F21, A18, K15
D14, K22, A24, P86, F21, A18, K15
D76, K22, A24, P86, S87, F21, K15
Epi#25
R249, R247, E197, E195
Epi#27
D172, E195, E197, K265
E197, E195, D172, K136
D172, E197, E195, K170
Epi#28
A18, D14, Q19, K15, E271, K12, Q17, S87, D76
V4, D14, Q17, K12, E271, K15, F21, A18, K22
Epi#29
L257, K265, L196, F261, E195
G53, N97, L96, F50, E54

Epi#30

G146, L241, S242, H238, K237, P239, L235

G146, L241, S236, H238, S242, P239, L235

Epi#33

K15, F21, P86, S87, A24, K27

K27, Y91, V45, S89, A24, K22

Epi#34

V4, P5, T3, G80, P40, S38, T211

V108, W113, T116, G118, R145, Y143, S244

V26, P239, S242, G146, R145, T115, T116

Epi#36

A52, A56, A48, V51, G102, Y104, S105, V108, A138, A134

A52, A56, A48, V51, G102, Y104, S103, V108, A134, A138

Epi#37

Y262, A194, L196, K265, Y256

Y263, R186, L257, K265, Y256

Y256, A254, L257, K265, Y262

Epi#40

R186, L257, A254, Y256, K265, S252

R186, L257, G258, Y256, K265, S260

Epi#41

Y256, L257, S260

Y256, L257, S259

Epi#42

L235, P239, S242, N248, R249, Q275

L241, P239, S242, Q245, R249, Q275

Epi#44

S132, Q137, D140, Y143, A144, A138, T133

V108, Q137, D140, Y143, A144, A138, T133

S173, Q137, D140, Y143, A144, A138, T133

Epi#48

Q19, K15, P9, P5, V4

E271, K15, P9, P5, V4

Protease B:

Epi#05

SAS: 454, Size 24.86: G189, A188, R164, P127, G125, S99

SAS: 452, Size 15.92: G189, A188, R164, P127, G125, S128

SAS: 451, Size 24.86: G157, A188, R164, P127, G125, S99

SAS: 449, Size 15.92: G157, A188, R164, P127, G125, S128

SAS: 445, Size 23.31: G189, A166, R164, P127, G125, S99

Epi#09

SAS: 446, Size 15.76: T254, G189, A166, R164, A188, S158

SAS: 312, Size 15.90: T22, G20, L21, R19, A15, S9

Epi#10

SAS: 460, Size 17.32: D175, N177, N179, S182, F183, G155, R180

SAS: 437, Size 16.70: D211, N212, N153, S182, F183, G155, R180

SAS: 424, Size 13.75: D175, N212, N153, S182, F183, G155, R180

SAS: 417, Size 16.70: D211, N212, N153, S154, F183, G155, R180

SAS: 404, Size 15.83: D175, N212, N153, S154, F183, G155, R180

Epi#12

SAS: 309, Size 13.46: P127, Y161, E134, P129

SAS: 292, Size 9.37: R164, Y161, E134, P129

SAS: 287, Size 18.66: P127, Y161, E134, N138

SAS: 284, Size 16.85: P127, Y161, E134, N167

SAS: 275, Size 11.53: S128, Y161, E134, P129

Epi#17

SAS: 275, Size 15.84: A188, S158, R164, S126

SAS: 225, Size 12.79: A156, S158, R164, S126

Epi#18

SAS: 444, Size 16.32: S250, K245, S259, L256, A188, T254, L251

SAS: 397, Size 14.14: S250, K245, S259, L256, G252, T254, L251

SAS: 397, Size 14.14: S250, K245, S259, L251, G252, T254, L256

SAS: 397, Size 14.14: S259, K245, S250, L251, G252, T254, L256

SAS: 396, Size 21.52: S158, R164, S126, V102, G100, S99, L124

Epi#19

SAS: 295, Size 15.06: D175, W6, S9, Q12, R10

SAS: 278, Size 21.23: E110, T141, S236, Q239, R241

Epi#23

SAS: 486, Size 19.88: R143, N114, E110, S139, Q135, A131

SAS: 473, Size 18.68: R19, N18, E265, L21, Q230, P233

SAS: 468, Size 15.74: R164, N167, E134, S139, Q135, A131

SAS: 463, Size 13.77: R164, N167, E134, S130, Q135, A131

SAS: 461, Size 21.98: R44, N42, E87, S24, Q230, P233

Epi#28

SAS: 520, Size 19.27: V102, Q107, W111, E110, Q135, S139, R143

SAS: 492, Size 24.70: V102, Q107, F49, E53, Q57, G46, R44

SAS: 480, Size 22.76: V50, Q107, W111, E110, Q135, S139, R143

SAS: 452, Size 19.08: V50, Q107, F49, E53, Q57, G46, R44

SAS: 441, Size 24.70: V102, Q107, E110, W111, F49, G46, R44

Epi#29

SAS: 239, Size 11.49: G20, N18, L21, E265

SAS: 224, Size 11.49: G20, R19, L21, E265

SAS: 179, Size 16.62: 14, P14, L21, E265

SAS: 175, Size 11.49: G20, K231, L21, E265

SAS: 153, Size 18.96: G25, Q230, L21, E265

Epi#30

SAS: 308, Size 24.27: G20, L21, A15, H17, S85, L73, P39

Epi#31

SAS: 363, Size 21.72: L256, R180, N178, R10, W6, V197, D211

SAS: 352, Size 22.95: L251, R180, N178, R10, W6, V197, D211

SAS: 350, Size 21.62: L256, R180, N178, R10, W6, V197, D175

SAS: 339, Size 17.75: L251, R180, N178, R10, W6, V197, D175

Epi#34

SAS: 430, Size 18.33: V238, W235, S236, G144, R143, S139, S142

SAS: 430, Size 18.33: V238, W235, S236, G144, R143, S142, S139

SAS: 420, Size 13.98: V238, W235, S236, G144, R143, S142, T141

SAS: 420, Size 13.98: V238, W235, S236, G144, R143, T141, S142

SAS: 352, Size 18.33: V238, W235, S236, G144, R143, S139, T141

Epi#37

SAS: 415, Size 23.06: T254, A188, L256, R180, N177

SAS: 374, Size 18.08: T254, A188, L256, R180, N179

SAS: 335, Size 19.96: T254, A188, L256, R180, N178

Epi#39

SAS: 425, Size 16.00: A166, E134, R164, P127, G125, L124

SAS: 421, Size 16.36: A131, E134, R164, P127, G125, L124

SAS: 400, Size 16.00: A166, E134, R164, P129, G125, L124

SAS: 396, Size 16.36: A131, E134, R164, P129, G125, L124

SAS: 359, Size 16.00: A166, E134, T132, P129, G125, L124

Epi#40

SAS: 358, Size 15.76: A166, G189, Y186, A188, T254

SAS: 352, Size 15.76: A166, G189, T254, A188, S158

SAS: 326, Size 11.62: A96, G59, T56, P54, S55

SAS: 322, Size 15.30: G98, G59, T56, P54, S55

SAS: 318, Size 17.81: A188, G189, Y186, A156, S182

Epi#42

SAS: 528, Size 16.22: L21, P14, S9, Q12, H17, R19, R269

Epi#44

SAS: 401, Size 15.10: L256, R180, Y186, S158, A188, T254

SAS: 393, Size 15.52: L256, R180, Y186, A188, G189, T254

SAS: 390, Size 18.46: L251, R180, Y186, S158, A188, T254

SAS: 382, Size 16.23: L251, R180, Y186, A188, G189, T254

SAS: 376, Size 22.23: V197, R180, Y186, S158, A188, T254

Epi#46

SAS: 559, Size 12.63: A15, R269, R19, P14, N18, G20

Epi#53

SAS: 298, Size 9.48: W235, S234, Q230, K231

SAS: 298, Size 18.05: W235, S234, Q239, K245

SAS: 289, Size 9.48: W235, P233, Q230, K231

SAS: 283, Size 9.61: W235, S234, Q239, K229

SAS: 255, Size 14.51: W235, S236, Q239, K245

ProteaseC:

Epi#05

SAS: 445, Size 23.34: G189, A166, R164, P127, G125, S99

SAS: 445, Size 24.90: G189, A188, R164, P127, G125, S99

SAS: 433, Size 24.90: G157, A188, R164, P127, G125, S99

SAS: 427, Size 15.89: G189, A188, R164, P127, G125, S128

SAS: 427, Size 15.50: G189, A166, R164, P127, G125, S128

Epi#09

SAS: 463, Size 15.74: T254, G189, A166, R164, A188, S158

SAS: 425, Size 15.74: D191, G189, A166, R164, A188, T254

SAS: 384, Size 13.57: D191, G189, A166, R164, A188, S158

Epi#10

SAS: 445, Size 17.28: D175, N177, N179, S182, F183, G155, R180

SAS: 431, Size 13.75: D175, N212, N153, S182, F183, G155, R180

SAS: 403, Size 15.83: D175, N212, N153, S154, F183, G155, R180

SAS: 387, Size 16.14: D175, N178, N179, S182, F183, G155, R180

SAS: 373, Size 16.76: D175, N212, N153, A156, F183, G155, R180

Epi#12

SAS: 292, Size 13.45: P127, Y161, E134, P129

SAS: 287, Size 9.30: R44, Y89, E87, N42

SAS: 284, Size 9.35: R164, Y161, E134, P129

SAS: 282, Size 9.35: R164, Y165, E134, P129

SAS: 272, Size 16.85: P127, Y161, E134, N167

Epi#16

SAS: 547, Size 20.59: R164, P129, Y165, G189, S158, N255, D191, A166, N167

SAS: 543, Size 23.80: R164, P129, Y165, G189, S158, N255, D191, A166, N138

Epi#17

SAS: 267, Size 15.84: A188, S158, R164, S126

SAS: 231, Size 12.82: A156, S158, R164, S126

Epi#18

SAS: 449, Size 16.85: S182, R180, L256, A188, T254, L251

SAS: 426, Size 21.97: S126, R164, S158, A188, T254, L256

SAS: 407, Size 15.92: S182, R180, L251, G252, T254, L256

SAS: 407, Size 15.92: S182, R180, L256, G252, T254, L251

SAS: 391, Size 18.26: S182, R180, L256, G252, S250, L251

Epi#19

SAS: 293, Size 15.04: D175, W6, S9, Q12, R10

SAS: 291, Size 17.13: D191, N242, S236, Q239, R241

SAS: 273, Size 21.24: E110, T141, S236, Q239, R241

Epi#23

SAS: 463, Size 19.84: R143, N114, E110, S139, Q135, A131

SAS: 451, Size 15.68: R164, N167, E134, S139, Q135, A131

SAS: 443, Size 21.95: R44, N42, E87, S24, Q230, P233

SAS: 440, Size 22.70: R143, N115, E110, S139, Q135, A131

SAS: 431, Size 15.11: R44, N42, E87, S85, L73, P39

Epi#28

SAS: 402, Size 18.79: G59, Q57, E53, F49, G46, R44

SAS: 384, Size 20.81: A96, Q57, E53, F49, G46, R44

SAS: 376, Size 18.79: A47, Q57, E53, F49, G46, R44

Epi#31

SAS: 348, Size 21.63: L256, R180, N178, R10, W6, V197, D175

SAS: 342, Size 17.75: L251, R180, N178, R10, W6, V197, D175

Epi#33

SAS: 399, Size 18.88: Q107, Y102, P129, S126, R164

SAS: 355, Size 15.95: Q135, Y165, P129, S126, R164

Epi#34

SAS: 424, Size 18.37: V238, W235, S236, G144, R143, S139, S142

SAS: 424, Size 18.37: V238, W235, S236, G144, R143, S142, S139

SAS: 408, Size 14.02: V238, W235, S236, G144, R143, S142, T141

SAS: 408, Size 14.02: V238, W235, S236, G144, R143, T141, S142

SAS: 346, Size 18.37: V238, W235, S236, G144, R143, T141, S139

Epi#37

SAS: 405, Size 23.05: T254, A188, L256, R180, N177

SAS: 364, Size 18.08: T254, A188, L256, R180, N179

SAS: 347, Size 19.96: T254, A188, L256, R180, N178

Epi#40

SAS: 368, Size 15.74: A166, G189, T254, A188, S158

SAS: 362, Size 15.74: A166, G189, Y186, A188, T254

SAS: 326, Size 17.80: A188, G189, Y186, A156, S182

SAS: 326, Size 23.72: A166, G189, Y186, A156, S182

SAS: 326, Size 17.80: G189, A188, Y186, A156, S182

Epi#41

SAS: 232, Size 19.49: P204, Y208, L211, V197, S210

Epi#44

SAS: 445, Size 22.71: V238, R241, D191, Y186, S158, A188, T254

SAS: 429, Size 21.14: V238, R241, D191, Y186, A188, G189, T254

SAS: 410, Size 22.71: V238, R241, D191, Y186, S158, G189, T254

SAS: 404, Size 23.33: V238, R241, D191, Y257, S250, G252, T254

SAS: 382, Size 23.33: V238, R241, D191, Y257, S253, G252, T254

Epi#46

SAS: 567, Size 12.67: A15, R269, R19, P14, N18, G20

Epi#53

SAS: 305, Size 9.43: W235, S234, Q230, K231

SAS: 303, Size 9.53: W235, S234, Q239, K229

SAS: 276, Size 9.43: W235, P233, Q230, K231

SAS: 259, Size 9.43: W235, S234, Q230, K229

SAS: 233, Size 9.53: W235, S236, Q239, K229

ProteaseD:

Epi#05

SAS: 453, Size 24.94: G189, A188, R164, P127, G125, S99

SAS: 449, Size 23.37: G189, A166, R164, P127, G125, S99

SAS: 442, Size 24.94: G157, A188, R164, P127, G125, S99

SAS: 439, Size 15.91: G189, A188, R164, P127, G125, S128

SAS: 435, Size 15.50: G189, A166, R164, P127, G125, S128

Epi#09

SAS: 448, Size 15.77: T254, G189, A166, R164, A188, S158

Epi#10

SAS: 460, Size 17.32: D175, N177, N179, S182, F183, G155, R180

SAS: 428, Size 13.76: D175, N212, N153, S182, F183, G155, R180

SAS: 403, Size 15.83: D175, N212, N153, S154, F183, G155, R180

SAS: 391, Size 16.15: D175, N178, N179, S182, F183, G155, R180

SAS: 372, Size 16.77: D175, N212, N153, A156, F183, G155, R180

Epi#12

SAS: 302, Size 13.47: P127, Y161, E134, P129

SAS: 290, Size 9.39: R164, Y161, E134, P129

SAS: 282, Size 18.68: P127, Y161, E134, N138

SAS: 280, Size 16.87: P127, Y161, E134, N167

SAS: 270, Size 13.10: R164, Y161, E134, N138

Epi#17

SAS: 286, Size 15.87: A188, S158, R164, S126

SAS: 250, Size 12.76: A156, S158, R164, S126

Epi#18

SAS: 446, Size 16.31: S250, K245, S259, L256, A188, T254, L251

SAS: 406, Size 14.13: S250, K245, S259, L256, G252, T254, L251

SAS: 406, Size 14.13: S250, K245, S259, L251, G252, T254, L256

SAS: 406, Size 14.13: S259, K245, S250, L251, G252, T254, L256

SAS: 388, Size 14.13: S250, K245, S259, L256, G252, T249, L251

Epi#19

SAS: 319, Size 15.07: D175, W6, S9, Q12, R10

SAS: 276, Size 21.28: E110, T141, S236, Q239, R241

Epi#23

SAS: 497, Size 19.86: R143, N114, E110, S139, Q135, A131

SAS: 487, Size 15.77: R164, N167, E134, S139, Q135, A131

SAS: 478, Size 13.78: R164, N167, E134, S130, Q135, A131

SAS: 477, Size 18.16: R143, N138, E134, S139, Q135, A131

SAS: 472, Size 22.70: R143, N115, E110, S139, Q135, A131

Epi#28

SAS: 554, Size 22.17: A101, Q107, I102, E134, Q135, S139, R143

SAS: 532, Size 19.36: 1102, Q107, W111, E110, Q135, S139, R143

SAS: 527, Size 22.79: V50, Q107, I102, E134, Q135, S139, R143

SAS: 509, Size 24.76: 1102, Q107, F49, E53, Q57, G46, R44

SAS: 508, Size 22.17: A101, Q107, W111, E110, Q135, S139, R143

Epi#31

SAS: 355, Size 21.56: L256, R180, N178, R10, W6, V197, D175

SAS: 352, Size 17.71: L251, R180, N178, R10, W6, V197, D175

Epi#34

SAS: 457, Size 18.37: V238, W235, S236, G144, R143, S139, S142

SAS: 457, Size 18.37: V238, W235, S236, G144, R143, S142, S139

SAS: 447, Size 14.02: V238, W235, S236, G144, R143, S142, T141

SAS: 447, Size 14.02: V238, W235, S236, G144, R143, T141, S142

SAS: 374, Size 18.37: V238, W235, S236, G144, R143, T141, S139

Epi#37

SAS: 397, Size 23.08: T254, A188, L256, R180, N177

SAS: 361, Size 18.08: T254, A188, L256, R180, N179

SAS: 328, Size 19.98: T254, A188, L256, R180, N178

Epi#39

SAS: 425, Size 16.36: A131, E134, R164, P127, G125, L124

SAS: 423, Size 16.02: A166, E134, R164, P127, G125, L124

SAS: 399, Size 16.36: A131, E134, R164, P129, G125, L124

SAS: 397, Size 16.02: A166, E134, R164, P129, G125, L124

SAS: 379, Size 16.36: A131, E134, T132, P129, G125, L124

Epi#40

SAS: 354, Size 15.77: A166, G189, T254, A188, S158

SAS: 351, Size 15.77: A166, G189, Y186, A188, T254

SAS: 334, Size 17.81: G189, A188, Y186, A156, S182

SAS: 334, Size 17.81: A188, G189, Y186, A156, S182

SAS: 330, Size 14.42: A166, G189, Y186, A188, S158

Epi#41

SAS: 217, Size 19.46: P204, Y208, L211, V197, S210

Epi#44

SAS: 407, Size 15.10: L256, R180, Y186, S158, A188, T254

SAS: 404, Size 18.45: L251, R180, Y186, S158, A188, T254

SAS: 387, Size 15.52: L256, R180, Y186, A188, G189, T254

SAS: 384, Size 16.23: L251, R180, Y186, A188, G189, T254

SAS: 373, Size 22.26: V197, R180, Y186, S158, A188, T254

Epi#46

SAS: 545, Size 12.69: A15, R269, R19, P14, N18, G20

Epi#53

SAS: 306, Size 18.06: W235, S234, Q239, K245

SAS: 277, Size 9.52: W235, S234, Q239, K229

SAS: 276, Size 9.46: W235, S234, Q230, K231

SAS: 268, Size 9.46: W235, P233, Q230, K231

SAS: 258, Size 14.50: W235, S236, Q239, K245

ProteaseE:

Epi#05

SAS: 461, Size 15.49: G189, A166, R164, P127, G125, S128

SAS: 459, Size 15.90: G189, A188, R164, P127, G125, S128

SAS: 435, Size 15.49: G189, A166, R164, P127, G125, S126

SAS: 433, Size 15.49: G189, A166, R164, P129, G125, S128

SAS: 433, Size 15.86: G189, A188, R164, P127, G125, S126

Epi#06

SAS: 518, Size 14.10: G189, A188, D157, S158, R164, P127

SAS: 490, Size 15.98: G189, A188, D157, S158, R164, P129

SAS: 460, Size 14.60: G155, A156, D157, S158, R164, P127

SAS: 432, Size 17.71: G155, A156, D157, S158, R164, P129

Epi#09

SAS: 482, Size 15.78: T254, G189, A166, R164, A188, S158

SAS: 311, Size 15.91: T22, G20, L21, R19, A15, S9

Epi#10

SAS: 455, Size 17.26: D175, N177, N179, S182, F183, G155, R180

SAS: 406, Size 13.76: D175, N212, N153, S182, F183, G155, R180

SAS: 383, Size 16.16: D175, N178, N179, S182, F183, G155, R180

SAS: 381, Size 15.82: D175, N212, N153, S154, F183, G155, R180

SAS: 347, Size 16.78: D175, N212, N153, A156, F183, G155, R180

Epi#12

SAS: 310, Size 13.48: P127, Y161, E134, P129

SAS: 306, Size 9.40: R164, Y161, E134, P129

SAS: 297, Size 9.40: R164, Y165, E134, P129

SAS: 285, Size 16.90: P127, Y161, E134, N167

SAS: 281, Size 18.68: P127, Y161, E134, N138

Epi#16

SAS: 673, Size 19.67: R164, P127, Y161, G125, S126, S154, D157, A188, N255

SAS: 664, Size 20.60: R164, P129, Y165, G189, S158, S154, D157, A188, N255

SAS: 645, Size 20.60: R164, P129, Y161, G125, S126, S154, D157, A188, N255

SAS: 636, Size 14.89: R164, P127, Y161, G125, S126, S154, D157, A156, N153

SAS: 627, Size 17.25: R164, P129, Y165, G189, S158, S154, D157, A156, N153

Epi#17

SAS: 305, Size 15.86: A188, S158, R164, S126

SAS: 270, Size 12.73: A156, S158, R164, S126

Epi#18

SAS: 590, Size 17.32: S250, K246, S259, L256, A188, T254, L251

SAS: 551, Size 16.26: S259, K246, S250, L251, G252, T254, L256

SAS: 551, Size 16.26: S250, K246, S259, L251, G252, T254, L256

SAS: 551, Size 16.26: S250, K246, S259, L256, G252, T254, L251

SAS: 518, Size 16.26: S250, K246, S259, L251, G252, S253, L256

Epi#23

SAS: 471, Size 19.86: R143, N114, E110, S139, Q135, A131

SAS: 467, Size 13.75: R164, N167, E134, S130, Q135, A131

SAS: 467, Size 15.76: R164, N167, E134, S139, Q135, A131

SAS: 451, Size 22.69: R143, N115, E110, S139, Q135, A131

SAS: 446, Size 19.99: R143, N138, E134, S130, Q135, A131

Epi#28

SAS: 505, Size 19.43: 1102, Q107, W111, E110, Q135, S139, R143

SAS: 500, Size 22.22: A101, Q107, W111, E110, Q135, S139, R143

SAS: 499, Size 24.79: 1102, Q107, F49, E53, Q57, G46, R44

SAS: 494, Size 24.56: A101, Q107, F49, E53, Q57, G46, R44

SAS: 441, Size 24.79: 1102, Q107, E110, W111, F49, G46, R44

Epi#29

SAS: 216, Size 9.94: 143, R44, L41, E87

SAS: 209, Size 10.85: L73, N42, L41, E87

SAS: 200, Size 13.98: G46, R44, L41, E87

SAS: 199, Size 11.98: G45, R44, L41, E87

SAS: 197, Size 19.08: 177, N74, L41, E87

Epi#30

SAS: 318, Size 24.25: G20, L21, A15, H17, S85, L73, P39

SAS: 277, Size 24.25: G20, L21, A15, H17, S85, L41, P39

SAS: 258, Size 21.05: G20, L21, A15, H17, S85, L73, L41

Epi#31

SAS: 377, Size 21.62: L256, R180, N178, R10, W6, V197, D175

SAS: 370, Size 17.72: L251, R180, N178, R10, W6, V197, D175

Epi#33

SAS: 388, Size 15.92: Q135, Y165, P129, S126, R164

Epi#34

SAS: 420, Size 18.35: V238, W235, S236, G144, R143, S139, S142

SAS: 411, Size 13.98: V238, W235, S236, G144, R143, S142, T141

SAS: 341, Size 18.35: V238, W235, S236, G144, R143, S139, T141

Epi#37

SAS: 412, Size 23.05: T254, A188, L256, R180, N177

SAS: 378, Size 18.07: T254, A188, L256, R180, N179

SAS: 340, Size 20.00: T254, A188, L256, R180, N178

Epi#39

SAS: 445, Size 16.04: A166, E134, R164, P127, G125, L124

SAS: 432, Size 16.40: A131, E134, R164, P127, G125, L124

SAS: 417, Size 16.04: A166, E134, R164, P129, G125, L124

SAS: 404, Size 16.40: A131, E134, R164, P129, G125, L124

SAS: 376, Size 16.04: A166, E134, T132, P129, G125, L124

Epi#40

SAS: 374, Size 15.78: A166, G189, T254, A188, S158

SAS: 334, Size 15.78: A166, G189, Y186, A188, T254

SAS: 317, Size 11.62: A96, G59, T56, P54, S55

SAS: 312, Size 15.30: G98, G59, T56, P54, S55

SAS: 307, Size 15.49: G189, A166, Y165, P129, S128

Epi#41

SAS: 234, Size 19.50: P204, Y208, L211, V197, S210

SAS: 189, Size 19.50: P204, Y208, L211, V197, S215

Epi#42

SAS: 549, Size 16.42: L21, P14, S9, Q12, H17, R19, R269

Epi#44

SAS: 398, Size 15.10: L256, R180, Y186, S158, A188, T254

SAS: 391, Size 18.47: L251, R180, Y186, S158, A188, T254

SAS: 372, Size 15.51: L256, R180, Y186, A188, G189, T254

SAS: 371, Size 12.26: L256, R180, Y257, S250, G252, T254

SAS: 367, Size 15.51: L256, R180, Y186, S158, G189, T254

Epi#46

SAS: 575, Size 12.75: A15, R269, R19, P14, N18, G20

Epi#47

SAS: 491, Size 19.28: G45, E87, I43, R44, L41, N42, P39, S206

Epi#53

SAS: 202, Size 9.12: W235, P233, K231

SAS: 199, Size 9.12: W235, S234, K231

SAS: 182, Size 6.73: W235, P233, K229

SAS: 179, Size 7.76: W235, S234, K229

SAS: 131, Size 8.39: W235, S236, K229

Properase:

