US 20150037872A1

(19) **United States**

(12) **Patent Application Publication**

Roggen et al.

(10) **Pub. No.: US 2015/0037872 A1**

(43) **Pub. Date: Feb. 5, 2015**

(54) PROTEIN VARIANTS HAVING MODIFIED IMMUNOGENICTY

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(21) Appl. No.: **14/514,652**

(22) Filed: **Oct. 15, 2014**

Related U.S. Application Data

(63) Continuation of application No. 13/936,300, filed on Jul. 8, 2013, now abandoned, which is a continuation of application No. 12/699,979, filed on Feb. 4, 2010, now abandoned, which is a continuation of application No. 09/957,806, filed on Sep. 21, 2001, now abandoned.

Publication Classification

(51) **Int. Cl.**

C12N 9/54 (2006.01)

(52) **U.S. Cl.**

CPC *C12N 9/54* (2013.01); *C12Y 304/21062* (2013.01); *A61K 39/00* (2013.01)

USPC **435/219**; 435/320.1; 435/254.11; 435/254.2; 435/348; 435/254.3; 435/254.7; 435/254.6; 435/254.8; 435/254.5; 435/254.4; 435/254.21; 435/254.22; 435/254.23; 435/419; 435/325; 435/362; 435/367; 435/352; 435/365; 435/252.3; 435/252.31; 435/252.35; 435/252.33

(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.

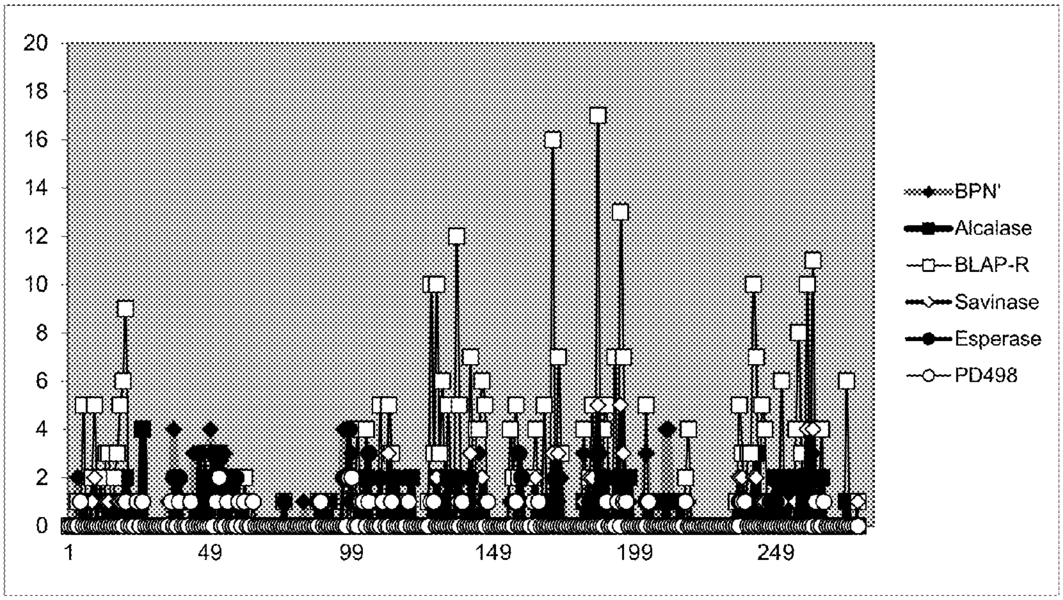
20
18
16
14
12
10
8
6
4
2
0
1
49
99
149
199
249
BPN'
Alcalase
BLAP-R
Savinase
Esperase
PD498

FIG. 1

PROTEIN VARIANTS HAVING MODIFIED IMMUNOGENICTY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 13/936,300 filed on Jul. 8, 2013 (now pending) which is a continuation of U.S. application Ser. No. 12/699,979 filed on Feb. 4, 2010 (now abandoned) which is a continuation of U.S. application Ser. No. 09/957,806 filed on Sep. 21, 2001, (now abandoned) 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 is 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 aicds 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 engineering. 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] FIG. **1** shows epitope mapping of Alcalase, Protease B, Savinase, Esperase, and PD498 as described in the Examples, and compared with epitope mapped BPN'.

DEFINITIONS

[0030] 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.

[0031] 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).

[0032] 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).

[0033] 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.

[0034] 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.

[0035] 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.

[0036] 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.

[0037] 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.

[0038] 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.

[0039] 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.

[0040] "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.

[0041] 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.

[0042] "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.

[0043] "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.

[0044] 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.

[0045] A "clone" is a population of cells derived from a single cell or common ancestor by mitosis.

[0046] "Homologous recombination" refers to the insertion of a forreign 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

[0047] 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.

[0048] 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

[0049] 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.

[0050] 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).

[0051] 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.

[0052] 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.

[0053] 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.

[0054] 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.

[0055] 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.

[0056] 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.

[0057] 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.

[0058] 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.

[0059] 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.

[0060] 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.

[0061] 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).

[0062] 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.

[0063] 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.

[0064] 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.

[0065] 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).

[0066] 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

[0067] 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.

[0068] 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.

[0069] 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.

[0070] 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).

[0071] 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.

[0072] 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

[0073] 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.

[0074] 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

[0075] 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.

[0076] Useful polyadenylation sequences for yeast host cells are described by Guo and Sherman, 1995, Molecular Cellular Biology 15:5983-5990.

[0077] Polyadenylation sequences are well known in the art for mammalian host cells such as SV40 or the adenovirus 5 Elb region.

Signal Sequences

[0078] 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.

[0079] 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.

[0080] 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.

[0081] 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).

[0082] For efficient secretion in yeast, a sequence encoding a leader peptide may also be inserted downstream of the signal sequence and uptream 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.

[0083] 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

[0084] 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.

[0085] 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 contain 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.

[0086] 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.

[0087] 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.

[0088] 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.

[0089] 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).

[0090] 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.

[0091] 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

[0092] 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.

[0093] 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.

[0094] 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).

[0095] The host cell may be a eukaryote, such as a mammalian cell, an insect cell, a plant cell or a fungal cell.

[0096] 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.

[0097] 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.

[0098] 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.*

[0099] 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).

[0100] 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).

[0101] 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 reticulatum, 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

[0102] 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.

[0103] 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).

[0104] 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

[0105] 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.

[0106] 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).

[0107] 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.

[0108] 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.

[0109] 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).

[0110] 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).

[0111] 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.

[0112] 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".

[0113] 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.

[0114] 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.

[0115] 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%.

[0116] The term "randomized library" of protein variants refers to a library with at least partially randomized composition of the members, e.g. protein variants.

[0117] 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.

[0118] 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.

[0119] 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.

[0120] 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).

[0121] 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.

[0122] "Anchor amino acids" are the individual amino acids of an epitope pattern.

[0123] "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.

[0124] "Environmental allergens" are protein allergens that are present naturally. They include pollen, dust mite allergens, pet allergens, food allergens, venoms, etc.

[0125] "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.

[0126] 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.

[0127] 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.

[0128] 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.

[0129] A "heteroepitope" is one that has been identified with distinct donor and acceptor proteins.

[0130] 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.

[0131] By the term "specific polyclonal antibodies" is meant polyclonal antibodies isolated according to their specificity for a certain antigen, e.g. the protein backbone.

[0132] 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.

[0133] The term "randomized library" of protein variants refers to a library with at least partially randomized composition of the members, e.g. protein variants.

[0134] '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.

[0135] 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.

[0136] In the present context the term “protein” is intended to cover oligopeptides, polypeptides as well as proteins as such.

DETAILED DESCRIPTION OF THE INVENTION

[0137] 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:

[0138] a) obtaining antibody binding peptide sequences,

[0139] b) using the sequences to localise epitope sequences on the 3-dimensional structure of parent protein,

[0140] c) defining an epitope area including amino acids situated within 5 Å from the epitope amino acids constituting the epitope sequence,

[0141] 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,

[0142] e) introducing the mutated DNA sequence into a suitable host, culturing said host and expressing the protein variant, and

[0143] 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

[0144] A first step of the method is to identify peptide sequences, which bind specifically to antibodies.

[0145] 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).

[0146] 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.

[0147] 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.

[0148] For the purpose of the present invention polyclonal antibodies are preferred in order to obtain a broader knowledge about the epitopes of a protein.

[0149] 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.

[0150] 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.

[0151] 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-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.

[0152] 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.

[0153] These coordinates can be found in databases (NCBI: http://www.ncbi.nlm.nih.gov/), determined experimentally using conventional methods (Ducruix and Giegé: 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.

[0154] 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.

[0155] 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.

[0156] 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.

[0157] 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.

[0158] In all cases, it is desirable that amino acids of the epitope sequence are surface exposed (as described below in Examples).

[0159] 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.

[0160] There are at least four ways to utilize the information about epitope sequences, which has been derived by the methods of this invention:

[0161] 1) reduce the allergenicity of a commercial protein using protein engineering.

[0162] 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).

[0163] 3) improve the immunotherapeutic effect of allergen vaccines.

[0164] 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.

[0165] 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.

[0166] 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

[0167] 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.

[0168] 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.

[0169] 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.

[0170] 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.

[0171] 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

[0172] 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

[0173] 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.

[0174] 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.

[0175] 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.

[0176] 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).

[0177] 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.

[0178] 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.

[0179] Accessible arginine residues in the polypeptide chain may be targeted by groups comprising two vicinal carbonyl groups.

[0180] 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.

[0181] 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.

[0182] 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.

[0183] 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.

[0184] Furthermore, isocyanates and isothiocyanates may be employed, yielding ureas and thioureas, respectively.

[0185] 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.

[0186] 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.

[0187] 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.

[0188] 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.

[0189] 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.

[0190] 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.

[0191] The coupling technique used in the examples is the N-succinimidyl carbonate conjugation technique descried in WO 90/13590 (Enzon).

[0192] 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.

[0193] 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

[0194] 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 (SEQ 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.

[0195] 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.

[0196] 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)).

[0197] 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).

[0198] 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.

[0199] 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.

[0200] 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

[0201] 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).

[0202] 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.

[0203] 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.

[0204] 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%.

[0205] Thus a first asessment 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

[0206] 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-ABELL-Ó). Also, several cellular assays 20 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).

[0207] 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

[0208] A wide variety of protein functionality assays are available in the literature.

[0209] Especially, those suitable for automated 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

[0210] 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.

[0211] 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.

[0212] 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.

[0213] 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

[0214] 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.

[0215] 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.

[0216] 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 A/S) 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.

[0217] 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.

[0218] 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).

[0219] 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.

[0220] 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.

[0221] 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.

[0222] 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.

[0223] 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.

[0224] 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.

[0225] 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

[0226] Further, a modified enzyme according to the invention may also be used in dishwashing detergents.

[0227] 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.

[0228] 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.

[0229] 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.

[0230] 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.

[0231] Other suitable organic builders include the higher molecular weight polymers and copolymers known to have builder properties, for example appropriate polyacrylic acid, polymaleic and polyacrylic/polymaleic acid copolymers and their salts.

[0232] 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.

[0233] 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.

[0234] 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.

[0235] 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.

[0236] 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

[0237] 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).

[0238] 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.

[0239] Further contemplated are oral care products such as dentifrice, oral washes, chewing gum.

[0240] 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.

[0241] 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

[0242] 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.

[0243] 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.

[0244] Proteases are also used in oral care products, especially for cleaning of dentures, but also in dentifrices.

[0245] Further, proteases are used in toiletries, bath and shower products, including shampoos, conditioners, lotions, creams, soap bars, toilet soaps, and liquid soaps.

Lipolytic Enzymes

[0246] 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.

[0247] 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.

[0248] Lipolytic enzymes are also effective ingredients in products for cleaning of contact lenses, where they remove lipid deposits from the lens surface.

Oxidoreductases

[0249] 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 SCN^- 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.

[0250] 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.

[0251] Anti-microbial systems comprising the combination of an oxidase and a peroxidase are know in the cleaning of contact lenses.

[0252] Another application of oxidoreductases is oxidative hair dyeing using oxidases, peroxidases and laccases.

[0253] 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, November 93, p. 48-52).

[0254] 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

[0255] 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.

[0256] Also other kinds of biofilm for instance the biofilm formed in lens cases can be removed by the action of glucanases.

Food and Feed

[0257] Further conjugated enzymes or polypeptides with reduced immunogenicity according to the invention may advantageously be used in the manufacturing of food and feed.

Proteases

[0258] 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.

[0259] 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.

[0260] Proteases are also used for modifying milk protein.

[0261] To coagulate casein in milk when producing cheese proteases such as rennet or chymosin may be used.

[0262] In the brewery industry proteases are used for brewing with unmalted cereals and for controlling the nitrogen content.

[0263] In animal feed products proteases are used so to speak to expand the animals digestion system.

Lipolytic Enzymes

[0264] 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.

[0265] 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.

[0266] 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

[0267] Further oxidoreductases with reduced immunogenicity according to the invention may advantageously be used in the manufacturing of food and feed.

[0268] 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.

[0269] 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

[0270] 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.

[0271] 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.

[0272] 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.

[0273] 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.

[0274] 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.

[0275] 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.

[0276] 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.

[0277] 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.

[0278] The glucose mixture can be made even sweeter by isomerization to fructose. For this an immobilized glucose isomerase can be used.

[0279] 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.

[0280] Furthermore dextranases are used to break down dextran in raw sugar juices and syrups.

[0281] In the alcohol industry alpha-amylases is advantageously being used for thinning of starch in distilling mashes.

[0282] In the brewing industry alpha-amylases is used for adjunct liquefaction.

[0283] In the dairy industry beta-galactosidases (lactase) is used when producing low lactose milk for persons suffering from lactose malabsorption.

[0284] When flavoured milk drinks are produced from lactase-treated milk, the addition of sugar can be reduced without reducing the sweetness of the product.

[0285] 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.

[0286] When producing ice cream made from lactase-treated milk (or whey) no lactose crystals will be formed and the defect, sandiness, will not occur.

[0287] 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).

[0288] 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

[0289] 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

[0290] Transglutaminases with reduced immunogenicity according to the invention may advantageously be used in the manufacturing of food and feed.

[0291] Transglutaminases has the ability to crosslinking protein.

[0292] 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).

[0293] 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).

[0294] 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).

[0295] 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

[0296] 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.

[0297] 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.

[0298] 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).

[0299] 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).

[0300] 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).

[0301] 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

[0302] Proteases are used for degumming and sand washing of silk.

Lipolytic Enzymes

[0303] 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

[0304] In bleach clean up of textiles catalases may serve to remove excess hydrogen peroxide.

Carbohydrases

[0305] 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”).

[0306] 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.

[0307] 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

[0308] 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

[0309] 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

[0310] 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.

[0311] 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.

[0312] 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.

[0313] 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

[0314] 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.

[0315] 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

[0316] 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.

[0317] 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.

[0318] Further, the knowledge about cross-reacting epitopes may improve vaccine development.

[0319] 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.

[0320] 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

[0321] 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.

[0322] 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.

[0323] 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.

[0324] Pharmaceutical polypeptides are potentially immunogenic as they are introduced into the circulatory system.

[0325] 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.

[0326] 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.

[0327] 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.

[0328] 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.

[0329] 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.

[0330] 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.

[0331] 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.

[0332] 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.

[0333] 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.

[0334] 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.*

[0335] 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

[0336] 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.

[0337] Examples include proteases selected from those classified under the Enzyme Classification (E.C.) numbers:

[0338] 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).

[0339] 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);

[0340] 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 (Actinidain), 3.4.22.30 (Caricain) and 3.4.22.31 (Ananain);

[0341] 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

[0342] 3.4.24 (i.e. so-called metalloendopeptidases), including 3.4.24.28 (Bacillolysin).

Serine Proteases

[0343] 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).

[0344] 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

[0345] 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

[0346] 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

[0347] 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).

[0348] Alternatively the term "parent subtilase" may be termed "wild type subtilase".

Modification(s) of a Subtilase Variant

[0349] 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

[0350] 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.

[0351] 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.

[0352] 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.).

[0353] 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.).

[0354] 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

[0355] 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.

[0356] 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).

[0357] 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.

[0358] 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).

[0359] 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).

[0360] 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.

[0361] Examples of lipases include lipases derived from the following microorganisms. The indicated patent publications are incorporated herein by reference:

[0362] *Humicola*, e.g. *H. brevispora, H. brevis* var. *thermoidea*.

[0363] *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.

[0364] *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).

[0365] *Geotricum*, e.g. *G. candidum* (Schimada et al., (1989), J. Biochem., 106, 383-388).

[0366] *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*.

[0367] *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).

[0368] 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).

[0369] 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.

[0370] 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.

[0371] 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

[0372] 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.

[0373] Examples include oxidoreductases selected from those classified under the Enzyme Classification (E.C.) numbers:

[0374] 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).

[0375] Said glucose oxidases may be derived from *Aspergillus niger*.

[0376] 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.

[0377] Bilirubin oxidases may be derived from *Myrothechecium verrucaria*.

[0378] The peroxidase may be derived from e.g. Soy bean, Horseradish or *Coprinus cinereus*.

[0379] 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*.

[0380] Specific examples of readily available commercial oxidoreductases include Gluzyme™ (enzyme available from Novozymes A/S). However, other oxidoreductases are available from others.

[0381] It is to be understood that also variants of oxidoreductases are contemplated as the parent enzyme.

[0382] The activity of oxidoreductases can be determined as described in "Methods of Enzymatic Analysis", third edition, 1984, Verlag Chemie, Weinheim, vol. 3.

Parent Carbohydrases

[0383] 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.

[0384] 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).

[0385] 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*.

[0386] 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.

[0387] 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

[0388] 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.

[0389] 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).

[0390] 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".

[0391] 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.

[0392] 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).

[0393] 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.

[0394] 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 Termamyl-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

[0395] 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).

[0396] 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).

[0397] 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.

[0398] 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.

[0399] It is to be understood that also carbohydrase variants are contemplated as the parent enzyme.

[0400] The activity of carbohydrases can be determined as described in "Methods of Enzymatic Analysis", third edition, 1984, Verlag Chemie, Weinheim, vol. 4.

Parent Transferases

[0401] 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.

[0402] 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.C 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).

[0403] In a preferred embodiment the parent transferase is a transglutaminase E.C 2.3.2.13 (Protein-glutamine μ-glutamyltransferase).

[0404] 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.

[0405] Examples of transglutaminases are described in the pending DK patent application no. 990/94 (Novo Nordisk A/S).

[0406] The parent transglutaminase may be of human, animal (e.g. bovine) or microbial origin.

[0407] 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).

[0408] It is to be understood that also transferase variants are contemplated as the parent enzyme.

[0409] 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

[0410] 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)).

[0411] Phytases are enzymes produced by microorganisms which catalyse the conversion of phytate to inositol and inorganic phosphorus

[0412] 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).

[0413] 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).

[0414] 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

[0415] 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

[0416] 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.

[0417] Contemplated isomerases include xylose/glucose Isomerase (5.3.1.5) including Sweetzyme®.

Environmental Allergens

[0418] The environmental allergens that are of interest for epitope mapping include allergens from pollen, dust mites, mammals, venoms, fungi, food items, and other plants.

[0419] 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).

[0420] 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.

[0421] 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.

[0422] 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.

[0423] Fungal allergens include those from *Alternaria* alt. and Cladospo. herb. such as Alt a1 and Cla h1.

[0424] Food allergens include but are not limited to those from milk (lactoglobulin), egg (ovalbumin), peanuts, hazelnuts, wheat (alpha-amylase inhibitor),

[0425] Other plant allergens include latex (hevea brasiliensis).

[0426] 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:

[0427] 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:

[0428] 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

[0429] Washing buffer PBS, 0.05% (v/v) Tween 20

[0430] Blocking buffer PBS, 2% (wt/v) Skim Milk powder

[0431] Dilution buffer PBS, 0.05% (v/v) Tween 20, 0.5% (wt/v) Skim Milk powder

[0432] Citrate buffer 0.1M, pH 5.0-5.2

[0433] Stop-solution (DMG-buffer)
[0434] Sodium Borate, borax (Sigma)
[0435] 3,3-Dimethyl glutaric acid (Sigma)
[0436] Tween 20: Poly oxyethylene sorbitan mono laurate (Merck cat no. 822184)
[0437] PMSF (phenyl methyl sulfonyl flouride) from Sigma
[0438] Succinyl-Alanine-Alanine-Proline-Phenylalanine-paranitro-anilide (Suc-AAPF-pNP) Sigma no. S-7388, Mw 624.6 g/mol.
[0439] mPEG (Fluke)

Coloring Substrate:

[0440] OPD: o-phenylene-diamine, (Kementec cat no. 4260)

Methods

Automatic Epitope Mapping

Implementation

[0441] 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).

[0442] 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:

[0443] A web server capable of running cgi-scripts, eg. Apache
[0444] Python 1.5 or later
[0445] Gnuplot 3.7 or later
[0446] DSSP, version July 1995

The Core Program

Inputs

[0447] 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)
DE (Asp or Glu allowed)
(*, ? or — in first or last position is allowed but obsolete. (— in first position is ignored.))

[0448] Examples of matching epitopes:

(SEQ ID NO: 41)
KAAKD,

(SEQ ID NO: 42)
KLASD,

(SEQ ID NO: 43)
KLYSD,

(SEQ ID NO: 44)
KLY-D,

R-M-D.

The Epitope Searching Algorithm

[0449] 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

[0450] 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

[0451] 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.

[0452] Depending on the type of input, the wrapper works in different modes:

[0453] Epitope consensus can be given directly or taken from a library

[0454] Input type can be a single PDB file or a collection of PDB file given as a ZIP-file.

[0455] Any of the four possible combinations are allowed.

[0456] The epitope library consists of a number of text files, each containing one epitope consensus sequence as specified above.

[0457] 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 conatined 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:

[0458] 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:

[0459] 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:

[0460] 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:

[0461] 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.

[0462] 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.

[0463] 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.

[0464] Unknown rat sera and a known rat IgE solution were diluted in dilution buffer: Typically 10×, 20× and 40× for the unknown sera, and ½ 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.

[0465] 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.

[0466] 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.

[0467] Similar determination of IgG can be performed using anti Rat-IgG and standard rat IgG reagents.

[0468] Similar determinations of IgG and IgE in mouse serum can be performed using the corresponding species-specific reagents.

Direct IgE Assay:

[0469] To determine the IgE binding capacity of protein variants one can use an assay, essentially as described above, but using sequential addition of the follwing 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.

[0470] 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 senstitized to the parent protein) provided that the species-specific anti-IgE capture antibodies are changed accordingly.