Epi#05

SAS: 456, Size 15.94: G189, A188, R164, P127, G125, S128

SAS: 453, Size 15.52: G189, A166, R164, P127, G125, S128

SAS: 451, Size 15.94: G157, A188, R164, P127, G125, S128

SAS: 427, Size 15.94: G189, A188, R164, P129, G125, S128

SAS: 424, Size 15.52: G189, A166, R164, P129, G125, S128

Epi#09

SAS: 480, Size 15.73: T254, G189, A166, R164, A188, S158

SAS: 302, Size 15.88: T22, G20, L21, R19, A15, S9

Epi#10

SAS: 470, Size 17.27: D175, N177, N179, S182, F183, G155, R180

SAS: 446, Size 13.75: D175, N212, N153, S182, F183, G155, R180

SAS: 420, Size 15.84: D175, N212, N153, S154, F183, G155, R180

SAS: 396, Size 16.09: D175, N178, N179, S182, F183, G155, R180

SAS: 380, Size 16.78: D175, N212, N153, A156, F183, G155, R180

Epi#12

SAS: 296, Size 9.36: R164, Y161, E134, P129

SAS: 295, Size 13.45: P127, Y161, E134, P129

SAS: 291, Size 9.36: R164, Y165, E134, P129

SAS: 271, Size 14.70: R164, Y161, E134, N102

SAS: 270, Size 13.45: P127, Y161, E134, N102

Epi#17

SAS: 283, Size 15.87: A188, S158, R164, S126

SAS: 241, Size 12.73: A156, S158, R164, S126

Epi#18

SAS: 474, Size 16.26: S250, K245, S259, L256, A188, T254, L251

SAS: 435, Size 14.14: S250, K245, S259, L256, G252, T254, L251

SAS: 398, Size 14.14: S259, K245, S250, L251, G252, S253, L256

Epi#19

SAS: 260, Size 21.26: E110, T141, S236, Q239, R241

Epi#23

SAS: 491, Size 19.86: R143, N114, E110, S139, Q135, A131

SAS: 482, Size 15.76: R164, N167, E134, S139, Q135, A131

SAS: 465, Size 22.69: R143, N115, E110, S139, Q135, A131

SAS: 462, Size 18.17: R143, N138, E134, S139, Q135, A131

SAS: 439, Size 18.17: R143, N138, E110, S139, Q135, A131

Epi#28

SAS: 445, Size 22.79: V50, Q107, W111, E110, Q135, S139, R143

SAS: 426, Size 19.06: V50, Q107, F49, E53, Q57, G46, R44

SAS: 370, Size 19.06: V50, Q107, E110, W111, F49, G46, R44

Epi#31

SAS: 347, Size 21.62: L256, R180, N178, R10, W6, V197, D175

SAS: 339, Size 17.74: L251, R180, N178, R10, W6, V197, D175

Epi#33

SAS: 368, Size 15.95: Q135, Y165, P129, S126, R164

Epi#34

SAS: 445, Size 18.39: V238, W235, S236, G144, R143, S139, S142

SAS: 436, Size 14.07: V238, W235, S236, G144, R143, S142, T141

SAS: 358, Size 18.39: V238, W235, S236, G144, R143, T141, S139

Epi#37

SAS: 415, Size 23.03: T254, A188, L256, R180, N177

SAS: 374, Size 18.04: T254, A188, L256, R180, N179

SAS: 341, Size 19.93: T254, A188, L256, R180, N178

Epi#39

SAS: 323, Size 11.55: A15, E265, H17, R19, P14, G20, L21

SAS: 238, Size 12.13: A15, E265, H17, T22, P14, G20, L21

Epi#40

SAS: 370, Size 15.73: A166, G189, T254, A188, S158

SAS: 360, Size 15.73: A166, G189, Y186, A188, T254

SAS: 324, Size 17.80: A188, G189, Y186, A156, S182

SAS: 321, Size 23.71: A166, G189, Y186, A156, S182

Epi#41

SAS: 228, Size 19.53: P204, Y208, L211, V197, S210

Epi#42

SAS: 554, Size 16.31: L21, P14, S9, Q12, H17, R19, R269

Epi#44

SAS: 406, Size 15.06: L256, R180, Y186, S158, A188, T254

SAS: 398, Size 18.38: L251, R180, Y186, S158, A188, T254

SAS: 395, Size 12.22: L256, R180, Y257, S250, G252, T254

SAS: 392, Size 15.49: L256, R180, Y186, A188, G189, T254

SAS: 387, Size 12.22: L251, R180, Y257, S250, G252, T254

Epi#46

SAS: 581, Size 12.65: A15, R269, R19, P14, N18, G20

Epi#53

SAS: 297, Size 18.06: W235, S234, Q239, K245

SAS: 283, Size 9.54: W235, S234, Q239, K229

SAS: 250, Size 9.46: W235, S234, Q230, K231

SAS: 249, Size 14.49: W235, S236, Q239, K245

SAS: 247, Size 9.46: W235, P233, Q230, K231

Release:

Epi#05

SAS: 461, Size 17.25: G158, A189, R165, P128, G126, S129

SAS: 439, Size 17.22: G158, A189, R165, P128, G126, S127

SAS: 436, Size 17.25: G158, A189, S159, P128, G126, S129

SAS: 420, Size 17.25: G158, A189, R165, P130, G126, S129

SAS: 414, Size 17.22: G158, A189, S159, P128, G126, S127

Epi#09

SAS: 510, Size 22.37: T22, G20, R19, A15, R270, A267, T250

SAS: 501, Size 22.37: L21, G20, R19, A15, R270, A267, T250

Epi#10

SAS: 458, Size 17.50: D176, N178, N180, S183, F184, G156, R181

SAS: 424, Size 13.68: D176, N213, N154, S183, F184, G156, R181

SAS: 407, Size 15.87: D176, N213, N154, S155, F184, G156, R181

SAS: 392, Size 16.18: D176, N179, N180, S183, F184, G156, R181

SAS: 362, Size 16.73: D176, N213, N154, A157, F184, G156, R181

Epi#12

SAS: 323, Size 9.38: R45, Y90, E88, N43

SAS: 312, Size 13.53: P128, Y162, E135, P130

SAS: 302, Size 9.46: R165, Y162, E135, P130

SAS: 296, Size 9.46: R165, Y166, E135, P130

SAS: 295, Size 13.19: T255, Y187, E190, S159

Epi#18

SAS: 431, Size 15.20: S251, K246, S260, L257, A189, T255, L252

SAS: 398, Size 14.35: S251, K246, S260, L252, G253, T255, L257

SAS: 378, Size 14.35: S251, K246, S260, L257, G253, T250, L252

Epi#19

SAS: 285, Size 21.53: E111, T142, S237, Q240, R242

SAS: 275, Size 12.58: D119, T142, S237, Q240, R242

Epi#23

SAS: 512, Size 22.29: R45, N43, E88, S24, Q231, P234

SAS: 476, Size 19.71: R144, N115, E111, S140, Q136, A132

SAS: 460, Size 13.83: R165, N168, E135, S131, Q136, A132

SAS: 455, Size 20.11: R144, N139, E135, S131, Q136, A132

SAS: 452, Size 15.83: R165, N168, E135, S140, Q136, A132

Epi#25

SAS: 293, Size 13.93: R45, K27, D119, E88

Epi#28

SAS: 502, Size 19.99: V103, Q108, W112, E111, Q136, S140, R144

SAS: 476, Size 21.74: V51, Q108, F50, E54, Q58, S37, R45

SAS: 472, Size 24.93: V103, Q108, F50, E54, Q58, G47, R45

SAS: 469, Size 23.18: V51, Q108, W112, E111, Q136, S140, R144

SAS: 439, Size 19.16: V51, Q108, F50, E54, Q58, G47, R45

Epi#31

SAS: 354, Size 21.73: L257, R181, N179, R10, W6, V198, D176

SAS: 348, Size 17.85: L252, R181, N179, R10, W6, V198, D176

Epi#33

SAS: 396, Size 22.75: Q201, Y204, P205, S37, R45

SAS: 379, Size 22.75: Q201, Y209, P205, S37, R45

SAS: 357, Size 18.39: H63, Y204, P205, S37, R45

Epi#34

SAS: 466, Size 13.97: V239, W236, S237, G145, R144, S143, T142

SAS: 463, Size 18.37: V239, W236, S237, G145, R144, S140, S143

SAS: 387, Size 18.37: V239, W236, S237, G145, R144, S140, T142

Epi#36

SAS: 206, Size 22.37: T250, A267, A15, G20, T22

Epi#37

SAS: 400, Size 22.59: T255, A189, L257, R181, N178

SAS: 359, Size 17.59: T255, A189, L257, R181, N180

SAS: 334, Size 19.35: T255, A189, L257, R181, N179

Epi#39

SAS: 464, Size 16.36: A167, E135, R165, P128, G126, L125

SAS: 444, Size 16.52: A132, E135, R165, P128, G126, L125

SAS: 441, Size 16.36: A167, E190, R165, P128, G126, L125

SAS: 441, Size 18.98: A189, E190, R165, P128, G126, L125

SAS: 423, Size 16.36: A167, E135, R165, P130, G126, L125

Epi#40

SAS: 324, Size 11.66: A97, G60, T57, P55, S56

SAS: 316, Size 17.09: G158, A189, Y187, A157, S183

SAS: 307, Size 14.92: G158, A157, Y187, A189, T255

SAS: 307, Size 15.34: G99, G60, T57, P55, S56

Epi#41

SAS: 222, Size 19.74: P205, Y209, L212, V198, S211

Epi#42

SAS: 544, Size 16.22: L21, P14, S9, Q12, H17, R19, R270

Epi#44

SAS: 421, Size 14.87: L257, R181, Y187, S159, A189, T255

SAS: 415, Size 18.81: L252, R181, Y187, S159, A189, T255

SAS: 389, Size 22.36: V198, R181, Y187, S159, A189, T255

SAS: 389, Size 21.81:144, R45, Y90, A48, V51, P52

SAS: 386, Size 19.16:144, R45, Y90, A48, V51, P55

Epi#46

SAS: 557, Size 14.54: A267, R270, R19, P14, N18, G20

SAS: 553, Size 12.63: A15, R270, R19, P14, N18, G20

SAS: 540, Size 13.10: A267, R270, R19, P14, N18, A15

SAS: 444, Size 14.54: A267, R270, R19, P14, G20, A15

Epi#47

SAS: 627, Size 16.22: A267, R270, A15, R19, L21, N18, P14, S9

SAS: 436, Size 15.11: A267, E266, A15, R19, L21, N18, P14, S9

Epi#51

SAS: 545, Size 21.66: L21, R19, H17, D75, S77, I78, S3, W6

SAS: 485, Size 21.66: L21, R19, H17, D75, Q2, I78, S3, W6

Epi#53

SAS: 328, Size 9.43: W236, S235, Q231, K232

SAS: 316, Size 9.43: W236, P234, Q231, K232

SAS: 301, Size 18.21: W236, S235, Q240, K246

SAS: 246, Size 14.68: W236, S237, Q240, K246

**[0598]** "SAS" is solvent accessible surface. "Size" is the total surface area of the epitope in A2.

Example 12

**[0599]** The object of this example is to provide evidence showing that subtilisins with an homology to BPN' of as low as 44.8% reveal a similar epitope distribution as BPN'.

**[0600]** Alcalase, Protease B, Savinase, Esperase, and PD498 (which range from 44.8% to 69.5% in sequence identity to BPN') were epitope mapped as described in the above example, and compared with epitope mapped BPN' (FIG. **1**).

**[0601]** The data in FIG. **1** show a significant overlap between the areas on the primary structure of the respective proteases. Overall, 6 regions were identified: 1-20, 35-65, 95-115, 130-145, 170-220, and 260-270.

**[0602]** Even better overlap between the epitope sequences can be found among proteins of higher sequence identity, such as within the Savinase-like subtilisins with more than 81% identity, preferably more than 85%, more preferably more than 90%, even more preferably more than 96% or most preferably more than 98% identity.

Example 13

Wash Performance

**[0603]** The following example provides results from a number of washing tests that were conducted under the conditions indicated

TABLE 9

Experimental conditions for evaluation of Subtilisin variants I44V.

| | |
|---|---|
| Detergent | OMO Acao |
| Detergent dose | 2.5 g/l |
| PH | 10.5 |
| Wash time | 14 min. |
| Temperature | 25° C. |
| Water hardness | 9° dH |
| Enzymes | Subtilisin variant I44V |
| Enzyme conc. | 10 nM |
| Test system | 150 ml glass beakers with a stirring rod |
| Textile/volume | 5 textile pieces (Ø 2.5 cm) in 50 ml detergent |
| Test material | EMPA117 from Center for Test materials, Holland |

TABLE 10

Experimental conditions for evaluation of Subtilisin variants Q12D.

| | |
|---|---|
| Detergent | Persil Powder |
| Detergent dose | 4 g/l |
| PH | 10.5 |
| Wash time | 20 min. |
| Temperature | 30° C. |
| Water hardness | 18° dH |
| Enzymes | Subtilisin variant Q12D |
| Enzyme conc. | 10 nM |
| Test system | 150 ml glass beakers with a stirring rod |
| Textile/volume | 5 textile pieces (Ø 2.5 cm) in 50 ml detergent |
| Test material | EMPA116 from Center for Test materials, Holland |

TABLE 11

Experimental conditions for evaluation of Subtilisin variants Q12D.

| | |
|---|---|
| Detergent | Tide |
| Detergent dose | 1 g/l |

TABLE 11-continued

Experimental conditions for evaluation of Subtilisin variants Q12D.

| | |
|---|---|
| PH | 10.5 |
| Wash time | 10 min. |
| Temperature | 25° C. |
| Water hardness | 6° dH |
| Enzymes | Subtilisin variant Q12D |
| Enzyme conc. | 10 nM |
| Test system | 150 ml glass beakers with a stirring rod |
| Textile/volume | 5 textile pieces (Ø 2.5 cm) in 50 ml detergent |
| Test material | EMPA117 from Center for Test materials, Holland |

**[0604]** pH is adjusted to 10.5 which is within the normal range for a powder detergent.

**[0605]** Water hardness was adjusted by adding $CaCl_2$ and $MgCl_2$ ($Ca^{2+}$:$Mg^{2+}$=2:1) to deionized water (see also Surfactants in Consumer Products—Theory, Technology and Application, Springer Verlag 1986). pH of the detergent solution was adjusted to pH 10.5 by addition of HCl.

**[0606]** Measurement of reflectance (R) on the test material was done at 460 nm using a Macbeth ColorEye 7000 photometer. The measurements were done according to the manufacturers protocol.

**[0607]** The wash performance of the variants was evaluated by calculating a performance factor:

$$P=(R_{Variant}-R_{Blank})/(R_{Savinase}-R_{Blank})$$

P: Performance factor

$R_{Variant}$: Reflectance of test material washed with variant

$R_{Savinase}$: Reflectance of test material washed with Savinase®

$R_{Blank}$: Reflectance of test material washed with no enzyme

**[0608]** The variants all have improved wash performance compared to Savinase®—i.e. P>1.

**[0609]** The variants can be divided into improvement classes designated with capital letters:

Class A: $1<P\leqq 1.5$

Class B: $1.5<P\leqq 2$

Class C: $P>2$

**[0610]**

TABLE 12

Subtilisin variants and improvement classes.

| Improvement class | Variants |
|---|---|
| C | I44V, Q12D |

**[0611]** As it can be seen from Table 12 SAVINASE® variants of the invention exhibits an improvement in wash performance.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 248

<210> SEQ ID NO 1
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: T. lanuginosus

<400> SEQUENCE: 1

Glu Val Ser Gln Asp Leu Phe Asn Gln Phe Asn Leu Phe Ala Gln Tyr

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1               5                   10                  15

Ser Ala Ala Ala Tyr Cys Gly Lys Asn Asn Asp Ala Pro Ala Gly Thr
            20                  25                  30

Asn Ile Thr Cys Thr Gly Asn Ala Cys Pro Glu Val Glu Lys Ala Asp
        35                  40                  45

Ala Thr Phe Leu Tyr Ser Phe Glu Asp Ser Gly Val Gly Asp Val Thr
    50                  55                  60

Gly Phe Leu Ala Leu Asp Asn Thr Asn Lys Leu Ile Val Leu Ser Phe
65                  70                  75                  80

Arg Gly Ser Arg Ser Ile Glu Asn Trp Ile Gly Asn Leu Asn Phe Asp
                85                  90                  95

Leu Lys Glu Ile Asn Asp Ile Cys Ser Gly Cys Arg Gly His Asp Gly
            100                 105                 110

Phe Thr Ser Ser Trp Arg Ser Val Ala Asp Thr Leu Arg Gln Lys Val
        115                 120                 125

Glu Asp Ala Val Arg Glu His Pro Asp Tyr Arg Val Val Phe Thr Gly
    130                 135                 140

His Ser Leu Gly Gly Ala Leu Ala Thr Val Ala Gly Ala Asp Leu Arg
145                 150                 155                 160

Gly Asn Gly Tyr Asp Ile Asp Val Phe Ser Tyr Gly Ala Pro Arg Val
                165                 170                 175

Gly Asn Arg Ala Phe Ala Glu Phe Leu Thr Val Gln Thr Gly Gly Thr
            180                 185                 190

Leu Tyr Arg Ile Thr His Thr Asn Asp Ile Val Pro Arg Leu Pro Pro
        195                 200                 205

Arg Glu Phe Gly Tyr Ser His Ser Ser Pro Glu Tyr Trp Ile Lys Ser
    210                 215                 220

Gly Thr Leu Val Pro Val Thr Arg Asn Asp Ile Val Lys Ile Glu Gly
225                 230                 235                 240

Ile Asp Ala Thr Gly Gly Asn Asn Gln Pro Asn Ile Pro Asp Ile Pro
                245                 250                 255

Ala His Leu Trp Tyr Phe Gly Leu Ile Gly Thr Cys Leu
            260                 265


<210> SEQ ID NO 2
<211> LENGTH: 481
<212> TYPE: PRT
<213> ORGANISM: Bacillus Halmapalus

<400> SEQUENCE: 2

Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp His Leu Pro Asn Asp
1               5                   10                  15

Gly Asn His Trp Asn Arg Leu Arg Asp Asp Ala Ser Asn Leu Arg Asn
            20                  25                  30

Arg Gly Ile Thr Ala Ile Trp Ile Pro Pro Ala Trp Lys Gly Thr Ser
        35                  40                  45

Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr Asp Leu Gly Glu
    50                  55                  60

Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Arg Ser Gln
65                  70                  75                  80

Leu Glu Ser Ala Ile His Ala Leu Lys Asn Asn Gly Val Gln Val Tyr
                85                  90                  95
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-continued

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Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp Ala Thr Glu Asn
            100                 105                 110

Val Leu Ala Val Glu Val Asn Pro Asn Asn Arg Asn Gln Glu Ile Ser
        115                 120                 125

Gly Asp Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp Phe Pro Gly Arg
    130                 135                 140

Gly Asn Thr Tyr Ser Asp Phe Lys Trp Arg Trp Tyr His Phe Asp Gly
145                 150                 155                 160

Val Asp Trp Asp Gln Ser Arg Gln Phe Gln Asn Arg Ile Tyr Lys Phe
                165                 170                 175

Arg Gly Asp Gly Lys Ala Trp Asp Trp Glu Val Asp Ser Glu Asn Gly
            180                 185                 190

Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Val Asp Met Asp His Pro Glu
        195                 200                 205

Val Val Asn Glu Leu Arg Arg Trp Gly Glu Trp Tyr Thr Asn Thr Leu
    210                 215                 220

Asn Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His Ile Lys Tyr Ser
225                 230                 235                 240

Phe Thr Arg Asp Trp Leu Thr His Val Arg Asn Ala Thr Gly Lys Glu
                245                 250                 255

Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu Gly Ala Leu Glu
            260                 265                 270

Asn Tyr Leu Asn Lys Thr Asn Trp Asn His Ser Val Phe Asp Val Pro
        275                 280                 285

Leu His Tyr Asn Leu Tyr Asn Ala Ser Asn Ser Gly Gly Asn Tyr Asp
    290                 295                 300

Met Ala Lys Leu Leu Asn Gly Thr Val Val Gln Lys His Pro Met His
305                 310                 315                 320

Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro Gly Glu Ser Leu
                325                 330                 335

Glu Ser Phe Val Gln Glu Trp Phe Lys Pro Leu Ala Tyr Ala Leu Ile
            340                 345                 350

Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr Gly Asp Tyr Tyr
        355                 360                 365

Gly Ile Pro Thr His Ser Val Pro Ala Met Lys Ala Lys Ile Asp Pro
    370                 375                 380

Ile Leu Glu Ala Arg Gln Asn Phe Ala Tyr Gly Thr Gln His Asp Tyr
385                 390                 395                 400

Phe Asp His His Asn Ile Ile Gly Trp Thr Arg Glu Gly Asn Thr Thr
                405                 410                 415

His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp Gly Pro Gly Gly
            420                 425                 430

Glu Lys Trp Met Tyr Val Gly Gln Asn Lys Ala Gly Gln Val Trp His
        435                 440                 445

Asp Ile Thr Gly Asn Lys Pro Gly Thr Val Thr Ile Asn Ala Asp Gly
    450                 455                 460

Trp Ala Asn Phe Ser Val Asn Gly Gly Ser Val Ser Ile Trp Val Lys
465                 470                 475                 480

Arg
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<210> SEQ ID NO 3

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<211> LENGTH: 504
<212> TYPE: PRT
<213> ORGANISM: Coprinus cinereus

<400> SEQUENCE: 3

```
Gln Ile Val Asn Ser Val Asp Thr Met Thr Leu Thr Asn Ala Asn Val
1               5                  10                  15

Ser Pro Asp Gly Phe Thr Arg Ala Gly Ile Leu Val Asn Gly Val His
            20                  25                  30

Gly Pro Leu Ile Arg Gly Gly Lys Asn Asp Asn Phe Glu Leu Asn Val
        35                  40                  45

Val Asn Asp Leu Asp Asn Pro Thr Met Leu Arg Pro Thr Ser Ile His
    50                  55                  60

Trp His Gly Leu Phe Gln Arg Gly Thr Asn Trp Ala Asn Gly Ala Asp
65                  70                  75                  80

Gly Val Asn Gln Cys Pro Ile Ser Pro Gly His Ala Phe Leu Tyr Lys
                85                  90                  95

Phe Thr Pro Ala Gly His Ala Gly Thr Phe Trp Tyr His Ser His Phe
            100                 105                 110

Gly Thr Gln Tyr Cys Asp Gly Leu Arg Gly Pro Met Val Ile Tyr Asp
        115                 120                 125

Asp Asn Asp Pro His Ala Ala Leu Tyr Asp Glu Asp Asp Glu Asn Thr
    130                 135                 140

Ile Ile Thr Leu Ala Asp Trp Tyr His Ile Pro Ala Pro Ser Ile Gln
145                 150                 155                 160

Gly Ala Ala Gln Pro Asp Ala Thr Leu Ile Asn Gly Lys Gly Arg Tyr
                165                 170                 175

Val Gly Gly Pro Ala Ala Glu Leu Ser Ile Val Asn Val Glu Gln Gly
            180                 185                 190

Lys Lys Tyr Arg Met Arg Leu Ile Ser Leu Ser Cys Asp Pro Asn Trp
        195                 200                 205

Gln Phe Ser Ile Asp Gly His Glu Leu Thr Ile Ile Glu Val Asp Gly
    210                 215                 220

Asn Leu Thr Glu Pro His Thr Val Asp Arg Leu Gln Ile Phe Thr Gly
225                 230                 235                 240

Gln Arg Tyr Ser Phe Val Leu Asp Ala Asn Gln Pro Val Asp Asn Tyr
                245                 250                 255

Trp Ile Arg Ala Gln Pro Asn Lys Gly Arg Asn Gly Leu Ala Gly Thr
            260                 265                 270

Phe Ala Asn Gly Val Asn Ser Ala Ile Leu Arg Tyr Ala Gly Ala Ala
        275                 280                 285

Asn Ala Asp Pro Thr Thr Ser Ala Asn Pro Asn Pro Ala Gln Leu Asn
    290                 295                 300

Glu Ala Asp Leu His Ala Leu Ile Asp Pro Ala Ala Pro Gly Ile Pro
305                 310                 315                 320

Thr Pro Gly Ala Ala Asn Val Asn Leu Arg Phe Gln Leu Gly Phe Ser
                325                 330                 335

Gly Gly Arg Phe Thr Ile Asn Gly Thr Ala Tyr Glu Ser Pro Ser Val
            340                 345                 350

Pro Thr Leu Leu Gln Ile Met Ser Gly Ala Gln Ser Ala Asn Asp Leu
        355                 360                 365

Leu Pro Ala Gly Ser Val Tyr Glu Leu Pro Arg Asn Gln Val Val Glu
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    370                 375                 380

Leu Val Val Pro Ala Gly Val Leu Gly Gly Pro His Pro Phe His Leu
385                 390                 395                 400

His Gly His Ala Phe Ser Val Val Arg Ser Ala Gly Ser Ser Thr Tyr
                405                 410                 415

Asn Phe Val Asn Pro Val Lys Arg Asp Val Val Ser Leu Gly Val Thr
            420                 425                 430

Gly Asp Glu Val Thr Ile Arg Phe Val Thr Asp Asn Pro Gly Pro Trp
        435                 440                 445

Phe Phe His Cys His Ile Glu Phe His Leu Met Asn Gly Leu Ala Ile
    450                 455                 460

Val Phe Ala Glu Asp Met Ala Asn Thr Val Asp Ala Asn Asn Pro Pro
465                 470                 475                 480

Val Glu Trp Ala Gln Leu Cys Glu Ile Tyr Asp Asp Leu Pro Pro Glu
                485                 490                 495

Ala Thr Ser Ile Gln Thr Val Val
            500


<210> SEQ ID NO 4
<211> LENGTH: 213
<212> TYPE: PRT
<213> ORGANISM: Humicola insolens

<400> SEQUENCE: 4

Ala Asp Gly Arg Ser Thr Arg Tyr Trp Asp Cys Cys Lys Pro Ser Cys
1               5                   10                  15

Gly Trp Ala Lys Lys Ala Pro Val Asn Gln Pro Val Phe Ser Cys Asn
            20                  25                  30

Ala Asn Phe Gln Arg Ile Thr Asp Phe Asp Ala Lys Ser Gly Cys Glu
        35                  40                  45

Pro Gly Gly Val Ala Tyr Ser Cys Ala Asp Gln Thr Pro Trp Ala Val
    50                  55                  60

Asn Asp Asp Phe Ala Leu Gly Phe Ala Ala Thr Ser Ile Ala Gly Ser
65                  70                  75                  80

Asn Glu Ala Gly Trp Cys Cys Ala Cys Tyr Glu Leu Thr Phe Thr Ser
                85                  90                  95

Gly Pro Val Ala Gly Lys Lys Met Val Val Gln Ser Thr Ser Thr Gly
            100                 105                 110

Gly Asp Leu Gly Ser Asn His Phe Asp Leu Asn Ile Pro Gly Gly Gly
        115                 120                 125

Val Gly Ile Phe Asp Gly Cys Thr Pro Gln Phe Gly Gly Leu Pro Gly
    130                 135                 140

Gln Arg Tyr Gly Gly Ile Ser Ser Arg Asn Glu Cys Asp Arg Phe Pro
145                 150                 155                 160

Asp Ala Leu Lys Pro Gly Cys Tyr Trp Arg Phe Asp Trp Phe Lys Asn
                165                 170                 175

Ala Asp Asn Pro Ser Phe Ser Phe Arg Gln Val Gln Cys Pro Ala Glu
            180                 185                 190

Leu Val Ala Arg Thr Gly Cys Arg Arg Asn Asp Asp Gly Asn Phe Pro
        195                 200                 205

Ala Val Gln Ile Pro
    210
```

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<210> SEQ ID NO 5
<211> LENGTH: 305
<212> TYPE: PRT
<213> ORGANISM: Humicola insolens

<400> SEQUENCE: 5

Met Arg Ser Ser Pro Leu Leu Pro Ser Ala Val Val Ala Ala Leu Pro
1 5 10 15

Val Leu Ala Leu Ala Ala Asp Gly Arg Ser Thr Arg Tyr Trp Asp Cys
20 25 30

Cys Lys Pro Ser Cys Gly Trp Ala Lys Lys Ala Pro Val Asn Gln Pro
35 40 45

Val Phe Ser Cys Asn Ala Asn Phe Gln Arg Ile Thr Asp Phe Asp Ala
50 55 60

Lys Ser Gly Cys Glu Pro Gly Gly Val Ala Tyr Ser Cys Ala Asp Gln
65 70 75 80

Thr Pro Trp Ala Val Asn Asp Asp Phe Ala Leu Gly Phe Ala Ala Thr
85 90 95

Ser Ile Ala Gly Ser Asn Glu Ala Gly Trp Cys Cys Ala Cys Tyr Glu
100 105 110

Leu Thr Phe Thr Ser Gly Pro Val Ala Gly Lys Lys Met Val Val Gln
115 120 125

Ser Thr Ser Thr Gly Gly Asp Leu Gly Ser Asn His Phe Asp Leu Asn
130 135 140

Ile Pro Gly Gly Gly Val Gly Ile Phe Asp Gly Cys Thr Pro Gln Phe
145 150 155 160

Gly Gly Leu Pro Gly Gln Arg Tyr Gly Gly Ile Ser Ser Arg Asn Glu
165 170 175

Cys Asp Arg Phe Pro Asp Ala Leu Lys Pro Gly Cys Tyr Trp Arg Phe
180 185 190

Asp Trp Phe Lys Asn Ala Asp Asn Pro Ser Phe Ser Phe Arg Gln Val
195 200 205

Gln Cys Pro Ala Glu Leu Val Ala Arg Thr Gly Cys Arg Arg Asn Asp
210 215 220

Asp Gly Asn Phe Pro Ala Val Gln Ile Pro Ser Ser Ser Thr Ser Ser
225 230 235 240

Pro Val Asn Gln Pro Thr Ser Thr Ser Thr Thr Ser Thr Ser Thr Thr
245 250 255

Ser Ser Pro Pro Val Gln Pro Thr Thr Pro Ser Gly Cys Thr Ala Glu
260 265 270

Arg Trp Ala Gln Cys Gly Gly Asn Gly Trp Ser Gly Cys Thr Thr Cys
275 280 285

Val Ala Gly Ser Thr Cys Thr Lys Ile Asn Asp Trp Tyr His Gln Cys
290 295 300

Leu
305

<210> SEQ ID NO 6
<211> LENGTH: 159
<212> TYPE: PRT
<213> ORGANISM: Betula pendula

<400> SEQUENCE: 6

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```
Gly Val Phe Asn Tyr Glu Thr Glu Thr Thr Ser Val Ile Pro Ala Ala
1               5                   10                  15

Arg Leu Phe Lys Ala Phe Ile Leu Asp Gly Asp Asn Leu Phe Pro Lys
            20                  25                  30

Val Ala Pro Gln Ala Ile Ser Ser Val Glu Asn Ile Glu Gly Asn Gly
        35                  40                  45

Gly Pro Gly Thr Ile Lys Lys Ile Ser Phe Pro Glu Gly Phe Pro Phe
    50                  55                  60

Lys Tyr Val Lys Asp Arg Val Asp Glu Val Asp His Thr Asn Phe Lys
65                  70                  75                  80

Tyr Asn Tyr Ser Val Ile Glu Gly Gly Pro Ile Gly Asp Thr Leu Glu
                85                  90                  95

Lys Ile Ser Asn Glu Ile Lys Ile Val Ala Thr Pro Asp Gly Gly Ser
            100                 105                 110

Ile Leu Lys Ile Ser Asn Lys Tyr His Thr Lys Gly Asp His Glu Val
        115                 120                 125

Lys Ala Glu Gln Val Lys Ala Ser Lys Glu Met Gly Glu Thr Leu Leu
    130                 135                 140

Arg Ala Val Glu Ser Tyr Leu Leu Ala His Ser Asp Ala Tyr Asn
145                 150                 155


<210> SEQ ID NO 7
<211> LENGTH: 129
<212> TYPE: PRT
<213> ORGANISM: Dermatophagoides farinae

<400> SEQUENCE: 7

Asp Gln Val Asp Val Lys Asp Cys Ala Asn Asn Glu Ile Lys Lys Val
1               5                   10                  15

Met Val Asp Gly Cys His Gly Ser Asp Pro Cys Ile Ile His Arg Gly
            20                  25                  30

Lys Pro Phe Thr Leu Glu Ala Leu Phe Asp Ala Asn Gln Asn Thr Lys
        35                  40                  45

Thr Ala Lys Ile Glu Ile Lys Ala Ser Leu Asp Gly Leu Glu Ile Asp
    50                  55                  60

Val Pro Gly Ile Asp Thr Asn Ala Cys His Phe Val Lys Cys Pro Leu
65                  70                  75                  80

Val Lys Gly Gln Gln Tyr Asp Ile Lys Tyr Thr Trp Asn Val Pro Lys
                85                  90                  95

Ile Ala Pro Lys Ser Glu Asn Val Val Val Thr Val Lys Leu Ile Gly
            100                 105                 110

Asp Asn Gly Val Leu Ala Cys Ala Ile Ala Thr His Gly Lys Ile Arg
        115                 120                 125

Asp


<210> SEQ ID NO 8
<211> LENGTH: 129
<212> TYPE: PRT
<213> ORGANISM: Dermatophagoides pteronyssinus

<400> SEQUENCE: 8

Ser Gln Val Asp Val Lys Asp Cys Ala Asn His Glu Ile Lys Lys Val
1               5                   10                  15

Leu Val Pro Gly Cys His Gly Ser Glu Pro Cys Ile Ile His Arg Gly
```

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```
            20                  25                  30

Lys Pro Phe Gln Leu Glu Ala Val Phe Glu Ala Asn Gln Asn Thr Lys
        35                  40                  45

Thr Ala Lys Ile Glu Ile Lys Ala Ser Ile Asp Gly Leu Glu Val Asp
    50                  55                  60

Val Pro Gly Ile Asp Pro Asn Ala Cys His Tyr Met Lys Cys Pro Leu
65                  70                  75                  80

Val Lys Gly Gln Gln Tyr Asp Ile Lys Tyr Thr Trp Asn Val Pro Lys
                85                  90                  95

Ile Ala Pro Lys Ser Glu Asn Val Val Val Thr Val Lys Val Met Gly
            100                 105                 110

Asp Asp Gly Val Leu Ala Cys Ala Ile Ala Thr His Ala Lys Ile Arg
        115                 120                 125

Asp


<210> SEQ ID NO 9
<211> LENGTH: 94
<212> TYPE: PRT
<213> ORGANISM: Phleum pratense

<400> SEQUENCE: 9

Val Pro Lys Val Thr Phe Thr Val Glu Lys Gly Ser Asn Glu Lys His
1               5                   10                  15

Leu Ala Val Leu Val Lys Tyr Glu Gly Asp Thr Met Ala Glu Val Glu
            20                  25                  30

Leu Arg Glu His Gly Ser Asp Glu Trp Val Ala Met Thr Lys Gly Glu
        35                  40                  45

Gly Gly Val Trp Thr Phe Asp Ser Glu Glu Pro Leu Gln Gly Pro Phe
    50                  55                  60

Asn Phe Arg Phe Leu Thr Glu Lys Gly Met Lys Asn Val Phe Asp Asp
65                  70                  75                  80

Val Val Pro Glu Lys Tyr Thr Ile Gly Ala Thr Tyr Ala Pro
                85                  90


<210> SEQ ID NO 10
<211> LENGTH: 338
<212> TYPE: PRT
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 10

Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu
1               5                   10                  15

His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp
            20                  25                  30

Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala
        35                  40                  45

Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His
    50                  55                  60

Gly Thr His Val Ala Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly
65                  70                  75                  80

Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu
                85                  90                  95

Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu
            100                 105                 110
```

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```
Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly
        115                 120                 125

Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala
    130                 135                 140

Ser Gly Val Val Val Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly
145                 150                 155                 160

Ser Ser Ser Thr Val Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala
                165                 170                 175

Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val
            180                 185                 190

Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr
        195                 200                 205

Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser
    210                 215                 220

Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn
225                 230                 235                 240

Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys
                245                 250                 255

Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala
            260                 265                 270

Ala Ala Gln Lys Ser Phe Pro Glu Val Val Gly Lys Thr Val Asp Gln
        275                 280                 285

Ala Arg Glu Tyr Phe Thr Leu His Tyr Pro Gln Tyr Asp Val Tyr Phe
    290                 295                 300

Leu Pro Glu Gly Ser Pro Val Thr Leu Asp Leu Arg Tyr Asn Arg Val
305                 310                 315                 320

Lys Val Phe Tyr Asn Pro Gly Thr Asn Val Val Asn His Val Pro His
                325                 330                 335

Val Gly
```

<210> SEQ ID NO 11
<211> LENGTH: 268
<212> TYPE: PRT
<213> ORGANISM: Bacillus lentus

<400> SEQUENCE: 11

```
Gln Thr Val Pro Trp Gly Ile Ser Phe Ile Asn Thr Gln Gln Ala His
1               5                   10                  15

Asn Arg Gly Ile Phe Gly Asn Gly Ala Arg Val Ala Val Leu Asp Thr
            20                  25                  30

Gly Ile Ala Ser His Pro Asp Leu Arg Ile Ala Gly Gly Ala Ser Phe
        35                  40                  45

Ile Ser Ser Glu Pro Ser Tyr His Asp Asn Asn Gly His Gly Thr His
    50                  55                  60

Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly
65                  70                  75                  80

Val Ala Pro Ser Ala Asp Leu Tyr Ala Val Lys Val Leu Asp Arg Asn
                85                  90                  95

Gly Ser Gly Ser Leu Ala Ser Val Ala Gln Gly Ile Glu Trp Ala Ile
            100                 105                 110

Asn Asn Asn Met His Ile Ile Asn Met Ser Leu Gly Ser Thr Ser Gly
        115                 120                 125
```

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```
Ser Ser Thr Leu Glu Leu Ala Val Asn Arg Ala Asn Asn Ala Gly Ile
    130                 135                 140

Leu Leu Val Gly Ala Ala Gly Asn Thr Gly Arg Gln Gly Val Asn Tyr
145                 150                 155                 160

Pro Ala Arg Tyr Ser Gly Val Met Ala Val Ala Ala Val Asp Gln Asn
                165                 170                 175

Gly Gln Arg Ala Ser Phe Ser Thr Tyr Gly Pro Glu Ile Glu Ile Ser
            180                 185                 190

Ala Pro Gly Val Asn Val Asn Ser Thr Tyr Thr Gly Asn Arg Tyr Val
        195                 200                 205

Ser Leu Ser Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Val Ala
    210                 215                 220

Ala Leu Val Lys Ser Arg Tyr Pro Ser Tyr Thr Asn Asn Gln Ile Arg
225                 230                 235                 240

Gln Arg Ile Asn Gln Thr Ala Thr Tyr Leu Gly Ser Pro Ser Leu Tyr
                245                 250                 255

Gly Asn Gly Leu Val His Ala Gly Arg Ala Thr Gln
            260                 265


<210> SEQ ID NO 12
<211> LENGTH: 150
<212> TYPE: PRT
<213> ORGANISM: Bos taurus

<400> SEQUENCE: 12

Ile Asp Pro Ser Lys Ile Pro Gly Glu Trp Arg Ile Ile Tyr Ala Ala
1               5                   10                  15

Ala Asp Asn Lys Asp Lys Ile Val Glu Gly Gly Pro Leu Arg Asn Tyr
            20                  25                  30

Tyr Arg Arg Ile Glu Cys Ile Asn Asp Cys Glu Ser Leu Ser Ile Thr
        35                  40                  45

Phe Tyr Leu Lys Asp Gln Gly Thr Cys Leu Leu Leu Thr Glu Val Ala
    50                  55                  60

Lys Arg Gln Glu Gly Tyr Val Tyr Val Leu Glu Phe Tyr Gly Thr Asn
65                  70                  75                  80

Thr Leu Glu Val Ile His Val Ser Glu Asn Met Leu Val Thr Tyr Val
                85                  90                  95

Glu Asn Tyr Asp Gly Glu Arg Ile Thr Lys Met Thr Glu Gly Leu Ala
            100                 105                 110

Lys Gly Thr Ser Phe Thr Pro Glu Glu Leu Glu Lys Tyr Gln Gln Leu
        115                 120                 125

Asn Ser Glu Arg Gly Val Pro Asn Glu Asn Ile Glu Asn Leu Ile Lys
    130                 135                 140

Thr Asp Asn Cys Pro Pro
145                 150


<210> SEQ ID NO 13
<211> LENGTH: 159
<212> TYPE: PRT
<213> ORGANISM: Equus caballus

<400> SEQUENCE: 13

Val Ala Ile Arg Asn Phe Asp Ile Ser Lys Ile Ser Gly Glu Trp Tyr
1               5                   10                  15

Ser Ile Phe Leu Ala Ser Asp Val Lys Glu Lys Ile Glu Glu Asn Gly
```

-continued

```
            20                  25                  30

Ser Met Arg Val Phe Val Asp Val Ile Arg Ala Leu Asp Asn Ser Ser
        35                  40                  45

Leu Tyr Ala Glu Tyr Gln Thr Lys Val Asn Gly Glu Cys Thr Glu Phe
    50                  55                  60

Pro Met Val Phe Asp Lys Thr Glu Glu Asp Gly Val Tyr Ser Leu Asn
65                  70                  75                  80

Tyr Asp Gly Tyr Asn Val Phe Arg Ile Ser Glu Phe Glu Asn Asp Glu
                85                  90                  95

His Ile Ile Leu Tyr Leu Val Asn Phe Asp Lys Asp Arg Pro Phe Gln
            100                 105                 110

Leu Phe Glu Phe Tyr Ala Arg Glu Pro Asp Val Ser Pro Glu Ile Lys
        115                 120                 125

Glu Glu Phe Val Lys Ile Val Gln Lys Arg Gly Ile Val Lys Glu Asn
    130                 135                 140

Ile Ile Asp Leu Thr Lys Ile Asp Arg Cys Phe Gln Leu Arg Gly
145                 150                 155


<210> SEQ ID NO 14
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 14

Ala Gln Thr Ile Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65                  70                  75                  80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145                 150                 155                 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
            180                 185                 190

Met Ala Pro Gly Val Asn Ile Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205

Ala Ser Asp Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
```

-continued

```
    210                 215                 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225                 230                 235                 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 15
<211> LENGTH: 129
<212> TYPE: PRT
<213> ORGANISM: Gallus gallus

<400> SEQUENCE: 15

Lys Val Phe Gly Arg Cys Glu Leu Ala Ala Ala Met Lys Arg His Gly
1               5                   10                  15

Leu Asp Asn Tyr Arg Gly Tyr Ser Leu Gly Asn Trp Val Cys Ala Ala
            20                  25                  30

Lys Phe Glu Ser Asn Phe Asn Thr Gln Ala Thr Asn Arg Asn Thr Asp
        35                  40                  45

Gly Ser Thr Asp Tyr Gly Ile Leu Gln Ile Asn Ser Arg Trp Trp Cys
    50                  55                  60

Asn Asp Gly Arg Thr Pro Gly Ser Arg Asn Leu Cys Asn Ile Pro Cys
65                  70                  75                  80

Ser Ala Leu Leu Ser Ser Asp Ile Thr Ala Ser Val Asn Cys Ala Lys
                85                  90                  95

Lys Ile Val Ser Asp Ala Asn Gly Met Asn Ala Trp Val Ala Trp Arg
            100                 105                 110

Asn Arg Cys Lys Gly Thr Asp Val Gln Ala Trp Ile Arg Gly Cys Arg
        115                 120                 125

Leu


<210> SEQ ID NO 16
<211> LENGTH: 260
<212> TYPE: PRT
<213> ORGANISM: Havea brasiliensis

<400> SEQUENCE: 16

Ser Trp Gln Thr Tyr Val Asp Asp His Leu Met Cys Asp Ile Asp Gly
1               5                   10                  15

His Arg Leu Thr Ala Ala Ala Ile Ile Gly His Asp Gly Ser Val Trp
            20                  25                  30

Ala Gln Ser Ser Ser Phe Pro Gln Phe Lys Ser Asp Glu Val Ala Ala
        35                  40                  45

Val Met Lys Asp Phe Asp Glu Pro Gly Ser Leu Ala Pro Thr Gly Leu
    50                  55                  60

His Leu Gly Gly Thr Lys Tyr Met Val Ile Gln Gly Glu Pro Gly Ala
65                  70                  75                  80

Val Ile Arg Gly Lys Lys Gly Ser Gly Gly Ile Thr Val Lys Arg Thr
                85                  90                  95

Gly Gln Ala Leu Ile Ile Gly Ile Tyr Asp Glu Pro Leu Thr Pro Gly
            100                 105                 110

Gln Cys Asn Met Ile Val Glu Arg Leu Gly Asp Tyr Leu Leu Asp Gln
        115                 120                 125
```

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```
Gly Leu Ser Trp Gln Thr Tyr Val Asp Asp His Leu Met Cys Asp Ile
    130                 135                 140

Asp Gly His Arg Leu Thr Ala Ala Ala Ile Ile Gly His Asp Gly Ser
145                 150                 155                 160

Val Trp Ala Gln Ser Ser Ser Phe Pro Gln Phe Lys Ser Asp Glu Val
                165                 170                 175

Ala Ala Val Met Lys Asp Phe Asp Glu Pro Gly Ser Leu Ala Pro Thr
            180                 185                 190

Gly Leu His Leu Gly Gly Thr Lys Tyr Met Val Ile Gln Gly Glu Pro
        195                 200                 205

Gly Ala Val Ile Arg Gly Lys Lys Gly Ser Gly Gly Ile Thr Val Lys
    210                 215                 220