Competitive ELISA (C-ELISA):

[0471] 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:

[0472] 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:

[0473] 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,     1  AQTVPYGIPLIKADKVQAQGFKGANVKVAVLDTGIQASHPDLNVVGGASFVAGEAYN-TD
BPN',         1  AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
                 ** ****   ***     **  * ****** * **  ***** * **** *  *  *  *

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

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

TABLE 1A-continued

Alignment of different proteases to the sequence of BPN'

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

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

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

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

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

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

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

PROTEASE B, 235 WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAATR (SEQ ID NO: 47)
BPN',       241 WTNTQVRSSLQNTTTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO: 46)
                * * * *  * ** * **    ** ** *  **

Esperase:
54.7% identity in 274 residues overlap; Score: 745.0; Gap frequency: 2.2%

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

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

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

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

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

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

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

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

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

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

TABLE 1A-continued

Alignment of different proteases to the sequence of BPN'

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

TABLE 1A-continued

Alignment of different proteases to the sequence of BPN'

```
PD498:
47.7% identity in 266 residues overlap; Score: 487.0; Gap frequency: 4.9%

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

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

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

PD498,     188  NDREASFSNYGTWVDVTAPGVNIASTVPNNGYSYMSGTSMASPHVAGLAALLASQGKN--
BPN',      183  SNQRASFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPNWT
                    ****  *   ** **** * ** * * *    *********** ***  *   *

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

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

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

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

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

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

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

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

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

Relase,     60  GNGHGTHVAGTIAALDNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWAGNNGMD
BPN',       61  DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
                 * ******** *** ***********  ********  ***  * *  * ***  * **

Relase,    120  VANLSLGSPSPSATLEQAVNSATSRGVLVVAASGNSGA----GSISYPARYANAMAVGAT
BPN',      121  VINMSLGGPSGSAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
                * * *** ** ** *  **  *   ** **** ** *         ** *    ****

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

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

TABLE 1A-continued

Alignment of different proteases to the sequence of BPN'

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

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

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

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

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

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

[0474] 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).

[0475] 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.

[0476] 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

[0477]

TABLE 1B

CLUSTAL W (1.7) multiple sequence alignment

Alignment of YAB protease to BPN': 55.3% identity

```
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)
       *:*.*:*. *:**:*:**::  :*.**:*.:**::
```

TABLE 1B-continued

CLUSTAL W (1.7) multiple sequence alignment

Alignment of Subtilisin sendai to BPN': 55.6% identity.