Arg Thr Gly Gln Ala Leu Ile Ile Gly Ile Tyr Asp Glu Pro Leu Thr
225                 230                 235                 240

Pro Gly Gln Cys Asn Met Ile Val Glu Arg Leu Gly Asp Tyr Leu Leu
                245                 250                 255

Asp Gln Gly Leu
            260


<210> SEQ ID NO 17
<211> LENGTH: 125
<212> TYPE: PRT
<213> ORGANISM: Acanthamoeba castellanii

<400> SEQUENCE: 17

Ser Trp Gln Thr Tyr Val Asp Thr Asn Leu Val Gly Thr Gly Ala Val
1               5                   10                  15

Thr Gln Ala Ala Ile Leu Gly Leu Asp Gly Asn Thr Trp Ala Thr Ser
            20                  25                  30

Ala Gly Phe Ala Val Thr Pro Ala Gln Gly Gln Thr Leu Ala Ser Ala
        35                  40                  45

Phe Asn Asn Ala Asp Pro Ile Arg Ala Ser Gly Phe Asp Leu Ala Gly
    50                  55                  60

Val His Tyr Val Thr Leu Arg Ala Asp Asp Arg Ser Ile Tyr Gly Lys
65                  70                  75                  80

Lys Gly Ser Ala Gly Val Ile Thr Val Lys Thr Ser Lys Ser Ile Leu
                85                  90                  95

Val Gly Val Tyr Asn Glu Lys Ile Gln Pro Gly Thr Ala Ala Asn Val
            100                 105                 110

Val Glu Lys Leu Ala Asp Tyr Leu Ile Gly Gln Gly Phe
        115                 120                 125


<210> SEQ ID NO 18
<211> LENGTH: 130
<212> TYPE: PRT
<213> ORGANISM: Arabidosis thaliana

<400> SEQUENCE: 18

Ser Trp Gln Ser Tyr Val Asp Asp His Leu Met Cys Asp Val Glu Gly
1               5                   10                  15

Asn His Leu Thr Ala Ala Ala Ile Leu Gly Gln Asp Gly Ser Val Trp
            20                  25                  30

Ala Gln Ser Ala Lys Phe Pro Gln Leu Lys Pro Gln Glu Ile Asp Gly
        35                  40                  45
```

-continued

```
Ile Lys Lys Asp Phe Glu Glu Pro Gly Phe Leu Ala Pro Thr Gly Leu
    50                  55                  60

Phe Leu Gly Gly Glu Lys Tyr Met Val Ile Gln Gly Glu Gln Gly Ala
65                  70                  75                  80

Val Ile Arg Gly Lys Lys Gly Pro Gly Gly Val Thr Ile Lys Lys Thr
                85                  90                  95

Asn Gln Ala Leu Val Phe Gly Phe Tyr Asp Glu Pro Met Thr Gly Gly
            100                 105                 110

Gln Cys Asn Leu Val Val Glu Arg Leu Gly Asp Tyr Leu Ile Glu Ser
        115                 120                 125

Glu Leu
    130


<210> SEQ ID NO 19
<211> LENGTH: 250
<212> TYPE: PRT
<213> ORGANISM: Acanthamoeba castellanii

<400> SEQUENCE: 19

Ser Trp Gln Thr Tyr Val Asp Thr Asn Leu Val Gly Thr Gly Ala Val
1               5                   10                  15

Thr Gln Ala Ala Ile Ile Gly His Asp Gly Asn Thr Trp Ala Thr Ser
            20                  25                  30

Ala Gly Phe Ala Val Ser Pro Ala Asn Gly Ala Ala Leu Ala Asn Ala
        35                  40                  45

Phe Lys Asp Ala Thr Ala Ile Arg Ser Asn Gly Phe Glu Leu Ala Gly
    50                  55                  60

Thr Arg Tyr Val Thr Ile Arg Ala Asp Asp Arg Ser Val Tyr Gly Lys
65                  70                  75                  80

Lys Gly Ser Ala Gly Val Ile Thr Val Lys Thr Ser Lys Ala Ile Leu
                85                  90                  95

Ile Gly Val Tyr Asn Glu Lys Ile Gln Pro Gly Thr Ala Ala Asn Val
            100                 105                 110

Val Glu Lys Leu Ala Asp Tyr Leu Ile Gly Gln Gly Phe Ser Trp Gln
        115                 120                 125

Thr Tyr Val Asp Thr Asn Leu Val Gly Thr Gly Ala Val Thr Gln Ala
    130                 135                 140

Ala Ile Ile Gly His Asp Gly Asn Thr Trp Ala Thr Ser Ala Gly Phe
145                 150                 155                 160

Ala Val Ser Pro Ala Asn Gly Ala Ala Leu Ala Asn Ala Phe Lys Asp
                165                 170                 175

Ala Thr Ala Ile Arg Ser Asn Gly Phe Glu Leu Ala Gly Thr Arg Tyr
            180                 185                 190

Val Thr Ile Arg Ala Asp Asp Arg Ser Val Tyr Gly Lys Lys Gly Ser
        195                 200                 205

Ala Gly Val Ile Thr Val Lys Thr Ser Lys Ala Ile Leu Ile Gly Val
    210                 215                 220

Tyr Asn Glu Lys Ile Gln Pro Gly Thr Ala Ala Asn Val Val Glu Lys
225                 230                 235                 240

Leu Ala Asp Tyr Leu Ile Gly Gln Gly Phe
                245                 250
```

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<210> SEQ ID NO 20
<211> LENGTH: 123
<212> TYPE: PRT
<213> ORGANISM: Betula pendula

<400> SEQUENCE: 20

Ser Trp Gln Thr Tyr Val Asp Glu His Leu Met Leu Ala Ala Ser Ala
1 5 10 15

Ile Val Gly His Asp Gly Ser Val Trp Ala Gln Ser Ser Ser Phe Pro
20 25 30

Gln Phe Lys Pro Gln Glu Ile Thr Gly Ile Met Lys Asp Phe Glu Glu
35 40 45

Pro Gly His Leu Ala Pro Thr Gly Leu His Leu Gly Gly Ile Lys Tyr
50 55 60

Met Val Ile Gln Gly Glu Ala Gly Ala Val Ile Arg Gly Lys Lys Gly
65 70 75 80

Ser Gly Gly Ile Thr Ile Lys Lys Thr Gly Gln Ala Leu Val Phe Gly
85 90 95

Ile Tyr Glu Glu Pro Val Thr Pro Gly Gln Cys Asn Met Val Val Glu
100 105 110

Arg Leu Gly Asp Tyr Leu Ile Asp Gln Gly Leu
115 120

<210> SEQ ID NO 21
<211> LENGTH: 40
<212> TYPE: PRT
<213> ORGANISM: Ambrosia trifida

<400> SEQUENCE: 21

Asp Asp Gly Leu Cys Tyr Glu Gly Thr Asn Cys Gly Lys Val Gly Lys
1 5 10 15

Tyr Cys Cys Ser Pro Ile Gly Lys Tyr Cys Val Cys Tyr Asp Ser Lys
20 25 30

Ala Ile Cys Asn Lys Asn Cys Thr
35 40

<210> SEQ ID NO 22
<211> LENGTH: 209
<212> TYPE: PRT
<213> ORGANISM: Vespula vulgaris

<400> SEQUENCE: 22

Ala Glu Ala Glu Phe Asn Asn Tyr Cys Lys Ile Lys Cys Leu Lys Gly
1 5 10 15

Gly Val His Thr Ala Cys Lys Tyr Gly Ser Leu Lys Pro Asn Cys Gly
20 25 30

Asn Lys Val Val Val Ser Tyr Gly Leu Thr Lys Gln Glu Lys Gln Asp
35 40 45

Ile Leu Lys Glu His Asn Asp Phe Arg Gln Lys Ile Ala Arg Gly Leu
50 55 60

Glu Thr Arg Gly Asn Pro Gly Pro Gln Pro Pro Ala Lys Asn Met Lys
65 70 75 80

Asn Leu Val Trp Asn Asp Glu Leu Ala Tyr Val Ala Gln Val Trp Ala
85 90 95

Asn Gln Cys Gln Tyr Gly His Asp Thr Cys Arg Asp Val Ala Lys Tyr
100 105 110

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```
Gln Val Gly Gln Asn Val Ala Leu Thr Gly Ser Thr Ala Ala Lys Tyr
        115                 120                 125

Asp Asp Pro Val Lys Leu Val Lys Met Trp Glu Asp Glu Val Lys Asp
    130                 135                 140

Tyr Asn Pro Lys Lys Lys Phe Ser Gly Asn Asp Phe Leu Lys Thr Gly
145                 150                 155                 160

His Tyr Thr Gln Met Val Trp Ala Asn Thr Lys Glu Val Gly Cys Gly
                165                 170                 175

Ser Ile Lys Tyr Ile Gln Glu Lys Trp His Lys His Tyr Leu Val Cys
            180                 185                 190

Asn Tyr Gly Pro Ser Gly Asn Phe Lys Asn Glu Glu Leu Tyr Gln Thr
        195                 200                 205

Lys
```

<210> SEQ ID NO 23
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 23

```
Ala Gln Thr Ile Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65                  70                  75                  80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145                 150                 155                 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
            180                 185                 190

Met Ala Pro Gly Val Asn Ile Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205

Ala Ser Asp Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
    210                 215                 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225                 230                 235                 240
```

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```
Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 24
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Bacillus lentus

<400> SEQUENCE: 24

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65                  70                  75                  80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145                 150                 155                 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
            180                 185                 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
    210                 215                 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225                 230                 235                 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 25
<211> LENGTH: 274
<212> TYPE: PRT
<213> ORGANISM: Bacillus licheniformis

<400> SEQUENCE: 25

Ala Gln Thr Val Pro Tyr Gly Ile Pro Leu Ile Lys Ala Asp Lys Val
1               5                   10                  15
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Gln Ala Gln Gly Phe Lys Gly Ala Asn Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Gln Ala Ser His Pro Asp Leu Asn Val Val Gly Gly Ala
        35                  40                  45

Ser Phe Val Ala Gly Glu Ala Tyr Asn Thr Asp Gly Asn Gly His Gly
    50                  55                  60

Thr His Val Ala Gly Thr Val Ala Ala Leu Asp Asn Thr Thr Gly Val
65                  70                  75                  80

Leu Gly Val Ala Pro Ser Val Ser Leu Tyr Ala Val Lys Val Leu Asn
                85                  90                  95

Ser Ser Gly Ser Gly Ser Tyr Ser Gly Ile Val Ser Gly Ile Glu Trp
            100                 105                 110

Ala Thr Thr Asn Gly Met Asp Val Ile Asn Met Ser Leu Gly Gly Ala
        115                 120                 125

Ser Gly Ser Thr Ala Met Lys Gln Ala Val Asp Asn Ala Tyr Ala Arg
    130                 135                 140

Gly Val Val Val Val Ala Ala Ala Gly Asn Ser Gly Ser Ser Gly Asn
145                 150                 155                 160

Thr Asn Thr Ile Gly Tyr Pro Ala Lys Tyr Asp Ser Val Ile Ala Val
                165                 170                 175

Gly Ala Val Asp Ser Asn Ser Asn Arg Ala Ser Phe Ser Ser Val Gly
            180                 185                 190

Ala Glu Leu Glu Val Met Ala Pro Gly Ala Gly Val Tyr Ser Thr Tyr
        195                 200                 205

Pro Thr Asn Thr Tyr Ala Thr Leu Asn Gly Thr Ser Met Ala Ser Pro
    210                 215                 220

His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn Leu
225                 230                 235                 240

Ser Ala Ser Gln Val Arg Asn Arg Leu Ser Ser Thr Ala Thr Tyr Leu
                245                 250                 255

Gly Ser Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Glu Ala Ala
            260                 265                 270

Ala Gln


<210> SEQ ID NO 26
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 26

Ala Gln Thr Ile Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65                  70                  75                  80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
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                85                  90                  95

Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145                 150                 155                 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
            180                 185                 190

Met Ala Pro Gly Val Asn Ile Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205

Ala Ser Asp Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
    210                 215                 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225                 230                 235                 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 27
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 27

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Arg Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65                  70                  75                  80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Ser Gly Ser Gly Ser Tyr Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110

Gly Asn Asn Gly Met His Val Ala Ser Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145                 150                 155                 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
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               165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
            180                 185                 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
    210                 215                 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225                 230                 235                 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Ala Arg
            260                 265


<210> SEQ ID NO 28
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 28

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asp Asn Ser Ile Gly Val Leu
65                  70                  75                  80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Ser Gly Ser Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145                 150                 155                 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
            180                 185                 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
    210                 215                 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225                 230                 235                 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
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                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 29
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 29

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65                  70                  75                  80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Ser Gly Gly Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Asp Ser Ile Ser
145                 150                 155                 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
            180                 185                 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
    210                 215                 220

Ala Val Leu Val Lys His Lys Asn Pro Ser Trp Ser Asn Val Arg Ile
225                 230                 235                 240

Arg Asp His Leu Lys Lys Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 30
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 30
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Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65                  70                  75                  80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Ala Gly Gly Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145                 150                 155                 160

Ala Pro Ala Ser Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Pro Gly Leu Asp Ile
            180                 185                 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
    210                 215                 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225                 230                 235                 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 31
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 31

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65                  70                  75                  80
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```
Gly Val Ala Pro Asn Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Ser Gly Gly Gly Ser Asn Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145                 150                 155                 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
            180                 185                 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
    210                 215                 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225                 230                 235                 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 32
<211> LENGTH: 270
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 32

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Asp Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala
        35                  40                  45

Ser Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly
    50                  55                  60

Thr His Val Ala Gly Thr Ile Ala Ala Leu Asp Asn Ser Ile Gly Val
65                  70                  75                  80

Leu Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly
                85                  90                  95

Ala Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp
            100                 105                 110

Ala Gly Asn Asn Gly Met Asp Val Ala Asn Leu Ser Leu Gly Ser Pro
        115                 120                 125

Ser Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg
    130                 135                 140

Gly Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile
145                 150                 155                 160
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Ser Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp
                165                 170                 175

Gln Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Glu Leu Asp
            180                 185                 190

Ile Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr
        195                 200                 205

Tyr Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly
    210                 215                 220

Ala Ala Ala Leu Val Leu Gln Lys Asn Pro Ser Trp Ser Asn Val Gln
225                 230                 235                 240

Ile Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn
                245                 250                 255

Leu Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265                 270


<210> SEQ ID NO 33
<211> LENGTH: 280
<212> TYPE: PRT
<213> ORGANISM: Bacillus species.

<400> SEQUENCE: 33

Trp Ser Pro Asn Asp Pro Tyr Tyr Ser Ala Tyr Gln Tyr Gly Pro Gln
1               5                   10                  15

Asn Thr Ser Thr Pro Ala Ala Trp Asp Val Thr Arg Gly Ser Ser Thr
            20                  25                  30

Gln Thr Val Ala Val Leu Asp Ser Gly Val Asp Tyr Asn His Pro Asp
        35                  40                  45

Leu Ala Arg Lys Val Ile Lys Gly Tyr Asp Phe Ile Asp Arg Asp Asn
    50                  55                  60

Asn Pro Met Asp Leu Asn Gly His Gly Thr His Val Ala Gly Thr Val
65                  70                  75                  80

Ala Ala Asp Thr Asn Asn Gly Ile Gly Val Ala Gly Met Ala Pro Asp
                85                  90                  95

Thr Lys Ile Leu Ala Val Arg Val Leu Asp Ala Asn Gly Ser Gly Ser
            100                 105                 110

Leu Asp Ser Ile Ala Ser Gly Ile Arg Tyr Ala Ala Asp Gln Gly Ala
        115                 120                 125

Lys Val Leu Asn Leu Ser Leu Gly Cys Glu Cys Asn Ser Thr Thr Leu
    130                 135                 140

Lys Ser Ala Val Asp Tyr Ala Trp Asn Lys Gly Ala Val Val Val Ala
145                 150                 155                 160

Ala Ala Gly Asn Asp Asn Val Ser Arg Thr Phe Gln Pro Ala Ser Tyr
                165                 170                 175

Pro Asn Ala Ile Ala Val Gly Ala Ile Asp Ser Asn Asp Arg Lys Ala
            180                 185                 190

Ser Phe Ser Asn Tyr Gly Thr Trp Val Asp Val Thr Ala Pro Gly Val
        195                 200                 205

Asn Ile Ala Ser Thr Val Pro Asn Asn Gly Tyr Ser Tyr Met Ser Gly
    210                 215                 220

Thr Ser Met Ala Ser Pro His Val Ala Gly Leu Ala Ala Leu Leu Ala
225                 230                 235                 240

Ser Gln Gly Lys Asn Asn Val Gln Ile Arg Gln Ala Ile Glu Gln Thr
                245                 250                 255
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Ala Asp Lys Ile Ser Gly Thr Gly Thr Asn Phe Lys Tyr Gly Lys Ile
260 265 270

Asn Ser Asn Lys Ala Val Arg Tyr
275 280

<210> SEQ ID NO 34
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Bacillus species.

<400> SEQUENCE: 34

Asn Gln Val Thr Pro Trp Gly Ile Thr Arg Val Gln Ala Pro Thr Ala
1 5 10 15

Trp Thr Arg Gly Tyr Thr Gly Thr Gly Val Arg Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Val Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Tyr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Val
65 70 75 80

Gly Val Ala Pro Asn Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asn Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Gln Trp Thr
100 105 110

Ala Gln Asn Asn Ile His Val Ala Asn Leu Ser Leu Gly Ser Pro Val
115 120 125

Gly Ser Gln Thr Leu Glu Leu Ala Val Asn Gln Ala Thr Asn Ala Gly
130 135 140

Val Leu Val Val Ala Ala Thr Gly Asn Asn Gly Ser Gly Thr Val Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Leu Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Thr Gly Leu Asn Ile
180 185 190

Val Ala Pro Gly Val Gly Ile Gln Ser Thr Tyr Pro Gly Asn Arg Tyr
195 200 205

Ala Ser Leu Ser Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Val
210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Thr Gln Ile
225 230 235 240

Arg Gln His Leu Thr Ser Thr Ala Thr Ser Leu Gly Asn Ser Asn Gln
245 250 255

Phe Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
260 265

<210> SEQ ID NO 35
<211> LENGTH: 268
<212> TYPE: PRT
<213> ORGANISM: Bacillus species.

<400> SEQUENCE: 35

Gln Thr Val Pro Trp Gly Ile Asn Arg Val Gln Ala Pro Ile Ala Gln
1 5 10 15

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Ser Arg Gly Phe Thr Gly Thr Gly Val Arg Val Ala Val Leu Asp Thr
            20                  25                  30

Gly Ile Ser Asn His Ala Asp Leu Arg Ile Arg Gly Gly Ala Ser Phe
        35                  40                  45

Val Pro Gly Glu Pro Asn Ile Ser Asp Gly Asn Gly His Gly Thr Gln
    50                  55                  60

Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly
65                  70                  75                  80

Val Ala Pro Asn Val Asp Leu Tyr Gly Val Lys Val Leu Gly Ala Ser
                85                  90                  95

Gly Ser Gly Ser Ile Ser Gly Ile Ala Gln Gly Leu Gln Trp Ala Ala
            100                 105                 110

Asn Asn Gly Met His Ile Ala Asn Met Ser Leu Gly Ser Ser Ala Gly
        115                 120                 125

Ser Ala Thr Met Glu Gln Ala Val Asn Gln Ala Thr Ala Ser Gly Val
    130                 135                 140

Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Asn Val Gly Phe
145                 150                 155                 160

Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln Asn
                165                 170                 175

Asn Asn Arg Ala Thr Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile Val
            180                 185                 190

Ala Pro Gly Val Gly Val Gln Ser Thr Val Pro Gly Asn Gly Tyr Ala
        195                 200                 205

Ser Phe Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Val Ala
    210                 215                 220

Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile Arg
225                 230                 235                 240

Asn His Leu Lys Asn Thr Ala Thr Asn Leu Gly Asn Thr Thr Gln Phe
                245                 250                 255

Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 36
<211> LENGTH: 471
<212> TYPE: PRT
<213> ORGANISM: Asergillus niger

<400> SEQUENCE: 36

Ala Thr Leu Asp Ser Trp Leu Ser Asn Glu Ala Thr Val Ala Arg Thr
1               5                   10                  15

Ala Ile Leu Asn Asn Ile Gly Ala Asp Gly Ala Trp Val Ser Gly Ala
            20                  25                  30

Asp Ser Gly Ile Val Val Ala Ser Pro Ser Thr Asp Asn Pro Asp Tyr
        35                  40                  45

Phe Tyr Thr Trp Thr Arg Asp Ser Gly Leu Val Leu Lys Thr Leu Val
    50                  55                  60

Asp Leu Phe Arg Asn Gly Asp Thr Ser Leu Leu Ser Thr Ile Glu Asn
65                  70                  75                  80

Tyr Ile Ser Ala Gln Ala Ile Val Gln Gly Ile Ser Asn Pro Ser Gly
                85                  90                  95

Asp Leu Ser Ser Gly Ala Gly Leu Gly Glu Pro Lys Phe Asn Val Asp
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            100                 105                 110

Glu Thr Ala Tyr Thr Gly Ser Trp Gly Arg Pro Gln Arg Asp Gly Pro
        115                 120                 125

Ala Leu Arg Ala Thr Ala Met Ile Gly Phe Gly Gln Trp Leu Leu Asp
    130                 135                 140

Asn Gly Tyr Thr Ser Thr Ala Thr Asp Ile Val Trp Pro Leu Val Arg
145                 150                 155                 160

Asn Asp Leu Ser Tyr Val Ala Gln Tyr Trp Asn Gln Thr Gly Tyr Asp
                165                 170                 175

Leu Trp Glu Glu Val Asn Gly Ser Ser Phe Phe Thr Ile Ala Val Gln
            180                 185                 190

His Arg Ala Leu Val Glu Gly Ser Ala Phe Ala Thr Ala Val Gly Ser
        195                 200                 205

Ser Cys Ser Trp Cys Asp Ser Gln Ala Pro Glu Ile Leu Cys Tyr Leu
    210                 215                 220

Gln Ser Phe Trp Thr Gly Ser Phe Ile Leu Ala Asn Phe Asp Ser Ser
225                 230                 235                 240

Arg Ser Gly Lys Asp Ala Asn Thr Leu Leu Gly Ser Ile His Thr Phe
                245                 250                 255

Asp Pro Glu Ala Ala Cys Asp Asp Ser Thr Phe Gln Pro Cys Ser Pro
            260                 265                 270

Arg Ala Leu Ala Asn His Lys Glu Val Val Asp Ser Phe Arg Ser Ile
        275                 280                 285

Tyr Thr Leu Asn Asp Gly Leu Ser Asp Ser Glu Ala Val Ala Val Gly
    290                 295                 300

Arg Tyr Pro Glu Asp Thr Tyr Tyr Asn Gly Asn Pro Trp Phe Leu Cys
305                 310                 315                 320

Thr Leu Ala Ala Ala Glu Gln Leu Tyr Asp Ala Leu Tyr Gln Trp Asp
                325                 330                 335

Lys Gln Gly Ser Leu Glu Val Thr Asp Val Ser Leu Asp Phe Phe Lys
            340                 345                 350

Ala Leu Tyr Ser Asp Ala Ala Thr Gly Thr Tyr Ser Ser Ser Ser Ser
        355                 360                 365

Thr Tyr Ser Ser Ile Val Asp Ala Val Lys Thr Phe Ala Asp Gly Phe
    370                 375                 380

Val Ser Ile Val Glu Thr His Ala Ala Ser Asn Gly Ser Met Ser Glu
385                 390                 395                 400

Gln Tyr Asp Lys Ser Asp Gly Glu Gln Leu Ser Ala Arg Asp Leu Thr
                405                 410                 415

Trp Ser Tyr Ala Ala Leu Leu Thr Ala Asn Asn Arg Arg Asn Ser Val
            420                 425                 430

Val Pro Ala Ser Trp Gly Glu Thr Ser Ala Ser Ser Val Pro Gly Thr
        435                 440                 445

Cys Ala Ala Thr Ser Ala Ile Gly Thr Tyr Ser Ser Val Thr Val Thr
    450                 455                 460

Ser Trp Pro Ser Ile Val Ala
465                 470


<210> SEQ ID NO 37
<211> LENGTH: 480
<212> TYPE: PRT
<213> ORGANISM: Bacillus species.
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<400> SEQUENCE: 37

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Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr Leu Pro Asn Asp
1               5                  10                  15

Gly Asn His Trp Asn Arg Leu Arg Ser Asp Ala Ser Asn Leu Lys Asp
            20                  25                  30

Lys Gly Ile Ser Ala Val Trp Ile Pro Pro Ala Trp Lys Gly Ala Ser
        35                  40                  45

Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr Asp Leu Gly Glu
    50                  55                  60

Phe Asn Gln Lys Gly Thr Ile Arg Thr Lys Tyr Gly Thr Arg Asn Gln
65                  70                  75                  80

Leu Gln Ala Ala Val Asn Ala Leu Lys Ser Asn Gly Ile Gln Val Tyr
                85                  90                  95

Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp Ala Thr Glu Met
            100                 105                 110

Val Arg Ala Val Glu Val Asn Pro Asn Asn Arg Asn Gln Glu Val Ser
        115                 120                 125

Gly Glu Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp Phe Pro Gly Arg
    130                 135                 140

Gly Asn Thr His Ser Asn Phe Lys Trp Arg Trp Tyr His Phe Asp Gly
145                 150                 155                 160

Val Asp Trp Asp Gln Ser Arg Lys Leu Asn Asn Arg Ile Tyr Lys Phe
                165                 170                 175

Arg Gly Asp Gly Lys Gly Trp Asp Trp Glu Val Asp Thr Glu Asn Gly
            180                 185                 190

Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Met Asp His Pro Glu
        195                 200                 205

Val Val Asn Glu Leu Arg Asn Trp Gly Val Trp Tyr Thr Asn Thr Leu
    210                 215                 220

Gly Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His Ile Lys Tyr Ser
225                 230                 235                 240

Phe Thr Arg Asp Trp Ile Asn His Val Arg Ser Ala Thr Gly Lys Asn
                245                 250                 255

Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu Gly Ala Ile Glu
            260                 265                 270

Asn Tyr Leu Asn Lys Thr Asn Trp Asn His Ser Val Phe Asp Val Pro
        275                 280                 285

Leu His Tyr Asn Leu Tyr Asn Ala Ser Lys Ser Gly Gly Asn Tyr Asp
    290                 295                 300

Met Arg Gln Ile Phe Asn Gly Thr Val Val Gln Arg His Pro Met His
305                 310                 315                 320

Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro Glu Glu Ala Leu
                325                 330                 335

Glu Ser Phe Val Glu Glu Trp Phe Lys Pro Leu Ala Tyr Ala Leu Thr
            340                 345                 350

Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr Gly Asp Tyr Tyr
        355                 360                 365

Gly Ile Pro Thr His Gly Val Pro Ala Met Lys Ser Lys Ile Asp Pro
    370                 375                 380

Ile Leu Glu Ala Arg Gln Lys Tyr Ala Tyr Gly Arg Gln Asn Asp Tyr
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385                 390                 395                 400

Leu Asp His His Asn Ile Ile Gly Trp Thr Arg Glu Gly Asn Thr Ala
                405                 410                 415

His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp Gly Ala Gly Gly
            420                 425                 430

Asn Lys Trp Met Phe Val Gly Arg Asn Lys Ala Gly Gln Val Trp Thr
        435                 440                 445

Asp Ile Thr Gly Asn Arg Ala Gly Thr Val Thr Ile Asn Ala Asp Gly
    450                 455                 460

Trp Gly Asn Phe Ser Val Asn Gly Gly Ser Val Ser Ile Trp Val Asn
465                 470                 475                 480


<210> SEQ ID NO 38
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa in position 3 denotes any amino acid

<400> SEQUENCE: 38

Arg Arg Xaa Ser
1


<210> SEQ ID NO 39
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct
<220> FEATURE:
<221> NAME/KEY: Misc_feature
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa in position 3 denotes any amino acid
<220> FEATURE:
<221> NAME/KEY: Misc_feature
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa in position 4 denotes any amino acid

<400> SEQUENCE: 39

Arg Arg Xaa Xaa Ser
1               5


<210> SEQ ID NO 40
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct
<220> FEATURE:
<221> NAME/KEY: Misc_feature
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa in position 3 denotes any amino acid
<220> FEATURE:
<221> NAME/KEY: Misc_feature
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa in position 4 denotes any amino acid
<220> FEATURE:
<221> NAME/KEY: Misc_feature
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa in position 5 denotes any amino acid
<220> FEATURE:
<221> NAME/KEY: Misc_feature
```

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<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa in position 8 denotes any amino acid
<220> FEATURE:
<221> NAME/KEY: Misc_feature
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa in position 9 denotes any amino acid
<220> FEATURE:
<221> NAME/KEY: Misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa in position 10 denotes any amino acid

<400> SEQUENCE: 40

Lys Arg Xaa Xaa Xaa Asp Glu Xaa Xaa Xaa Tyr
1 5 10

<210> SEQ ID NO 41
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 41

Lys Ala Ala Lys Asp
1 5

<210> SEQ ID NO 42
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 42

Lys Leu Ala Ser Asp
1 5

<210> SEQ ID NO 43
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 43

Lys Leu Tyr Ser Asp
1 5

<210> SEQ ID NO 44
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 44

Lys Leu Tyr Asp
1

<210> SEQ ID NO 45
<211> LENGTH: 274
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 45

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```
Ala Gln Thr Val Pro Tyr Gly Ile Pro Leu Ile Lys Ala Asp Lys Val
1               5                   10                  15

Gln Ala Gln Gly Phe Lys Gly Ala Asn Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Gln Ala Ser His Pro Asp Leu Asn Val Val Gly Gly Ala
        35                  40                  45

Ser Phe Val Ala Gly Glu Ala Tyr Asn Thr Asp Gly Asn Gly His Gly
    50                  55                  60

Thr His Val Ala Gly Thr Val Ala Ala Leu Asp Asn Thr Thr Gly Val
65                  70                  75                  80

Leu Gly Val Ala Pro Ser Val Ser Leu Tyr Ala Val Lys Val Leu Asn
                85                  90                  95

Ser Ser Gly Ser Gly Ser Tyr Ser Gly Ile Val Ser Gly Ile Glu Trp
            100                 105                 110

Ala Thr Thr Asn Gly Met Asp Val Ile Asn Met Ser Leu Gly Gly Ala
        115                 120                 125

Ser Gly Ser Thr Ala Met Lys Gln Ala Val Asp Asn Ala Tyr Ala Arg
    130                 135                 140

Gly Val Val Val Val Ala Ala Ala Gly Asn Ser Gly Ser Ser Gly Asn
145                 150                 155                 160

Thr Asn Thr Ile Gly Tyr Pro Ala Lys Tyr Asp Ser Val Ile Ala Val
                165                 170                 175

Gly Ala Val Asp Ser Asn Ser Asn Arg Ala Ser Phe Ser Ser Val Gly
            180                 185                 190

Ala Glu Leu Glu Val Met Ala Pro Gly Ala Gly Val Tyr Ser Thr Tyr
        195                 200                 205

Pro Thr Asn Thr Tyr Ala Thr Leu Asn Gly Thr Ser Met Ala Ser Pro
    210                 215                 220

His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn Leu
225                 230                 235                 240

Ser Ala Ser Gln Val Arg Asn Arg Leu Ser Ser Thr Ala Thr Tyr Leu
                245                 250                 255

Gly Ser Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Glu Ala Ala
            260                 265                 270

Ala Gln


<210> SEQ ID NO 46
<211> LENGTH: 275
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 46

Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu
1               5                   10                  15

His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp
            20                  25                  30

Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala
        35                  40                  45

Ser Met Val Pro Ser Glu Thr Pro Asn Phe Gln Asp Asp Asn Ser His
    50                  55                  60

Gly Thr His Val Ala Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly
65                  70                  75                  80
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```
Val Leu Gly Val Ala Pro Ser Ser Ala Leu Tyr Ala Val Lys Val Leu
                85                  90                  95

Gly Asp Ala Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu
            100                 105                 110

Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly
        115                 120                 125

Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala
    130                 135                 140

Ser Gly Val Val Val Val Ala Ala Ala Gly Asn Glu Gly Ser Thr Gly
145                 150                 155                 160

Ser Ser Ser Thr Val Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala
                165                 170                 175

Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val
            180                 185                 190

Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr
        195                 200                 205

Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser
    210                 215                 220

Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn
225                 230                 235                 240

Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Gln Asn Thr Thr Thr Lys
                245                 250                 255

Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala
            260                 265                 270

Ala Ala Gln
        275


<210> SEQ ID NO 47
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 47

Ala Gln Thr Ile Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65                  70                  75                  80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145                 150                 155                 160
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```
Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
            180                 185                 190

Met Ala Pro Gly Val Asn Ile Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205

Ala Ser Asp Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
    210                 215                 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225                 230                 235                 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 48
<211> LENGTH: 268
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 48

Gln Thr Val Pro Trp Gly Ile Ser Phe Ile Asn Thr Gln Gln Ala His
1               5                   10                  15

Asn Arg Gly Ile Phe Gly Asn Gly Ala Arg Val Ala Val Leu Asp Thr
            20                  25                  30

Gly Ile Ala Ser His Pro Asp Leu Arg Ile Ala Gly Gly Ala Ser Phe
        35                  40                  45

Ile Ser Ser Glu Pro Ser Tyr His Asp Asn Asn Gly His Gly Thr His
    50                  55                  60

Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly
65                  70                  75                  80

Val Ala Pro Ser Ala Asp Leu Tyr Ala Val Lys Val Leu Asp Arg Asn
                85                  90                  95

Gly Ser Gly Ser Leu Ala Ser Val Ala Gln Gly Ile Glu Trp Ala Ile
            100                 105                 110

Asn Asn Asn Met His Ile Ile Asn Met Ser Leu Gly Ser Thr Ser Gly
        115                 120                 125

Ser Ser Thr Leu Glu Leu Ala Val Asn Arg Ala Asn Asn Ala Gly Ile
    130                 135                 140

Leu Leu Val Gly Ala Ala Gly Asn Thr Gly Arg Gln Gly Val Asn Tyr
145                 150                 155                 160

Pro Ala Arg Tyr Ser Gly Val Met Ala Val Ala Ala Val Asp Gln Asn
                165                 170                 175

Gly Gln Arg Ala Ser Phe Ser Thr Tyr Gly Pro Glu Ile Glu Ile Ser
            180                 185                 190

Ala Pro Gly Val Asn Val Asn Ser Thr Tyr Thr Gly Asn Arg Tyr Val
        195                 200                 205

Ser Leu Ser Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Val Ala
    210                 215                 220

Ala Leu Val Lys Ser Arg Tyr Pro Ser Tyr Thr Asn Asn Gln Ile Arg
225                 230                 235                 240

Gln Arg Ile Asn Gln Thr Ala Thr Tyr Leu Gly Ser Pro Ser Leu Tyr
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                245                 250                 255

Gly Asn Gly Leu Val His Ala Gly Arg Ala Thr Gln
            260                 265


<210> SEQ ID NO 49
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 49

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Arg Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65                  70                  75                  80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Ser Gly Ser Gly Ser Tyr Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110

Gly Asn Asn Gly Met His Val Ala Ser Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145                 150                 155                 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
            180                 185                 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
    210                 215                 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225                 230                 235                 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Ala Arg
            260                 265


<210> SEQ ID NO 50
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 50

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
```

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```
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asp Asn Ser Ile Gly Val Leu
65                  70                  75                  80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Ser Gly Ser Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145                 150                 155                 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
            180                 185                 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
    210                 215                 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225                 230                 235                 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 51
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 51

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65                  70                  75                  80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Ser Gly Gly Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110
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```
Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Asp Ser Ile Ser
145                 150                 155                 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
            180                 185                 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
    210                 215                 220

Ala Val Leu Val Lys His Lys Asn Pro Ser Trp Ser Asn Val Arg Ile
225                 230                 235                 240

Arg Asp His Leu Lys Lys Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 52
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 52

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65                  70                  75                  80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Ala Gly Gly Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145                 150                 155                 160

Ala Pro Ala Ser Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Pro Gly Leu Asp Ile
            180                 185                 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205
```

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```
Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
    210                 215                 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225                 230                 235                 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 53
<211> LENGTH: 271
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 53

Trp Ser Pro Asn Asp Pro Tyr Tyr Ser Ala Tyr Gln Tyr Gly Pro Gln
1               5                   10                  15

Asn Thr Ser Thr Pro Ala Ala Trp Asp Val Thr Arg Gly Ser Ser Thr
            20                  25                  30

Gln Thr Val Ala Val Leu Asp Ser Gly Val Asp Tyr Asn His Pro Asp
        35                  40                  45

Leu Ala Arg Lys Val Ile Lys Gly Tyr Asp Phe Ile Asp Arg Asp Asn
    50                  55                  60

Asn Pro Met Asp Leu Asn Gly His Gly Thr His Val Ala Gly Thr Val
65                  70                  75                  80

Ala Ala Asp Thr Asn Asn Gly Ile Gly Val Ala Gly Met Ala Pro Asp
                85                  90                  95

Thr Lys Ile Leu Ala Val Arg Val Leu Asp Ala Asn Gly Ser Gly Ser
            100                 105                 110

Leu Asp Ser Ile Ala Ser Gly Ile Arg Tyr Ala Ala Asp Gln Gly Ala
        115                 120                 125

Lys Val Leu Asn Leu Ser Leu Gly Cys Glu Cys Asn Ser Thr Thr Leu
    130                 135                 140

Lys Ser Ala Val Asp Tyr Ala Trp Asn Lys Gly Ala Val Val Val Ala
145                 150                 155                 160

Ala Ala Gly Asn Asp Asn Val Ser Arg Thr Phe Gln Pro Ala Ser Tyr
                165                 170                 175

Pro Asn Ala Ile Ala Val Gly Ala Ile Asp Ser Asn Asp Arg Lys Ala
            180                 185                 190

Ser Phe Ser Asn Tyr Gly Thr Trp Val Asp Val Thr Ala Pro Gly Val
        195                 200                 205

Asn Ile Ala Ser Thr Val Pro Asn Asn Gly Tyr Ser Tyr Met Ser Gly
    210                 215                 220

Thr Ser Met Ala Ser Pro His Val Ala Gly Leu Ala Ala Leu Leu Ala
225                 230                 235                 240

Ser Gln Gly Lys Asn Asn Val Gln Ile Arg Gln Ala Ile Glu Gln Thr
                245                 250                 255

Ala Asp Lys Ile Ser Gly Thr Gly Thr Asn Phe Lys Tyr Gly Lys
            260                 265                 270


<210> SEQ ID NO 54
<211> LENGTH: 269
<212> TYPE: PRT
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-continued

<213> ORGANISM: Bacillus

<400> SEQUENCE: 54

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Asn Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Ser Gly Gly Gly Ser Asn Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
260 265

<210> SEQ ID NO 55
<211> LENGTH: 270
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 55

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Asp Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala
35 40 45

Ser Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly
50 55 60

Thr His Val Ala Gly Thr Ile Ala Ala Leu Asp Asn Ser Ile Gly Val

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```
65                  70                  75                  80

Leu Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly
                85                  90                  95

Ala Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp
            100                 105                 110

Ala Gly Asn Asn Gly Met Asp Val Ala Asn Leu Ser Leu Gly Ser Pro
        115                 120                 125

Ser Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg
    130                 135                 140

Gly Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile
145                 150                 155                 160

Ser Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp
                165                 170                 175

Gln Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Glu Leu Asp
            180                 185                 190

Ile Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr
        195                 200                 205

Tyr Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly
    210                 215                 220

Ala Ala Ala Leu Val Leu Gln Lys Asn Pro Ser Trp Ser Asn Val Gln
225                 230                 235                 240

Ile Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn
                245                 250                 255

Leu Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265                 270


<210> SEQ ID NO 56
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 56

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1               5                   10                  15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65                  70                  75                  80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
            100                 105                 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
        115                 120                 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
    130                 135                 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145                 150                 155                 160
```

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```
Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
            180                 185                 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
        195                 200                 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
    210                 215                 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225                 230                 235                 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                245                 250                 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 57
<211> LENGTH: 268
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 57

Gln Thr Val Pro Trp Gly Ile Asn Arg Val Gln Ala Pro Ile Ala Gln
1               5                   10                  15

Ser Arg Gly Phe Thr Gly Thr Gly Val Arg Val Ala Val Leu Asp Thr
            20                  25                  30

Gly Ile Ser Asn His Ala Asp Leu Arg Ile Arg Gly Gly Ala Ser Phe
        35                  40                  45

Val Pro Gly Glu Pro Asn Ile Ser Asp Gly Asn Gly His Gly Thr Gln
    50                  55                  60

Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly
65                  70                  75                  80

Val Ala Pro Asn Val Asp Leu Tyr Gly Val Lys Val Leu Gly Ala Ser
                85                  90                  95

Gly Ser Gly Ser Ile Ser Gly Ile Ala Gln Gly Leu Gln Trp Ala Ala
            100                 105                 110

Asn Asn Gly Met His Ile Ala Asn Met Ser Leu Gly Ser Ser Ala Gly
        115                 120                 125

Ser Ala Thr Met Glu Gln Ala Val Asn Gln Ala Thr Ala Ser Gly Val
    130                 135                 140

Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Asn Val Gly Phe
145                 150                 155                 160

Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln Asn
                165                 170                 175

Asn Asn Arg Ala Thr Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile Val
            180                 185                 190

Ala Pro Gly Val Gly Val Gln Ser Thr Val Pro Gly Asn Gly Tyr Ala
        195                 200                 205

Ser Phe Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Val Ala
    210                 215                 220

Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile Arg
225                 230                 235                 240

Asn His Leu Lys Asn Thr Ala Thr Asn Leu Gly Asn Thr Thr Gln Phe
                245                 250                 255
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Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 58
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 58

Asn Gln Val Thr Pro Trp Gly Ile Thr Arg Val Gln Ala Pro Thr Ala
1               5                   10                  15

Trp Thr Arg Gly Tyr Thr Gly Thr Gly Val Arg Val Ala Val Leu Asp
            20                  25                  30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Val Ser
        35                  40                  45

Phe Val Pro Gly Glu Pro Ser Tyr Gln Asp Gly Asn Gly His Gly Thr
    50                  55                  60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Val
65                  70                  75                  80

Gly Val Ala Pro Asn Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                85                  90                  95

Asn Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Gln Trp Thr
            100                 105                 110

Ala Gln Asn Asn Ile His Val Ala Asn Leu Ser Leu Gly Ser Pro Val
        115                 120                 125

Gly Ser Gln Thr Leu Glu Leu Ala Val Asn Gln Ala Thr Asn Ala Gly
    130                 135                 140

Val Leu Val Val Ala Ala Thr Gly Asn Asn Gly Ser Gly Thr Val Ser
145                 150                 155                 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Leu Ala Val Gly Ala Thr Asp Gln
                165                 170                 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Thr Gly Leu Asn Ile
            180                 185                 190

Val Ala Pro Gly Val Gly Ile Gln Ser Thr Tyr Pro Gly Asn Arg Tyr
        195                 200                 205

Ala Ser Leu Ser Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Val
    210                 215                 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Thr Gln Ile
225                 230                 235                 240

Arg Gln His Leu Thr Ser Thr Ala Thr Ser Leu Gly Asn Ser Asn Gln
                245                 250                 255

Phe Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265


<210> SEQ ID NO 59
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 59

Val Gly Val Tyr Gly Asp Thr Ser Ala
1               5


<210> SEQ ID NO 60
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<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 60

Leu Gln Cys Val Gly Ser
1 5

<210> SEQ ID NO 61
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 61

Lys Arg Phe Ala Asn Thr Glu Leu Ala
1 5

<210> SEQ ID NO 62
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 62

Leu Asp Gln Ile Phe Phe Thr Arg Trp
1 5

<210> SEQ ID NO 63
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 63

Phe Asn Asp Ala Phe Phe Val Lys Met
1 5

<210> SEQ ID NO 64
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 64

Ala Asn Ile Pro Ile Trp Ser Arg Ser Ala
1 5 10

<210> SEQ ID NO 65
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 65

Arg Gln Ser Thr Asp Phe Gly Thr Thr
1 5

<210> SEQ ID NO 66
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 66

Val Gln Val Tyr Gly Asp Thr Ser Ala
1 5

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<210> SEQ ID NO 67
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 67

Arg Arg Phe Ser Asn Ala Thr Arg Ala
1 5

<210> SEQ ID NO 68
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 68

Cys Thr Ala Arg Leu Arg Ala Gly Asn Ala Cys Gly
1 5 10

<210> SEQ ID NO 69
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 69

Leu Asp Gln Ile Phe Phe Thr Arg Trp
1 5

<210> SEQ ID NO 70
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 70

Glu Gln Ile Phe Phe Thr Ser Gly Leu
1 5

<210> SEQ ID NO 71
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 71

Gly Arg Phe Ser Asn Ser Lys Phe Lys
1 5

<210> SEQ ID NO 72
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 72

Ala Val Leu Arg Asp Cys
1 5

<210> SEQ ID NO 73
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 73

Leu Gln Cys Val Gly Ser
1 5

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<210> SEQ ID NO 74
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 74

Leu Arg Gln Cys Asn Glu Arg Cys Val
1 5

<210> SEQ ID NO 75
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 75

Ser Pro Val Thr Lys Arg Ala Ser Leu Lys Ile Asp Ser Lys Lys
1 5 10 15

<210> SEQ ID NO 76
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 76

Arg Gln Ser Thr Asp Phe Gly Thr Thr
1 5

<210> SEQ ID NO 77
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 77

Phe Cys Thr Asn Asn Cys Glu Leu Ser
1 5

<210> SEQ ID NO 78
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 78

Asp Phe His Val Lys Tyr Ala Ala Gln
1 5

<210> SEQ ID NO 79
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 79

Val Ala Gln Tyr Lys Ala Leu Pro Val Val Leu Glu Asn Ala
1 5 10

<210> SEQ ID NO 80
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 80

Ala Ala Tyr Pro Asp Val
1 5

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<210> SEQ ID NO 81
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 81

Glu Gln Ile Phe Phe Thr Ser Gly Leu
1 5

<210> SEQ ID NO 82
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 82

Val Asp Ala Ala Phe
1 5

<210> SEQ ID NO 83
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 83

Ala Val Leu Arg Asp Cys
1 5

<210> SEQ ID NO 84
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 84

Arg Ala Phe Arg Arg Asn Ala Asn Trp
1 5

<210> SEQ ID NO 85
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 85

Cys Thr Ala Arg Leu Arg Ala Gly Asn Ala Cys Gly
1 5 10

<210> SEQ ID NO 86
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 86

Thr Phe His Asp Ala Pro Ala Leu Gln
1 5

<210> SEQ ID NO 87
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 87

Cys Thr Ala Arg Val Val Ala Leu Gly Val Cys Gly

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1 5 10

<210> SEQ ID NO 88
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 88

Gly Arg Phe Ser Asn Ser Lys Phe Lys
1 5

<210> SEQ ID NO 89
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 89

Arg Arg Phe Ala Asn Asp His Thr Arg
1 5

<210> SEQ ID NO 90
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 90

Lys Arg Phe Ala Asn Thr Glu Pro Ala
1 5

<210> SEQ ID NO 91
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 91

Tyr Lys Val Ser Ala Leu
1 5

<210> SEQ ID NO 92
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 92

Thr Gly Lys Tyr Val Ser
1 5

<210> SEQ ID NO 93
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 93

Gln Arg Pro Pro Arg Tyr Glu Leu Glu
1 5

<210> SEQ ID NO 94
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 94

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Glu Leu Glu Tyr Arg Pro Pro Arg Gln
1 5