```
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)
       *:***:*. * .*:*.**:*  :*.**:*.:**::
```

[0478] These alignements reveal that that homology between various subtilisin proteases ranges between 100% and 40%.

[0479] Unless specified, subtilisin sequences and positions mentioned in the present invention, are given in the BPN' numeration, and can be converted by alignement as described above (Tables 1A and 1B).

[0480] 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

[0481] 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

[0482] Three dimensional structural models of the subtilisins properase, relase, 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 ($\bar{\bar{S}}$ali, 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:

[0483] 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 "1 tib", available in Structural Classification of Proteins (SCOP) on the Internet.

Amylase:

[0484] 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.

[0485] 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.

[0486] 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:

[0487] 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:

[0488] 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	ProteaseB	Esperase	ProteaseC	ProteaseD	ProteaseE	ProteaseA	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

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	ProteaseB	Esperase	ProteaseC	ProteaseD	ProteaseE	ProteaseA	Properase	Relase	Savinase	PD498
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
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

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	ProteaseB	Esperase	ProteaseC	ProteaseD	ProteaseE	ProteaseA	Properase	Relase	Savinase	PD498
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
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

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	Pro-perase	Relase	Savinase	PD498
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
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	0	Q	0	Q	0	0	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

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	Pro-perase	Relase	Savinase	PD498
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
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

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	ProteaseB	Esperase	ProteaseC	ProteaseD	ProteaseE	ProteaseA	Properase	Relase	Savinase	PD498
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										
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

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	ProteaseB	Esperase	ProteaseC	ProteaseD	ProteaseE	ProteaseA	Properase	Relase	Savinase	PD498
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
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

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	Pro-perase	Relase	Savinase	PD498
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
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

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	Pro-perase	Relase	Savinase	PD498
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
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

[0489] 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).

[0490] 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.

[0491] 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.

[0492] 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.

[0493] 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.

[0494] 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.

[0495] 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 identi-fication	Epitope pattern	Donor	Acceptor	Epitope Sequence (BPN')	Epitope #	IgG	IgE
VQVYGDTSA (SEQ ID NO: 59)	Phage display	Q > Y > D >	Savinase	savinase	Q206 V81 Y214 G80 D41 T208	sav1.1	Ra	
LQCVGS (SEQ ID NO: 60)	Protein fragments		a-amylase inhibitor	savinase	L21 Q236 V26 G25 S24	sav19.1		Hu
KRFANTELA (SEQ ID NO: 61)	Phage display	R/K R F > N	Savinase	savinase	K251 R247 A174 N173	sav6.1	Ra-Mo	Mo
LDQIFFTRW (SEQ ID NO: 62)	Phage display	D/E Q I F F T	Savinase	savinase	L42/L75 D41 Q2 I79	sav5.1	Ra	
FNDAFFVKM (SEQ ID NO: 63)	Phage display		Savinase	savinase	N185 D181 A187 F189 V203	sav11.0	Ra	
ANIPIWSRSA (SEQ ID NO: 64)	Phage display	> R S A	Savinase	savinase	R145 S144 A142	sav3.2-lac1.0-lip4.0-pd5.0	Ra	
ANIPIWSRSA (SEQ ID NO: 64)	Phage display	> R S A	Savinase	savinase	S188 R186 S190 A179	sav3.1-lac1.0-lip4.0-pd5.0	Ra	
RQSTDFGTT (SEQ ID NO: 65)	Phage display	R Q > > D/E	Savinase	savinase	R186 Q191 S156	sav2.2	Ra	
VQVYGDTSA (SEQ ID NO: 66)	Phage display	Q > Y > D >	Savinase	savinase	Q191 Y192 G193/A194/G195 D197 S265	sav1.2	Ra	
RRFSNATRA (SEQ ID NO: 67)	Phage display	R/K R F > N	Savinase	savinase	K251 R247 A174 N173	sav6.1	Ra-Mo	Mo
CTARLRA GNACG (SEQ ID NO: 68)	Phage display	A R > A	Savinase	savinase	A172/A169 R170 A194 G193 N261	sav10.4	Ra	
LDQIFFTRW (SEQ ID NO: 69)	Phage display	D/E Q I F F T	Savinase	savinase	D60 Q59 I44/I35	sav5.2	Ra	

TABLE 2-continued

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

Antibody binding peptide	Method of identi-fication	Epitope pattern	Donor	Acceptor	Epitope Sequence (BPN')	Epitope #	IgG	IgE
LDQIFFTRW (SEQ ID NO: 69)	Phage display	D/E Q I F F T	Savinase	savinase	L42/L75 D41 Q2 179	sav5.1	Ra	
EQIFFTSGL (SEQ ID NO: 70)	Phage display	D/E Q I F F T	Savinase	savinase	E112 Q109 179	sav5.4	Ra	
GRFSNSKFK (SEQ ID NO: 71)	Phage display	L > G R S	Savinase	savinase	L196 G195 R170 S163	sav9.2-je4.0-lip5.1-5.2	Ra	
AVLRDC (SEQ ID NO: 72)	Protein fragments		a-amylase inhibitor	savinase	A254 V268 L267 R10 D181	sav18.1-pd18.1-18.2		Hu
LQCVGS (SEQ ID NO: 73)	Protein fragments		a-amylase inhibitor	savinase	L217 Q206 V81 G80 S3	sav19.2		Hu
LRQCNERCV (SEQ ID NO: 74)	Phage display	R Q > > D/E	Savinase	savinase	L267 R10 Q12 N269 E271 R275	sav2.1	Ra	
SPVTKRASL KIDSKK (SEQ ID NO: 75)	Protein fragments		Der p II	savinase	A88 587/T22 L233 K235 I246	sav16.0-pd7.0		Hu
RQSTDFGTT (SEQ ID NO: 76)	Phage display	R Q > > D/E	Savinase	savinase	R247 Q245 S240/S242	sav2.3	Ra	
FCTNNCELS (SEQ ID NO: 77)	Phage display	N > > E L	Savinase	savinase	T143 N173 N140 E136 L135	sav7.2	Ra	
FCTNNCELS (SEQ ID NO: 77)	Phage display	N > > E L	Savinase	savinase	N117 N116 E112 L111	sav7.1	Ra	
DFHVKYAAQ (SEQ ID NO: 78)	Phage display		Savinase	savinase		sav8.0	Ra	
VAQYKAL PVVLENA (SEQ ID NO: 79)	Protein fragments		Fel d I	savinase	L135 P168 V139 L111 E112 N116	sav12.0-pd8.0		Hu
AAYPDV (SEQ ID NO: 80)	Protein fragments	A > > > > Y P >	a-amylase inhibitor	savinase	A215 Y214 P40 D41 V81	sav13.0-pd13.1-13.2		Hu
EQIFFTSGL (SEQ ID NO: 81)	Phage display	D/E Q I F F T	Savinase	savinase	E271 Q12 i8	sav5.3	Ra	
VDAAF (SEQ ID NO: 82)	Protein fragments		Poa p IX	savinase	V203 D181 A179 A187 F189	sav15.0-pd12.0	Hu	
AVLRDC (SEQ ID NO: 83)	Protein fragments		a-amylase inhibitor	savinase	A232 V234 L250 R247 D197	sav18.2-pd18.1-18.2		Hu

TABLE 2-continued

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

Antibody binding peptide	Method of identi-fication	Epitope pattern	Donor	Acceptor	Epitope Sequence (BPN')	Epitope #	IgG	IgE
RAFRRNANW (SEQ ID NO: 84)	Phage display	A R > A	Savinase	savinase	A272/A273 R275 R19 N18 A15/A16	sav10.1	Ra	
CTARLRA GNACG (SEQ ID NO: 85)	Phage display	A R > A	Savinase	savinase	A15/A16 R19 L21 R275 A272 A273 N269	sav10.2	Ra	
TFHDAPALQ (SEQ ID NO: 86)	Phage display		Savinase	savinase	H39 D41 A74/ A73 P86 A88 L90	sav4.0	Ra	
CTARVV ALGVCG (SEQ ID NO: 87)	Phage display	A R > A	Savinase	savinase	R145 V147 V149 A151 L124/L126 G127	sav10.3	Ra	
GRFSNSKFK (SEQ ID NO: 88)	Phage display	L > G R S	Savinase	savinase	L148 G146 R145 S144/S141 N140	sav9.1-je4.0-lip5.1-5.2	Ra	
RRFANDHTR (SEQ ID NO: 89)	Phage display	R/K R F > N	savinase	savinase	K27 R45 N43 D41 H39 T38/T213	sav6.2	Ra	
KRFANTEPA (SEQ ID NO: 90)	Phage display	R/K R F > N	savinase	savinase	K251 R247 A174 N173	sav6.1	Ra-Mo	Mo
YKVSAL (SEQ ID NO: 91)	Protein fragments		a-amylase inhibitor	savinase	Y91 K27 V26 S24 G23 L21	sav14.0-pd14.0		Hu
TGKYVS (SEQ ID NO: 92)	Protein fragments		a-amylase inhibitor	savinase	S24 G25 K27 Y91 V93	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>>>>YP>	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	
>KL>>	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	
KQS	Poa p IX	pd498	A142 A147 V148 K120 Q27 S24/S25	pd2.3	Hu	
KQS	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
>KL>>	pd498	pd498	N56/N55 K46 L91 A29/A119 T28	pd4.3	Ra	
>KL>>	pd498	pd498	N240/N243 K239 L233/L234 A16 T21 R22	pd4.1	Ra	
>KL>>	Poa p IX	pd498	Y37 K46 L91 A114 Y113	pd4.5	Hu	
>KL>>	pd498	pd498	N240/N243 K239 L233/L234 A16 T21 R22	pd4.1	Ra	
YI>KL	pd498	pd498	Y113 I111 A108/A138 K136 L135	pd3.1	Ra	
KQS	pd498	pd498	A115 K145 N243 N240 K239 Q237 S236	pd2.1	Ra	
>RY>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

TABLE 3-continued

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

Epitope pattern	Donor	Acceptor	Epitope Sequence (BPN')	Epitope #	IgG	IgE
YI>KL	pd498	pd498	Y276 I246 K239 L234 S236	pd3.2	Ra	
>KL>>	pd498	pd498	N240/N243 K239 L233/L234 R22 P86	pd4.2	Ra	
A>>>>YP>	a-amylase inhibitor	pd498	*3aA Y2 P14 D18 V19	pd13.1		Hu
KQS	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
AR>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	
>RY>K/R	pd498	pd498	R94 R53 Y48 P57 K46 L91	pd1.4-lac2.0	Ra	
>RY>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
>RY>K/R	pd498	pd498	S109/S137 R112 Y141 N144 K145	pd1.3-lac2.0	Ra	
>RY>K/R	pd498	pd498	T162 R161 Y192 N191 K186	pd1.2-lac2.0	Ra	
>RY>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
>RSA	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	
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	

TABLE 4-continued

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

Antibody binding peptide	Method of identi-fication	Epitope pattern	Donor	Acceptor	Epitope Sequence	Epitope #	IgG	IgE
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 (SEQ 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 (SEQ 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	

TABLE 4-continued

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

Antibody binding peptide	Method of identi-fication	Epitope pattern	Donor	Acceptor	Epitope Sequence	Epitope #	IgG	IgE
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
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 identi-fication	Epitope pattern	Donor	Acceptor	Epitope Sequence	Epitope #	IgG	IgE
ARIDPRGPS (SEQ ID NO: 123)	Phage display	A > I D P R/K	amylase	amylase	A380 K381 I382 D383 P384 R389	je1.1	Ra	
ARIDPRHGS (SEQ ID NO: 124)	Phage display	A > I D P R/K	amylase	amylase	A380 K381 I382 D383 P384 R389	je1.1	Ra	
CSVAKIDPRTCG (SEQ ID NO: 125)	Phage display	A > I D P R/K	amylase	amylase	A109 K138 D140 P142 R144	je1.2	Ra	
CSVAKIDPRTCG (SEQ ID NO: 125)	Phage display	A > I D P R/K	amylase	amylase	A380 K381 I382 D383 P384 R389	je1.1	Ra	
AKIDPKPDT (SEQ ID NO: 126)	Phage display	A > I D P R/K	amylase	amylase	A109 K138 D140 P142 R144	je1.2	Ra	
AKIDPKPDT (SEQ ID NO: 126)	Phage display	A > I D P R/K	amylase	amylase	A380 K381 I382 D383 P384 R389	je1.1	Ra	
ARIDPRHGS (SEQ ID NO: 127)	Phage display	A > I D P R/K	amylase	amylase	A109 K138 D140 P142 R144	je1.2	Ra	
QIYNDTGPT (SEQ ID NO: 128)	Phage display	Q > Y > D >	amylase	amylase	Q390 L386 Y368 N367 D366	je2.4	Ra	
QIYNDTGPT (SEQ ID NO: 128)	Phage display	Q > Y > D >	amylase	amylase	Q170 I173 Y196 D195	je2.3	Ra	
QIYNDTGPT (SEQ ID NO: 128)	Phage display	Q > Y > D >	amylase	amylase	Q357 I352 Y349 D366	je2.2	Ra	
QIYNDTGPT (SEQ ID NO: 128)	Phage display	Q > Y > D >	amylase	amylase	Q331 I370 Y368 N367 D366	je2.1	Ra	
CGSATIDPRQCG (SEQ ID NO: 129)	Phage display	A > I D P R/K	amylase	amylase	A109 K138 D140 P142 R144	je1.2	Ra	
CNADNQMYPQCG (SEQ ID NO: 130)	Phage display	A > > > > Y P >	amylase	amylase	N29 A27 D26/D25 Y8 P41/P42	je3.1	Ra	
ARIDPRGPS (SEQ ID NO: 131)	Phage display	A > I D P R/K	amylase	amylase	A109 K138 D140 P142 R144	je1.2	Ra	
CGSATIDPRQCG (SEQ ID NO: 132)	Phage display	A > I D P R/K	amylase	amylase	A380 K381 I382 D383 P384 R389	je1.1	Ra	

TABLE 5-continued

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

Antibody binding peptide	Method of identification	Epitope pattern	Donor	Acceptor	Epitope Sequence	Epitope #	IgG	IgE
CDADSSGYPLCG (SEQ ID NO: 133)	Phage display	A > > > > Y P >	amylase	amylase	A107/A109 D108 Y57 P41/42	je3.3	Ra	
QLYGDEQLP (SEQ ID NO: 134)	Phage display	Q > Y > D >	amylase	amylase	Q331 I370 Y368/Y367 D366	je2.1	Ra	
QLYGDEQLP (SEQ ID NO: 134)	Phage display	Q > Y > D >	amylase	amylase	Q357 I352 Y349 D366	je2.2	Ra	
QLYGDEQLP (SEQ ID NO: 134)	Phage display	Q > Y > D >	amylase	amylase	Q170 I173 Y196 D195	je2.3	Ra	
QLYGDEQLP (SEQ ID NO: 134)	Phage display	Q > Y > D >	amylase	amylase	Q390 L386 Y368/Y367 D366	je2.4	Ra	
RYAQIDPRW (SEQ ID NO: 135)	Phage display	A > I D P R/K	amylase	amylase	A380 K381 I382 D383 P384 R389	je1.1	Ra	
RYAQIDPRW (SEQ ID NO: 135)	Phage display	A > I D P R/K	amylase	amylase	A109 K138 D140 P142 R144	je1.2	Ra	
GEFNLGRSS (SEQ ID NO: 136)	Phage display	L > G R S	amylase	amylase	L88 G92 R31 S28	je4.1-sav9.0-lip5.1-5.2	Ra	
CNADSWGYPRCG (SEQ ID NO: 137)	Phage display	A > > > > Y P >	amylase	amylase	N29 A27 D26/D25 Y8 P41/P42	je3.1	Ra	
CNADNQMYPQCG (SEQ ID NO: 138)	Phage display	A > > > > Y P >	amylase	amylase	N102 A233 D232 Y54 P41/P42	je3.2	Ra	
CNADSWGYPRCG (SEQ ID NO: 137)	Phage display	A > > > > Y P >	amylase	amylase	N102 A233 D232 Y54 P41/P42	je3.2	Ra	
GEFNLGRSS (SEQ ID NO: 139)	Phage display	L > G R S	amylase	amylase	L62 G63/G76 R78 S79	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	

TABLE 6-continued

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

Antibody binding peptide	Method of identi-fication	Epitope pattern	Donor	Acceptor	Epitope Sequence	Epitope #	IgG	IgE
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	
	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		car6.1	Ra	
	Phage display	> P > R D T G	laccase	carezyme	A1 R4 R7 A177 N176	car5.0	Ra	
	Phage display	> R Y > K/R	pd498	carezyme	D178 P180 R4 D2 S183 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	

TABLE 6-continued

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

Antibody binding peptide	Method of identi-fication	Epitope pattern	Donor	Acceptor	Epitope Sequence	Epitope #	IgG	IgE
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 identi-fication	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	

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
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	
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	
PQSDAGWM (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	
PQSDAGWM (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	

TABLE 7-continued

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

Antibody binding peptide	Method of identi-fication	Epitope pattern	Donor	Acceptor	Epitope Sequence	Epitope #	IgG	IgE
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

[0496] 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).

[0497] 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.

[0498] 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.

[0499] 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 I79 and L42 D41 Q2 I79. A shorthand notation for such a situation is: L42/L75 D41 Q2 I79.

[0500] 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).

[0501] The identified epitope sequences are shown in Tables 2-7.

Birch Allergen:

[0502] 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

[0503] 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.

[0504] 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						
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	

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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											
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										

[0505] 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										

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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	A115	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			
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

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	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						
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		

[0506] 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				

-continued

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			
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		

[0507] 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		
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

[0508] 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:

[0509] 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 InsightII from Molecular Simulations Inc.

[0510] 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).

[0511] Substitutions of one or more residue (s) within 18 Å, prefereably 15 Å, more prefereably 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.

[0512] Residues involved in epitope is 2, preferably 3 and more prefereably 4

Example 5

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

[0513] 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.).

[0514] 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.

[0515] 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.

[0516] Variants with reduced antibody binding capacity were further evaluated in the mouse SC animal model (See methods section).

[0517] 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

[0518] Hot-spots or epitopes were mutated using techniques known to the expert in the field (e.g. site-directed mutagenesis, error-prone PCR).

[0519] 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.

[0520] 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).

[0521] Variants with reduced antibody binding capacity were further evaluated in animal studies.

[0522] 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.

[0523] 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 R170 A194 G193 N261

Pattern: A R>R>A>N

Antibody: IgG1+IgE

Backbone: Savinase

[0524] The variant carried the mutation R170F.

[0525] 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.

[0526] 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

[0527] The variant carried the mutation S216W.

[0528] 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.

[0529] 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 N N>E L

Antibody: IgG1

Backbone: Savinase

[0530] The variant carried the mutation E136R.

[0531] 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.

[0532] Mouse studies revealed a dramatic increase in specific IgG1 levels, compared to savinase backbone ($p<0.01$). The IgE levels were not significantly affected.

[0533] 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.

[0534] 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 R170 A194 G193 N261

Pattern: A R>R>A>N

Antibody: IgG1+IgE

Backbone: Savinase

[0535] 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.

[0536] The variant was different at position Y167 by the mutation Y1671.

[0537] 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.

[0538] 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 N N>E L

Antibody: IgG1

Backbone: Savinase

[0539] Epitope area: V10A, I107, 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.

[0540] 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).

[0541] 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.

[0542] 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

[0543] 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.

[0544] 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.

[0545] The conjugate was stored at −20° C., in the above described buffer.

[0546] 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

[0547] 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).

[0548] 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

[0549] Modification: bis-NHS-PEG1000

[0550] 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

[0551] For this example the epitope sequences were determined in four environmental allergens (Bet v1; Der f2; Der p2 and Phl p2), based on their structures (1 btv.pdb; 1 ahm.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 (Phl p2 from *Phleum pratense*).

[0552] 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.

[0553] The critical parameters used in this screening included:

[0554] i) a maximal distance betweenthe alpha-carbon atoms of subsequent amino acids,

[0555] ii) a minimal accessability of the amino acid of 20 Å2,

[0556] iii) the largest maximal distance between the most distinct amino acids should be less than 25 Å,

[0557] iv) the 5 best epitopes were taken,

[0558] v) the minimal homology with the epitope pattern of interest was 80%

[0559] In this way a number of potential epitopes are identified. The epitopes are sorted according to total surface accessible area, and certain entries removed:

[0560] 1) Epitopes that contain the same protein surface residue more than once. These are artefacts generated by the described algorithm.

[0561] 2) Epitopes which are "too big", i.e. where a distance between any two residues in the epitope exceeds a given threshold.

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, I104, 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, 113, 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

I123, 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

K96, P99, D129, I28, 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

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

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

Phl 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
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

[0562]

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	Position												
Number	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
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

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	
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)	ACLPT VWY- (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
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

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	Position												
Number	1	2	3	4	5	6	7	8	9	10	11	12	
29	E	RF-	L	KRQHNGP (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	Fl-	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-	AP K-	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)								

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	Position											
Number	1	2	3	4	5	6	7	8	9	10	11	12
49	KN	Q-	TMC	WYC-	Q	Q	FP-	VP-	L	W-	D	
50	PST	STAPLWV (SEQ ID NO: 242)	W	WY-	RHD							
51	WH	TSKH RQG (SEQ ID NO: 243)	LIRKGP (SEQ ID NO: 244)	DSRTQ GKH- (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

[0563] For this example the third-generation epitope sequences were determined in further 11 environmental allergens (Bosd2, Equc1, Gald-4-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 Phl p2, were redetermined.

[0564] 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 C 1, a major horse allergen aslo 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).

[0565] 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.

[0566] The critical parameters used in this screening included:

[0567] i) a maximal distance between the alpha-carbon atoms of subsequent amino acids,

[0568] ii) a minimal accessability of the amino acid of 20 Å2,

[0569] iii) the largest maximal distance between the most distinct amino acids should be less than 25 Å,

[0570] iv) the best epitope were taken,

[0571] v) the homology with the epitope pattern of interest was 100%

[0572] In this way a number of potential epitopes are identified. The epitopes are sorted according to total surface accessible area, and certain entries removed:

[0573] a. Epitopes that contain the same protein surface residue more than once.

[0574] These are artefacts generated by the described algorithm.

[0575] b. Epitopes which are "too big", i.e. where a distance between any two residues in the epitope exceeds a given threshold.

[0576] 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, I7, Y76

L134, K130, H92, I7, Y76

L128, K130, H92, I91, 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, I110, G107, Y83, T85, E81, V69

R109, I110, 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, I167, Q158, G161

R131, P132, D45, I167, 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, I88, 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

L116, N111, P106, K80, K81, N101, S83, Q105, T108

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

L24, S47, D53, A57, V68, R71, L70, R56, N51, N50, R75

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, I55, D53, N50

R71, R56, I55, 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

K80, H66, D61, T44, P39, T28, W29

K80, H66, D61, T38, P39, T28, W29

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Epi#01

P109, P89, K86, R84, N116, Y106, Q114, T111

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, I127, 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

V74, P62, M73, G88, P89, Y106, T111

Epi#37

T111, V15, L117, R121, Y125

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

L116, N111, P106, K80, K81, N101, S83, Q105, T108

L116, N111, P106, K80, K81, N101, S83, Q105, T108

Epi#02

T53, N58, S57, R56, T69, Y67, R66, V68

T53, K50, A52, R56, T69, Y67, R66, V68

T53, K50, A72, R56, T69, Y67, R66, V68

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, 1104

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

T53, N58, R56, S57, F60, R66, K81

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

Q105, P106, Y78, R71, S57, N58, A54, A44, D51

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

Epi#22

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

Profillin-Brich Pollen:

Epi#01

L124, N118, P114, K88, K73, H68, Y74, R86, T95

Epi#02

T113, N118, Q116, P114, R86, V76

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

K45, P46, D31, I49, Q47, G32

K45, P46, D31, I49, K54, G60

K45, P46, D31, I49, K54, G82

K45, P46, D31, I49, T50, G51

Epi#16

Q116, P114, Y108, M12, S39, S40, A23, A24, D8

Q116, P114, Y108, M12, Q37, S40, A23, A24, D8

R86, P114, Y108, M12, S39, S40, A23, A24, D8

Epi#22

D126, L133, D130, Y127, E122

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

E109, G90, E110, P114, R86, E80, Q78

E57, K54, E58, P59, F56, A81, Q78

E58, G60, E57, P59, F56, E80, Q78

Epi#25

R86, K88, I107, E109, E110

R86, K88, I77, E80, V84

R86, K88, I107, E109, V112

Epi#27

57, E58, D55, K54

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, I107

A23, L11, A36, S39, M12, I10

Epi#37

Y127, R123, L124, K97, N118

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

I27, W35, A36, L11, Q37, S39, M12, I107, T95

Epi#44

I77, R86, P114, Y108, S91, V112, P111

V120, Q116, P114, Y108, S91, V112, P111

L22, Q116, P114, Y108, S91, V112, T113

L22, Q116, P114, Y108, A23, V112, P111

Epi#47

I129, Y127, E122, M119, R123, L124, N118, R86, P114

L133, Y127, E122, M119, R123, L124, N118, R86, P114

Epi#48

E122, Q116, P114, P111, V112

S91, K88, P114, P111, V112

Epi#50

H10, Y6, W3, S2, T5

H10, Y6, W3, T5, S39

Epi#51

K73, H68, K45, Q47, P46, S33, W35

Q101, H30, D31, T50, K45, Q47, H68

Rag Weed Pollen5:

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: I116, 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: I23, 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

[0577] “SAS” is solvent accessible surface. “Size” is the total suface area of the epitope in Å2.

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, I28, 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

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, 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

Phlp2:

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

[0578] 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.

[0579] 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.

[0580] The critical parameters used in this screening included:

[0581] i) a maximal distance between the alpha-carbon atoms of subsequent amino acids,

[0582] ii) a minimal accessability of the amino acid of 20 Å2,

[0583] iii) the largest maximal distance between the most distinct amino acids should be less than 25 Å

[0584] iv) the best epitope were taken,

[0585] v) the homology with the epitope pattern of interest was 100%

[0586] In this way a number of potential epitopes are identified. The epitopes are sorted according to total surface accessible area, and certain entries removed:

[0587] 1) Epitopes that contain the same protein surface residue more than once. These are artefacts generated by the described algorithm.

[0588] 2) Epitopes which are "too big", i.e. where a distance between any two residues in the epitope exceeds a given threshold.

[0589] The subtilisin sequences and positions mentioned in the following are not given in the BPN' numeration but in the subtilisins own numeration (see the alignement as described above in Tables 1A and 1B).

[0590] 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, I91, 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

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Epi#45

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Epi#48

S211, D214, P218, P446, G447

E259, K337, P258, P434, V432

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Epi#51

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T390, H391, E408, S405, I288, K404, W317

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Epi#02

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Epi#03

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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

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G127, A152, N155, T164, G160, S158, S188, Y262

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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

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T55, A48, N57, S37, F58, G46, K43

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Epi#11

K136, I108, Q103, V51, D98

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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, 1115, K141, A144

Epi#16

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Epi#17

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Epi#18

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N184, R186, S188, G157, S158, T159, S162

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Epi19

E156, N155, S188, Q185, R186, L257

E156, N155, S188, Q185, R186, G258

E156, N155, S188, Q185, R186, A187

Epi#22

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D197, S260, D259, Y263, K256

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N155, E156, S188, Q185, A187

Epi#24

E156, G166, E195, P194, S260, L257, K256

D259, G264, E195, P194, S260, L257, K256

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Epi#25

K141, I115, D120, N25

K141, I115, D120, N118

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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, 1205, D181

L267, Q10, N184, R186, F189, V203, 1205, D181

Epi#33

K213, Y214, P210, S38, S37, K43

Q59, F58, V44, S38, S37, K43

Epi#34

W106, P52, M50, G47, P56, T55, S53

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I115, W113, M50, V51, P52, T55, S53

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Epi#35

A99, L96, S49, M50, I108

A99, L96, S49, M50, I107

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

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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

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Epi#51

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Q245, H238, Q275, K237, P239, T242, W241

Q275, H238, Q245, T242, R247, T244, W241

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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, I131

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

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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

I131, 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

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D67, P213, F68, N65, D66, V64

R185, P208, F207, N_2O_6, D204, G205

Epi#46

A195, R200, R201, P23, N202, G205

A191, R200, R201, P23, N202, G205

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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

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Epi#50

D172, Y168, W62, V64, P213

D42, W18, A43, T39

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D66, W173, W62, V64, P213

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Epi#51

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R4, H119, D2, R185, P208, Q186, W85

R4, H119, D2, T95, G97, K175, W173

Epi#52

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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

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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

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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

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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

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Epi#19

I21, N18, Q15, Q275, R19, G20

I21, N18, Q15, Q275, R237, G20

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Epi#23

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R98, N61, E54, Y58, F50, P55

R98, N61, E54, S57, F50, P55

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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

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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

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Epi#34

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Epi#37

T134, A108, L137, R141, N144

Y256, A254, L257, R186, N183

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Epi#38

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Epi#40

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R186, L262, G264, Y256, P260, S259

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R186, L257, G264, Y256, P260, S261

Epi#41

P260, Y256, L257, S259

Epi#42

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Epi#44

S53, R98, D97, Y58, S57, A48, P55

S53, R98, D97, Y58, S38, G211, T210

Epi#45

R19, H17, F22, N24, D89, G25

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Epi#03

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Epi#04

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R259, S211, Q302, R234, N299, A301

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Epi#05

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Epi#06

G286, N289, D291, T293, S295, P292

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A288, N289, D291, T293, S295, P292

Epi#07

G214, T294, D291, R283, V253, P252, D254

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Epi#08

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Epi#09

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Epi#13

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Epi#15

N366, P370, D367, I358, Q363, A471

N366, P370, D367, I358, Q363, G361

R379, P378, D326, I319, T321, G323

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R283, P292, Y256, G214, Q251, D254, D291, A290, N289

Epi#17

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A411, S413, R409, S414

A306, S410, R409, S414

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Epi#19

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Epi#23

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R339, N460, E348, S351, L369, P370

R339, N460, E348, S351, L369, A365

R339, N460, E348, S351, L369, P350

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Epi#24

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Epi#25

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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

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Epi#29

G390, Q332, L329, R330, E435

V374, N366, L369, E348

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G344, Q332, L333, R330, E435

Epi#30

G412, N304, A306, H309, I312, P314, V419

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Epi#31

L329, Q332, N343, R330, F331, V386, D434

L333, Q332, N343, R330, F331, V386, D434

L58, Q501, N54, R59, F112, M459, F456, D205

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Epi#33

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Epi#34

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Epi#35

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A362, A471, A476, V474, G361, S360, Q357, P350, A371, A365

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A288, A285, A287, V253, Y256, S295, A296, W257

Epi#37

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Epi#38

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L356, G81, E348, A371, V374, L369, N366, P370, S349

Epi#39

A411, E435, T432, P395, G393, L392

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Epi#40

R330, L333, G390, T432, A411, S414

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R330, L333, G390, T432, A411, T415

Epi#41

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Epi#46

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Epi#47

I212, S211, R234, L303, A301, N299, P300, P298

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Epi#48

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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

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Epi#51

D307, H309, E228, T218, P229, T231, H230

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Epi#52

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Epi#02

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Epi#03

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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

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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

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Epi#07

G145, T147, D150, S149, R213, V208, P205, D201

Epi#08

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K305, D400, A304, H401, F399

Epi#09

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Epi#10

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Epi#11

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Epi#12

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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

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Epi#16

Q313, P316, Y357, R353, Q395, D397, D400, A304, N308

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Epi#17

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Epi#18

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Epi#19

D16, N50, S48, Q49, R72, G69

D25, N21, Q80, Q18, R24, A87

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Epi#22

D461, A460, W463, W433

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K478, N417, E410, N439, Q438, A460

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Epi#24

E332, G331, E335, P330, S372, A375, K379

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Epi#25

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Epi#27

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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

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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