<210> SEQ ID NO 95
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 95

His Glu Tyr Asp Met Arg Val Ala Trp
1 5

<210> SEQ ID NO 96
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 96

His Glu Tyr Pro Met Asp Phe His Leu
1 5

<210> SEQ ID NO 97
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 97

Ser Glu Tyr Ser Met Ser Ile Thr Pro
1 5

<210> SEQ ID NO 98
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 98

Cys Val Trp Pro Ala His Ala Pro Leu Ser Cys Gly
1 5 10

<210> SEQ ID NO 99
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 99

Cys Ser Trp Pro Ser Pro Ala Pro Leu Ser Cys Gly
1 5 10

<210> SEQ ID NO 100
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 100

Cys Asp Phe Pro Leu His Ala Pro Leu Ser Cys Gly
1 5 10

<210> SEQ ID NO 101
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 101

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Cys Leu Phe Pro Ser Pro Ala Pro Arg Ser Cys Gly
1 5 10

<210> SEQ ID NO 102
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 102

Cys Asp Gly Pro Ala Pro Ala Pro Trp Ser Cys Gly
1 5 10

<210> SEQ ID NO 103
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 103

Cys Ser Phe Pro Leu Pro Ala Pro Arg Ser Cys Gly
1 5 10

<210> SEQ ID NO 104
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 104

Cys Val Tyr Pro Ser Pro Ala Pro Trp Ser Cys Gly
1 5 10

<210> SEQ ID NO 105
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 105

Pro Glu Tyr Thr Met Asn Ala Leu Ser
1 5

<210> SEQ ID NO 106
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 106

Cys Ser Arg Ser Ala Lys Gly Ala Arg Leu Cys Gly
1 5 10

<210> SEQ ID NO 107
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 107

Leu Glu Tyr Pro Met Ser Ala Ser Gln
1 5

<210> SEQ ID NO 108
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

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<400> SEQUENCE: 108

Arg Lys Leu Thr Leu Ser Gly Arg Ser
1 5

<210> SEQ ID NO 109
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 109

Arg Lys Leu Thr Leu Ser Gly Arg Ser
1 5

<210> SEQ ID NO 110
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 110

Ser Tyr Gly Ala Pro Ala Thr Pro Ala Ala
1 5 10

<210> SEQ ID NO 111
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 111

Pro Ala Ala Gly Tyr Thr Pro Ala Ala Pro
1 5 10

<210> SEQ ID NO 112
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 112

Tyr Lys Leu Ala Tyr
1 5

<210> SEQ ID NO 113
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 113

Lys Tyr Asp Asp Tyr Val Ala Thr Leu Ser
1 5 10

<210> SEQ ID NO 114
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 114

Glu Val Lys Ala Thr Pro Ala Gly Glu Leu
1 5 10

<210> SEQ ID NO 115
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

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<400> SEQUENCE: 115

Cys Gly Tyr Ser Asn Ala Gln Gly Val Asp Tyr Trp Ile
1 5 10

<210> SEQ ID NO 116
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 116

Val Pro Gly Ile Asp Pro Asn Ala Cys His Tyr Met Lys Cys
1 5 10

<210> SEQ ID NO 117
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 117

Ser Pro Val Thr Lys Arg Ala Ser Leu Lys Ile Asp Ser Lys Lys
1 5 10 15

<210> SEQ ID NO 118
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 118

Ile Met Ser Ala Leu Ala Met Val Tyr Leu Gly Ala Lys
1 5 10

<210> SEQ ID NO 119
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 119

Glu Leu Gly Val Arg Glu
1 5

<210> SEQ ID NO 120
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 120

Gly Cys Arg Lys Glu Val
1 5

<210> SEQ ID NO 121
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Thermomyces

<400> SEQUENCE: 121

Leu Arg Ser Val Tyr Gln
1 5

<210> SEQ ID NO 122
<211> LENGTH: 6
<212> TYPE: PRT

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<213> ORGANISM: Thermomyces

<400> SEQUENCE: 122

Ser Gly Pro Trp Ser Trp
1 5

<210> SEQ ID NO 123
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 123

Ala Arg Ile Asp Pro Arg Gly Pro Ser
1 5

<210> SEQ ID NO 124
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 124

Ala Arg Ile Asp Pro Arg His Gly Ser
1 5

<210> SEQ ID NO 125
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 125

Cys Ser Val Ala Lys Ile Asp Pro Arg Thr Cys Gly
1 5 10

<210> SEQ ID NO 126
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 126

Ala Lys Ile Asp Pro Lys Pro Asp Thr
1 5

<210> SEQ ID NO 127
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 127

Ala Arg Ile Asp Pro Arg His Gly Ser
1 5

<210> SEQ ID NO 128
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 128

Gln Ile Tyr Asn Asp Thr Gly Pro Thr
1 5

<210> SEQ ID NO 129
<211> LENGTH: 12

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<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 129

Cys Gly Ser Ala Thr Ile Asp Pro Arg Gln Cys Gly
1 5 10

<210> SEQ ID NO 130
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 130

Cys Asn Ala Asp Asn Gln Met Tyr Pro Gln Cys Gly
1 5 10

<210> SEQ ID NO 131
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 131

Ala Arg Ile Asp Pro Arg Gly Pro Ser
1 5

<210> SEQ ID NO 132
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 132

Cys Gly Ser Ala Thr Ile Asp Pro Arg Gln Cys Gly
1 5 10

<210> SEQ ID NO 133
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 133

Cys Asp Ala Asp Ser Ser Gly Tyr Pro Leu Cys Gly
1 5 10

<210> SEQ ID NO 134
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 134

Gln Leu Tyr Gly Asp Glu Gln Leu Pro
1 5

<210> SEQ ID NO 135
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 135

Arg Tyr Ala Gln Ile Asp Pro Arg Trp
1 5

<210> SEQ ID NO 136

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<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 136

Gly Glu Phe Asn Leu Gly Arg Ser Ser
1 5

<210> SEQ ID NO 137
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 137

Cys Asn Ala Asp Ser Trp Gly Tyr Pro Arg Cys Gly
1 5 10

<210> SEQ ID NO 138
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 138

Cys Asn Ala Asp Asn Gln Met Tyr Pro Gln Cys Gly
1 5 10

<210> SEQ ID NO 139
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 139

Gly Glu Phe Asn Leu Gly Arg Ser Ser
1 5

<210> SEQ ID NO 140
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Humicola

<400> SEQUENCE: 140

Cys Val His Ala Gly Pro Arg Ala Gly Thr Cys Gly
1 5 10

<210> SEQ ID NO 141
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Humicola

<400> SEQUENCE: 141

Cys Leu Ser Gly Pro Leu Ala Gly Arg Val Cys Gly
1 5 10

<210> SEQ ID NO 142
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Humicola

<400> SEQUENCE: 142

Cys Arg Ile Ser Pro Trp Tyr Ser Val Pro Cys Gly
1 5 10

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<210> SEQ ID NO 143
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Humicola

<400> SEQUENCE: 143

Cys Leu Ser Gly Pro Ala Ala Gly Gln Ser Cys Gly
1 5 10

<210> SEQ ID NO 144
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Humicola

<400> SEQUENCE: 144

Cys Ile Thr Arg Gly Thr Arg Ala Gly Trp Cys Gly
1 5 10

<210> SEQ ID NO 145
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Humicola

<400> SEQUENCE: 145

Cys Leu Ser Gly Pro Leu Ala Gly Arg Val Cys Gly
1 5 10

<210> SEQ ID NO 146
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Humicola

<400> SEQUENCE: 146

Cys Leu Thr Ala Gly Pro Ser Ala Gly Tyr Cys Gly
1 5 10

<210> SEQ ID NO 147
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Humicola

<400> SEQUENCE: 147

Cys Tyr Thr Thr Gly Arg Leu Ala Gly Leu Cys Gly
1 5 10

<210> SEQ ID NO 148
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Humicola

<400> SEQUENCE: 148

Cys Val His Ser Gly Pro Arg Ala Gly Tyr Cys Gly
1 5 10

<210> SEQ ID NO 149
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Humicola

<400> SEQUENCE: 149

Cys Val His Ala Gly Pro Arg Ala Gly Thr Cys Gly
1 5 10

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<210> SEQ ID NO 150
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Humicola

<400> SEQUENCE: 150

Cys Val His Ser Gly Leu Ser Arg Arg Leu Leu Arg
1 5 10

<210> SEQ ID NO 151
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Humicola

<400> SEQUENCE: 151

Cys Val Thr Arg Gly Pro Asn Ala Gly Ser Cys Gly
1 5 10

<210> SEQ ID NO 152
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Humicola

<400> SEQUENCE: 152

Cys Leu Thr Ala Gly Pro Ser Ala Gly Tyr Cys Gly
1 5 10

<210> SEQ ID NO 153
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Humicola

<400> SEQUENCE: 153

Cys Ile Thr Ser Gly Pro Arg Ala Gly Asn Cys Gly
1 5 10

<210> SEQ ID NO 154
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 154

Pro Gln Ser Asp Pro Gly Glu Ser Gln
1 5

<210> SEQ ID NO 155
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 155

Trp Pro Lys Ser Asp Ala Gly Asp Ser
1 5

<210> SEQ ID NO 156
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 156

Pro Gln Ser Asp Ala Gly Val Val Met
1 5

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<210> SEQ ID NO 157
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 157

Asp Pro Val Arg Asp Thr Gly Ala Gly
1 5

<210> SEQ ID NO 158
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 158

Gly Pro Ser Arg Asp Ala Gly Leu Leu
1 5

<210> SEQ ID NO 159
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 159

Pro Ala Ser Asp Ala Gly Arg Gly Pro
1 5

<210> SEQ ID NO 160
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 160

Pro Arg Asp Ser Thr Gly Leu Ala Leu
1 5

<210> SEQ ID NO 161
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 161

Pro Gln Ser Asp Pro Gly Glu Ser Gln
1 5

<210> SEQ ID NO 162
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 162

Arg Tyr Pro Phe Leu Arg Ala Thr Asn
1 5

<210> SEQ ID NO 163
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 163

Gly Ala Ala Arg Asp Ala Arg Ser Ala

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1 5

<210> SEQ ID NO 164
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 164

Pro Arg Ser Asp Thr Gly Phe Gly Ser
1 5

<210> SEQ ID NO 165
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 165

Leu Pro Arg Ser Asp Pro Gly Gly Arg
1 5

<210> SEQ ID NO 166
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 166

Asp Pro Ala Arg Asp Thr Gly Asp Val
1 5

<210> SEQ ID NO 167
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 167

Ala Pro Lys Ser Asp Asn Gly Ile Thr
1 5

<210> SEQ ID NO 168
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 168

Pro Lys Ser Asp Pro Gly Thr Asn Trp
1 5

<210> SEQ ID NO 169
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 169

Pro Arg Thr Asp Pro Gly Trp Leu Ala
1 5

<210> SEQ ID NO 170
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 170

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Leu Pro Arg Ser Asp Pro Gly Gly Arg
1 5

<210> SEQ ID NO 171
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 171

Pro Ser Ser Asp Pro Gly Ala Arg Ser
1 5

<210> SEQ ID NO 172
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 172

His Val Phe Asp Lys Asn Val Thr Arg
1 5

<210> SEQ ID NO 173
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 173

Pro Arg Ser Asp Pro Gly Thr Pro Thr
1 5

<210> SEQ ID NO 174
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 174

Pro Arg Asp Ser Thr Gly Leu Ala Leu
1 5

<210> SEQ ID NO 175
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 175

Pro Arg Asp Ser Thr Gly Leu Ala Leu
1 5

<210> SEQ ID NO 176
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 176

Pro Ser Ser Asp Pro Gly Ala Arg Ser
1 5

<210> SEQ ID NO 177
<211> LENGTH: 9
<212> TYPE: PRT
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<400> SEQUENCE: 177

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Pro Lys Ser Asp Pro Gly Thr Asn Trp
1 5

<210> SEQ ID NO 178
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 178

Trp Pro Lys Ser Asp Ala Gly Asp Ser
1 5

<210> SEQ ID NO 179
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 179

Pro Gln Ser Asp Ala Gly Val Val Met
1 5

<210> SEQ ID NO 180
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 180

Gly Pro Ser Arg Asp Ala Gly Leu Leu
1 5

<210> SEQ ID NO 181
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 181

Pro Ala Ser Asp Ala Gly Arg Gly Pro
1 5

<210> SEQ ID NO 182
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 182

Ala Pro Lys Ser Asp Asn Gly Ile Thr
1 5

<210> SEQ ID NO 183
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 183

Trp Pro Lys Ser Asp Ala Gly Asp Ser
1 5

<210> SEQ ID NO 184
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

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<400> SEQUENCE: 184

Pro Gln Ser Asp Ala Gly Val Val Met
1 5

<210> SEQ ID NO 185
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 185

Gly Pro Ser Arg Asp Ala Gly Leu Leu
1 5

<210> SEQ ID NO 186
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 186

Pro Ala Ser Asp Ala Gly Arg Gly Pro
1 5

<210> SEQ ID NO 187
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 187

Ala Pro Lys Ser Asp Asn Gly Ile Thr
1 5

<210> SEQ ID NO 188
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 188

Asp Pro Val Arg Asp Thr Gly Ala Gly
1 5

<210> SEQ ID NO 189
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 189

Pro Arg Ser Asp Thr Gly Phe Gly Ser
1 5

<210> SEQ ID NO 190
<211> LENGTH: 9
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<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 190

Asp Pro Ala Arg Asp Thr Gly Asp Val
1 5

<210> SEQ ID NO 191
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

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<400> SEQUENCE: 191

Asp Pro Val Arg Asp Thr Gly Ala Gly
1 5

<210> SEQ ID NO 192
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 192

Pro Arg Ser Asp Thr Gly Phe Gly Ser
1 5

<210> SEQ ID NO 193
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 193

Asp Pro Ala Arg Asp Thr Gly Asp Val
1 5

<210> SEQ ID NO 194
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 194

Asp Pro Val Arg Asp Thr Gly Ala Gly
1 5

<210> SEQ ID NO 195
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 195

Pro Arg Ser Asp Thr Gly Phe Gly Ser
1 5

<210> SEQ ID NO 196
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 196

Asp Pro Ala Arg Asp Thr Gly Asp Val
1 5

<210> SEQ ID NO 197
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 197

Ala Leu Pro Gln Ser
1 5

<210> SEQ ID NO 198
<211> LENGTH: 4
<212> TYPE: PRT

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<213> ORGANISM: Betula

<400> SEQUENCE: 198

Ala Gly Ile Leu
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<210> SEQ ID NO 199
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 199

Ala Asn Arg Thr Val
1 5

<210> SEQ ID NO 200
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 200

Gly Ile Leu Val Tyr
1 5

<210> SEQ ID NO 201
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 201

Ala Ser Thr Arg
1

<210> SEQ ID NO 202
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 202

Arg Asn Ala Phe Leu Ser
1 5

<210> SEQ ID NO 203
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 203

Lys Arg Gln Ser Ala
1 5

<210> SEQ ID NO 204
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 204

Ser Thr Arg Cys
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<210> SEQ ID NO 205
<211> LENGTH: 6

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<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 205

Asn Arg Gly Leu Thr Val
1 5

<210> SEQ ID NO 206
<211> LENGTH: 4
<212> TYPE: PRT
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<400> SEQUENCE: 206

Ser Thr Ala Asn
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<210> SEQ ID NO 207
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<400> SEQUENCE: 207

Ala Ile Leu Val
1

<210> SEQ ID NO 208
<211> LENGTH: 5
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<400> SEQUENCE: 208

Glu Asn Arg Ser Val
1 5

<210> SEQ ID NO 209
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 209

Asp Gly Asn Thr
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<210> SEQ ID NO 210
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<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 210

Asp Glu Cys Thr
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<210> SEQ ID NO 211
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 211

Phe Trp Tyr Gly Leu
1 5

<210> SEQ ID NO 212

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<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 212

Leu Arg Trp Ala
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<210> SEQ ID NO 213
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 213

Arg Lys Gln Thr
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<210> SEQ ID NO 214
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 214

Gln Arg Ser Trp
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<210> SEQ ID NO 215
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 215

Val Leu Ser Phe Asn
1 5

<210> SEQ ID NO 216
<211> LENGTH: 6
<212> TYPE: PRT
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<400> SEQUENCE: 216

Ala Glu His Asn Pro Thr
1 5

<210> SEQ ID NO 217
<211> LENGTH: 5
<212> TYPE: PRT
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<400> SEQUENCE: 217

Ala Gly Leu Lys Met
1 5

<210> SEQ ID NO 218
<211> LENGTH: 4
<212> TYPE: PRT
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<400> SEQUENCE: 218

Glu Asp Lys Trp
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<210> SEQ ID NO 219
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 219

Ala Cys Leu Pro Thr Val Trp Tyr
1 5

<210> SEQ ID NO 220
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 220

Ala Ser Leu Pro Met
1 5

<210> SEQ ID NO 221
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 221

Ser Tyr Leu Asn
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<210> SEQ ID NO 222
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 222

Ala Glu Leu Phe Pro Arg
1 5

<210> SEQ ID NO 223
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 223

Thr Ser Phe Arg
1

<210> SEQ ID NO 224
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 224

Ala Pro Ser Gly
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<210> SEQ ID NO 225
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 225

Cys Phe Ile Lys Leu Trp
1 5

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<210> SEQ ID NO 226
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 226

Phe Ile Lys Leu Trp
1 5

<210> SEQ ID NO 227
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 227

Ala Gly Ile Leu Val
1 5

<210> SEQ ID NO 228
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 228

Lys Arg Gln His Asn Gly Pro
1 5

<210> SEQ ID NO 229
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 229

Gly Ile Leu Val
1

<210> SEQ ID NO 230
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 230

Ile Lys Leu Pro Gln Ser
1 5

<210> SEQ ID NO 231
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 231

Leu Ile Met Asn
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<210> SEQ ID NO 232
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 232

Thr Tyr Ala Pro
1

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<210> SEQ ID NO 233
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<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 233

Ile Leu Val Ser
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<210> SEQ ID NO 234
<211> LENGTH: 5
<212> TYPE: PRT
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<400> SEQUENCE: 234

His Asn Gln Gly Cys
1 5

<210> SEQ ID NO 235
<211> LENGTH: 5
<212> TYPE: PRT
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<400> SEQUENCE: 235

Ala Val Leu Cys Tyr
1 5

<210> SEQ ID NO 236
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<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 236

Leu Phe Gln Ala
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<210> SEQ ID NO 237
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 237

Ala Ile Leu Met Asn Val
1 5

<210> SEQ ID NO 238
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 238

Ala Gly Ser Tyr Leu Glu
1 5

<210> SEQ ID NO 239
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 239

Leu Ile Ala Gly Val Ser

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1 5

<210> SEQ ID NO 240
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 240

Lys His Gln Asp
1

<210> SEQ ID NO 241
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 241

Ser His Gln Glu
1

<210> SEQ ID NO 242
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 242

Ser Thr Ala Pro Leu Trp Val
1 5

<210> SEQ ID NO 243
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 243

Thr Ser Lys His Arg Gln Gly
1 5

<210> SEQ ID NO 244
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 244

Leu Ile Arg Lys Gly Pro
1 5

<210> SEQ ID NO 245
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 245

Asp Ser Arg Thr Gln Gly Lys His
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<210> SEQ ID NO 246
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 246

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Asp Glu Lys Gln His Thr
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<210> SEQ ID NO 247
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 247

Arg Lys Gln Asp Thr
1               5


<210> SEQ ID NO 248
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 248

Ser Thr Arg Glu
1
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**1-134**. (canceled)

**135**. A protease variant having modified immunogenicity as compared to a parent protease, obtainable by a method comprising the steps of:

(a) obtaining antibody binding peptide sequences,

(b) using the sequences to localise epitope sequences on the 3-dimensional structure of the parent protein,

(c) defining an epitope area including amino acids situated within 5 Å from the epitope amino acids constituting the epitope sequence,

(d) changing one or more of the amino acids defining the epitope area of the parent protein by genetic engineering mutations of a DNA sequence encoding the parent protein,

(e) introducing the mutated DNA sequence into a suitable host, culturing said host and expressing the protein variant, and

(f) evaluating the immunogenicity of the protein variant using the parent protein as reference.

**136**. The protease variant of claim **135**, wherein the protease is a subtilisin comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO: 10:

Position −6 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion;

Position −5 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion;

Position −4 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion;

Position −2 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion;

Position 3a to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion;

Position 28a to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion;

Position 44a to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion;

Position 44b to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion;

Position 139 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion;

Position 148 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion;

Position 149 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion;

Position 264a to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion.