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Epi#40

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Epi#45

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Epi#46

Y392, R387, R33, P382, G450, G454

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Epi#47

S83, S79, E82, I85, R24, A87, N90, R31, S28

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S372, H371, P374, P369, V373

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Epi#02

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Epi#04

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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

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Epi#07

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G29, T332, S330, D95, S331, R28, V26, P21, D25

Epi#08

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Epi#09

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Epi#10

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Epi#15

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Epi#18

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Epi#22

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Epi#27

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Epi#28

I259, D257, Q254, E253, K267, F266, S260, R189

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Epi#29

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Epi#30

G110, N107, A106, H71, L68, L104, L112

G108, N107, A106, H71, L68, P214, V213

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Epi#35

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Epi#37

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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

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

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

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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

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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

[0591] G456, A459, R458, T461, G460, T452, T463, V450

[0592] G456, A459, R458, T452, G460, T461, T463, G448

Epi#06

[0593] 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

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, N_2O_4, Q206

A15, S9, R10, W6, N_2O_4, Q182

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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, 1165, 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, A_2O_3, 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: I4, 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: I102, Q107, W111, E110, Q135, S139, R143

SAS: 527, Size 22.79: V50, Q107, I102, E134, Q135, S139, R143

SAS: 509, Size 24.76: I102, 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: I102, Q107, W111, E110, Q135, S139, R143

SAS: 500, Size 22.22: A101, Q107, W111, E110, Q135, S139, R143

SAS: 499, Size 24.79: I102, Q107, F49, E53, Q57, G46, R44

SAS: 494, Size 24.56: A101, Q107, F49, E53, Q57, G46, R44

SAS: 441, Size 24.79: I102, Q107, E110, W111, F49, G46, R44

Epi#29

SAS: 216, Size 9.94: I43, 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: I77, 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

Relase:

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

[0594] 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: I44, 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

[0595] "SAS" is solvent accessible surface. "Size" is the total suface area of the epitope in Å2.

Example 12

[0596] 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'.

[0597] 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**).

[0598] 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.

[0599] 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

[0600] 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 Testmaterials, 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 Testmaterials, Holland

TABLE 11

Experimental conditions for evaluation of Subtilisin variants Q12D.

Detergent	Tide
Detergent dose	1 g/l
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 Testmaterials, Holland

[0601] pH is adjusted to 10.5 which is within the normal range for a powder detergent.

[0602] 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.

[0603] 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.

[0604] 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_{BIank}: Reflectance of test material washed with no enzyme

[0605] The variants all have improved wash performance compared to Savinase®—i.e. P>1.

[0606] The variants can be divided into improvement classes designated with capital letters:

Class A: $1<P\leq 1.5$

Class B: $1.5<P\leq 2$

Class C: $P>2$

[0607]

TABLE 12

Subtilisin variants and improvement classes.

Improvement class	Variants
C	I44V, Q12D

[0608] As it can be seen from Table 12 SAVINASE® variants of the invention exhibits an improvement in wash performance.

APPENDIX D

3D Structure of Esperase

ATOM	1	N	GLN	A	2	24.343	43.495	26.356	1.00	26.00	7
ATOM	2	NE2	GLN	A	2	25.686	39.582	30.163	1.00	20.88	7
ATOM	3	OE1	GLN	A	2	23.497	39.261	29.938	1.00	23.07	8
ATOM	4	CD	GLN	A	2	24.448	40.036	29.883	1.00	23.09	6
ATOM	5	CG	GLN	A	2	24.420	41.507	29.607	1.00	23.93	6
ATOM	6	CB	GLN	A	2	24.309	41.801	28.125	1.00	23.06	6
ATOM	7	CA	GLN	A	2	23.999	43.235	27.778	1.00	25.53	6
ATOM	8	C	GLN	A	2	24.957	44.096	28.566	1.00	28.66	6
ATOM	9	O	GLN	A	2	26.126	44.049	28.148	1.00	31.97	8
ATOM	10	N	THR	A	3	24.538	44.857	29.557	1.00	25.20	7
ATOM	11	CG2	THR	A	3	24.948	47.593	29.045	1.00	32.60	6
ATOM	12	OG1	THR	A	3	23.634	46.905	30.890	1.00	33.76	8
ATOM	13	CB	THR	A	3	24.979	47.085	30.464	1.00	26.52	6
ATOM	14	CA	THR	A	3	25.508	45.643	30.316	1.00	24.44	6
ATOM	15	C	THR	A	3	25.551	45.035	31.717	1.00	23.97	6
ATOM	16	O	THR	A	3	24.566	44.377	32.092	1.00	27.28	8
ATOM	17	N	VAL	A	4	26.585	45.366	32.449	1.00	24.67	7
ATOM	18	CG2	VAL	A	4	28.377	43.274	33.058	1.00	22.99	6
ATOM	19	CG1	VAL	A	4	28.147	43.784	35.492	1.00	22.90	6
ATOM	20	CB	VAL	A	4	28.128	44.351	34.069	1.00	24.23	6
ATOM	21	CA	VAL	A	4	26.694	44.897	33.837	1.00	24.05	6
ATOM	22	C	VAL	A	4	26.445	46.114	34.776	1.00	22.35	6
ATOM	23	O	VAL	A	4	27.323	47.015	34.816	1.00	24.67	8
ATOM	24	N	PRO	A	5	25.365	46.082	35.507	1.00	21.36	7
ATOM	25	CD	PRO	A	5	24.284	45.039	35.492	1.00	16.33	6
ATOM	26	CG	PRO	A	5	23.100	45.761	36.119	1.00	19.38	6
ATOM	27	CB	PRO	A	5	23.741	46.724	37.115	1.00	17.69	6
ATOM	28	CA	PRO	A	5	25.049	47.159	36.454	1.00	17.81	6
ATOM	29	C	PRO	A	5	26.231	47.367	37.382	1.00	24.17	6
ATOM	30	O	PRO	A	5	26.903	46.375	37.763	1.00	19.11	8
ATOM	31	N	TRP	A	6	26.505	48.602	37.832	1.00	21.75	7
ATOM	32	CD2	TRP	A	6	26.928	50.889	41.509	1.00	18.89	6
ATOM	33	CE3	TRP	A	6	27.995	50.522	42.349	1.00	19.68	6
ATOM	34	CZ3	TRP	A	6	27.789	50.639	43.721	1.00	18.65	6
ATOM	35	CH2	TRP	A	6	26.582	51.111	44.306	1.00	18.90	6
ATOM	36	CZ2	TRP	A	6	25.524	51.469	43.465	1.00	18.51	6
ATOM	37	CE2	TRP	A	6	25.705	51.348	42.088	1.00	24.32	6
ATOM	38	NE1	TRP	A	6	24.852	51.593	41.020	1.00	22.59	7
ATOM	39	CD1	TRP	A	6	25.420	51.300	39.828	1.00	14.24	6
ATOM	40	CG	TRP	A	6	26.698	50.865	40.074	1.00	17.07	6
ATOM	41	CB	TRP	A	6	27.702	50.382	39.095	1.00	19.96	6
ATOM	42	CA	TRP	A	6	27.668	48.899	38.677	1.00	19.10	6
ATOM	43	C	TRP	A	6	27.699	48.015	39.926	1.00	20.24	6
ATOM	44	O	TRP	A	6	28.865	47.719	40.230	1.00	19.68	8
ATOM	45	N	GLY	A	7	26.553	47.779	40.554	1.00	19.54	7
ATOM	46	CA	GLY	A	7	26.573	47.016	41.827	1.00	15.44	6
ATOM	47	C	GLY	A	7	27.075	45.596	41.634	1.00	21.44	6
ATOM	48	O	GLY	A	7	27.733	45.067	42.534	1.00	20.88	8

APPENDIX D-continued

3D Structure of Esperase											
ATOM	49	N	ILE	A	8	26.862	44.983	40.482	1.00	19.17	7
ATOM	50	CD1	ILE	A	8	24.548	42.180	39.852	1.00	19.08	6
ATOM	51	CG1	ILE	A	8	25.219	43.020	38.790	1.00	17.53	6
ATOM	52	CB	ILE	A	8	26.746	43.093	38.871	1.00	23.00	6
ATOM	53	CG2	ILE	A	8	27.338	41.799	38.350	1.00	22.68	6
ATOM	54	CA	ILE	A	8	27.325	43.598	40.192	1.00	23.07	6
ATOM	55	C	ILE	A	8	28.853	43.585	40.232	1.00	22.71	6
ATOM	56	O	ILE	A	8	29.462	42.674	40.821	1.00	21.85	8
ATOM	57	N	SER	A	9	29.527	44.534	39.631	1.00	19.30	7
ATOM	58	OG	SER	A	9	31.089	45.298	37.438	1.00	28.25	8
ATOM	59	CB	SER	A	9	31.514	45.590	38.718	1.00	24.45	6
ATOM	60	CA	SER	A	9	30.986	44.532	39.663	1.00	18.00	6
ATOM	61	C	SER	A	9	31.431	45.071	41.000	1.00	18.16	6
ATOM	62	O	SER	A	9	32.543	44.676	41.351	1.00	21.78	8
ATOM	63	N	PHE	A	10	30.702	45.961	41.617	1.00	17.83	7
ATOM	64	CD2	PHE	A	10	31.780	49.344	44.181	1.00	23.83	6
ATOM	65	CE2	PHE	A	10	32.100	50.259	45.170	1.00	27.32	6
ATOM	66	CZ	PHE	A	10	31.514	50.266	46.431	1.00	21.18	6
ATOM	67	CE1	PHE	A	10	30.563	49.309	46.768	1.00	29.76	6
ATOM	68	CD1	PHE	A	10	30.188	48.429	45.759	1.00	23.23	6
ATOM	69	CG	PHE	A	10	30.778	48.438	44.521	1.00	18.74	6
ATOM	70	CB	PHE	A	10	30.285	47.522	43.455	1.00	17.70	6
ATOM	71	CA	PHE	A	10	31.270	46.528	42.864	1.00	20.00	6
ATOM	72	C	PHE	A	10	31.457	45.396	43.870	1.00	22.92	6
ATOM	73	O	PHE	A	10	32.357	45.569	44.723	1.00	24.39	8
ATOM	74	N	ILE	A	11	30.614	44.376	43.829	1.00	19.21	7
ATOM	75	CD1	ILE	A	11	27.476	41.276	44.648	1.00	14.26	6
ATOM	76	CG1	ILE	A	11	28.743	41.954	44.149	1.00	18.25	6
ATOM	77	CB	ILE	A	11	29.500	42.669	45.229	1.00	23.27	6
ATOM	78	CG2	ILE	A	11	28.762	43.839	45.866	1.00	21.09	6
ATOM	79	CA	ILE	A	11	30.789	43.259	44.739	1.00	20.52	6
ATOM	80	C	ILE	A	11	31.715	42.170	44.172	1.00	21.46	6
ATOM	81	O	ILE	A	11	31.783	41.155	44.840	1.00	20.99	8
ATOM	82	N	ASN	A	12	32.378	42.329	43.056	1.00	21.03	7
ATOM	83	ND2	ASN	A	12	35.345	43.095	44.578	1.00	30.69	7
ATOM	84	OD1	ASN	A	12	36.135	42.268	42.569	1.00	35.13	8
ATOM	85	CG	ASN	A	12	35.390	42.276	43.541	1.00	25.00	6
ATOM	86	CB	ASN	A	12	34.450	41.092	43.449	1.00	21.03	6
ATOM	87	CA	ASN	A	12	33.340	41.412	42.463	1.00	23.98	6
ATOM	88	C	ASN	A	12	32.735	40.088	41.978	1.00	24.79	6
ATOM	89	O	ASN	A	12	33.438	39.085	42.118	1.00	23.07	8
ATOM	90	N	THR	A	13	31.520	40.204	41.505	1.00	20.38	7
ATOM	91	CG2	THR	A	13	28.654	38.417	39.642	1.00	15.01	6
ATOM	92	OG1	THR	A	13	28.704	40.013	41.326	1.00	22.51	8
ATOM	93	CB	THR	A	13	29.488	39.474	40.308	1.00	19.67	6
ATOM	94	CA	THR	A	13	30.810	39.083	40.956	1.00	20.28	6
ATOM	95	C	THR	A	13	31.671	38.384	39.892	1.00	21.19	6
ATOM	96	O	THR	A	13	31.605	37.158	39.791	1.00	23.59	8
ATOM	97	N	GLN	A	14	32.334	39.049	39.028	1.00	20.22	7
ATOM	98	NE2	GLN	A	14	32.431	41.889	38.600	1.00	33.33	7
ATOM	99	OE1	GLN	A	14	31.706	42.497	36.548	1.00	50.01	8
ATOM	100	CD	GLN	A	14	32.245	41.660	37.297	1.00	52.65	6
ATOM	101	CG	GLN	A	14	32.764	40.430	36.555	1.00	52.84	6
ATOM	102	CB	GLN	A	14	33.857	39.542	37.128	1.00	28.62	6
ATOM	103	CA	GLN	A	14	33.138	38.429	37.955	1.00	32.46	6
ATOM	104	C	GLN	A	14	34.201	37.476	38.497	1.00	31.89	6
ATOM	105	O	GLN	A	14	34.509	36.571	37.705	1.00	27.29	8
ATOM	106	N	GLN	A	15	34.744	37.757	39.679	1.00	23.92	7
ATOM	107	NE2	GLN	A	15	38.511	39.924	42.603	1.00	44.05	7
ATOM	108	OE1	GLN	A	15	37.542	38.314	43.749	1.00	38.30	8
ATOM	109	CD	GLN	A	15	37.762	38.831	42.664	1.00	40.79	6
ATOM	110	CG	GLN	A	15	37.188	38.390	41.331	1.00	34.24	6
ATOM	111	CB	GLN	A	15	36.297	37.200	41.508	1.00	24.39	6
ATOM	112	CA	GLN	A	15	35.728	36.783	40.170	1.00	22.62	6
ATOM	113	C	GLN	A	15	35.042	35.443	40.384	1.00	29.48	6
ATOM	114	O	GLN	A	15	35.749	34.432	40.285	1.00	31.32	8
ATOM	115	N	ALA	A	16	33.762	35.385	40.769	1.00	23.78	7
ATOM	116	CB	ALA	A	16	31.804	34.146	41.761	1.00	18.00	6
ATOM	117	CA	ALA	A	16	33.069	34.097	40.925	1.00	21.90	6
ATOM	118	C	ALA	A	16	32.825	33.561	39.502	1.00	26.74	6
ATOM	119	O	ALA	A	16	32.967	32.352	39.191	1.00	30.41	8
ATOM	120	N	HIS	A	17	32.281	34.385	38.577	1.00	30.64	7
ATOM	121	CD2	HIS	A	17	29.257	34.877	38.233	1.00	22.07	6
ATOM	122	NE2	HIS	A	17	28.016	35.453	38.259	1.00	25.33	7
ATOM	123	CE1	HIS	A	17	27.909	36.328	37.220	1.00	20.45	6

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3D Structure of Esperase

ATOM	124	ND1	HIS	A	17	29.020	36.372	36.515	1.00	24.91	7
ATOM	125	CG	HIS	A	17	29.849	35.428	37.109	1.00	22.09	6
ATOM	126	CB	HIS	A	17	31.222	35.150	36.543	1.00	19.27	6
ATOM	127	CA	HIS	A	17	31.865	33.972	37.219	1.00	19.98	6
ATOM	128	C	HIS	A	17	33.073	33.367	36.512	1.00	29.30	6
ATOM	129	O	HIS	A	17	32.959	32.347	35.823	1.00	27.69	8
ATOM	130	N	ASN	A	18	34.191	34.028	36.705	1.00	28.18	7
ATOM	131	ND2	ASN	A	18	36.859	36.788	35.613	1.00	45.93	7
ATOM	132	OD1	ASN	A	18	35.325	35.559	34.498	1.00	40.29	8
ATOM	133	CG	ASN	A	18	36.220	35.663	35.347	1.00	40.01	6
ATOM	134	CB	ASN	A	18	36.641	34.520	36.270	1.00	30.63	6
ATOM	135	CA	ASN	A	18	35.432	33.605	36.085	1.00	27.13	6
ATOM	136	C	ASN	A	18	35.838	32.250	36.577	1.00	35.11	6
ATOM	137	O	ASN	A	18	36.705	31.803	35.846	1.00	35.07	8
ATOM	138	N	ARG	A	19	35.399	31.756	37.675	1.00	32.73	7
ATOM	139	NH2	ARG	A	19	35.515	32.617	44.021	1.00	53.72	7
ATOM	140	NH1	ARG	A	19	37.640	32.800	43.686	1.00	51.43	7
ATOM	141	CZ	ARG	A	19	36.530	32.120	43.307	1.00	57.69	6
ATOM	142	NE	ARG	A	19	36.207	31.186	42.351	1.00	42.98	7
ATOM	143	CD	ARG	A	19	37.338	31.011	41.450	1.00	46.84	6
ATOM	144	CG	ARG	A	19	37.117	31.155	39.995	1.00	33.34	6
ATOM	145	CB	ARG	A	19	35.800	30.421	39.724	1.00	26.86	6
ATOM	146	CA	ARG	A	19	35.773	30.449	38.180	1.00	24.16	6
ATOM	147	C	ARG	A	19	34.635	29.545	37.735	1.00	32.80	6
ATOM	148	O	ARG	A	19	34.691	28.447	38.295	1.00	38.37	8
ATOM	149	N	GLY	A	20	33.659	29.890	36.943	1.00	26.10	7
ATOM	150	CA	GLY	A	20	32.569	28.978	36.587	1.00	22.13	6
ATOM	151	C	GLY	A	20	31.546	28.912	37.702	1.00	34.41	6
ATOM	152	O	GLY	A	20	30.872	27.856	37.735	1.00	28.59	8
ATOM	153	N	ILE	A	21	31.493	29.934	38.591	1.00	29.96	7
ATOM	154	CD1	ILE	A	21	33.459	29.632	41.814	1.00	41.54	6
ATOM	155	CG1	ILE	A	21	32.100	29.052	41.506	1.00	25.19	6
ATOM	156	CB	ILE	A	21	30.975	29.986	41.122	1.00	26.29	6
ATOM	157	CG2	ILE	A	21	29.844	29.735	42.107	1.00	19.84	6
ATOM	158	CA	ILE	A	21	30.460	29.794	39.684	1.00	32.15	6
ATOM	159	C	ILE	A	21	29.284	30.745	39.329	1.00	27.88	6
ATOM	160	O	ILE	A	21	29.528	31.975	39.238	1.00	25.54	8
ATOM	161	N	PHE	A	22	28.130	30.216	39.043	1.00	22.71	7
ATOM	162	CD2	PHE	A	22	28.593	30.211	35.689	1.00	27.44	6
ATOM	163	CE2	PHE	A	22	29.621	30.567	34.823	1.00	24.36	6
ATOM	164	CZ	PHE	A	22	29.741	31.905	34.446	1.00	33.93	6
ATOM	165	CE1	PHE	A	22	28.872	32.884	34.911	1.00	27.82	6
ATOM	166	CD1	PHE	A	22	27.870	32.510	35.793	1.00	28.92	6
ATOM	167	CG	PHE	A	22	27.724	31.192	36.172	1.00	28.03	6
ATOM	168	CB	PHE	A	22	26.658	30.789	37.118	1.00	24.21	6
ATOM	169	CA	PHE	A	22	26.950	30.969	38.613	1.00	26.09	6
ATOM	170	C	PHE	A	22	25.683	30.711	39.409	1.00	25.39	6
ATOM	171	O	PHE	A	22	24.665	31.302	38.981	1.00	24.97	8
ATOM	172	N	GLY	A	23	25.607	29.924	40.467	1.00	18.81	7
ATOM	173	CA	GLY	A	23	24.363	29.724	41.148	1.00	18.46	6
ATOM	174	C	GLY	A	23	23.503	28.543	40.757	1.00	19.87	6
ATOM	175	O	GLY	A	23	22.414	28.258	41.288	1.00	21.97	8
ATOM	176	N	ASN	A	24	24.176	27.813	39.877	1.00	24.80	7
ATOM	177	ND2	ASN	A	24	24.193	25.603	36.454	1.00	54.67	7
ATOM	178	OD1	ASN	A	24	23.354	24.090	38.041	1.00	52.66	8
ATOM	179	CG	ASN	A	24	24.034	25.056	37.655	1.00	54.67	6
ATOM	180	CB	ASN	A	24	24.770	26.009	38.589	1.00	32.23	6
ATOM	181	CA	ASN	A	24	23.593	26.534	39.395	1.00	25.92	6
ATOM	182	C	ASN	A	24	23.179	25.638	40.552	1.00	25.32	6
ATOM	183	O	ASN	A	24	23.976	25.322	41.465	1.00	30.34	8
ATOM	184	N	GLY	A	25	21.885	25.306	40.580	1.00	24.65	7
ATOM	185	CA	GLY	A	25	21.465	24.504	41.725	1.00	28.29	6
ATOM	186	C	GLY	A	25	20.845	25.160	42.938	1.00	26.14	6
ATOM	187	O	GLY	A	25	20.160	24.516	43.717	1.00	27.35	8
ATOM	188	N	ALA	A	26	21.025	26.469	43.065	1.00	33.36	7
ATOM	189	CB	ALA	A	26	21.389	28.357	44.440	1.00	22.66	6
ATOM	190	CA	ALA	A	26	20.451	27.216	44.226	1.00	21.52	6
ATOM	191	C	ALA	A	26	19.024	27.532	43.905	1.00	18.32	6
ATOM	192	O	ALA	A	26	18.702	27.928	42.773	1.00	24.15	8
ATOM	193	N	ARG	A	27	18.210	27.375	44.899	1.00	19.06	7
ATOM	194	NH2	ARG	A	27	15.995	22.073	47.281	1.00	46.56	7
ATOM	195	NH1	ARG	A	27	16.803	22.004	45.047	1.00	39.77	7
ATOM	196	CZ	ARG	A	27	16.017	22.485	46.012	1.00	48.33	6
ATOM	197	NE	ARG	A	27	15.098	23.456	45.820	1.00	41.99	7
ATOM	198	CD	ARG	A	27	15.075	24.160	44.559	1.00	36.91	6

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3D Structure of Esperase

ATOM	199	CG	ARG	A	27	16.301	25.064	44.358	1.00	29.21	6
ATOM	200	CB	ARG	A	27	15.999	26.369	45.132	1.00	26.05	6
ATOM	201	CA	ARG	A	27	16.785	27.590	44.764	1.00	19.90	6
ATOM	202	C	ARG	A	27	16.462	28.820	45.623	1.00	24.82	6
ATOM	203	O	ARG	A	27	16.484	28.798	46.855	1.00	23.36	8
ATOM	204	N	VAL	A	28	16.090	29.902	44.963	1.00	21.58	7
ATOM	205	CG2	VAL	A	28	18.212	31.847	44.971	1.00	20.76	6
ATOM	206	CG1	VAL	A	28	16.584	33.595	45.659	1.00	24.41	6
ATOM	207	CB	VAL	A	28	16.756	32.246	44.948	1.00	18.33	6
ATOM	208	CA	VAL	A	28	15.821	31.208	45.600	1.00	20.58	6
ATOM	209	C	VAL	A	28	14.369	31.568	45.504	1.00	16.41	6
ATOM	210	O	VAL	A	28	13.904	31.628	44.344	1.00	22.07	8
ATOM	211	N	ALA	A	29	13.724	31.792	46.617	1.00	15.89	7
ATOM	212	CB	ALA	A	29	11.536	31.675	47.718	1.00	16.94	6
ATOM	213	CA	ALA	A	29	12.322	32.248	46.580	1.00	21.50	6
ATOM	214	C	ALA	A	29	12.353	33.820	46.734	1.00	19.32	6
ATOM	215	O	ALA	A	29	13.042	34.312	47.649	1.00	19.70	8
ATOM	216	N	VAL	A	30	11.770	34.530	45.806	1.00	18.83	7
ATOM	217	CG2	VAL	A	30	13.356	36.406	44.142	1.00	17.28	6
ATOM	218	CG1	VAL	A	30	11.680	38.150	44.538	1.00	19.61	6
ATOM	219	CB	VAL	A	30	11.885	36.649	44.450	1.00	19.02	6
ATOM	220	CA	VAL	A	30	11.590	35.993	45.824	1.00	21.94	6
ATOM	221	C	VAL	A	30	10.211	36.329	46.406	1.00	17.79	6
ATOM	222	O	VAL	A	30	9.239	36.104	45.639	1.00	16.80	8
ATOM	223	N	LEU	A	31	10.136	36.740	47.677	1.00	16.21	7
ATOM	224	CD2	LEU	A	31	8.443	35.115	51.734	1.00	18.64	6
ATOM	225	CD1	LEU	A	31	9.392	34.230	49.510	1.00	18.41	6
ATOM	226	CG	LEU	A	31	8.513	35.233	50.228	1.00	27.95	6
ATOM	227	CB	LEU	A	31	8.841	36.689	49.787	1.00	17.41	6
ATOM	228	CA	LEU	A	31	8.837	37.091	48.332	1.00	17.17	6
ATOM	229	C	LEU	A	31	8.609	38.573	48.053	1.00	23.39	6
ATOM	230	O	LEU	A	31	9.245	39.436	48.649	1.00	19.56	8
ATOM	231	N	ASP	A	32	7.756	38.918	47.142	1.00	20.33	7
ATOM	232	OD2	ASP	A	32	8.509	42.872	45.463	1.00	17.46	8
ATOM	233	OD1	ASP	A	32	10.355	42.272	46.272	1.00	18.58	8
ATOM	234	CG	ASP	A	32	9.249	41.959	45.903	1.00	17.91	6
ATOM	235	CB	ASP	A	32	8.780	40.509	45.770	1.00	17.55	6
ATOM	236	CA	ASP	A	32	7.544	40.265	46.640	1.00	18.05	6
ATOM	237	C	ASP	A	32	6.259	40.407	45.874	1.00	16.34	6
ATOM	238	O	ASP	A	32	5.265	39.719	46.233	1.00	18.95	8
ATOM	239	N	THR	A	33	6.345	41.337	44.922	1.00	18.08	7
ATOM	240	CG2	THR	A	33	5.111	44.100	44.539	1.00	15.20	6
ATOM	241	OG1	THR	A	33	6.078	43.108	42.626	1.00	15.34	8
ATOM	242	CB	THR	A	33	5.050	42.995	43.536	1.00	17.62	6
ATOM	243	CA	THR	A	33	5.068	41.559	44.165	1.00	19.10	6
ATOM	244	C	THR	A	33	4.876	40.503	43.046	1.00	21.43	6
ATOM	245	O	THR	A	33	3.956	40.703	42.210	1.00	19.77	8
ATOM	246	N	GLY	A	34	5.747	39.519	42.979	1.00	19.23	7
ATOM	247	CA	GLY	A	34	5.694	38.503	41.928	1.00	18.38	6
ATOM	248	C	GLY	A	34	6.872	38.646	41.034	1.00	17.22	6
ATOM	249	O	GLY	A	34	7.711	39.459	41.383	1.00	18.99	8
ATOM	250	N	ILE	A	35	6.974	37.882	39.956	1.00	17.46	7
ATOM	251	CD1	ILE	A	35	10.899	35.757	40.596	1.00	15.13	6
ATOM	252	CG1	ILE	A	35	9.791	36.828	40.462	1.00	14.72	6
ATOM	253	CB	ILE	A	35	9.166	36.970	39.068	1.00	15.03	6
ATOM	254	CG2	ILE	A	35	10.243	37.326	38.068	1.00	15.97	6
ATOM	255	CA	ILE	A	35	8.048	37.960	38.978	1.00	14.81	6
ATOM	256	C	ILE	A	35	7.360	37.965	37.617	1.00	17.66	6
ATOM	257	O	ILE	A	35	6.554	37.071	37.431	1.00	21.48	8
ATOM	258	N	ALA	A	37	7.565	38.985	36.818	1.00	17.09	7
ATOM	259	CB	ALA	A	37	6.974	40.415	34.895	1.00	19.79	6
ATOM	260	CA	ALA	A	37	6.929	39.026	35.522	1.00	19.65	6
ATOM	261	C	ALA	A	37	7.799	38.217	34.551	1.00	17.88	6
ATOM	262	O	ALA	A	37	9.037	38.066	34.604	1.00	21.23	8
ATOM	263	N	SER	A	38	7.062	37.689	33.589	1.00	16.80	7
ATOM	264	OG	SER	A	38	7.219	35.805	30.632	1.00	30.69	8
ATOM	265	CB	SER	A	38	6.656	36.129	31.852	1.00	24.32	6
ATOM	266	CA	SER	A	38	7.794	36.946	32.527	1.00	20.02	6
ATOM	267	C	SER	A	38	8.554	38.064	31.824	1.00	20.83	6
ATOM	268	O	SER	A	38	8.026	39.138	31.556	1.00	21.16	8
ATOM	269	N	HIS	A	39	9.788	37.876	31.449	1.00	16.67	7
ATOM	270	CD2	HIS	A	39	11.839	42.154	31.855	1.00	18.50	6
ATOM	271	NE2	HIS	A	39	12.849	42.828	31.229	1.00	17.78	7
ATOM	272	CE1	HIS	A	39	13.757	41.990	30.654	1.00	19.11	6
ATOM	273	ND1	HIS	A	39	13.250	40.817	30.899	1.00	18.95	7

APPENDIX D-continued

3D Structure of Esperase											
ATOM	274	CG	HIS	A	39	12.108	40.809	31.630	1.00	18.98	6
ATOM	275	CB	HIS	A	39	11.359	39.557	32.049	1.00	18.97	6
ATOM	276	CA	HIS	A	39	10.744	38.721	30.858	1.00	19.12	6
ATOM	277	C	HIS	A	39	11.775	37.948	30.062	1.00	17.80	6
ATOM	278	O	HIS	A	39	12.355	37.014	30.570	1.00	20.73	8
ATOM	279	N	PRO	A	40	12.200	38.418	28.889	1.00	21.00	7
ATOM	280	CG	PRO	A	40	12.293	39.449	26.786	1.00	21.21	6
ATOM	281	CD	PRO	A	40	11.597	39.542	28.113	1.00	18.96	6
ATOM	282	CB	PRO	A	40	13.560	38.729	26.913	1.00	19.67	6
ATOM	283	CA	PRO	A	40	13.254	37.823	28.100	1.00	22.54	6
ATOM	284	C	PRO	A	40	14.534	37.614	28.909	1.00	24.98	6
ATOM	285	O	PRO	A	40	15.326	36.689	28.538	1.00	23.15	8
ATOM	286	N	ASP	A	41	14.864	38.402	29.921	1.00	21.23	7
ATOM	287	OD2	ASP	A	41	19.022	40.411	31.203	1.00	23.14	8
ATOM	288	OD1	ASP	A	41	18.902	38.575	30.179	1.00	20.45	8
ATOM	289	CG	ASP	A	41	18.278	39.474	30.706	1.00	21.49	6
ATOM	290	CB	ASP	A	41	16.801	39.675	30.849	1.00	17.52	6
ATOM	291	CA	ASP	A	41	16.149	38.300	30.623	1.00	18.20	6
ATOM	292	C	ASP	A	41	16.007	37.531	31.930	1.00	16.57	6
ATOM	293	O	ASP	A	41	16.990	37.609	32.687	1.00	21.11	8
ATOM	294	N	LEU	A	42	14.877	36.908	32.100	1.00	16.23	7
ATOM	295	CD2	LEU	A	42	15.154	37.970	35.800	1.00	20.71	6
ATOM	296	CD1	LEU	A	42	12.728	38.634	35.680	1.00	18.04	6
ATOM	297	CG	LEU	A	42	13.906	38.079	34.940	1.00	22.07	6
ATOM	298	CB	LEU	A	42	13.573	36.743	34.250	1.00	19.04	6
ATOM	299	CA	LEU	A	42	14.688	36.119	33.316	1.00	18.11	6
ATOM	300	C	LEU	A	42	14.147	34.706	33.035	1.00	22.16	6
ATOM	301	O	LEU	A	42	13.321	34.478	32.117	1.00	24.54	8
ATOM	302	N	ARG	A	43	14.426	33.731	33.856	1.00	20.59	7
ATOM	303	NH2	ARG	A	43	16.861	27.990	36.107	1.00	53.82	7
ATOM	304	NH1	ARG	A	43	14.504	27.483	36.114	1.00	58.81	7
ATOM	305	CZ	ARG	A	43	15.623	27.968	35.534	1.00	59.96	6
ATOM	306	NE	ARG	A	43	15.539	28.580	34.285	1.00	59.26	7
ATOM	307	CD	ARG	A	43	14.187	29.098	33.890	1.00	53.79	6
ATOM	308	CG	ARG	A	43	14.538	30.144	32.891	1.00	38.80	6
ATOM	309	CB	ARG	A	43	14.893	31.393	33.636	1.00	20.63	6
ATOM	310	CA	ARG	A	43	13.780	32.413	33.764	1.00	21.97	6
ATOM	311	C	ARG	A	43	13.120	32.158	35.092	1.00	20.02	6
ATOM	312	O	ARG	A	43	13.858	32.194	36.102	1.00	24.03	8
ATOM	313	N	ILE	A	44	11.867	31.959	35.226	1.00	20.63	7
ATOM	314	CD1	ILE	A	44	8.902	34.679	35.796	1.00	25.57	6
ATOM	315	CG1	ILE	A	44	10.068	33.881	36.368	1.00	29.55	6
ATOM	316	CB	ILE	A	44	9.746	32.360	36.490	1.00	24.21	6
ATOM	317	CG2	ILE	A	44	8.902	31.922	37.662	1.00	21.80	6
ATOM	318	CA	ILE	A	44	11.103	31.670	36.445	1.00	20.36	6
ATOM	319	C	ILE	A	44	10.838	30.166	36.550	1.00	28.98	6
ATOM	320	O	ILE	A	44	10.177	29.571	35.695	1.00	23.55	8
ATOM	321	N	ALA	A	45	11.322	29.549	37.602	1.00	27.19	7
ATOM	322	CB	ALA	A	45	12.254	27.427	38.711	1.00	18.19	6
ATOM	323	CA	ALA	A	45	11.176	28.111	37.907	1.00	25.70	6
ATOM	324	C	ALA	A	45	9.799	27.798	38.418	1.00	25.04	6
ATOM	325	O	ALA	A	45	9.394	26.706	38.033	1.00	28.94	8
ATOM	326	N	GLY	A	46	9.044	28.597	39.089	1.00	20.03	7
ATOM	327	CA	GLY	A	46	7.719	28.282	39.555	1.00	16.95	6
ATOM	328	C	GLY	A	46	7.400	29.295	40.624	1.00	22.67	6
ATOM	329	O	GLY	A	46	8.103	30.327	40.564	1.00	21.98	8
ATOM	330	N	GLY	A	47	6.408	29.068	41.382	1.00	22.31	7
ATOM	331	CA	GLY	A	47	6.038	30.017	42.427	1.00	21.33	6
ATOM	332	C	GLY	A	47	4.601	29.839	42.841	1.00	25.87	6
ATOM	333	O	GLY	A	47	3.918	28.882	42.428	1.00	25.43	8
ATOM	334	N	ALA	A	48	4.055	30.737	43.620	1.00	20.53	7
ATOM	335	CB	ALA	A	48	2.815	29.944	45.442	1.00	20.90	6
ATOM	336	CA	ALA	A	48	2.713	30.745	44.144	1.00	20.50	6
ATOM	337	C	ALA	A	48	2.326	32.203	44.460	1.00	29.20	6
ATOM	338	O	ALA	A	48	3.178	33.083	44.532	1.00	25.97	8
ATOM	339	N	SER	A	49	1.068	32.454	44.688	1.00	22.19	7
ATOM	340	OG	SER	A	49	−0.986	35.495	44.409	1.00	27.17	8
ATOM	341	CB	SER	A	49	−0.441	34.225	43.938	1.00	26.70	6
ATOM	342	CA	SER	A	49	0.478	33.712	45.013	1.00	22.03	6
ATOM	343	C	SER	A	49	−0.307	33.577	46.315	1.00	31.92	6
ATOM	344	O	SER	A	49	−1.067	32.591	46.360	1.00	26.97	8
ATOM	345	N	PHE	A	50	−0.097	34.588	47.147	1.00	22.91	7
ATOM	346	CD2	PHE	A	50	−0.049	32.109	50.111	1.00	31.06	6
ATOM	347	CE2	PHE	A	50	0.409	30.786	49.993	1.00	23.47	6
ATOM	348	CZ	PHE	A	50	1.692	30.585	49.509	1.00	26.37	6

APPENDIX D-continued

3D Structure of Esperase

ATOM	349	CE1	PHE	A	50	2.459	31.650	49.044	1.00	27.36	6
ATOM	350	CD1	PHE	A	50	1.909	32.920	49.123	1.00	25.18	6
ATOM	351	CG	PHE	A	50	0.659	33.206	49.640	1.00	27.18	6
ATOM	352	CB	PHE	A	50	0.068	34.581	49.654	1.00	20.39	6
ATOM	353	CA	PHE	A	50	−0.814	34.627	48.416	1.00	20.79	6
ATOM	354	C	PHE	A	50	−1.699	35.845	48.217	1.00	26.50	6
ATOM	355	O	PHE	A	50	−2.095	36.380	49.255	1.00	33.21	8
ATOM	356	N	ILE	A	51	−2.067	36.337	47.068	1.00	25.81	7
ATOM	357	CD1	ILE	A	51	−0.964	39.394	48.263	1.00	25.15	6
ATOM	358	CG1	ILE	A	51	−0.838	39.160	46.744	1.00	26.10	6
ATOM	359	CB	ILE	A	51	−2.155	38.659	46.174	1.00	28.46	6
ATOM	360	CG2	ILE	A	51	−2.994	39.906	45.884	1.00	26.35	6
ATOM	361	CA	ILE	A	51	−2.870	37.563	46.980	1.00	25.17	6
ATOM	362	C	ILE	A	51	−4.111	37.059	46.276	1.00	22.13	6
ATOM	363	O	ILE	A	51	−4.019	36.809	45.075	1.00	26.47	8
ATOM	364	N	SER	A	52	−5.211	36.990	46.985	1.00	31.96	7
ATOM	365	OG	SER	A	52	−7.326	37.187	48.213	1.00	55.96	8
ATOM	366	CB	SER	A	52	−7.637	36.283	47.168	1.00	40.98	6
ATOM	367	CA	SER	A	52	−6.416	36.494	46.288	1.00	36.15	6
ATOM	368	C	SER	A	52	−6.840	37.320	45.088	1.00	41.46	6
ATOM	369	O	SER	A	52	−7.334	36.657	44.131	1.00	42.48	8
ATOM	370	N	SER	A	53	−6.711	38.640	45.097	1.00	34.99	7
ATOM	371	OG	SER	A	53	−6.064	41.220	44.420	1.00	45.24	8
ATOM	372	CB	SER	A	53	−7.345	40.753	44.027	1.00	36.41	6
ATOM	373	CA	SER	A	53	−7.166	39.272	43.832	1.00	32.42	6
ATOM	374	C	SER	A	53	−6.198	39.008	42.704	1.00	28.79	6
ATOM	375	O	SER	A	53	−6.518	39.427	41.610	1.00	30.59	8
ATOM	376	N	GLU	A	54	−5.089	38.335	42.931	1.00	26.60	7
ATOM	377	OE2	GLU	A	54	−2.266	42.297	42.536	1.00	28.17	8
ATOM	378	OE1	GLU	A	54	−0.866	41.124	41.290	1.00	25.34	8
ATOM	379	CD	GLU	A	54	−1.988	41.335	41.716	1.00	26.67	6
ATOM	380	CG	GLU	A	54	−3.245	40.511	41.554	1.00	33.12	6
ATOM	381	CB	GLU	A	54	−2.993	39.046	41.906	1.00	30.53	6
ATOM	382	CA	GLU	A	54	−4.147	38.053	41.836	1.00	27.17	6
ATOM	383	C	GLU	A	54	−3.550	36.669	41.985	1.00	29.10	6
ATOM	384	O	GLU	A	54	−2.499	36.360	42.543	1.00	31.16	8
ATOM	385	N	PRO	A	55	−4.303	35.698	41.531	1.00	28.22	7
ATOM	386	CG	PRO	A	55	−6.256	34.510	40.919	1.00	32.87	6
ATOM	387	CD	PRO	A	55	−5.638	35.901	40.877	1.00	27.93	6
ATOM	388	CB	PRO	A	55	−5.108	33.565	40.980	1.00	25.50	6
ATOM	389	CA	PRO	A	55	−3.921	34.295	41.596	1.00	27.69	6
ATOM	390	C	PRO	A	55	−2.652	33.893	40.869	1.00	26.18	6
ATOM	391	O	PRO	A	55	−2.111	32.861	41.284	1.00	29.26	8
ATOM	392	N	SER	A	57	−2.177	34.589	39.865	1.00	23.03	7
ATOM	393	OG	SER	A	57	0.204	34.676	37.165	1.00	24.28	8
ATOM	394	CB	SER	A	57	−1.012	34.882	37.811	1.00	17.78	6
ATOM	395	CA	SER	A	57	−0.933	34.228	39.178	1.00	17.61	6
ATOM	396	C	SER	A	57	0.231	34.769	40.022	1.00	23.28	6
ATOM	397	O	SER	A	57	0.077	35.788	40.730	1.00	23.01	8
ATOM	398	N	TYR	A	58	1.401	34.208	39.978	1.00	21.42	7
ATOM	399	OH	TYR	A	58	5.286	30.151	36.865	1.00	33.08	8
ATOM	400	CD2	TYR	A	58	4.751	33.134	38.858	1.00	20.82	6
ATOM	401	CE2	TYR	A	58	5.242	32.389	37.792	1.00	27.67	6
ATOM	402	CZ	TYR	A	58	4.847	31.036	37.806	1.00	30.71	6
ATOM	403	CE1	TYR	A	58	4.098	30.504	38.847	1.00	24.64	6
ATOM	404	CD1	TYR	A	58	3.650	31.337	39.884	1.00	30.01	6
ATOM	405	CG	TYR	A	58	3.956	32.697	39.898	1.00	24.45	6
ATOM	406	CB	TYR	A	58	3.496	33.547	41.049	1.00	19.56	6
ATOM	407	CA	TYR	A	58	2.579	34.707	40.656	1.00	22.41	6
ATOM	408	C	TYR	A	58	3.245	35.769	39.795	1.00	18.11	6
ATOM	409	O	TYR	A	58	4.272	36.323	40.134	1.00	19.48	8
ATOM	410	N	HIS	A	59	2.819	36.120	38.608	1.00	19.19	7
ATOM	411	CD2	HIS	A	59	2.574	34.690	35.084	1.00	24.45	6
ATOM	412	NE2	HIS	A	59	3.570	33.918	34.542	1.00	23.56	7
ATOM	413	CE1	HIS	A	59	4.820	34.391	34.635	1.00	23.74	6
ATOM	414	ND1	HIS	A	59	4.689	35.505	35.318	1.00	27.94	7
ATOM	415	CG	HIS	A	59	3.333	35.753	35.529	1.00	23.77	6
ATOM	416	CB	HIS	A	59	2.907	37.006	36.276	1.00	23.35	6
ATOM	417	CA	HIS	A	59	3.464	37.096	37.717	1.00	23.68	6
ATOM	418	C	HIS	A	59	3.223	38.478	38.330	1.00	16.77	6
ATOM	419	O	HIS	A	59	2.112	38.802	38.813	1.00	20.69	8
ATOM	420	N	ASP	A	60	4.262	39.225	38.217	1.00	17.78	7
ATOM	421	OD2	ASP	A	60	7.207	42.684	39.352	1.00	16.87	8
ATOM	422	OD1	ASP	A	60	5.224	42.870	40.299	1.00	17.98	8
ATOM	423	CG	ASP	A	60	6.005	42.319	39.583	1.00	15.82	6

APPENDIX D-continued

3D Structure of Esperase

ATOM	424	CB	ASP	A	60	5.713	41.108	38.718	1.00	20.17	6
ATOM	425	CA	ASP	A	60	4.257	40.615	38.746	1.00	19.60	6
ATOM	426	C	ASP	A	60	3.449	41.628	37.887	1.00	16.78	6
ATOM	427	O	ASP	A	60	3.755	41.641	36.688	1.00	17.17	8
ATOM	428	N	ASN	A	61	2.553	42.321	38.565	1.00	16.17	7
ATOM	429	ND2	ASN	A	61	−0.712	41.216	38.409	1.00	21.25	7
ATOM	430	OD1	ASN	A	61	0.074	41.753	36.354	1.00	22.89	8
ATOM	431	CG	ASN	A	61	−0.126	42.022	37.543	1.00	19.95	6
ATOM	432	CB	ASN	A	61	0.343	43.358	38.057	1.00	18.61	6
ATOM	433	CA	ASN	A	61	1.837	43.400	37.853	1.00	18.92	6
ATOM	434	C	ASN	A	61	2.346	44.793	38.274	1.00	22.66	6
ATOM	435	O	ASN	A	61	1.893	45.845	37.801	1.00	23.21	8
ATOM	436	N	ASN	A	62	3.297	44.887	39.186	1.00	19.85	7
ATOM	437	ND2	ASN	A	62	3.761	48.155	42.016	1.00	22.91	7
ATOM	438	OD1	ASN	A	62	5.928	47.387	41.972	1.00	21.51	8
ATOM	439	CG	ASN	A	62	4.708	47.221	41.809	1.00	24.07	6
ATOM	440	CB	ASN	A	62	4.074	45.934	41.266	1.00	15.90	6
ATOM	441	CA	ASN	A	62	3.942	46.038	39.781	1.00	17.18	6
ATOM	442	C	ASN	A	62	5.262	46.370	39.149	1.00	21.56	6
ATOM	443	O	ASN	A	62	5.450	47.489	38.652	1.00	23.34	8
ATOM	444	N	GLY	A	63	6.219	45.499	39.274	1.00	16.07	7
ATOM	445	CA	GLY	A	63	7.560	45.696	38.775	1.00	15.56	6
ATOM	446	C	GLY	A	63	8.566	45.526	39.928	1.00	13.16	6
ATOM	447	O	GLY	A	63	9.705	45.220	39.576	1.00	14.42	8
ATOM	448	N	HIS	A	64	8.181	45.732	41.170	1.00	14.55	7
ATOM	449	CD2	HIS	A	64	9.944	47.365	45.114	1.00	19.41	6
ATOM	450	NE2	HIS	A	64	10.615	47.068	46.239	1.00	17.69	7
ATOM	451	CE1	HIS	A	64	10.371	45.792	46.555	1.00	17.59	6
ATOM	452	ND1	HIS	A	64	9.605	45.312	45.607	1.00	19.22	7
ATOM	453	CG	HIS	A	64	9.334	46.232	44.659	1.00	17.77	6
ATOM	454	CB	HIS	A	64	8.428	45.991	43.484	1.00	13.22	6
ATOM	455	CA	HIS	A	64	9.195	45.658	42.241	1.00	17.90	6
ATOM	456	C	HIS	A	64	9.902	44.259	42.331	1.00	17.60	6
ATOM	457	O	HIS	A	64	11.161	44.161	42.393	1.00	15.99	8
ATOM	458	N	GLY	A	65	9.081	43.180	42.309	1.00	16.44	7
ATOM	459	CA	GLY	A	65	9.616	41.816	42.380	1.00	14.82	6
ATOM	460	C	GLY	A	65	10.479	41.481	41.172	1.00	14.51	6
ATOM	461	O	GLY	A	65	11.471	40.769	41.349	1.00	17.10	8
ATOM	462	N	THR	A	66	10.099	41.938	39.997	1.00	14.08	7
ATOM	463	CG2	THR	A	66	10.799	41.935	36.263	1.00	16.28	6
ATOM	464	OG1	THR	A	66	8.783	41.636	37.548	1.00	16.38	8
ATOM	465	CB	THR	A	66	10.092	42.160	37.567	1.00	13.88	6
ATOM	466	CA	THR	A	66	10.851	41.608	38.787	1.00	11.82	6
ATOM	467	C	THR	A	66	12.223	42.209	38.848	1.00	17.20	6
ATOM	468	O	THR	A	66	13.251	41.729	38.360	1.00	15.82	8
ATOM	469	N	HIS	A	67	12.283	43.430	39.440	1.00	16.72	7
ATOM	470	CD2	HIS	A	67	14.672	47.526	38.936	1.00	14.06	6
ATOM	471	NE2	HIS	A	67	15.894	48.068	39.341	1.00	15.93	7
ATOM	472	CE1	HIS	A	67	16.222	47.455	40.502	1.00	16.28	6
ATOM	473	ND1	HIS	A	67	15.270	46.657	40.870	1.00	14.20	7
ATOM	474	CG	HIS	A	67	14.288	46.658	39.897	1.00	13.11	6
ATOM	475	CB	HIS	A	67	13.142	45.733	40.058	1.00	13.83	6
ATOM	476	CA	HIS	A	67	13.524	44.275	39.602	1.00	17.85	6
ATOM	477	C	HIS	A	67	14.489	43.467	40.555	1.00	12.74	6
ATOM	478	O	HIS	A	67	15.676	43.217	40.217	1.00	14.79	8
ATOM	479	N	VAL	A	68	13.875	43.184	41.692	1.00	15.52	7
ATOM	480	CG2	VAL	A	68	13.554	43.532	44.544	1.00	16.01	6
ATOM	481	CG1	VAL	A	68	14.397	41.111	44.868	1.00	15.56	6
ATOM	482	CB	VAL	A	68	13.732	42.126	43.930	1.00	17.25	6
ATOM	483	CA	VAL	A	68	14.631	42.373	42.702	1.00	18.13	6
ATOM	484	C	VAL	A	68	15.115	41.029	42.063	1.00	13.97	6
ATOM	485	O	VAL	A	68	16.303	40.718	42.241	1.00	15.56	8
ATOM	486	N	ALA	A	69	14.226	40.381	41.343	1.00	16.97	7
ATOM	487	CB	ALA	A	69	13.385	38.483	40.044	1.00	15.14	6
ATOM	488	CA	ALA	A	69	14.625	39.104	40.683	1.00	20.11	6
ATOM	489	C	ALA	A	69	15.800	39.240	39.746	1.00	19.97	6
ATOM	490	O	ALA	A	69	16.716	38.370	39.765	1.00	18.07	8
ATOM	491	N	GLY	A	70	15.860	40.297	38.929	1.00	16.08	7
ATOM	492	CA	GLY	A	70	16.915	40.521	37.962	1.00	13.42	6
ATOM	493	C	GLY	A	70	18.248	40.803	38.624	1.00	17.11	6
ATOM	494	O	GLY	A	70	19.301	40.458	38.069	1.00	18.05	8
ATOM	495	N	THR	A	71	18.251	41.364	39.834	1.00	16.82	7
ATOM	496	CG2	THR	A	71	20.803	42.713	42.461	1.00	11.71	6
ATOM	497	OG1	THR	A	71	19.044	43.833	41.152	1.00	19.96	8
ATOM	498	CB	THR	A	71	19.494	42.605	41.692	1.00	17.79	6

APPENDIX D-continued

3D Structure of Esperase

ATOM	499	CA	THR	A	71	19.570	41.620	40.463	1.00	18.16	6
ATOM	500	C	THR	A	71	20.085	40.254	40.907	1.00	16.28	6
ATOM	501	O	THR	A	71	21.302	40.022	40.823	1.00	20.35	8
ATOM	502	N	ILE	A	72	19.224	39.377	41.381	1.00	17.87	7
ATOM	503	CD1	ILE	A	72	16.919	37.403	44.477	1.00	15.03	6
ATOM	504	CG1	ILE	A	72	18.141	37.904	43.767	1.00	16.72	6
ATOM	505	CB	ILE	A	72	18.628	37.243	42.500	1.00	22.03	6
ATOM	506	CG2	ILE	A	72	19.096	35.809	42.923	1.00	18.50	6
ATOM	507	CA	ILE	A	72	19.708	38.025	41.767	1.00	18.21	6
ATOM	508	C	ILE	A	72	20.158	37.194	40.536	1.00	18.25	6
ATOM	509	O	ILE	A	72	21.223	36.584	40.501	1.00	17.34	8
ATOM	510	N	ALA	A	73	19.308	37.143	39.514	1.00	18.67	7
ATOM	511	CB	ALA	A	73	18.850	34.961	38.811	1.00	21.72	6
ATOM	512	CA	ALA	A	73	19.600	36.258	38.384	1.00	20.55	6
ATOM	513	C	ALA	A	73	19.220	36.650	36.993	1.00	21.64	6
ATOM	514	O	ALA	A	73	18.847	35.677	36.292	1.00	21.02	8
ATOM	515	N	ALA	A	74	19.351	37.891	36.551	1.00	19.57	7
ATOM	516	CB	ALA	A	74	19.407	39.748	34.855	1.00	16.43	6
ATOM	517	CA	ALA	A	74	19.129	38.268	35.176	1.00	17.51	6
ATOM	518	C	ALA	A	74	20.182	37.387	34.423	1.00	21.22	6
ATOM	519	O	ALA	A	74	21.379	37.294	34.773	1.00	18.12	8
ATOM	520	N	LEU	A	75	19.625	36.759	33.380	1.00	19.89	7
ATOM	521	CD2	LEU	A	75	18.684	33.287	32.938	1.00	20.44	6
ATOM	522	CD1	LEU	A	75	17.370	34.159	30.853	1.00	22.84	6
ATOM	523	CG	LEU	A	75	18.279	34.390	32.036	1.00	23.72	6
ATOM	524	CB	LEU	A	75	19.491	35.129	31.487	1.00	22.59	6
ATOM	525	CA	LEU	A	75	20.421	35.799	32.558	1.00	21.45	6
ATOM	526	C	LEU	A	75	21.644	36.353	31.885	1.00	22.38	6
ATOM	527	O	LEU	A	75	21.691	37.506	31.413	1.00	21.99	8
ATOM	528	N	ASN	A	76	22.678	35.519	31.836	1.00	23.39	7
ATOM	529	ND2	ASN	A	76	27.453	34.761	31.699	1.00	31.91	7
ATOM	530	OD1	ASN	A	76	26.466	36.466	30.730	1.00	26.97	8
ATOM	531	CG	ASN	A	76	26.339	35.407	31.355	1.00	33.84	6
ATOM	532	CB	ASN	A	76	24.992	34.941	31.890	1.00	18.81	6
ATOM	533	CA	ASN	A	76	23.966	35.823	31.226	1.00	22.81	6
ATOM	534	C	ASN	A	76	23.762	35.565	29.728	1.00	32.51	6
ATOM	535	O	ASN	A	76	23.757	34.402	29.350	1.00	27.52	8
ATOM	536	N	ASN	A	77	23.499	36.553	28.890	1.00	29.68	7
ATOM	537	ND2	ASN	A	77	19.501	36.639	28.267	1.00	20.91	7
ATOM	538	OD1	ASN	A	77	21.260	38.058	28.176	1.00	23.61	8
ATOM	539	CG	ASN	A	77	20.739	36.958	28.001	1.00	23.21	6
ATOM	540	CB	ASN	A	77	21.698	36.006	27.290	1.00	24.11	6
ATOM	541	CA	ASN	A	77	23.184	36.392	27.455	1.00	29.10	6
ATOM	542	C	ASN	A	77	23.597	37.625	26.699	1.00	23.45	6
ATOM	543	O	ASN	A	77	24.554	38.269	27.092	1.00	26.46	8
ATOM	544	N	SER	A	78	22.917	37.914	25.631	1.00	23.85	7
ATOM	545	OG	SER	A	78	23.826	38.128	22.933	1.00	51.66	8
ATOM	546	CB	SER	A	78	22.726	38.836	23.468	1.00	38.99	6
ATOM	547	CA	SER	A	78	23.343	39.124	24.902	1.00	28.32	6
ATOM	548	C	SER	A	78	22.590	40.392	25.196	1.00	26.17	6
ATOM	549	O	SER	A	78	22.848	41.406	24.556	1.00	30.79	8
ATOM	550	N	ILE	A	79	21.553	40.260	25.994	1.00	26.87	7
ATOM	551	CD1	ILE	A	79	17.234	39.484	26.505	1.00	22.48	6
ATOM	552	CG1	ILE	A	79	18.723	39.666	26.593	1.00	23.59	6
ATOM	553	CB	ILE	A	79	19.291	40.851	25.835	1.00	29.56	6
ATOM	554	CG2	ILE	A	79	19.401	40.371	24.400	1.00	25.83	6
ATOM	555	CA	ILE	A	79	20.675	41.390	26.218	1.00	22.47	6
ATOM	556	C	ILE	A	79	20.590	41.758	27.679	1.00	22.23	6
ATOM	557	O	ILE	A	79	21.096	41.041	28.498	1.00	21.05	8
ATOM	558	N	GLY	A	80	19.921	42.847	27.901	1.00	21.78	7
ATOM	559	CA	GLY	A	80	19.579	43.296	29.237	1.00	20.74	6
ATOM	560	C	GLY	A	80	20.731	43.409	30.215	1.00	22.30	6
ATOM	561	O	GLY	A	80	21.767	43.988	29.848	1.00	24.00	8
ATOM	562	N	VAL	A	81	20.534	42.884	31.415	1.00	20.72	7
ATOM	563	CG2	VAL	A	81	19.687	43.194	34.148	1.00	16.10	6
ATOM	564	CG1	VAL	A	81	20.666	45.283	33.070	1.00	19.66	6
ATOM	565	CB	VAL	A	81	20.938	43.844	33.561	1.00	21.57	6
ATOM	566	CA	VAL	A	81	21.616	43.067	32.414	1.00	18.79	6
ATOM	567	C	VAL	A	81	22.121	41.681	32.721	1.00	24.48	6
ATOM	568	O	VAL	A	81	21.953	40.670	32.065	1.00	22.82	8
ATOM	569	N	LEU	A	82	22.797	41.495	33.827	1.00	26.20	7
ATOM	570	CD2	LEU	A	82	27.235	39.378	34.412	1.00	20.59	6
ATOM	571	CD1	LEU	A	82	25.342	37.924	33.896	1.00	22.30	6
ATOM	572	CG	LEU	A	82	25.740	39.235	34.558	1.00	22.25	6
ATOM	573	CB	LEU	A	82	24.947	40.464	34.054	1.00	20.75	6

APPENDIX D-continued

3D Structure of Esperase											
ATOM	574	CA	LEU	A	82	23.431	40.297	34.339	1.00	21.39	6
ATOM	575	C	LEU	A	82	23.171	40.165	35.847	1.00	19.49	6
ATOM	576	O	LEU	A	82	23.528	41.144	36.502	1.00	23.34	8
ATOM	577	N	GLY	A	83	22.671	39.066	36.348	1.00	20.69	7
ATOM	578	CA	GLY	A	83	22.457	38.949	37.770	1.00	17.03	6
ATOM	579	C	GLY	A	83	23.782	38.468	38.350	1.00	17.15	6
ATOM	580	O	GLY	A	83	24.759	38.085	37.729	1.00	17.70	8
ATOM	581	N	VAL	A	84	23.723	38.456	39.683	1.00	21.38	7
ATOM	582	CG2	VAL	A	84	24.533	39.699	42.307	1.00	17.59	6
ATOM	583	CG1	VAL	A	84	25.675	37.585	42.933	1.00	18.61	6
ATOM	584	CB	VAL	A	84	24.568	38.197	42.032	1.00	19.33	6
ATOM	585	CA	VAL	A	84	24.791	37.919	40.537	1.00	18.94	6
ATOM	586	C	VAL	A	84	24.883	36.373	40.292	1.00	19.93	6
ATOM	587	O	VAL	A	84	26.024	35.890	40.194	1.00	18.82	8
ATOM	588	N	ALA	A	85	23.766	35.668	40.255	1.00	19.98	7
ATOM	589	CB	ALA	A	85	23.136	33.645	41.452	1.00	16.16	6
ATOM	590	CA	ALA	A	85	23.717	34.185	40.149	1.00	23.42	6
ATOM	591	C	ALA	A	85	22.819	33.819	38.945	1.00	15.76	6
ATOM	592	O	ALA	A	85	21.669	33.420	39.123	1.00	17.91	8
ATOM	593	N	PRO	A	86	23.320	34.080	37.739	1.00	19.61	7
ATOM	594	CG	PRO	A	86	24.802	34.328	35.990	1.00	22.42	6
ATOM	595	CD	PRO	A	86	24.691	34.594	37.481	1.00	17.62	6
ATOM	596	CB	PRO	A	86	23.412	34.286	35.395	1.00	18.97	6
ATOM	597	CA	PRO	A	86	22.527	33.884	36.525	1.00	22.90	6
ATOM	598	C	PRO	A	86	21.982	32.494	36.282	1.00	25.04	6
ATOM	599	O	PRO	A	86	21.044	32.392	35.510	1.00	25.03	8
ATOM	600	N	SER	A	87	22.550	31.531	36.954	1.00	21.61	7
ATOM	601	OG	SER	A	87	23.828	29.588	35.364	1.00	24.93	8
ATOM	602	CB	SER	A	87	23.195	29.132	36.539	1.00	21.86	6
ATOM	603	CA	SER	A	87	22.079	30.144	36.789	1.00	25.33	6
ATOM	604	C	SER	A	87	21.253	29.730	37.973	1.00	27.17	6
ATOM	605	O	SER	A	87	20.806	28.602	37.975	1.00	26.19	8
ATOM	606	N	ALA	A	88	20.892	30.516	38.966	1.00	23.05	7
ATOM	607	CB	ALA	A	88	20.108	31.154	41.189	1.00	18.32	6
ATOM	608	CA	ALA	A	88	20.051	30.084	40.053	1.00	22.79	6
ATOM	609	C	ALA	A	88	18.628	29.760	39.608	1.00	21.41	6
ATOM	610	O	ALA	A	88	18.106	30.259	38.608	1.00	25.76	8
ATOM	611	N	ASP	A	89	17.896	28.967	40.323	1.00	19.89	7
ATOM	612	OD2	ASP	A	89	16.801	26.516	38.434	1.00	31.22	8
ATOM	613	OD1	ASP	A	89	17.282	25.428	40.116	1.00	44.17	8
ATOM	614	CG	ASP	A	89	16.662	26.363	39.689	1.00	32.29	6
ATOM	615	CB	ASP	A	89	16.007	27.380	40.585	1.00	26.99	6
ATOM	616	CA	ASP	A	89	16.475	28.764	40.089	1.00	22.99	6
ATOM	617	C	ASP	A	89	15.649	29.788	40.846	1.00	26.13	6
ATOM	618	O	ASP	A	89	15.605	29.765	42.092	1.00	23.54	8
ATOM	619	N	LEU	A	90	14.876	30.620	40.201	1.00	23.36	7
ATOM	620	CD2	LEU	A	90	14.764	35.038	38.890	1.00	23.98	6
ATOM	621	CD1	LEU	A	90	15.677	34.244	41.144	1.00	23.31	6
ATOM	622	CG	LEU	A	90	14.540	34.313	40.203	1.00	32.59	6
ATOM	623	CB	LEU	A	90	14.110	32.873	39.878	1.00	29.22	6
ATOM	624	CA	LEU	A	90	14.041	31.659	40.828	1.00	22.24	6
ATOM	625	C	LEU	A	90	12.643	31.203	41.002	1.00	19.26	6
ATOM	626	O	LEU	A	90	12.017	30.724	40.038	1.00	20.76	8
ATOM	627	N	TYR	A	91	12.125	31.476	42.174	1.00	17.22	7
ATOM	628	OH	TYR	A	91	12.321	25.105	41.504	1.00	31.21	8
ATOM	629	CD2	TYR	A	91	10.097	27.804	42.484	1.00	24.84	6
ATOM	630	CE2	TYR	A	91	10.565	26.613	41.969	1.00	22.93	6
ATOM	631	CZ	TYR	A	91	11.917	26.318	42.020	1.00	31.94	6