**137**. The protease variant of claim **135**, wherein the protease is a subtilisin comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO: 10:

Position −1 to G, V, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, H;

Position 1 to V, L, I, W, M, F, Y, S, T, R;

Position 2 to G, V, I, M, F, N, Q, Y, S, T, H;

Position 3 to W, M, F, N, Q, Y, S, D, E, R, H;

Position 4 to V, L, W, M, F, Y, R;

Position 5 to V, L, I, W, M, F, N, Q, Y, T, R, H;

Position 6 to G, V, L, I, W, P, M, N, Q, T, D, E, R, H;

Position 9 to G, V, L, I, W, P, M, F, Q, Y, S, T, R, H;

Position 10 to G, A, V, I, W, P, M, N, Q, Y, S, T, D, E, R;

Position 12 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E;

Position 14 to V, L, I, W, P, M, F, N, Q, Y, T, R, H;

Position 15 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, E, H;

Position 17 to G, A, V, I, W, P, M, F, Y, H;

Position 18 to G, A, L, I, W, P, M, F, N, Q, Y, T, D, E, H;

Position 19 to A, V, I, W, M, F, N, Y, S, T, D, R, H;

Position 20 to G, V, L, I, W, M, F, N, Q, Y, S, T, D, E;

Position 21 to G, V, I, W, N, Q, Y, S, T, D, E, R, H;

Position 22 to G, V, L, I, W, M, F, Y, S, T;

Position 24 to G, V, L, I, W, M, F, N, Q, Y, S, D, E, R;

Position 25 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H;

Position 27 to G, L, I, W, P, M, F, Y, T, H;

Position 38 to V, L, I, W, M, F, N, Q, Y, T, H;

Position 39 to G, A, V, L, I, W, M, F, N, Q, Y, T, D, E, R, H;

Position 40 to V, L, I, W, M, F, N, Q, Y, T, R, H;

Position 42 to G, A, L, W, C, M, F, N, Q, Y, S, T, D, E, R, H;

Position 43 to G, L, H;

Position 44 to G, V, L, I, W, P, M, F, Y, S, T;
Position 45 to G, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H;
Position 46 to G, A, L, I, W, P, M, F, Y, H;
Position 47 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H;
Position 48 to A, L, I, P, M, F, N, Y, D, H;
Position 49 to G, A, V, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H;
Position 50 to G, A, W, M, N, Q, Y, S, T, D, E, H;
Position 51 to V, L, I, W, M, F, N, Y, R;
Position 52 to V, L, I, W, M, F, Y, S, T, R;
Position 53 to A, V, L, I, W, M, F, N, Q, Y, S, D, E, H;
Position 54 to V, L, I, W, M, F, S, R;
Position 55 to G, A, V, L, I, W, C, M, F, N, Q, Y, T, D, E, R, K, H;
Position 56 to G, V, L, I, W, M, F, N, Q, Y, S, T, H;
Position 57 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 58 to L, W, M, F, N, Y, R;
Position 59 to A, V, L, I, C, T, H;
Position 61 to V, L, I, W, M, F, Y;
Position 62 to G, A, L, W, M, F, N, Y, R;
Position 64 to G, V, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 75 to L;
Position 79 to I;
Position 80 to G;
Position 87 to A, V, L, I, W, M, F, Q, Y, S, T, D, E, H;
Position 89 to G, V, L, I, W, P, F, N, Y, T, E;
Position 91 to G, A, V, L, I, W, P, M, N, Y, S, T, D, E, R, H;
Position 98 to A;
Position 99 to V, L, I, W, M, F, Q, Y, H;
Position 100 to G, V, L, I, W, M, F, Y, R, H;
Position 101 to V, I, W, M, F, N, Q, Y, H;
Position 102 to V, L, I, W, M, F, Y, R, H, G;
Position 108 to I;
Position 109 to N,
Position 112 to E;
Position 113 to W;
Position 115 to I;
Position 117 to N;
Position 118 to N;
Position 126 to L;
Position 127 to G, A, V, I, W, M, F, Y, R, H, L;
Position 128 to I, W;
Position 129 to W;
Position 130 to W, F, Y, R;
Position 131 to W, Y, R;
Position 132 to L, W, M, F, Y, S, H;
Position 133 to A, L, I, W, M, F, Y, R;
Position 134 to L, I, W, F, N, Q, Y, R, H;
Position 136 to G, A, W, P, N, Y, S, T, D, E, H;
Position 137 to G, A, V, I, W, P, M, N, Y, H;
Position 140 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, H;
Position 141 to G, V, L, I, W, P, M, F, Q, S, D, E, H;
Position 143 to V, L, I, P, M, F, N, Y, R;
Position 144 to L, W, P, M, F, N, Q, Y, S, D, E, R, H;
Position 145 to G, V, L, I, W, M, F, Q, Y, D, E, R, H;
Position 146 to G, A, W, L, I, W, M, F, N, Q, Y, T, D, E, R, H;
Position 155 to V, L, I, W, M, F, Y, R;
Position 156 to V, I, W, F, R;
Position 157 to G, A, V, L, I, W, M, F, Y, T, R, H;
Position 158 to V, L, I, W, M, F, Y;
Position 159 to A, W, M, Y, T, R, H;
Position 160 to W, M, F, Y, R, H;
Position 161 to I, W, M, F, Y, H;
Position 167 to R, K;
Position 171 to D;
Position 172 to G, A, V, L, I, S, T, H;
Position 173 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, E, H;
Position 181 to G, A, V, L, I, W, C, M, F, Q, Y, T, D, R, K, H;
Position 182 to A, V, L, I, W, C, M, F, N, Q, Y, S, T, D, E, H;
Position 183 to G, A, V, L, W, C, M, F, N, Q, Y, S, T, E, R, H;
Position 184 to A, V, L, I, W, C, M, F, N, Q, Y, T, E, H;
Position 185 to G, A, V, L, I, W, C, M, F, N, Q, Y, T, E, H;
Position 186 to G, A, V, L, W, M, F, N, Q, Y, S, T, D, E, R, H;
Position 188 to G, A, V, L, W, F, S, R, K;
Position 189 to W, F;
Position 191 to A, V, L, I, W, M, F, Y, T, R, H;
Position 192 to G, L, I, W, M, N, Q, Y, S, T, D, R, H;
Position 194 to W, N, Q, Y, D, H;
Position 195 to W, P, Y;
Position 196 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H;
Position 203 to V, F, Y, R, H;
Position 204 to I, W, M, Y, H;
Position 206 to F;
Position 209 to Y, R;
Position 210 to W, F, Y;
Position 211 to L, W, M, F, Y, H;
Position 212 to V, L, I, W, M, F, Y, T, R, H;
Position 214 to W, Y, R;
Position 215 to A, L, I, W, M, F, Y;
Position 216 to A, L, I, W, M, F, Y, R;
Position 217 to W, R;
Position 218 to G, A, L, W, P, M, F, Y, R, H;
Position 221 to S;
Position 236 to S;
Position 240 to N;
Position 241 to W;
Position 243 to N;
Position 245 to Q;
Position 247 to G, V, I, W, P, F, Y, S, T, R;
Position 248 to W, P, F, Y, E, R, H;
Position 249 to L, W, P, F, S, D, E, H;
Position 251 to G, L, I, W, P, M, F, Y, H;
Position 252 to G, A, W, P, N, Q, Y, T, E, R, H;
Position 254 to G, V, L, I, W, M, F, N, Q, Y, S, D, E, R, H;
Position 255 to G, L, W, M, F, N, Y, T, D, H;
Position 256 to G, A, V, L, I, W, M, F, Q, Y, S, T, D, H;
Position 257 to G, A, L, I, W, C, M, F, N, Q, Y, S, T, D, E, K, H;
Position 258 to G, A, V, L, I, W, C, M, F, N, Q, Y, S, T, E, K, H;
Position 259 to A, V, I, W, M, F, N, Q, Y, S, T, E, R;
Position 260 to L, I, W, M, F, Y, T, H;
Position 261 to L, N, S, H;
Position 262 to G, A, V, L, I, W, P, F, N, Q, Y, T, D, E, R, H;
Position 263 to G, A, V, L, I, P, C, M, N, Q, Y, S, T, R, K;
Position 265 to V, L, I, W, M, F, Y;
Position 269 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, E, R, H;

Position 271 to A, L, I, W, P, M, F, N, Y, S, T, R, H;
Position 272 to G, A, V, L, I, W, P, M, F, N, Q, Y, T, D, E, H;
Position 275 to G, A, V, L, I, W, M, F, N, Y, T, D.

**138**. The protease variant of claim **135**, wherein the protease is a subtilisin comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO: 10:

Position −1 to Deletion;
Position 9 to Insertion, deletion;
Position 10 to Insertion, deletion;
Position 12 to Insertion, deletion;
Position 14 to Insertion, deletion;
Position 15 to Insertion, deletion;
Position 17 to Insertion, deletion;
Position 18 to Insertion, deletion;
Position 19 to Insertion, deletion;
Position 20 to Insertion, deletion;
Position 21 to Insertion, deletion;
Position 22 to Insertion, deletion;
Position 24 to Insertion, deletion;
Position 25 to Insertion, deletion;
Position 46 to Insertion, deletion;
Position 47 to Insertion, deletion;
Position 48 to Insertion, deletion;
Position 49 to Insertion, deletion;
Position 50 to Insertion, deletion;
Position 51 to Insertion, deletion;
Position 52 to Insertion, deletion;
Position 53 to Insertion, deletion;
Position 54 to Insertion, deletion;
Position 55 to Insertion, deletion;
Position 58 to Insertion, deletion;
Position 59 to Insertion, deletion;
Position 61 to Insertion, deletion;
Position 64 to Insertion, deletion;
Position 78 to Insertion:
Position 80 to Insertion;
Position 91 to Insertion, deletion;
Position 98 to Deletion;
Position 99 to Deletion;
Position 102 to Deletion;
Position 105 to Insertion;
Position 108 to Insertion;
Position 109 to Insertion;
Position 112 to Insertion;
Position 113 to Insertion;
Position 115 to Insertion;
Position 116 to Insertion;
Position 117 to Insertion;
Position 118 to Insertion;
Position 131 to Deletion;
Position 134 to Insertion, deletion;
Position 136 to Insertion, deletion;
Position 137 to Insertion, deletion;
Position 140 to Insertion, deletion;
Position 141 to Insertion, deletion;
Position 143 to Insertion, deletion;
Position 144 to Insertion, deletion;
Position 145 to Insertion, deletion;
Position 146 to Insertion, deletion;
Position 171 to Deletion;
Position 172 to Deletion;
Position 173 to Deletion;
Position 181 to Deletion;
Position 182 to Deletion;
Position 183 to Deletion;
Position 184 to Deletion;
Position 185 to Deletion;
Position 186 to Deletion;
Position 188 to Deletion;
Position 189 to Deletion;
Position 191 to Deletion;
Position 192 to Deletion;
Position 195 to Deletion;
Position 196 to Insertion, deletion;
Position 221 to Insertion;
Position 236 to Insertion;
Position 237 to Insertion;
Position 238 to Insertion;
Position 239 to Insertion;
Position 240 to Insertion;
Position 241 to Insertion;
Position 242 to Insertion;
Position 243 to Insertion;
Position 244 to Insertion;
Position 245 to Insertion;
Position 247 to Insertion, deletion;
Position 248 to Insertion, deletion;
Position 249 to Insertion, deletion;
Position 251 to Insertion, deletion;
Position 252 to Insertion, deletion;
Position 254 to Insertion, deletion;
Position 255 to Insertion, deletion;
Position 256 to Insertion, deletion;
Position 257 to Insertion, deletion;
Position 258 to Insertion, deletion;
Position 259 to Insertion, deletion;
Position 260 to Insertion, deletion;
Position 261 to Insertion, deletion;
Position 262 to Insertion, deletion;
Position 263 to Insertion, deletion;
Position 265 to Insertion, deletion;
Position 269 to Insertion, deletion;
Position 271 to Insertion, deletion;
Position 272 to Insertion, deletion;
Position 275 to Insertion, deletion.

**139**. The protease variant of claim **135**, wherein the protease is a subtilisin comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO: 10:

Position 7 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H;
Position 8 to G, A, L, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 13 to G, L, I, W, P, M, F, N, Q, Y, S, D, E, H;
Position 16 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, D, E, R, H;
Position 23 to G, A, V, L, I, W, M, F, Y, E, R, H;
Position 26 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H;
Position 28 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 29 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 33 to V, L, I, W, C, M, F, N, Q, Y, R, H;
Position 35 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 36 to V, L, I, W, P, M, F, N, Y, S, T, R, H;

Position 37 to L, I, W, M, F, N, Q, Y, S, R, H;
Position 41 to G, V, L, I, W, M, F, N, Q, Y, S, T, R, H;
Position 60 to G, A, V, L, I, W, C, M, F, Q, Y, T, D, R, K, H;
Position 63 to G, A, V, L, I, W, M, F, Y, T, R, H;
Position 73 to A;
Position 74 to A;
Position 81 to V;
Position 82 to L;
Position 86 to G, A, V, L, I, W, M, F, N, Q, Y, T, D, E, R, H;
Position 88 to A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H;
Position 92 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 93 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 94 to G, V, L, I, W, P, M, F, N, Y, T, D, E, K, H;
Position 96 to L, W, F, Y, R, K;
Position 97 to V, L, W, C, M, F, Y, H;
Position 111 to I;
Position 114 to A;
Position 119 to M;
Position 124 to M;
Position 135 to G, L, P, C, N, Q, T, R, H;
Position 138 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H;
Position 142 to G, A, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 147 to G, A, V, L, W, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 151 to G, V, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 162 to I, W, F, Y, R;
Position 163 to V, W, M, F, H;
Position 168 to G, V, L, I, W, C, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 169 to C, E, F, G, H, I, K, L, M, N, Q, R, T, V, W, Y;
Position 174 to G, A, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 176 to G, A, V, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 179 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 187 to A, V, L, I, W, M, F, Y, R;
Position 190 to G, A, V, L, I, W, C, M, F, N, Q, Y, S, T, R, K, H;
Position 193 to G, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H;
Position 197 to G, V, L, I, W, P, M, F, Q, Y, S, T, H;
Position 198 to G, A, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 205 to W, F, Y, R, K;
Position 208 to A, V, L, I, W, C, M, F, Y, T, R, K, H;
Position 219 to G, A, V, L, I, W, F, Y, R, H;
Position 222 to M;
Position 232 to A;
Position 233 to L;
Position 234 to I;
Position 250 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H;
Position 267 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H;
Position 268 to G, V, L, I, W, C, M, N, Q, Y, S, T, D, E, R, K, H;
Position 270 to G, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 273 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H;
Position 274 to W, P, M, F, N, Q, Y, T, D, E, R, H.

**140**. The protease variant of claim **135**, wherein the protease is a subtilisin comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO: 10:
Position 13 to Insertion, deletion;
Position 16 to Insertion, deletion;
Position 23 to Insertion, deletion;
Position 26 to Insertion, deletion;
Position 28 to Insertion, deletion;
Position 29 to Insertion, deletion;
Position 35 to Deletion;
Position 60 to Insertion, deletion;
Position 63 to Insertion;
Position 81 to Insertion;
Position 82 to Insertion;
Position 92 to Insertion, deletion;
Position 93 to Insertion, deletion;
Position 94 to Insertion, deletion;
Position 96 to Deletion,
Position 106 to Insertion,
Position 111 to Insertion,
Position 114 to Insertion,
Position 119 to Insertion,
Position 124 to Insertion,
Position 138 to Insertion, deletion;
Position 142 to Insertion, deletion;
Position 147 to Insertion, deletion;
Position 151 to Insertion, deletion;
Position 174 to Insertion, deletion;
Position 176 to Insertion, deletion;
Position 179 to Insertion, deletion;
Position 187 to Deletion;
Position 190 to Deletion;
Position 193 to Deletion;
Position 197 to Insertion, deletion;
Position 198 to Insertion, deletion;
Position 232 to Insertion,
Position 233 to Insertion,
Position 234 to Insertion,
Position 246 to Insertion,
Position 250 to Insertion, deletion;
Position 267 to Insertion, deletion;
Position 268 to Insertion, deletion;
Position 270 to Insertion, deletion;
Position 273 to Insertion, deletion.

**141**. The protease variant of claim **135**, wherein the protease is a savinase-like subtilisin comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO: 10:
Position 2 to G, V, I, M, F, N, Q, Y, S, T, H,
Position 3 to W, M, F, N, Q, Y, S, D, E, R, H,
Position 4 to V, L, W, M, F, Y, R,
Position 6 to G, V, L, I, W, P, M, N, Q, T, D, E, R, H,
Position 9 to G, V, L, I, W, P, M, F, Q, Y, S, T, R, H, insertion, deletion,
Position 10 to G, A, V, I, W, P, M, N, Q, Y, S, T, D, E, R, insertion, deletion,
Position 12 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, insertion, deletion,
Position 14 to V, L, I, W, P, M, F, N, Q, Y, T, R, H, insertion, deletion,

Position 15 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, E, H, insertion, deletion,
Position 17 to G, A, V, I, W, P, M, F, Y, H, insertion, deletion,
Position 18 to G, A, L, I, W, P, M, F, N, Q, Y, T, D, E, H, insertion, deletion,
Position 19 to A, V, I, W, M, F, N, Y, S, T, D, R, H, insertion, deletion,
Position 20 to G, V, L, I, W, M, F, N, Q, Y, S, T, D, E, insertion, deletion,
Position 21 to G, V, I, W, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 22 to G, V, L, I, W, M, F, Y, S, T, insertion, deletion,
Position 24 to G, V, L, I, W, M, F, N, Q, Y, S, D, E, R, insertion, deletion,
Position 25 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 27 to G, L, I, W, P, M, F, Y, T, H,
Position 37 to L, I, W, M, F, N, Q, Y, S, R, H,
Position 40 to V, L, I, W, M, F, N, Q, Y, T, R, H,
Position 42 to G, A, L, W, C, M, F, N, Q, Y, S, T, D, E, R, H,
Position 43 to G, L, H,
Position 44 to G, V, L, I, W, P, M, F, Y, S, T,
Position 45 to G, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H,
Position 46 to G, A, L, I, W, P, M, F, Y, H, insertion, deletion,
Position 47 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 48 to A, L, I, P, M, F, N, Y, D, H, insertion, deletion,
Position 50 to G, A, W, M, N, Q, Y, S, T, D, E, H, insertion, deletion,
Position 51 to V, L, I, W, M, F, N, Y, R, deletion, insertion,
Position 54 to V, L, I, W, M, F, S, R, deletion, insertion,
Position 55 to G, A, V, L, I, W, C, M, F, N, Q, Y, T, D, E, R, K, H, deletion, insertion,
Position 57 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, K, H,
Position 58 to L, W, M, F, N, Y, R, insertion, deletion,
Position 59 to A, V, L, I, C, T, H, insertion, deletion,
Position 61 to V, L, I, W, M, F, Y, insertion, deletion,
Position 64 to G, V, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 75 to L,
Position 78 to insertion,
Position 79 to I,
Position 87 to A, V, L, I, W, M, F, Q, Y, S, T, D, E, H,
Position 89 to G, V, L, I, W, P, F, N, Y, T, E,
Position 91 to G, A, V, L, I, W, P, M, N, Y, S, T, D, E, R, H, insertion, deletion,
Position 98 to A, deletion,
Position 100 to G, V, L, I, W, M, F, Y, R, H,
Position 101 to V, I, W, M, F, N, Q, Y, H,
Position 102 to V, L, I, W, M, F, Y, R, H, G, deletion,
Position 109 to N, insertion,
Position 112 to E, insertion,
Position 113 to W, insertion,
Position 116 to insertion,
Position 117 to N, insertion,
Position 126 to L,
Position 127 to G, A, V, I, W, M, F, Y, R, H, L,
Position 128 to I, W,
Position 129 to W,
Position 130 to W, F, Y, R,
Position 131 to W, Y, R, deletion,
Position 132 to L, W, M, F, Y, S, H,
Position 133 to A, L, I, W, M, F, Y, R,
Position 134 to L, I, W, F, N, Q, Y, R, H, insertion, deletion,
Position 136 to G, A, W, P, N, Y, S, T, D, E, H, insertion, deletion,
Position 137 to G, A, V, I, W, P, M, N, Y, H, insertion, deletion,
Position 140 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, H, insertion, deletion,
Position 141 to G, V, L, I, W, P, M, F, Q, S, D, E, H, insertion, deletion,
Position 143 to V, L, I, P, M, F, N, Y, R, insertion, deletion,
Position 144 to L, W, P, M, F, N, Q, Y, S, D, E, R, H, insertion, deletion,
Position 145 to G, V, L, I, W, M, F, Q, Y, D, E, R, H, insertion, deletion,
Position 146 to G, A, W, L, I, W, M, F, N, Q, Y, T, D, E, R, H, insertion, deletion,
Position 155 to V, L, I, W, M, F, Y, R,
Position 156 to V, I, W, F, R,
Position 157 to G, A, V, L, I, W, M, F, Y, T, R, H,
Position 158 to V, L, I, W, M, F, Y,
Position 160 to W, M, F, Y, R, H,
Position 161 to I, W, M, F, Y, H,
Position 167 to R, K,
Position 170 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
Position 171 to D, deletion,
Position 172 to G, A, V, L, I, S, T, H, deletion,
Position 173 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, E, H, deletion,
Position 181 to G, A, V, L, I, W, C, M, F, Q, Y, T, D, R, K, H, deletion,
Position 183 to G, A, V, L, W, C, M, F, N, Q, Y, S, T, E, R, H, deletion,
Position 184 to A, V, L, I, W, C, M, F, N, Q, Y, T, E, H, deletion,
Position 185 to G, A, V, L, I, W, C, M, F, N, Q, Y, T, E, H, deletion,
Position 186 to G, A, V, L, W, M, F, N, Q, Y, S, T, D, E, R, H, deletion,
Position 188 to G, A, V, L, W, F, S, R, K, deletion,
Position 189 to W, F, deletion,
Position 191 to A, V, L, I, W, M, F, Y, T, R, H, deletion,
Position 192 to G, L, I, W, M, N, Q, Y, S, T, D, R, H, deletion,
Position 194 to W, N, Q, Y, D, H,
Position 195 to W, P, Y, deletion,
Position 197 to G, V, L, I, W, P, M, F, Q, Y, S, T, H, insertion, deletion,
Position 203 to V, F, Y, R, H,
Position 206 to F,
Position 209 to Y, R,
Position 210 to W, F, Y,
Position 212 to V, L, I, W, M, F, Y, T, R, H,
Position 214 to W, Y, R,
Position 216 to A, L, I, W, M, F, Y, R,
Position 217 to W, R,
Position 218 to G, A, L, W, P, M, F, Y, R, H,
Position 221 to S, insertion,
Position 236 to S, insertion,
Position 237 to insertion,
Position 239 to insertion,
Position 240 to N, insertion,

Position 241 to W, insertion,
Position 242 to insertion,
Position 244 to insertion,
Position 245 to Q, insertion,
Position 247 to G, V, I, W, P, F, Y, S, T, R, insertion, deletion,
Position 248 to W, P, F, Y, E, R, H, insertion, deletion,
Position 251 to G, L, I, W, P, M, F, Y, H, insertion, deletion,
Position 252 to G, A, W, P, N, Q, Y, T, E, R, H, insertion, deletion,
Position 255 to G, L, W, M, F, N, Y, T, D, H, insertion, deletion,
Position 256 to G, A, V, L, I, W, M, F, Q, Y, S, T, D, H, insertion, deletion,
Position 257 to G, A, L, I, W, C, M, F, N, Q, Y, S, T, D, E, K, H, insertion, deletion,
Position 258 to G, A, V, L, I, W, C, M, F, N, Q, Y, S, T, E, K, H, insertion, deletion,
Position 259 to A, V, I, W, M, F, N, Q, Y, S, T, E, R, insertion, deletion,
Position 260 to L, I, W, M, F, Y, T, H, insertion, deletion,
Position 261 to L, N, S, H, insertion, deletion,
Position 262 to G, A, V, L, I, W, P, F, N, Q, Y, T, D, E, R, H, insertion, deletion,
Position 263 to G, A, V, L, I, P, C, M, N, Q, Y, S, T, R, K, insertion, deletion,
Position 265 to V, L, I, W, M, F, Y, insertion, deletion,
Position 271 to A, L, I, W, P, M, F, N, Y, S, T, R, H, insertion, deletion,
Position 272 to G, A, V, L, I, W, P, M, F, N, Q, Y, T, D, E, H, insertion, deletion,
Position 275 to G, A, V, L, I, W, M, F, N, Y, T, D, insertion, deletion.