ATOM	632	CE1	TYR	A	91	12.863	27.261	42.476	1.00	23.17	6
ATOM	633	CD1	TYR	A	91	12.382	28.442	43.022	1.00	19.76	6
ATOM	634	CG	TYR	A	91	11.026	28.729	43.006	1.00	22.41	6
ATOM	635	CB	TYR	A	91	10.551	30.077	43.551	1.00	22.69	6
ATOM	636	CA	TYR	A	91	10.755	31.167	42.437	1.00	17.72	6
ATOM	637	C	TYR	A	91	10.023	32.465	42.832	1.00	21.10	6
ATOM	638	O	TYR	A	91	10.483	33.128	43.740	1.00	21.02	8
ATOM	639	N	ALA	A	92	8.955	32.776	42.133	1.00	23.09	7
ATOM	640	CB	ALA	A	92	7.352	34.205	40.926	1.00	14.26	6
ATOM	641	CA	ALA	A	92	8.067	33.911	42.258	1.00	21.27	6
ATOM	642	C	ALA	A	92	7.090	33.619	43.378	1.00	19.16	6
ATOM	643	O	ALA	A	92	6.104	32.928	43.143	1.00	21.07	8
ATOM	644	N	VAL	A	93	7.184	34.197	44.567	1.00	19.51	7
ATOM	645	CG2	VAL	A	93	7.656	32.310	46.567	1.00	21.27	6
ATOM	646	CG1	VAL	A	93	5.678	33.194	47.960	1.00	19.09	6
ATOM	647	CB	VAL	A	93	6.745	33.478	46.928	1.00	18.62	6
ATOM	648	CA	VAL	A	93	6.141	34.036	45.629	1.00	17.35	6

APPENDIX D-continued

3D Structure of Esperase

ATOM	649	C	VAL	A	93	5.534	35.446	45.836	1.00	18.48	6
ATOM	650	O	VAL	A	93	6.166	36.320	46.491	1.00	17.69	8
ATOM	651	N	LYS	A	94	4.359	35.587	45.326	1.00	15.95	7
ATOM	652	NZ	LYS	A	94	0.341	38.732	40.786	1.00	16.98	7
ATOM	653	CE	LYS	A	94	1.380	38.435	41.794	1.00	17.51	6
ATOM	654	CD	LYS	A	94	0.902	38.548	43.246	1.00	18.13	6
ATOM	655	CG	LYS	A	94	1.857	38.317	44.368	1.00	19.09	6
ATOM	656	CB	LYS	A	94	2.668	37.038	44.233	1.00	16.57	6
ATOM	657	CA	LYS	A	94	3.611	36.817	45.392	1.00	21.78	6
ATOM	658	C	LYS	A	94	3.007	36.982	46.792	1.00	25.09	6
ATOM	659	O	LYS	A	94	1.985	36.358	47.139	1.00	21.82	8
ATOM	660	N	VAL	A	95	3.600	37.907	47.568	1.00	20.23	7
ATOM	661	CG2	VAL	A	95	5.283	38.661	50.019	1.00	20.17	6
ATOM	662	CG1	VAL	A	95	4.360	36.294	49.917	1.00	25.66	6
ATOM	663	CB	VAL	A	95	4.009	37.779	49.976	1.00	30.09	6
ATOM	664	CA	VAL	A	95	3.030	38.216	48.885	1.00	21.11	6
ATOM	665	C	VAL	A	95	2.623	39.696	48.987	1.00	24.66	6
ATOM	666	O	VAL	A	95	2.177	40.080	50.064	1.00	23.19	8
ATOM	667	N	LEU	A	96	2.818	40.511	47.962	1.00	23.27	7
ATOM	668	CD2	LEU	A	96	3.997	43.237	50.138	1.00	25.60	6
ATOM	669	CD1	LEU	A	96	5.970	43.494	48.659	1.00	20.15	6
ATOM	670	CG	LEU	A	96	4.751	42.698	48.975	1.00	22.84	6
ATOM	671	CB	LEU	A	96	3.706	42.779	47.891	1.00	20.75	6
ATOM	672	CA	LEU	A	96	2.451	41.918	47.920	1.00	23.08	6
ATOM	673	C	LEU	A	96	1.703	42.036	46.589	1.00	23.01	6
ATOM	674	O	LEU	A	96	2.061	41.403	45.579	1.00	21.24	8
ATOM	675	N	ASP	A	97	0.689	42.897	46.551	1.00	23.27	7
ATOM	676	OD2	ASP	A	97	−2.600	45.183	46.914	1.00	34.41	8
ATOM	677	OD1	ASP	A	97	−0.584	45.765	46.103	1.00	29.86	8
ATOM	678	CG	ASP	A	97	−1.488	44.950	46.240	1.00	30.57	6
ATOM	679	CB	ASP	A	97	−1.555	43.475	45.731	1.00	26.33	6
ATOM	680	CA	ASP	A	97	−0.137	43.056	45.358	1.00	23.04	6
ATOM	681	C	ASP	A	97	0.478	44.050	44.362	1.00	19.75	6
ATOM	682	O	ASP	A	97	1.581	44.509	44.552	1.00	20.60	8
ATOM	683	N	ARG	A	98	−0.293	44.333	43.361	1.00	21.05	7
ATOM	684	NH2	ARG	A	98	−6.414	46.513	41.337	1.00	61.54	7
ATOM	685	NH1	ARG	A	98	−5.383	46.580	39.258	1.00	61.06	7
ATOM	686	CZ	ARG	A	98	−5.345	46.297	40.563	1.00	59.40	6
ATOM	687	NE	ARG	A	98	−4.287	45.797	41.191	1.00	43.41	7
ATOM	688	CD	ARG	A	98	−3.085	45.642	40.374	1.00	30.97	6
ATOM	689	CG	ARG	A	98	−2.099	45.874	41.477	1.00	23.76	6
ATOM	690	CB	ARG	A	98	−0.838	45.175	41.048	1.00	25.56	6
ATOM	691	CA	ARG	A	98	0.109	45.190	42.254	1.00	25.82	6
ATOM	692	C	ARG	A	98	0.420	46.628	42.667	1.00	23.93	6
ATOM	693	O	ARG	A	98	1.088	47.281	41.838	1.00	23.91	8
ATOM	694	N	ASN	A	99	−0.032	46.924	43.851	1.00	23.90	7
ATOM	695	ND2	ASN	A	99	−1.713	49.748	42.838	1.00	28.85	7
ATOM	696	OD1	ASN	A	99	−3.264	48.712	44.128	1.00	39.99	8
ATOM	697	CG	ASN	A	99	−2.098	49.125	43.955	1.00	32.07	6
ATOM	698	CB	ASN	A	99	−1.056	48.862	45.047	1.00	28.96	6
ATOM	699	CA	ASN	A	99	0.209	48.265	44.383	1.00	30.38	6
ATOM	700	C	ASN	A	99	1.392	48.195	45.301	1.00	30.88	6
ATOM	701	O	ASN	A	99	1.809	49.252	45.800	1.00	30.12	8
ATOM	702	N	GLY	A	100	1.910	47.022	45.541	1.00	24.81	7
ATOM	703	CA	GLY	A	100	3.112	46.938	46.388	1.00	21.34	6
ATOM	704	C	GLY	A	100	2.730	46.700	47.825	1.00	26.62	6
ATOM	705	O	GLY	A	100	3.572	46.651	48.719	1.00	30.05	8
ATOM	706	N	SER	A	101	1.455	46.465	47.998	1.00	25.04	7
ATOM	707	OG	SER	A	101	−1.086	47.063	50.195	1.00	52.71	8
ATOM	708	CB	SER	A	101	−0.288	47.078	49.079	1.00	33.36	6
ATOM	709	CA	SER	A	101	1.004	46.287	49.369	1.00	28.75	6
ATOM	710	C	SER	A	101	0.669	44.899	49.843	1.00	37.54	6
ATOM	711	O	SER	A	101	0.182	44.154	49.006	1.00	29.65	8
ATOM	712	N	GLY	A	102	0.852	44.455	51.064	1.00	35.37	7
ATOM	713	CA	GLY	A	102	0.402	43.090	51.473	1.00	42.38	6
ATOM	714	C	GLY	A	102	0.311	43.081	53.009	1.00	41.95	6
ATOM	715	O	GLY	A	102	0.662	44.081	53.674	1.00	51.09	8
ATOM	716	N	SER	A	103	−0.061	42.076	53.725	1.00	30.23	7
ATOM	717	OG	SER	A	103	−1.367	40.088	54.944	1.00	40.84	8
ATOM	718	CB	SER	A	103	−1.220	41.179	55.778	1.00	31.04	6
ATOM	719	CA	SER	A	103	−0.076	41.926	55.156	1.00	29.72	6
ATOM	720	C	SER	A	103	1.057	41.013	55.610	1.00	31.65	6
ATOM	721	O	SER	A	103	1.642	40.294	54.835	1.00	34.54	8
ATOM	722	N	LEU	A	104	1.319	41.101	56.870	1.00	28.22	7
ATOM	723	CD2	LEU	A	104	4.090	42.177	60.461	1.00	51.24	6

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3D Structure of Esperase

ATOM	724	CD1	LEU	A	104	4.621	42.281	58.095	1.00	41.73	6
ATOM	725	CG	LEU	A	104	4.001	41.439	59.150	1.00	39.07	6
ATOM	726	CB	LEU	A	104	2.654	40.887	58.817	1.00	38.11	6
ATOM	727	CA	LEU	A	104	2.397	40.307	57.444	1.00	31.06	6
ATOM	728	C	LEU	A	104	1.894	38.866	57.408	1.00	35.45	6
ATOM	729	O	LEU	A	104	2.809	38.009	57.345	1.00	34.06	8
ATOM	730	N	ALA	A	105	0.578	38.666	57.355	1.00	30.73	7
ATOM	731	CB	ALA	A	105	−1.345	37.170	57.302	1.00	28.85	6
ATOM	732	CA	ALA	A	105	0.171	37.260	57.244	1.00	32.26	6
ATOM	733	C	ALA	A	105	0.492	36.695	55.838	1.00	30.41	6
ATOM	734	O	ALA	A	105	0.790	35.495	55.764	1.00	26.17	8
ATOM	735	N	SER	A	106	0.370	37.484	54.767	1.00	26.36	7
ATOM	736	OG	SER	A	106	0.908	38.945	52.353	1.00	47.12	8
ATOM	737	CB	SER	A	106	0.078	37.776	52.335	1.00	28.12	6
ATOM	738	CA	SER	A	106	0.695	36.929	53.429	1.00	27.72	6
ATOM	739	C	SER	A	106	2.174	36.648	53.385	1.00	24.51	6
ATOM	740	O	SER	A	106	2.586	35.664	52.760	1.00	25.93	8
ATOM	741	N	VAL	A	107	3.021	37.452	54.025	1.00	22.96	7
ATOM	742	CG2	VAL	A	107	5.113	39.633	53.921	1.00	23.30	6
ATOM	743	CG1	VAL	A	107	6.747	37.936	54.918	1.00	22.54	6
ATOM	744	CB	VAL	A	107	5.292	38.352	54.742	1.00	23.47	6
ATOM	745	CA	VAL	A	107	4.467	37.209	54.117	1.00	22.96	6
ATOM	746	C	VAL	A	107	4.792	35.863	54.775	1.00	27.46	6
ATOM	747	O	VAL	A	107	5.638	35.148	54.247	1.00	22.03	8
ATOM	748	N	ALA	A	108	4.152	35.572	55.895	1.00	26.22	7
ATOM	749	CB	ALA	A	108	3.431	34.340	57.872	1.00	22.56	6
ATOM	750	CA	ALA	A	108	4.291	34.320	56.623	1.00	22.04	6
ATOM	751	C	ALA	A	108	3.862	33.098	55.769	1.00	23.82	6
ATOM	752	O	ALA	A	108	4.541	32.073	55.760	1.00	25.45	8
ATOM	753	N	GLN	A	109	2.798	33.159	55.019	1.00	26.10	7
ATOM	754	NE2	GLN	A	109	−1.990	31.648	53.180	1.00	56.51	7
ATOM	755	OE1	GLN	A	109	−1.807	33.819	52.964	1.00	52.89	8
ATOM	756	CD	GLN	A	109	−1.363	32.789	53.524	1.00	52.62	6
ATOM	757	CG	GLN	A	109	−0.163	32.492	54.418	1.00	23.57	6
ATOM	758	CB	GLN	A	109	1.020	32.469	53.458	1.00	19.24	6
ATOM	759	CA	GLN	A	109	2.302	32.153	54.141	1.00	21.53	6
ATOM	760	C	GLN	A	109	3.302	31.924	53.060	1.00	23.82	6
ATOM	761	O	GLN	A	109	3.633	30.801	52.709	1.00	24.29	8
ATOM	762	N	GLY	A	110	3.955	32.956	52.566	1.00	26.56	7
ATOM	763	CA	GLY	A	110	5.010	32.793	51.539	1.00	21.54	6
ATOM	764	C	GLY	A	110	6.193	32.057	52.065	1.00	18.77	6
ATOM	765	O	GLY	A	110	6.890	31.359	51.328	1.00	20.70	8
ATOM	766	N	ILE	A	111	6.506	32.348	53.333	1.00	19.34	7
ATOM	767	CD1	ILE	A	111	8.879	34.550	56.483	1.00	19.97	6
ATOM	768	CG1	ILE	A	111	8.799	33.646	55.221	1.00	25.91	6
ATOM	769	CB	ILE	A	111	8.041	32.300	55.338	1.00	21.06	6
ATOM	770	CG2	ILE	A	111	9.069	31.422	56.004	1.00	19.42	6
ATOM	771	CA	ILE	A	111	7.639	31.695	54.014	1.00	20.08	6
ATOM	772	C	ILE	A	111	7.287	30.164	54.171	1.00	28.01	6
ATOM	773	O	ILE	A	111	8.174	29.356	53.925	1.00	19.72	8
ATOM	774	N	GLU	A	112	6.057	29.853	54.534	1.00	26.23	7
ATOM	775	OE2	GLU	A	112	5.242	26.589	57.599	1.00	55.41	8
ATOM	776	OE1	GLU	A	112	5.307	28.380	59.130	1.00	58.68	8
ATOM	777	CD	GLU	A	112	5.032	27.876	57.981	1.00	57.74	6
ATOM	778	CG	GLU	A	112	4.340	28.653	56.863	1.00	54.59	6
ATOM	779	CB	GLU	A	112	4.264	28.406	55.355	1.00	26.07	6
ATOM	780	CA	GLU	A	112	5.632	28.463	54.721	1.00	26.47	6
ATOM	781	C	GLU	A	112	5.651	27.787	53.384	1.00	24.57	6
ATOM	782	O	GLU	A	112	6.181	26.678	53.335	1.00	27.03	8
ATOM	783	N	TRP	A	113	5.345	28.415	52.295	1.00	20.47	7
ATOM	784	CD2	TRP	A	113	5.939	28.229	47.577	1.00	23.15	6
ATOM	785	CE3	TRP	A	113	7.244	28.726	47.644	1.00	22.83	6
ATOM	786	CZ3	TRP	A	113	8.109	28.544	46.587	1.00	22.30	6
ATOM	787	CH2	TRP	A	113	7.680	27.910	45.424	1.00	22.04	6
ATOM	788	CZ2	TRP	A	113	6.378	27.441	45.332	1.00	20.63	6
ATOM	789	CE2	TRP	A	113	5.543	27.598	46.399	1.00	19.44	6
ATOM	790	NE1	TRP	A	113	4.261	27.215	46.619	1.00	22.83	7
ATOM	791	CD1	TRP	A	113	3.821	27.559	47.869	1.00	19.44	6
ATOM	792	CG	TRP	A	113	4.847	28.192	48.511	1.00	20.85	6
ATOM	793	CB	TRP	A	113	4.744	28.731	49.896	1.00	20.61	6
ATOM	794	CA	TRP	A	113	5.385	27.849	50.973	1.00	18.92	6
ATOM	795	C	TRP	A	113	6.817	27.518	50.681	1.00	22.62	6
ATOM	796	O	TRP	A	113	7.102	26.484	50.055	1.00	23.67	8
ATOM	797	N	ALA	A	114	7.790	28.387	50.988	1.00	23.59	7
ATOM	798	CB	ALA	A	114	10.199	29.314	50.947	1.00	21.64	6

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3D Structure of Esperase

ATOM	799	CA	ALA	A	114	9.208	28.145	50.684	1.00	20.45	6
ATOM	800	C	ALA	A	114	9.720	26.925	51.508	1.00	24.40	6
ATOM	801	O	ALA	A	114	10.656	26.271	51.084	1.00	22.37	8
ATOM	802	N	ILE	A	115	9.263	26.665	52.696	1.00	21.85	7
ATOM	803	CD1	ILE	A	115	8.887	27.137	57.080	1.00	21.98	6
ATOM	804	CG1	ILE	A	115	9.735	26.862	55.832	1.00	22.19	6
ATOM	805	CB	ILE	A	115	9.187	25.725	54.945	1.00	36.67	6
ATOM	806	CG2	ILE	A	115	9.445	24.332	55.597	1.00	26.39	6
ATOM	807	CA	ILE	A	115	9.712	25.557	53.533	1.00	23.50	6
ATOM	808	C	ILE	A	115	9.183	24.244	52.881	1.00	22.01	6
ATOM	809	O	ILE	A	115	9.979	23.385	52.509	1.00	23.68	8
ATOM	810	N	ASN	A	116	7.904	24.294	52.591	1.00	23.13	7
ATOM	811	ND2	ASN	A	116	5.718	22.906	53.985	1.00	35.86	7
ATOM	812	OD1	ASN	A	116	4.028	23.976	53.170	1.00	43.01	8
ATOM	813	CG	ASN	A	116	5.117	23.420	52.940	1.00	31.13	6
ATOM	814	CB	ASN	A	116	5.859	23.287	51.643	1.00	20.42	6
ATOM	815	CA	ASN	A	116	7.327	23.166	51.910	1.00	19.55	6
ATOM	816	C	ASN	A	116	7.917	22.893	50.561	1.00	29.30	6
ATOM	817	O	ASN	A	116	7.758	21.709	50.183	1.00	30.79	8
ATOM	818	N	ASN	A	117	8.452	23.795	49.801	1.00	22.00	7
ATOM	819	ND2	ASN	A	117	6.020	24.758	48.002	1.00	20.38	7
ATOM	820	OD1	ASN	A	117	6.621	23.594	46.231	1.00	25.41	8
ATOM	821	CG	ASN	A	117	6.944	24.266	47.222	1.00	21.80	6
ATOM	822	CB	ASN	A	117	8.400	24.593	47.462	1.00	19.43	6
ATOM	823	CA	ASN	A	117	8.993	23.648	48.467	1.00	18.42	6
ATOM	824	C	ASN	A	117	10.488	23.572	48.529	1.00	16.67	6
ATOM	825	O	ASN	A	117	11.080	23.586	47.448	1.00	23.59	8
ATOM	826	N	ASN	A	118	10.994	23.449	49.770	1.00	24.36	7
ATOM	827	ND2	ASN	A	118	14.257	20.977	49.784	1.00	46.79	7
ATOM	828	OD1	ASN	A	118	11.956	20.616	50.768	1.00	42.51	8
ATOM	829	CG	ASN	A	118	12.926	20.992	50.037	1.00	53.99	6
ATOM	830	CB	ASN	A	118	12.676	22.017	48.931	1.00	40.09	6
ATOM	831	CA	ASN	A	118	12.463	23.293	49.763	1.00	25.20	6
ATOM	832	C	ASN	A	118	13.436	24.264	49.061	1.00	29.14	6
ATOM	833	O	ASN	A	118	14.413	23.816	48.416	1.00	23.06	8
ATOM	834	N	MET	A	119	13.069	25.539	49.345	1.00	24.91	7
ATOM	835	CE	MET	A	119	11.345	26.688	45.875	1.00	25.32	6
ATOM	836	SD	MET	A	119	12.390	28.044	46.482	1.00	24.14	16
ATOM	837	CG	MET	A	119	11.874	27.979	48.232	1.00	19.15	6
ATOM	838	CB	MET	A	119	13.167	27.925	49.032	1.00	21.49	6
ATOM	839	CA	MET	A	119	13.931	26.603	48.812	1.00	20.74	6
ATOM	840	C	MET	A	119	15.198	26.587	49.594	1.00	19.07	6
ATOM	841	O	MET	A	119	15.184	26.188	50.752	1.00	23.02	8
ATOM	842	N	HIS	A	120	16.296	27.065	49.124	1.00	18.99	7
ATOM	843	CD2	HIS	A	120	18.647	24.610	49.083	1.00	30.88	6
ATOM	844	NE2	HIS	A	120	18.706	23.671	48.118	1.00	24.71	7
ATOM	845	CE1	HIS	A	120	18.992	24.314	46.957	1.00	28.15	6
ATOM	846	ND1	HIS	A	120	19.000	25.611	47.103	1.00	29.83	7
ATOM	847	CG	HIS	A	120	18.816	25.840	48.415	1.00	26.54	6
ATOM	848	CB	HIS	A	120	18.805	27.181	49.043	1.00	20.56	6
ATOM	849	CA	HIS	A	120	17.517	27.249	49.902	1.00	19.50	6
ATOM	850	C	HIS	A	120	17.618	28.675	50.536	1.00	24.81	6
ATOM	851	O	HIS	A	120	18.213	28.839	51.568	1.00	18.22	8
ATOM	852	N	ILE	A	121	17.096	29.668	49.807	1.00	20.09	7
ATOM	853	CD1	ILE	A	121	20.650	31.060	48.208	1.00	18.27	6
ATOM	854	CG1	ILE	A	121	19.750	31.034	49.431	1.00	19.89	6
ATOM	855	CB	ILE	A	121	18.384	31.719	49.200	1.00	24.43	6
ATOM	856	CG2	ILE	A	121	18.411	33.247	49.285	1.00	19.92	6
ATOM	857	CA	ILE	A	121	17.296	31.108	50.101	1.00	27.30	6
ATOM	858	C	ILE	A	121	15.996	31.862	49.892	1.00	18.34	6
ATOM	859	O	ILE	A	121	15.345	31.498	48.913	1.00	21.09	8
ATOM	860	N	ILE	A	122	15.641	32.603	50.895	1.00	16.71	7
ATOM	861	CD1	ILE	A	122	11.953	31.536	53.181	1.00	22.89	6
ATOM	862	CG1	ILE	A	122	12.837	31.911	51.979	1.00	24.32	6
ATOM	863	CB	ILE	A	122	13.522	33.267	52.001	1.00	22.36	6
ATOM	864	CG2	ILE	A	122	12.472	34.387	52.058	1.00	22.28	6
ATOM	865	CA	ILE	A	122	14.414	33.410	50.792	1.00	17.89	6
ATOM	866	C	ILE	A	122	14.873	34.891	50.714	1.00	20.53	6
ATOM	867	O	ILE	A	122	15.632	35.335	51.596	1.00	18.10	8
ATOM	868	N	ASN	A	123	14.457	35.638	49.735	1.00	24.14	7
ATOM	869	ND2	ASN	A	123	14.634	39.722	47.933	1.00	17.66	7
ATOM	870	OD1	ASN	A	123	16.741	39.208	47.968	1.00	16.54	8
ATOM	871	CG	ASN	A	123	15.601	38.839	48.002	1.00	18.32	6
ATOM	872	CB	ASN	A	123	15.217	37.352	48.089	1.00	17.61	6
ATOM	873	CA	ASN	A	123	14.771	37.063	49.516	1.00	16.49	6

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3D Structure of Esperase

ATOM	874	C	ASN	A	123	13.519	37.846	49.924	1.00	16.16	6
ATOM	875	O	ASN	A	123	12.473	37.561	49.364	1.00	15.99	8
ATOM	876	N	MET	A	124	13.682	38.631	51.003	1.00	17.91	7
ATOM	877	CE	MET	A	124	12.625	37.065	55.122	1.00	18.43	6
ATOM	878	SD	MET	A	124	10.961	37.279	54.473	1.00	25.22	16
ATOM	879	CG	MET	A	124	11.393	37.747	52.785	1.00	22.98	6
ATOM	880	CB	MET	A	124	12.092	39.072	52.786	1.00	16.34	6
ATOM	881	CA	MET	A	124	12.517	39.473	51.413	1.00	19.71	6
ATOM	882	C	MET	A	124	12.848	40.994	51.279	1.00	22.29	6
ATOM	883	O	MET	A	124	13.425	41.612	52.209	1.00	17.93	8
ATOM	884	N	SER	A	125	12.669	41.567	50.101	1.00	19.47	7
ATOM	885	OG	SER	A	125	14.523	42.940	48.182	1.00	18.33	8
ATOM	886	CB	SER	A	125	13.198	43.275	48.457	1.00	15.97	6
ATOM	887	CA	SER	A	125	12.942	43.032	49.909	1.00	18.46	6
ATOM	888	C	SER	A	125	11.655	43.750	50.350	1.00	20.28	6
ATOM	889	O	SER	A	125	10.902	44.316	49.570	1.00	19.39	8
ATOM	890	N	LEU	A	126	11.297	43.695	51.624	1.00	17.62	7
ATOM	891	CD2	LEU	A	126	8.102	40.862	51.658	1.00	24.63	6
ATOM	892	CD1	LEU	A	126	8.622	41.714	53.877	1.00	23.93	6
ATOM	893	CG	LEU	A	126	8.997	41.757	52.422	1.00	25.53	6
ATOM	894	CB	LEU	A	126	8.916	43.187	51.871	1.00	28.42	6
ATOM	895	CA	LEU	A	126	10.051	44.199	52.184	1.00	26.68	6
ATOM	896	C	LEU	A	126	10.270	44.487	53.671	1.00	21.12	6
ATOM	897	O	LEU	A	126	11.254	44.020	54.240	1.00	20.64	8
ATOM	898	N	GLY	A	127	9.505	45.329	54.335	1.00	22.92	7
ATOM	899	CA	GLY	A	127	9.794	45.637	55.735	1.00	23.96	6
ATOM	900	C	GLY	A	127	8.602	46.346	56.347	1.00	29.15	6
ATOM	901	O	GLY	A	127	7.718	46.926	55.745	1.00	30.52	8
ATOM	902	N	SER	A	128	8.499	46.244	57.635	1.00	22.96	7
ATOM	903	OG	SER	A	128	5.648	45.725	59.563	1.00	44.80	8
ATOM	904	CB	SER	A	128	6.579	45.564	58.544	1.00	31.06	6
ATOM	905	CA	SER	A	128	7.422	46.809	58.423	1.00	26.75	6
ATOM	906	C	SER	A	128	8.089	47.306	59.704	1.00	29.54	6
ATOM	907	O	SER	A	128	9.118	46.792	60.156	1.00	25.89	8
ATOM	908	N	THR	A	129	7.438	48.299	60.299	1.00	33.31	7
ATOM	909	CG2	THR	A	129	7.743	51.258	60.493	1.00	30.94	6
ATOM	910	OG1	THR	A	129	6.191	50.069	61.840	1.00	40.54	8
ATOM	911	CB	THR	A	129	7.555	50.360	61.680	1.00	32.41	6
ATOM	912	CA	THR	A	129	8.018	48.915	61.506	1.00	32.74	6
ATOM	913	C	THR	A	129	7.714	48.005	62.673	1.00	32.62	6
ATOM	914	O	THR	A	129	8.427	48.117	63.667	1.00	36.81	8
ATOM	915	N	SER	A	130	6.757	47.138	62.480	1.00	30.40	7
ATOM	916	OG	SER	A	130	4.251	46.613	62.921	1.00	60.10	8
ATOM	917	CB	SER	A	130	5.130	46.585	64.070	1.00	57.43	6
ATOM	918	CA	SER	A	130	6.491	46.151	63.545	1.00	33.34	6
ATOM	919	C	SER	A	130	6.372	44.754	62.914	1.00	41.55	6
ATOM	920	O	SER	A	130	6.086	44.558	61.706	1.00	40.64	8
ATOM	921	N	GLY	A	131	6.541	43.773	63.782	1.00	36.39	7
ATOM	922	CA	GLY	A	131	6.503	42.373	63.329	1.00	34.64	6
ATOM	923	C	GLY	A	131	5.234	41.724	63.822	1.00	35.04	6
ATOM	924	O	GLY	A	131	4.273	42.468	64.031	1.00	42.75	8
ATOM	925	N	SER	A	132	5.179	40.422	63.893	1.00	37.30	7
ATOM	926	OG	SER	A	132	3.196	38.497	62.627	1.00	39.73	8
ATOM	927	CB	SER	A	132	2.876	39.643	63.376	1.00	35.53	6
ATOM	928	CA	SER	A	132	3.986	39.723	64.382	1.00	30.05	6
ATOM	929	C	SER	A	132	4.556	38.374	64.813	1.00	31.11	6
ATOM	930	O	SER	A	132	5.572	37.836	64.411	1.00	32.00	8
ATOM	931	N	SER	A	133	3.842	37.734	65.695	1.00	32.96	7
ATOM	932	OG	SER	A	133	2.307	36.218	67.376	1.00	54.62	8
ATOM	933	CB	SER	A	133	3.700	36.342	67.576	1.00	47.70	6
ATOM	934	CA	SER	A	133	4.331	36.440	66.195	1.00	35.90	6
ATOM	935	C	SER	A	133	4.149	35.380	65.111	1.00	39.43	6
ATOM	936	O	SER	A	133	4.847	34.366	65.010	1.00	33.00	8
ATOM	937	N	THR	A	134	3.180	35.667	64.251	1.00	37.16	7
ATOM	938	CG2	THR	A	134	1.470	34.464	61.014	1.00	42.89	6
ATOM	939	OG1	THR	A	134	0.694	35.406	63.113	1.00	55.08	8
ATOM	940	CB	THR	A	134	1.813	35.282	62.246	1.00	54.29	6
ATOM	941	CA	THR	A	134	2.940	34.724	63.144	1.00	39.11	6
ATOM	942	C	THR	A	134	4.213	34.729	62.288	1.00	34.90	6
ATOM	943	O	THR	A	134	4.693	33.638	61.945	1.00	31.77	8
ATOM	944	N	LEU	A	135	4.600	35.994	62.058	1.00	30.88	7
ATOM	945	CD2	LEU	A	135	7.189	39.568	59.758	1.00	28.02	6
ATOM	946	CD1	LEU	A	135	7.086	37.378	58.627	1.00	30.72	6
ATOM	947	CG	LEU	A	135	7.166	38.073	59.953	1.00	28.29	6
ATOM	948	CB	LEU	A	135	5.946	37.672	60.799	1.00	30.19	6

APPENDIX D-continued

3D Structure of Esperase

ATOM	949	CA	LEU	A	135	5.796	36.201	61.203	1.00	29.37	6
ATOM	950	C	LEU	A	135	7.077	35.635	61.777	1.00	27.29	6
ATOM	951	O	LEU	A	135	7.958	35.025	61.154	1.00	28.89	8
ATOM	952	N	GLU	A	136	7.230	35.860	63.081	1.00	28.97	7
ATOM	953	OE2	GLU	A	136	8.946	36.631	67.630	1.00	43.17	8
ATOM	954	OE1	GLU	A	136	11.229	36.190	67.562	1.00	56.47	8
ATOM	955	CD	GLU	A	136	10.073	36.210	67.125	1.00	51.60	6
ATOM	956	CG	GLU	A	136	9.871	35.664	65.729	1.00	37.60	6
ATOM	957	CB	GLU	A	136	8.518	35.957	65.118	1.00	30.71	6
ATOM	958	CA	GLU	A	136	8.494	35.399	63.696	1.00	25.88	6
ATOM	959	C	GLU	A	136	8.483	33.887	63.747	1.00	25.04	6
ATOM	960	O	GLU	A	136	9.527	33.244	63.636	1.00	29.33	8
ATOM	961	N	LEU	A	137	7.287	33.373	64.028	1.00	26.02	7
ATOM	962	CD2	LEU	A	137	4.017	29.845	64.773	1.00	54.85	6
ATOM	963	CD1	LEU	A	137	6.341	29.084	65.226	1.00	54.59	6
ATOM	964	CG	LEU	A	137	5.487	30.026	64.399	1.00	53.18	6
ATOM	965	CB	LEU	A	137	5.909	31.481	64.461	1.00	42.31	6
ATOM	966	CA	LEU	A	137	7.330	31.886	64.051	1.00	29.41	6
ATOM	967	C	LEU	A	137	7.745	31.326	62.696	1.00	31.98	6
ATOM	968	O	LEU	A	137	8.491	30.301	62.593	1.00	32.69	8
ATOM	969	N	ALA	A	138	7.170	31.984	61.676	1.00	27.96	7
ATOM	970	CB	ALA	A	138	6.457	32.131	59.296	1.00	25.09	6
ATOM	971	CA	ALA	A	138	7.450	31.547	60.295	1.00	24.67	6
ATOM	972	C	ALA	A	138	8.946	31.641	60.041	1.00	26.68	6
ATOM	973	O	ALA	A	138	9.651	30.736	59.482	1.00	24.43	8
ATOM	974	N	VAL	A	139	9.509	32.777	60.481	1.00	24.43	7
ATOM	975	CG2	VAL	A	139	10.805	35.468	59.644	1.00	23.18	6
ATOM	976	CG1	VAL	A	139	12.736	34.458	60.955	1.00	25.26	6
ATOM	977	CB	VAL	A	139	11.240	34.427	60.639	1.00	23.72	6
ATOM	978	CA	VAL	A	139	10.946	32.963	60.179	1.00	24.64	6
ATOM	979	C	VAL	A	139	11.785	31.875	60.847	1.00	22.27	6
ATOM	980	O	VAL	A	139	12.734	31.316	60.296	1.00	24.72	8
ATOM	981	N	ASN	A	140	11.486	31.593	62.118	1.00	27.66	7
ATOM	982	ND2	ASN	A	140	11.683	32.285	66.008	1.00	42.32	7
ATOM	983	OD1	ASN	A	140	13.425	32.414	64.611	1.00	36.78	8
ATOM	984	CG	ASN	A	140	12.388	31.851	64.974	1.00	40.71	6
ATOM	985	CB	ASN	A	140	11.762	30.648	64.308	1.00	38.24	6
ATOM	986	CA	ASN	A	140	12.215	30.570	62.870	1.00	28.09	6
ATOM	987	C	ASN	A	140	12.048	29.142	62.314	1.00	23.74	6