**142**. The protease variant of claim **141**, wherein the savinase-like subtilisin comprises one or more of the following substitutions corresponding to any of the following in SEQ ID NO: 10:

Position 6 to G, V, L, I, W, P, M, N, Q, T, D, E, R, H,
Position 9 to G, V, L, I, W, P, M, F, Q, Y, S, T, R, H, insertion, deletion,
Position 10 to G, A, V, I, W, P, M, N, Q, Y, S, T, D, E, R, insertion, deletion,
Position 14 to V, L, I, W, P, M, F, N, Q, Y, T, R, H, insertion, deletion,
Position 15 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, E, H, insertion, deletion,
Position 17 to G, A, V, I, W, P, M, F, Y, H, insertion, deletion,
Position 18 to G, A, L, I, W, P, M, F, N, Q, Y, T, D, E, H, insertion, deletion,
Position 19 to A, V, I, W, M, F, N, Y, S, T, D, R, H, insertion, deletion,
Position 20 to G, V, L, I, W, M, F, N, Q, Y, S, T, D, E, insertion, deletion,
Position 21 to G, V, I, W, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 37 to L, I, W, M, F, N, Q, Y, S, R, H,
Position 43 to G, L, H,
Position 45 to G, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H,
Position 47 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 50 to G, A, W, M, N, Q, Y, S, T, D, E, H, insertion, deletion,
Position 51 to V, L, I, W, M, F, N, Y, R, deletion, insertion,
Position 54 to V, L, I, W, M, F, S, R, deletion, insertion,
Position 59 to A, V, L, I, C, T, H, insertion, deletion,
Position 89 to G, V, L, I, W, P, F, N, Y, T, E,
Position 91 to G, A, V, L, I, W, P, M, N, Y, S, T, D, E, R, H, insertion, deletion,
Position 101 to V, I, W, M, F, N, Q, Y, H,
Position 109 to N, insertion,
Position 112 to E, insertion,
Position 113 to W, insertion,
Position 127 to G, A, V, I, W, M, F, Y, R, H, L,
Position 128 to I, W,
Position 129 to W,
Position 130 to W, F, Y, R,
Position 131 to W, Y, R, deletion,
Position 133 to A, L, I, W, M, F, Y, R,
Position 136 to G, A, W, P, N, Y, S, T, D, E, H, insertion, deletion,
Position 137 to G, A, V, I, W, P, M, N, Y, H, insertion, deletion,
Position 140 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, H, insertion, deletion,
Position 141 to G, V, L, I, W, P, M, F, Q, S, D, E, H, insertion, deletion,
Position 143 to V, L, I, P, M, F, N, Y, R, insertion, deletion,
Position 144 to L, W, P, M, F, N, Q, Y, S, D, E, R, H, insertion, deletion,
Position 145 to G, V, L, I, W, M, F, Q, Y, D, E, R, H, insertion, deletion,
Position 146 to G, A, W, L, I, W, M, F, N, Q, Y, T, D, E, R, H, insertion, deletion,
Position 155 to V, L, I, W, M, F, Y, R,
Position 157 to G, A, V, L, I, W, M, F, Y, T, R, H,
Position 158 to V, L, I, W, M, F, Y,
Position 160 to W, M, F, Y, R, H,
Position 161 to I, W, M, F, Y, H,
Position 167 to R, K,
Position 170 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
Position 171 to D, deletion,
Position 172 to G, A, V, L, I, S, T, H, deletion,
Position 173 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, E, H, deletion,
Position 181 to G, A, V, L, I, W, C, M, F, Q, Y, T, D, R, K, H, deletion,
Position 184 to A, V, L, I, W, C, M, F, N, Q, Y, T, E, H, deletion,
Position 185 to G, A, V, L, I, W, C, M, F, N, Q, Y, T, E, H, deletion,
Position 186 to G, A, V, L, W, M, F, N, Q, Y, S, T, D, E, R, H, deletion,
Position 188 to G, A, V, L, W, F, S, R, K, deletion,
Position 189 to W, F, deletion,
Position 192 to G, L, I, W, M, N, Q, Y, S, T, D, R, H, deletion,
Position 194 to W, N, Q, Y, D, H,
Position 195 to W, P, Y, deletion,
Position 197 to G, V, L, I, W, P, M, F, Q, Y, S, T, H, insertion, deletion,
Position 203 to V, F, Y, R, H,
Position 210 to W, F, Y,
Position 218 to G, A, L, W, P, M, F, Y, R, H,
Position 236 to S, insertion,
Position 237 to insertion,
Position 239 to insertion,
Position 240 to N, insertion,

Position 241 to W, insertion,
Position 242 to insertion,
Position 244 to insertion,
Position 245 to Q, insertion,
Position 247 to G, V, I, W, P, F, Y, S, T, R, insertion, deletion,
Position 251 to G, L, I, W, P, M, F, Y, H, insertion, deletion,
Position 255 to G, L, W, M, F, N, Y, T, D, H, insertion, deletion,
Position 256 to G, A, V, L, I, W, M, F, Q, Y, S, T, D, H, insertion, deletion,
Position 257 to G, A, L, I, W, C, M, F, N, Q, Y, S, T, D, E, K, H, insertion, deletion,
Position 258 to G, A, V, L, I, W, C, M, F, N, Q, Y, S, T, E, K, H, insertion, deletion,
Position 260 to L, I, W, M, F, Y, T, H, insertion, deletion,
Position 262 to G, A, V, L, I, W, P, F, N, Q, Y, T, D, E, R, H, insertion, deletion,
Position 265 to V, L, I, W, M, F, Y, insertion, deletion,
Position 271 to A, L, I, W, P, M, F, N, Y, S, T, R, H, insertion, deletion,
Position 272 to G, A, V, L, I, W, P, M, F, N, Q, Y, T, D, E, H, insertion, deletion,
Position 275 to G, A, V, L, I, W, M, F, N, Y, T, D, insertion, deletion.

**143**. The savinase-like subtilisin of claim **141**, wherein the subtilisin has at least 81% homology to SEQ ID NO: 24.

**144**. The savinase-like subtilisin of claim **141**, wherein the subtilisin has any of the amino acid sequence of SEQ ID NO: 24, 26, 27, 28, 29, 30, 31, 32, 34, 35.

**145**. The protein variant of claim **135**, wherein the protease is a savinase-like subtilisin comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO: 10:

Position 8 to G, A, L, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H,
Position 16 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, D, E, R, H, insertion, deletion,
Position 23 to G, A, V, L, I, W, M, F, Y, E, R, H, insertion, deletion,
Position 26 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 35 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, K, H, deletion,
Position 38 to V, L, I, W, M, F, N, Q, Y, T, H,
Position 39 to G, A, V, L, I, W, M, F, N, Q, Y, T, D, E, R, H,
Position 41 to G, V, L, I, W, M, F, N, Q, Y, S, T, R, H,
Position 60 to G, A, V, L, I, W, C, M, F, Q, Y, T, D, R, K, H, insertion, deletion,
Position 73 to A,
Position 74 to A,
Position 80 to G, insertion,
Position 81 to V, insertion,
Position 86 to G, A, V, L, I, W, M, F, N, Q, Y, T, D, E, R, H,
Position 88 to A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H,
Position 90 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion,
Position 93 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 108 to I, insertion,
Position 111 to I, insertion,
Position 124 to M, insertion,
Position 135 to G, L, P, C, N, Q, T, R, H,
Position 142 to G, A, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 147 to G, A, V, L, W, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 148 to G, A, V, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 149 to G, A, V, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 151 to G, V, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 163 to V, W, M, F, H,
Position 168 to G, V, L, I, W, C, M, F, N, Q, Y, S, T, D, E, R, K, H,
Position 169 to C, E, F, G, H, I, K, L, M, N, Q, R, T, V, W, Y,
Position 174 to G, A, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 179 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 190 to G, A, V, L, I, W, C, M, F, N, Q, Y, S, T, R, K, H, deletion,
Position 193 to G, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H, deletion,
Position 196 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 208 to A, V, L, I, W, C, M, F, Y, T, R, K, H,
Position 213 to N, oN, E,
Position 215 to A, L, I, W, M, F, Y,
Position 232 to A, insertion,
Position 233 to L, insertion,
Position 234 to I, insertion,
Position 246 to insertion,
Position 250 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 254 to G, V, L, I, W, M, F, N, Q, Y, S, D, E, R, H, insertion, deletion,
Position 267 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 268 to G, V, L, I, W, C, M, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 269 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, E, R, H, insertion, deletion,
Position 273 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion.

**146**. The savinase-like subtilisin of claim **145**, wherein the subtilisin has at least 81% homology to SEQ ID NO: 24.

**147**. The savinase-like subtilisin of claim **146**, wherein the subtilisin has any of the amino acid sequence of SEQ ID NO: 24, 26, 27, 28, 29, 30, 31, 32, 34, 35.

**148**. The protease variant of claim **135** having modified immunogenicity as compared to its parent protein having at least 81% homology to SEQ ID NO: 25 comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO: 25:

Position 21 to G, V, I, W, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 27 to G, L, I, W, P, M, F, Y, T, H,
Position 50 to G, A, W, M, N, Q, Y, S, T, D, E, H, insertion, deletion,
Position 52 to V, L, I, W, M, F, Y, S, T, R, deletion, insertion,
Position 55 to G, A, V, L, I, W, C, M, F, N, Q, Y, T, D, E, R, K, H, deletion, insertion,
Position 129 to W,
Position 133 to A, L, I, W, M, F, Y, R,
Position 172 to G, A, V, L, I, S, T, H, deletion,

Position 186 to G, A, V, L, W, M, F, N, Q, Y, S, T, D, E, R, H, deletion,
Position 194 to W, N, Q, Y, D, H,
Position 195 to W, P, Y, deletion,
Position 197 to G, V, L, I, W, P, M, F, Q, Y, S, T, H, insertion, deletion,
Position 242 to insertion,
Position 249 to L, W, P, F, S, D, E, H, insertion, deletion,
Position 252 to G, A, W, P, N, Q, Y, T, E, R, H, insertion, deletion,
Position 254 to G, V, L, I, W, M, F, N, Q, Y, S, D, E, R, H, insertion, deletion,
Position 257 to G, A, L, I, W, C, M, F, N, Q, Y, S, T, D, E, K, H, insertion, deletion,
Position 260 to L, I, W, M, F, Y, T, H, insertion, deletion,
Position 265 to V, L, I, W, M, F, Y, insertion, deletion,

with the proviso that the amino acids of the parent enzyme are substituted to another amino acid.

**149**. The protein variant of claim **135** having modified immunogenicity as compared to its parent protein having at least 81% homology to SEQ ID NO: 10 comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO: 10:

Position 4 to V, L, W, M, F, Y, R,
Position 38 to V, L, I, W, M, F, N, Q, Y, T, H,
Position 40 to V, L, I, W, M, F, N, Q, Y, T, R, H,
Position 43 to G, L, H,
Position 47 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 49 to G, A, V, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 54 to V, L, I, W, M, F, S, R, deletion, insertion,
Position 96 to L, W, F, Y, R, K, deletion,
Position 99 to V, L, I, W, M, F, Q, Y, H, deletion,
Position 113 to W, insertion,
Position 131 to W, Y, R, deletion,
Position 133 to A, L, I, W, M, F, Y, R,
Position 137 to G, A, V, I, W, P, M, N, Y, H, insertion, deletion,
Position 141 to G, V, L, I, W, P, M, F, Q, S, D, E, H, insertion, deletion,
Position 144 to L, W, P, M, F, N, Q, Y, S, D, E, R, H, insertion, deletion,
Position 170 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
Position 173 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, E, H, deletion,
Position 181 to G, A, V, L, I, W, C, M, F, Q, Y, T, D, R, K, H, deletion,
Position 185 to G, A, V, L, I, W, C, M, F, N, Q, Y, T, E, H, deletion,
Position 186 to G, A, V, L, W, M, F, N, Q, Y, S, T, D, E, R, H, deletion,
Position 188 to G, A, V, L, W, F, S, R, K, deletion,
Position 194 to W, N, Q, Y, D, H,
Position 203 to V, F, Y, R, H,
Position 210 to W, F, Y,
Position 211 to L, W, M, F, Y, H,
Position 257 to G, A, L, I, W, C, M, F, N, Q, Y, S, T, D, E, K, H, insertion, deletion,
Position 261 to L, N, S, H, insertion, deletion,
Position 262 to G, A, V, L, I, W, P, F, N, Q, Y, T, D, E, R, H, insertion, deletion,
Position 265 to V, L, I, W, M, F, Y, insertion, deletion.

with the proviso that the amino acids of the parent enzyme are substituted to another amino acid.

**150**. The protein variant of claim **135** having modified immunogenicity as compared to its parent protein having at least 81% homology to SEQ ID NO: 11 comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO: 11:

Position 38 to V, L, I, W, M, F, N, Q, Y, T, H,
Position 40 to V, L, I, W, M, F, N, Q, Y, T, R, H,
Position 45 to G, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H,
Position 47 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 49 to G, A, V, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 50 to G, A, W, M, N, Q, Y, S, T, D, E, H, insertion, deletion,
Position 52 to V, L, I, W, M, F, Y, S, T, R, deletion, insertion,
Position 53 to A, V, L, I, W, M, F, N, Q, Y, S, D, E, H, deletion, insertion,
Position 56 to G, V, L, I, W, M, F, N, Q, Y, S, T, H,
Position 58 to L, W, M, F, N, Y, R, insertion, deletion,
Position 96 to L, W, F, Y, R, K, deletion,
Position 97 to V, L, W, C, M, F, Y, H,
Position 98 to A, deletion,
Position 105 to insertion,
Position 109 to N, insertion,
Position 113 to W, insertion,
Position 115 to I, insertion,
Position 133 to A, L, I, W, M, F, Y, R,
Position 136 to G, A, W, P, N, Y, S, T, D, E, H, insertion, deletion,
Position 137 to G, A, V, I, W, P, M, N, Y, H, insertion, deletion,
Position 141 to G, V, L, I, W, P, M, F, Q, S, D, E, H, insertion, deletion,
Position 158 to V, L, I, W, M, F, Y,
Position 159 to A, W, M, Y, T, R, H,
Position 172 to G, A, V, L, I, S, T, H, deletion,
Position 186 to G, A, V, L, W, M, F, N, Q, Y, S, T, D, E, R, H, deletion,
Position 189 to W, F, deletion,
Position 192 to G, L, I, W, M, N, Q, Y, S, T, D, R, H, deletion,
Position 195 to W, P, Y, deletion,
Position 197 to G, V, L, I, W, P, M, F, Q, Y, S, T, H, insertion, deletion,
Position 257 to G, A, L, I, W, C, M, F, N, Q, Y, S, T, D, E, K, H, insertion, deletion,
Position 261 to L, N, S, H, insertion, deletion,
Position 265 to V, L, I, W, M, F, Y, insertion, deletion,

with the proviso that the amino acids of the parent enzyme are substituted to another amino acid.

**151**. The protein variant of claim **135** having modified immunogenicity as compared to its parent protein having at least 81% homology to SEQ ID NO: 33 comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO: 33:

Position −6 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, Y, insertion, deletion,
Position −5 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, Y, insertion, deletion,
Position −4 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y, insertion, deletion,

Position −2 to A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion,
Position −1 to G, V, L, I, W, C, M, F, N, Q, Y, S, T, D, E, R, H, deletion,
Position 1 to V, L, I, W, M, F, Y, S, T, R,
Position 2 to G, V, I, M, F, N, Q, Y, S, T, H,
Position 3a to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion,
Position 5 to V, L, I, W, M, F, N, Q, Y, T, R, H,
Position 6 to G, V, L, I, W, P, M, N, Q, T, D, E, R, H,
Position 7 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H,
Position 8 to G, A, L, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H,
Position 10 to G, A, V, I, W, P, M, N, Q, Y, S, T, D, E, R, insertion, deletion,
Position 12 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, insertion, deletion,
Position 13 to G, L, I, W, P, M, F, N, Q, Y, S, D, E, H, insertion, deletion,
Position 14 to V, L, I, W, P, M, F, N, Q, Y, T, R, H, insertion, deletion,
Position 15 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, E, H, insertion, deletion,
Position 16 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, D, E, R, H, insertion, deletion,
Position 17 to G, A, V, I, W, P, M, F, Y, H, insertion, deletion,
Position 18 to G, A, L, I, W, P, M, F, N, Q, Y, T, D, E, H, insertion, deletion,
Position 19 to A, V, I, W, M, F, N, Y, S, T, D, R, H, insertion, deletion,
Position 21 to G, V, I, W, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 22 to G, V, L, I, W, M, F, Y, S, T, insertion, deletion,
Position 23 to G, A, V, L, I, W, M, F, Y, E, R, H, insertion, deletion,
Position 24 to G, V, L, I, W, M, F, N, Q, Y, S, D, E, R, insertion, deletion,
Position 25 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 26 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 27 to G, L, I, W, P, M, F, Y, T, H,
Position 28 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 28a to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion,
Position 29 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 33 to V, L, I, W, C, M, F, N, Q, Y, R, H,
Position 35 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, K, H, deletion,
Position 37 to L, I, W, M, F, N, Q, Y, S, R, H,
Position 40 to V, L, I, W, M, F, N, Q, Y, T, R, H,
Position 42 to G, A, L, W, C, M, F, N, Q, Y, S, T, D, E, R, H,
Position 43 to G, L, H,
Position 44 to G, V, L, I, W, P, M, F, Y, S, T,
Position 44a to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion,
Position 44b to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion,
Position 46 to G, A, L, I, W, P, M, F, Y, H, insertion, deletion,
Position 48 to A, L, I, P, M, F, N, Y, D, H, insertion, deletion,
Position 51 to V, L, I, W, M, F, N, Y, R, deletion, insertion,
Position 52 to V, L, I, W, M, F, Y, S, T, R, deletion, insertion,
Position 53 to A, V, L, I, W, M, F, N, Q, Y, S, D, E, H, deletion, insertion,
Position 55 to G, A, V, L, I, W, C, M, F, N, Q, Y, T, D, E, R, K, H, deletion, insertion,
Position 56 to G, V, L, I, W, M, F, N, Q, Y, S, T, H,
Position 57 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, K, H,
Position 58 to L, W, M, F, N, Y, R, insertion, deletion,
Position 61 to V, L, I, W, M, F, Y, insertion, deletion,
Position 64 to G, V, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 75 to L,
Position 81 to insertion,
Position 86 to G, A, V, L, I, W, M, F, N, Q, Y, T, D, E, R, H,
Position 87 to A, V, L, I, W, M, F, Q, Y, S, T, D, E, H,
Position 88 to A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H,
Position 89 to G, V, L, I, W, P, F, N, Y, T, E,
Position 91 to G, A, V, L, I, W, P, M, N, Y, S, T, D, E, R, H, insertion, deletion,
Position 92 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 94 to G, V, L, I, W, P, M, F, N, Y, T, D, E, K, H, insertion, deletion,
Position 96 to L, W, F, Y, R, K, deletion,
Position 97 to V, L, W, C, M, F, Y, H,
Position 98 to deletion,
Position 101 to V, I, W, M, F, N, Q, Y, H,
Position 102 to V, L, I, W, M, F, Y, R, H, G, deletion,
Position 108 to I, insertion,
Position 109 to N, insertion,
Position 111 to insertion,
Position 112 to E, insertion,
Position 113 to W, insertion,
Position 114 to insertion,
Position 115 to I, insertion,
Position 117 to N, insertion,
Position 118 to N, insertion,
Position 119 to M, insertion,
Position 127 to G, A, V, I, W, M, F, Y, R, H, L,
Position 133 to A, L, I, W, M, F, Y, R,
Position 134 to L, I, W, F, N, Q, Y, R, H, insertion, deletion,
Position 135 to G, L, P, C, N, Q, T, R, H,
Position 136 to G, A, W, P, N, Y, S, T, D, E, H, insertion, deletion,
Position 137 to G, A, V, I, W, P, M, N, Y, H, insertion, deletion,
Position 138 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 139 to G, A, V, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 141 to G, V, L, I, W, P, M, F, Q, S, D, E, H, insertion, deletion,
Position 142 to G, A, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 144 to L, W, P, M, F, N, Q, Y, S, D, E, R, H, insertion, deletion,
Position 145 to G, V, L, I, W, M, F, Q, Y, D, E, R, H, insertion, deletion,
Position 146 to G, A, W, L, I, W, M, F, N, Q, Y, T, D, E, R, H, insertion, deletion,
Position 147 to G, A, V, L, W, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,

Position 148 to G, A, V, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 156 to V, I, W, F, R,
Position 158 to V, L, I, W, M, F, Y,
Position 160 to W, M, F, Y, R, H,
Position 161 to I, W, M, F, Y, H,
Position 162 to I, W, F, Y, R,
Position 163 to V, W, M, F, H,
Position 167 to R, K,
Position 169 to C, E, F, G, H, I, K, L, M, N, Q, R, T, V, W, Y,
Position 170 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion,
Position 171 to D, deletion,
Position 174 to G, A, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 176 to G, A, V, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 182 to A, V, L, I, W, C, M, F, N, Q, Y, S, T, D, E, H, deletion,
Position 186 to G, A, V, L, W, M, F, N, Q, Y, S, T, D, E, R, H, deletion,
Position 188 to G, A, V, L, W, F, S, R, K, deletion,
Position 191 to A, V, L, I, W, M, F, Y, T, R, H, deletion,
Position 192 to G, L, I, W, M, N, Q, Y, S, T, D, R, H, deletion,
Position 193 to G, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H, deletion,
Position 194 to W, N, Q, Y, D, H,
Position 195 to W, P, Y, deletion,
Position 196 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 197 to G, V, L, I, W, P, M, F, Q, Y, S, T, H, insertion, deletion,
Position 198 to G, A, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 203 to V, F, Y, R, H,
Position 205 to W, F, Y, R, K,
Position 215 to A, L, I, W, M, F, Y,
Position 216 to A, L, I, W, M, F, Y, R,
Position 217 to W, R,
Position 219 to G, A, V, L, I, W, F, Y, R, H,
Position 233 to insertion,
Position 234 to I, insertion,
Position 236 to insertion,
Position 237 to insertion,
Position 238 to insertion,
Position 239 to insertion,
Position 240 to insertion,
Position 243 to insertion,
Position 246 to insertion,
Position 247 to G, V, I, W, P, F, Y, S, T, R, insertion, deletion,
Position 249 to L, W, P, F, S, D, E, H, insertion, deletion,
Position 252 to G, A, W, P, N, Q, Y, T, E, R, H, insertion, deletion,
Position 254 to G, V, L, I, W, M, F, N, Q, Y, S, D, E, R, H, insertion, deletion,
Position 262 to G, A, V, L, I, W, P, F, N, Q, Y, T, D, E, R, H, insertion, deletion,
Position 264a to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion,
Position 270 to G, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 273 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 274 to W, P, M, F, N, Q, Y, T, D, E, R, H,
Position 275 to G, A, V, L, I, W, M, F, N, Y, T, D, insertion, deletion,
Position 276 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion,

with the proviso that the amino acids of the parent enzyme are substituted to another amino acid.

**152**. The protein variant of claim **135** having modified immunogenicity as compared to its parent protein having at least 81% homology to SEQ ID NO: 33 comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO: 33:

Position 5 to V, L, I, W, M, F, N, Q, Y, T, R, H,
Position 22 to G, V, L, I, W, M, F, Y, S, T, insertion, deletion,
Position 26 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H, insertion, deletion,
Position 28 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 37 to L, I, W, M, F, N, Q, Y, S, R, H,
Position 40 to V, L, I, W, M, F, N, Q, Y, T, R, H,
Position 44 to G, V, L, I, W, P, M, F, Y, S, T,
Position 51 to V, L, I, W, M, F, N, Y, R, deletion, insertion,
Position 52 to V, L, I, W, M, F, Y, S, T, R, deletion, insertion,
Position 55 to G, A, V, L, I, W, C, M, F, N, Q, Y, T, D, E, R, K, H, deletion, insertion,
Position 58 to L, W, M, F, N, Y, R, insertion, deletion,
Position 61 to V, L, I, W, M, F, Y, insertion, deletion,
Position 64 to G, V, L, I, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H, insertion, deletion,
Position 87 to A, V, L, I, W, M, F, Q, Y, S, T, D, E, H,
Position 97 to V, L, W, C, M, F, Y, H,
Position 98 to deletion,
Position 101 to V, I, W, M, F, N, Q, Y, H,
Position 102 to V, L, I, W, M, F, Y, R, H, G, deletion,
Position 109 to N, insertion,
Position 112 to E, insertion,
Position 118 to N, insertion,
Position 127 to G, A, V, I, W, M, F, Y, R, H, L,
Position 137 to G, A, V, I, W, P, M, N, Y, H, insertion, deletion,
Position 146 to G, A, W, L, I, W, M, F, N, Q, Y, T, D, E, R, H, insertion, deletion,
Position 156 to V, I, W, F, R,
Position 158 to V, L, I, W, M, F, Y,
Position 161 to I, W, M, F, Y, H,
Position 188 to G, A, V, L, W, F, S, R, K, deletion,
Position 192 to G, L, I, W, M, N, Q, Y, S, T, D, R, H, deletion,
Position 194 to W, N, Q, Y, D, H,
Position 195 to W, P, Y, deletion,
Position 203 to V, F, Y, R, H,
Position 216 to A, L, I, W, M, F, Y, R,
Position 236 to insertion,
Position 237 to insertion,
Position 262 to G, A, V, L, I, W, P, F, N, Q, Y, T, D, E, R, H, insertion, deletion,
Position 264a to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion,

with the proviso that the amino acids of the parent enzyme are substituted to another amino acid.

**153**. A subtilisin variant comprising one or more of the insertions, substitutions and/or deletions in any of the positions of claim **136**.

**154**. The variant of claim **153**, wherein the subtilisin has at least 80% homology to SEQ ID NO; 10.

**155**. A composition comprising the protein variant of claim **135**.

**156**. A DNA construct comprising a DNA sequence encoding a protein variant of claim **135**.

**157**. An expression vector comprising a DNA construct of claim **156**.

**158**. A host cell which is capable of expressing a polypeptide and comprising a DNA construct of claim **156**.

**159**. A host cell which is capable of expressing a polypeptide and which is transformed by an expression vector of claim **157**.

**160**. The host cell of claim **159**, which is a fungal cell, an insect cell, a mammalian cell, or a plant cell.

**161**. A method of producing a protein variant having reduced immunogenicity as compared to the parent protein, comprising:

(a) culturing the host of claim **159** in a suitable culture medium to obtain expression and secretion of the protein into the medium, followed by

(b) isolation of the protein from the culture medium.

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