ATOM	988	O	ASN	A	140	13.079	28.438	62.234	1.00	27.56	8
ATOM	989	N	ARG	A	141	10.819	28.818	61.934	1.00	29.30	7
ATOM	990	NH2	ARG	A	141	6.667	24.020	60.976	1.00	62.35	7
ATOM	991	NH1	ARG	A	141	7.366	25.245	59.341	1.00	62.64	7
ATOM	992	CZ	ARG	A	141	6.619	25.314	60.452	1.00	61.47	6
ATOM	993	NE	ARG	A	141	6.129	26.266	61.285	1.00	59.61	7
ATOM	994	CD	ARG	A	141	6.849	27.392	61.861	1.00	48.63	6
ATOM	995	CG	ARG	A	141	8.296	26.951	62.044	1.00	33.19	6
ATOM	996	CB	ARG	A	141	9.203	27.214	60.872	1.00	26.71	6
ATOM	997	CA	ARG	A	141	10.629	27.489	61.338	1.00	24.61	6
ATOM	998	C	ARG	A	141	11.475	27.428	60.116	1.00	28.36	6
ATOM	999	O	ARG	A	141	12.111	26.409	59.919	1.00	30.57	8
ATOM	1000	N	ALA	A	142	11.510	28.420	59.220	1.00	28.76	7
ATOM	1001	CB	ALA	A	142	12.125	29.617	57.121	1.00	22.79	6
ATOM	1002	CA	ALA	A	142	12.326	28.336	57.992	1.00	22.45	6
ATOM	1003	C	ALA	A	142	13.799	28.193	58.312	1.00	23.46	6
ATOM	1004	O	ALA	A	142	14.580	27.473	57.674	1.00	26.21	8
ATOM	1005	N	ASN	A	143	14.220	28.995	59.297	1.00	27.87	7
ATOM	1006	ND2	ASN	A	143	17.784	30.625	61.839	1.00	41.96	7
ATOM	1007	OD1	ASN	A	143	18.187	30.679	59.745	1.00	34.24	8
ATOM	1008	CG	ASN	A	143	17.322	30.588	60.596	1.00	29.11	6
ATOM	1009	CB	ASN	A	143	15.871	30.329	60.523	1.00	29.69	6
ATOM	1010	CA	ASN	A	143	15.635	29.021	59.743	1.00	30.16	6
ATOM	1011	C	ASN	A	143	15.953	27.666	60.335	1.00	30.12	6
ATOM	1012	O	ASN	A	143	17.010	27.136	59.946	1.00	31.87	8
ATOM	1013	N	ASN	A	144	15.008	27.125	61.112	1.00	29.34	7
ATOM	1014	ND2	ASN	A	144	15.977	26.890	64.048	1.00	45.86	7
ATOM	1015	OD1	ASN	A	144	13.874	26.581	64.829	1.00	57.91	8
ATOM	1016	CG	ASN	A	144	14.771	26.309	63.974	1.00	57.11	6
ATOM	1017	CB	ASN	A	144	14.450	25.359	62.806	1.00	44.00	6
ATOM	1018	CA	ASN	A	144	15.299	25.781	61.618	1.00	29.75	6
ATOM	1019	C	ASN	A	144	15.282	24.762	60.497	1.00	40.41	6
ATOM	1020	O	ASN	A	144	15.968	23.716	60.573	1.00	42.44	8
ATOM	1021	N	ALA	A	145	14.528	25.050	59.457	1.00	34.04	7
ATOM	1022	CB	ALA	A	145	13.330	24.281	57.390	1.00	26.85	6
ATOM	1023	CA	ALA	A	145	14.483	24.121	58.327	1.00	20.42	6

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3D Structure of Esperase

ATOM	1024	C	ALA	A	145	15.731	24.288	57.552	1.00	23.85	6
ATOM	1025	O	ALA	A	145	15.664	23.663	56.514	1.00	30.91	8
ATOM	1026	N	GLY	A	146	16.740	25.040	57.840	1.00	26.51	7
ATOM	1027	CA	GLY	A	146	17.921	25.100	56.958	1.00	22.88	6
ATOM	1028	C	GLY	A	146	17.767	26.214	55.904	1.00	27.41	6
ATOM	1029	O	GLY	A	146	18.735	26.130	55.122	1.00	24.39	8
ATOM	1030	N	ILE	A	147	16.707	27.049	55.889	1.00	21.34	7
ATOM	1031	CD1	ILE	A	147	13.320	27.096	53.722	1.00	23.01	6
ATOM	1032	CG1	ILE	A	147	14.789	27.060	54.041	1.00	22.99	6
ATOM	1033	CB	ILE	A	147	15.321	28.439	54.332	1.00	26.62	6
ATOM	1034	CG2	ILE	A	147	15.232	29.384	53.135	1.00	23.14	6
ATOM	1035	CA	ILE	A	147	16.730	28.111	54.845	1.00	26.00	6
ATOM	1036	C	ILE	A	147	17.500	29.398	55.235	1.00	18.99	6
ATOM	1037	O	ILE	A	147	17.385	29.727	56.411	1.00	20.20	8
ATOM	1038	N	LEU	A	148	18.230	30.007	54.320	1.00	20.50	7
ATOM	1039	CD2	LEU	A	148	21.996	32.963	53.094	1.00	21.59	6
ATOM	1040	CD1	LEU	A	148	21.187	32.871	55.483	1.00	21.52	6
ATOM	1041	CG	LEU	A	148	20.849	32.729	54.004	1.00	21.01	6
ATOM	1042	CB	LEU	A	148	20.076	31.416	53.699	1.00	21.28	6
ATOM	1043	CA	LEU	A	148	18.874	31.288	54.622	1.00	18.16	6
ATOM	1044	C	LEU	A	148	17.890	32.403	54.204	1.00	21.69	6
ATOM	1045	O	LEU	A	148	17.385	32.443	53.053	1.00	18.87	8
ATOM	1046	N	LEU	A	149	17.504	33.244	55.115	1.00	19.79	7
ATOM	1047	CD2	LEU	A	149	13.039	33.698	56.361	1.00	21.21	6
ATOM	1048	CD1	LEU	A	149	14.937	32.303	57.044	1.00	29.79	6
ATOM	1049	CG	LEU	A	149	14.430	33.273	55.986	1.00	23.63	6
ATOM	1050	CB	LEU	A	149	15.412	34.443	55.914	1.00	19.13	6
ATOM	1051	CA	LEU	A	149	16.580	34.382	54.989	1.00	18.47	6
ATOM	1052	C	LEU	A	149	17.403	35.669	54.993	1.00	22.25	6
ATOM	1053	O	LEU	A	149	18.294	35.913	55.802	1.00	19.26	8
ATOM	1054	N	VAL	A	150	17.140	36.501	53.974	1.00	21.30	7
ATOM	1055	CG2	VAL	A	150	19.747	36.476	52.518	1.00	19.59	6
ATOM	1056	CG1	VAL	A	150	19.570	38.785	52.177	1.00	22.93	6
ATOM	1057	CB	VAL	A	150	18.710	37.578	52.402	1.00	20.01	6
ATOM	1058	CA	VAL	A	150	17.846	37.764	53.660	1.00	20.55	6
ATOM	1059	C	VAL	A	150	16.751	38.844	53.547	1.00	18.11	6
ATOM	1060	O	VAL	A	150	15.817	38.657	52.756	1.00	18.48	8
ATOM	1061	N	GLY	A	151	16.896	39.886	54.338	1.00	16.89	7
ATOM	1062	CA	GLY	A	151	15.849	40.980	54.289	1.00	20.73	6
ATOM	1063	C	GLY	A	151	16.402	42.404	54.347	1.00	16.63	6
ATOM	1064	O	GLY	A	151	17.563	42.678	54.734	1.00	16.14	8
ATOM	1065	N	ALA	A	152	15.614	43.322	53.807	1.00	17.20	7
ATOM	1066	CB	ALA	A	152	14.900	45.297	52.755	1.00	14.94	6
ATOM	1067	CA	ALA	A	152	15.998	44.737	53.682	1.00	14.71	6
ATOM	1068	C	ALA	A	152	15.895	45.381	55.071	1.00	13.99	6
ATOM	1069	O	ALA	A	152	14.892	45.173	55.788	1.00	17.68	8
ATOM	1070	N	ALA	A	153	16.952	46.133	55.387	1.00	16.31	7
ATOM	1071	CB	ALA	A	153	18.293	47.552	56.901	1.00	17.15	6
ATOM	1072	CA	ALA	A	153	16.956	46.875	56.681	1.00	16.19	6
ATOM	1073	C	ALA	A	153	15.860	47.945	56.800	1.00	22.55	6
ATOM	1074	O	ALA	A	153	15.313	48.113	57.913	1.00	22.09	8
ATOM	1075	N	GLY	A	154	15.484	48.543	55.690	1.00	16.09	7
ATOM	1076	CA	GLY	A	154	14.427	49.555	55.683	1.00	18.21	6
ATOM	1077	C	GLY	A	154	15.049	50.809	55.066	1.00	14.46	6
ATOM	1078	O	GLY	A	154	16.263	50.930	54.899	1.00	16.40	8
ATOM	1079	N	ASN	A	155	14.113	51.674	54.663	1.00	20.62	7
ATOM	1080	ND2	ASN	A	155	13.511	51.960	50.428	1.00	16.52	7
ATOM	1081	OD1	ASN	A	155	15.360	51.538	51.718	1.00	19.81	8
ATOM	1082	CG	ASN	A	155	14.233	52.033	51.537	1.00	17.87	6
ATOM	1083	CB	ASN	A	155	13.765	52.902	52.677	1.00	18.24	6
ATOM	1084	CA	ASN	A	155	14.551	52.936	53.989	1.00	17.90	6
ATOM	1085	C	ASN	A	155	14.159	54.123	54.891	1.00	24.83	6
ATOM	1086	O	ASN	A	155	13.733	55.098	54.292	1.00	22.47	8
ATOM	1087	N	THR	A	156	14.154	53.978	56.193	1.00	20.39	7
ATOM	1088	CG2	THR	A	156	12.287	53.113	58.276	1.00	23.08	6
ATOM	1089	OG1	THR	A	156	14.307	54.076	59.118	1.00	23.01	8
ATOM	1090	CB	THR	A	156	13.124	54.367	58.402	1.00	23.69	6
ATOM	1091	CA	THR	A	156	13.714	54.997	57.116	1.00	24.79	6
ATOM	1092	C	THR	A	156	14.848	56.011	57.320	1.00	29.93	6
ATOM	1093	O	THR	A	156	14.402	57.042	57.813	1.00	27.99	8
ATOM	1094	N	GLY	A	157	16.086	55.856	57.005	1.00	20.16	7
ATOM	1095	CA	GLY	A	157	17.154	56.785	57.245	1.00	25.10	6
ATOM	1096	C	GLY	A	157	17.486	57.000	58.723	1.00	29.14	6
ATOM	1097	O	GLY	A	157	18.377	57.810	58.961	1.00	33.04	8
ATOM	1098	N	ARG	A	160	16.904	56.334	59.657	1.00	25.62	7

APPENDIX D-continued

3D Structure of Esperase										
ATOM	1099	NH2	ARG	A	160	10.330	58.682	62.645	1.00	60.34	7
ATOM	1100	NH1	ARG	A	160	12.170	59.527	63.732	1.00	59.53	7
ATOM	1101	CZ	ARG	A	160	11.711	58.643	62.754	1.00	59.28	6
ATOM	1102	NE	ARG	A	160	12.583	57.864	61.970	1.00	57.95	7
ATOM	1103	CD	ARG	A	160	13.994	58.266	62.165	1.00	51.11	6
ATOM	1104	CG	ARG	A	160	15.060	57.898	61.220	1.00	42.14	6
ATOM	1105	CB	ARG	A	160	15.570	56.502	61.634	1.00	31.02	6
ATOM	1106	CA	ARG	A	160	17.041	56.392	61.112	1.00	28.11	6
ATOM	1107	C	ARG	A	160	17.381	55.048	61.710	1.00	30.03	6
ATOM	1108	O	ARG	A	160	17.398	54.049	60.983	1.00	27.84	8
ATOM	1109	N	GLN	A	161	17.535	55.017	63.000	1.00	26.77	7
ATOM	1110	NE2	GLN	A	161	19.350	52.013	67.864	1.00	60.74	7
ATOM	1111	OE1	GLN	A	161	20.262	53.735	66.798	1.00	59.57	8
ATOM	1112	CD	GLN	A	161	19.355	52.904	66.883	1.00	58.69	6
ATOM	1113	CG	GLN	A	161	18.232	52.759	65.891	1.00	34.77	6
ATOM	1114	CB	GLN	A	161	18.519	53.945	64.970	1.00	30.48	6
ATOM	1115	CA	GLN	A	161	17.801	53.757	63.664	1.00	23.33	6
ATOM	1116	C	GLN	A	161	16.520	52.971	63.833	1.00	29.67	6
ATOM	1117	O	GLN	A	161	15.474	53.589	63.955	1.00	29.09	8
ATOM	1118	N	GLY	A	162	16.517	51.663	63.859	1.00	24.53	7
ATOM	1119	CA	GLY	A	162	15.351	50.793	64.031	1.00	20.53	6
ATOM	1120	C	GLY	A	162	15.104	49.941	62.796	1.00	26.19	6
ATOM	1121	O	GLY	A	162	14.288	50.249	61.907	1.00	22.33	8
ATOM	1122	N	VAL	A	165	15.844	48.832	62.774	1.00	25.37	7
ATOM	1123	CG2	VAL	A	165	18.242	47.376	61.823	1.00	20.11	6
ATOM	1124	CG1	VAL	A	165	16.767	45.785	60.528	1.00	21.35	6
ATOM	1125	CB	VAL	A	165	16.841	46.808	61.703	1.00	22.43	6
ATOM	1126	CA	VAL	A	165	15.776	47.891	61.618	1.00	20.88	6
ATOM	1127	C	VAL	A	165	14.383	47.384	61.360	1.00	24.44	6
ATOM	1128	O	VAL	A	165	13.793	46.948	62.359	1.00	22.51	8
ATOM	1129	N	ASN	A	166	13.847	47.458	60.151	1.00	20.59	7
ATOM	1130	ND2	ASN	A	166	11.804	49.622	59.063	1.00	37.01	7
ATOM	1131	OD1	ASN	A	166	11.291	48.862	57.045	1.00	40.47	8
ATOM	1132	CG	ASN	A	166	11.691	48.612	58.213	1.00	36.75	6
ATOM	1133	CB	ASN	A	166	12.084	47.201	58.564	1.00	18.42	6
ATOM	1134	CA	ASN	A	166	12.480	46.925	60.012	1.00	20.41	6
ATOM	1135	C	ASN	A	166	12.430	45.397	60.220	1.00	27.19	6
ATOM	1136	O	ASN	A	166	13.394	44.641	60.213	1.00	20.29	8
ATOM	1137	N	TYR	A	167	11.219	44.939	60.323	1.00	23.03	7
ATOM	1138	OH	TYR	A	167	10.922	44.540	66.485	1.00	45.50	8
ATOM	1139	CD2	TYR	A	167	9.715	44.838	63.205	1.00	34.30	6
ATOM	1140	CE2	TYR	A	167	10.084	45.141	64.501	1.00	27.99	6
ATOM	1141	CZ	TYR	A	167	10.625	44.092	65.233	1.00	48.07	6
ATOM	1142	CE1	TYR	A	167	10.871	42.802	64.754	1.00	30.09	6
ATOM	1143	CD1	TYR	A	167	10.582	42.588	63.401	1.00	27.59	6
ATOM	1144	CG	TYR	A	167	9.959	43.576	62.657	1.00	30.84	6
ATOM	1145	CB	TYR	A	167	9.537	43.461	61.197	1.00	25.40	6
ATOM	1146	CA	TYR	A	167	10.830	43.562	60.383	1.00	22.25	6
ATOM	1147	C	TYR	A	167	10.479	43.048	58.968	1.00	26.82	6
ATOM	1148	O	TYR	A	167	9.785	43.740	58.230	1.00	28.17	8
ATOM	1149	N	PRO	A	168	10.803	41.830	58.559	1.00	24.12	7
ATOM	1150	CG	PRO	A	168	11.069	39.952	57.192	1.00	21.03	6
ATOM	1151	CD	PRO	A	168	10.376	41.337	57.220	1.00	18.66	6
ATOM	1152	CB	PRO	A	168	11.014	39.509	58.639	1.00	20.70	6
ATOM	1153	CA	PRO	A	168	11.468	40.788	59.357	1.00	21.08	6
ATOM	1154	C	PRO	A	168	12.960	40.862	59.456	1.00	22.02	6
ATOM	1155	O	PRO	A	168	13.492	39.981	60.180	1.00	21.94	8
ATOM	1156	N	ALA	A	169	13.657	41.831	58.841	1.00	15.90	7
ATOM	1157	CB	ALA	A	169	15.736	42.908	58.091	1.00	17.37	6
ATOM	1158	CA	ALA	A	169	15.106	41.851	58.949	1.00	15.97	6
ATOM	1159	C	ALA	A	169	15.607	41.947	60.374	1.00	21.06	6
ATOM	1160	O	ALA	A	169	16.752	41.565	60.663	1.00	21.07	8
ATOM	1161	N	ARG	A	170	14.833	42.498	61.289	1.00	21.46	7
ATOM	1162	NH2	ARG	A	170	13.387	47.123	67.747	1.00	60.78	7
ATOM	1163	NH1	ARG	A	170	13.043	47.610	65.444	1.00	49.63	7
ATOM	1164	CZ	ARG	A	170	13.604	46.896	66.440	1.00	59.33	6
ATOM	1165	NE	ARG	A	170	14.377	45.776	66.226	1.00	56.52	7
ATOM	1166	CD	ARG	A	170	14.143	45.240	64.921	1.00	35.28	6
ATOM	1167	CG	ARG	A	170	15.134	44.173	64.633	1.00	26.86	6
ATOM	1168	CB	ARG	A	170	14.382	43.573	63.430	1.00	22.20	6
ATOM	1169	CA	ARG	A	170	15.339	42.683	62.653	1.00	22.58	6
ATOM	1170	C	ARG	A	170	15.423	41.335	63.390	1.00	26.44	6
ATOM	1171	O	ARG	A	170	16.298	41.175	64.268	1.00	24.76	8
ATOM	1172	N	TYR	A	171	14.601	40.421	63.006	1.00	21.88	7
ATOM	1173	OH	TYR	A	171	8.238	39.587	63.993	1.00	30.27	8

APPENDIX D-continued

3D Structure of Esperase

ATOM	1174	CD2	TYR	A	171	11.260	38.551	62.366	1.00	23.79	6
ATOM	1175	CE2	TYR	A	171	9.930	38.895	62.534	1.00	26.08	6
ATOM	1176	CZ	TYR	A	171	9.544	39.258	63.827	1.00	22.66	6
ATOM	1177	CE1	TYR	A	171	10.437	39.256	64.849	1.00	22.42	6
ATOM	1178	CD1	TYR	A	171	11.754	38.908	64.657	1.00	25.00	6
ATOM	1179	CG	TYR	A	171	12.190	38.520	63.397	1.00	20.82	6
ATOM	1180	CB	TYR	A	171	13.614	38.157	63.120	1.00	20.76	6
ATOM	1181	CA	TYR	A	171	14.662	39.115	63.666	1.00	21.12	6
ATOM	1182	C	TYR	A	171	16.019	38.496	63.429	1.00	23.66	6
ATOM	1183	O	TYR	A	171	16.595	38.612	62.377	1.00	19.53	8
ATOM	1184	N	SER	A	172	16.590	37.805	64.409	1.00	21.25	7
ATOM	1185	OG	SER	A	172	18.439	36.736	66.290	1.00	40.47	8
ATOM	1186	CB	SER	A	172	17.643	36.002	65.430	1.00	30.04	6
ATOM	1187	CA	SER	A	172	17.855	37.114	64.343	1.00	18.01	6
ATOM	1188	C	SER	A	172	17.972	36.098	63.241	1.00	18.07	6
ATOM	1189	O	SER	A	172	19.076	35.857	62.794	1.00	24.44	8
ATOM	1190	N	GLY	A	173	16.849	35.497	62.953	1.00	20.49	7
ATOM	1191	CA	GLY	A	173	16.895	34.520	61.849	1.00	25.76	6
ATOM	1192	C	GLY	A	173	17.065	35.136	60.466	1.00	28.21	6
ATOM	1193	O	GLY	A	173	17.142	34.299	59.561	1.00	24.65	8
ATOM	1194	N	VAL	A	174	17.037	36.454	60.298	1.00	22.37	7
ATOM	1195	CG2	VAL	A	174	14.544	37.187	59.094	1.00	21.22	6
ATOM	1196	CG1	VAL	A	174	15.868	38.353	57.251	1.00	16.19	6
ATOM	1197	CB	VAL	A	174	15.853	37.860	58.711	1.00	19.69	6
ATOM	1198	CA	VAL	A	174	17.081	37.002	58.950	1.00	18.39	6
ATOM	1199	C	VAL	A	174	18.302	37.804	58.794	1.00	20.62	6
ATOM	1200	O	VAL	A	174	18.537	38.493	59.767	1.00	20.79	8
ATOM	1201	N	MET	A	175	19.071	37.843	57.763	1.00	19.50	7
ATOM	1202	CE	MET	A	175	24.752	37.650	55.722	1.00	18.78	6
ATOM	1203	SD	MET	A	175	23.178	36.899	55.344	1.00	28.69	16
ATOM	1204	CG	MET	A	175	22.276	37.473	56.764	1.00	30.99	6
ATOM	1205	CB	MET	A	175	21.073	38.209	56.458	1.00	18.24	6
ATOM	1206	CA	MET	A	175	20.269	38.719	57.673	1.00	19.14	6
ATOM	1207	C	MET	A	175	19.808	40.085	57.181	1.00	19.60	6
ATOM	1208	O	MET	A	175	19.243	40.124	56.075	1.00	19.68	8
ATOM	1209	N	ALA	A	176	19.998	41.141	57.911	1.00	20.50	7
ATOM	1210	CB	ALA	A	176	19.374	43.342	58.912	1.00	17.16	6
ATOM	1211	CA	ALA	A	176	19.559	42.523	57.638	1.00	18.51	6
ATOM	1212	C	ALA	A	176	20.608	43.146	56.758	1.00	15.58	6
ATOM	1213	O	ALA	A	176	21.802	43.226	57.028	1.00	18.20	8
ATOM	1214	N	VAL	A	177	20.119	43.546	55.592	1.00	17.19	7
ATOM	1215	CG2	VAL	A	177	20.837	41.990	53.335	1.00	16.29	6
ATOM	1216	CG1	VAL	A	177	21.783	44.025	52.249	1.00	12.13	6
ATOM	1217	CB	VAL	A	177	20.739	43.505	53.233	1.00	14.48	6
ATOM	1218	CA	VAL	A	177	21.011	44.188	54.618	1.00	18.22	6
ATOM	1219	C	VAL	A	177	20.828	45.734	54.489	1.00	19.81	6
ATOM	1220	O	VAL	A	177	19.728	46.259	54.253	1.00	16.83	8
ATOM	1221	N	ALA	A	178	21.957	46.444	54.565	1.00	17.27	7
ATOM	1222	CB	ALA	A	178	23.054	48.386	55.452	1.00	14.79	6
ATOM	1223	CA	ALA	A	178	22.035	47.894	54.418	1.00	18.82	6
ATOM	1224	C	ALA	A	178	22.445	48.215	52.970	1.00	17.05	6
ATOM	1225	O	ALA	A	178	23.095	47.447	52.260	1.00	16.34	8
ATOM	1226	N	ALA	A	179	22.014	49.381	52.483	1.00	18.05	7
ATOM	1227	CB	ALA	A	179	21.168	50.710	50.548	1.00	14.34	6
ATOM	1228	CA	ALA	A	179	22.317	49.940	51.148	1.00	17.14	6
ATOM	1229	C	ALA	A	179	23.496	50.901	51.162	1.00	16.16	6
ATOM	1230	O	ALA	A	179	23.525	51.777	52.044	1.00	18.65	8
ATOM	1231	N	VAL	A	180	24.451	50.812	50.317	1.00	14.26	7
ATOM	1232	CG2	VAL	A	180	27.438	49.981	49.469	1.00	17.76	6
ATOM	1233	CG1	VAL	A	180	26.913	50.487	51.890	1.00	16.51	6
ATOM	1234	CB	VAL	A	180	26.964	50.989	50.462	1.00	17.24	6
ATOM	1235	CA	VAL	A	180	25.609	51.616	50.075	1.00	17.31	6
ATOM	1236	C	VAL	A	180	25.586	52.187	48.644	1.00	22.88	6
ATOM	1237	O	VAL	A	180	24.947	51.671	47.675	1.00	20.50	8
ATOM	1238	N	ASP	A	181	26.291	53.321	48.446	1.00	25.29	7
ATOM	1239	OD2	ASP	A	181	27.098	57.308	48.607	1.00	32.67	8
ATOM	1240	OD1	ASP	A	181	28.547	55.806	48.232	1.00	27.26	8
ATOM	1241	CG	ASP	A	181	27.399	56.184	48.028	1.00	26.15	6
ATOM	1242	CB	ASP	A	181	26.254	55.570	47.285	1.00	24.05	6
ATOM	1243	CA	ASP	A	181	26.408	54.054	47.131	1.00	22.55	6
ATOM	1244	C	ASP	A	181	27.687	53.624	46.461	1.00	28.00	6
ATOM	1245	O	ASP	A	181	28.393	52.695	46.923	1.00	23.27	8
ATOM	1246	N	GLN	A	182	28.038	54.220	45.348	1.00	23.99	7
ATOM	1247	NE2	GLN	A	182	28.625	56.347	43.392	1.00	59.18	7
ATOM	1248	OE1	GLN	A	182	26.424	55.625	43.700	1.00	51.62	8

APPENDIX D-continued

3D Structure of Esperase

ATOM	1249	CD	GLN	A	182	27.579	55.498	43.211	1.00	57.87	6
ATOM	1250	CG	GLN	A	182	28.188	54.342	42.400	1.00	36.22	6
ATOM	1251	CB	GLN	A	182	29.458	54.178	43.178	1.00	29.07	6
ATOM	1252	CA	GLN	A	182	29.220	53.827	44.637	1.00	20.63	6
ATOM	1253	C	GLN	A	182	30.498	54.185	45.347	1.00	22.86	6
ATOM	1254	O	GLN	A	182	31.519	53.726	44.882	1.00	27.70	8
ATOM	1255	N	ASN	A	183	30.450	54.957	46.362	1.00	27.14	7
ATOM	1256	ND2	ASN	A	183	30.750	58.651	46.572	1.00	47.15	7
ATOM	1257	OD1	ASN	A	183	32.423	57.377	45.640	1.00	47.21	8
ATOM	1258	CG	ASN	A	183	31.593	57.633	46.532	1.00	47.14	6
ATOM	1259	CB	ASN	A	183	31.436	56.677	47.691	1.00	32.89	6
ATOM	1260	CA	ASN	A	183	31.656	55.252	47.134	1.00	31.50	6
ATOM	1261	C	ASN	A	183	31.698	54.274	48.330	1.00	33.88	6
ATOM	1262	O	ASN	A	183	32.459	54.594	49.245	1.00	32.01	8
ATOM	1263	N	GLY	A	184	30.838	53.306	48.492	1.00	23.02	7
ATOM	1264	CA	GLY	A	184	30.887	52.499	49.688	1.00	23.48	6
ATOM	1265	C	GLY	A	184	30.322	53.209	50.879	1.00	26.52	6
ATOM	1266	O	GLY	A	184	30.461	52.723	52.013	1.00	30.46	8
ATOM	1267	N	GLN	A	185	29.568	54.273	50.751	1.00	27.03	7
ATOM	1268	NE2	GLN	A	185	30.258	58.823	51.467	1.00	60.06	7
ATOM	1269	OE1	GLN	A	185	31.633	57.570	53.078	1.00	61.27	8
ATOM	1270	CD	GLN	A	185	30.896	57.806	52.089	1.00	58.99	6
ATOM	1271	CG	GLN	A	185	30.465	56.526	51.381	1.00	54.79	6
ATOM	1272	CB	GLN	A	185	29.023	56.422	51.884	1.00	24.50	6
ATOM	1273	CA	GLN	A	185	29.012	54.889	51.969	1.00	24.00	6
ATOM	1274	C	GLN	A	185	27.518	54.587	52.026	1.00	20.62	6
ATOM	1275	O	GLN	A	185	26.870	54.488	51.012	1.00	21.50	8
ATOM	1276	N	ARG	A	186	27.110	54.610	53.277	1.00	20.28	7
ATOM	1277	NH2	ARG	A	186	21.131	56.372	57.060	1.00	27.55	7
ATOM	1278	NH1	ARG	A	186	22.904	57.626	57.258	1.00	35.29	7
ATOM	1279	CZ	ARG	A	186	22.478	56.400	57.225	1.00	38.65	6
ATOM	1280	NE	ARG	A	186	23.030	55.217	57.167	1.00	30.56	7
ATOM	1281	CD	ARG	A	186	24.081	54.434	56.803	1.00	26.76	6
ATOM	1282	CG	ARG	A	186	24.037	54.280	55.356	1.00	19.72	6
ATOM	1283	CB	ARG	A	186	25.484	54.482	55.024	1.00	17.77	6
ATOM	1284	CA	ARG	A	186	25.719	54.343	53.529	1.00	19.83	6
ATOM	1285	C	ARG	A	186	24.849	55.249	52.697	1.00	29.56	6
ATOM	1286	O	ARG	A	186	25.067	56.444	52.788	1.00	26.52	8
ATOM	1287	N	ALA	A	187	23.822	54.843	52.015	1.00	19.87	7
ATOM	1288	CB	ALA	A	187	22.098	54.655	50.429	1.00	22.61	6
ATOM	1289	CA	ALA	A	187	22.847	55.634	51.325	1.00	21.48	6
ATOM	1290	C	ALA	A	187	22.107	56.312	52.498	1.00	23.68	6
ATOM	1291	O	ALA	A	187	21.762	55.850	53.579	1.00	20.22	8
ATOM	1292	N	SER	A	188	21.706	57.586	52.332	1.00	22.67	7
ATOM	1293	OG	SER	A	188	19.942	59.678	51.654	1.00	31.32	8
ATOM	1294	CB	SER	A	188	20.789	59.773	52.799	1.00	27.54	6
ATOM	1295	CA	SER	A	188	21.069	58.367	53.386	1.00	26.75	6
ATOM	1296	C	SER	A	188	19.792	57.706	53.819	1.00	22.34	6
ATOM	1297	O	SER	A	188	19.413	58.002	54.966	1.00	22.84	8
ATOM	1298	N	PHE	A	189	19.037	56.941	53.001	1.00	22.24	7
ATOM	1299	CD2	PHE	A	189	17.852	56.033	49.969	1.00	17.59	6
ATOM	1300	CE2	PHE	A	189	18.514	55.372	48.951	1.00	24.96	6
ATOM	1301	CZ	PHE	A	189	18.791	54.030	49.053	1.00	21.12	6
ATOM	1302	CE1	PHE	A	189	18.335	53.422	50.233	1.00	19.91	6
ATOM	1303	CD1	PHE	A	189	17.679	54.049	51.248	1.00	19.31	6
ATOM	1304	CG	PHE	A	189	17.414	55.447	51.104	1.00	24.18	6
ATOM	1305	CB	PHE	A	189	16.754	56.211	52.243	1.00	17.91	6
ATOM	1306	CA	PHE	A	189	17.738	56.340	53.411	1.00	19.41	6
ATOM	1307	C	PHE	A	189	17.900	54.995	54.158	1.00	13.56	6
ATOM	1308	O	PHE	A	189	16.915	54.531	54.699	1.00	19.77	8
ATOM	1309	N	SER	A	190	19.127	54.513	54.121	1.00	17.30	7
ATOM	1310	OG	SER	A	190	20.958	51.491	54.627	1.00	18.49	8
ATOM	1311	CB	SER	A	190	20.614	52.728	54.152	1.00	19.55	6
ATOM	1312	CA	SER	A	190	19.310	53.198	54.732	1.00	19.59	6
ATOM	1313	C	SER	A	190	19.165	53.145	56.233	1.00	19.58	6
ATOM	1314	O	SER	A	190	19.993	53.714	56.959	1.00	22.91	8
ATOM	1315	N	THR	A	191	18.230	52.366	56.775	1.00	20.03	7
ATOM	1316	CG2	THR	A	191	16.453	50.951	59.871	1.00	19.20	6
ATOM	1317	OG1	THR	A	191	15.685	51.953	57.813	1.00	23.24	8
ATOM	1318	CB	THR	A	191	16.775	51.284	58.421	1.00	18.08	6
ATOM	1319	CA	THR	A	191	17.970	52.140	58.186	1.00	19.69	6
ATOM	1320	C	THR	A	191	19.214	51.465	58.784	1.00	26.64	6
ATOM	1321	O	THR	A	191	19.971	50.764	58.083	1.00	20.74	8
ATOM	1322	N	TYR	A	192	19.509	51.785	60.037	1.00	24.10	7
ATOM	1323	OH	TYR	A	192	20.579	57.242	62.652	1.00	42.72	8

APPENDIX D-continued

3D Structure of Esperase

ATOM	1324	CD2	TYR	A	192	21.008	54.515	60.307	1.00	27.03	6
ATOM	1325	CE2	TYR	A	192	20.670	55.799	60.760	1.00	28.72	6
ATOM	1326	CZ	TYR	A	192	20.864	56.031	62.103	1.00	37.26	6
ATOM	1327	CE1	TYR	A	192	21.348	55.083	63.015	1.00	36.10	6
ATOM	1328	CD1	TYR	A	192	21.652	53.820	62.541	1.00	25.25	6
ATOM	1329	CG	TYR	A	192	21.516	53.550	61.169	1.00	22.50	6
ATOM	1330	CB	TYR	A	192	21.910	52.154	60.684	1.00	25.18	6
ATOM	1331	CA	TYR	A	192	20.708	51.213	60.683	1.00	17.72	6
ATOM	1332	C	TYR	A	192	20.258	50.806	62.081	1.00	17.55	6
ATOM	1333	O	TYR	A	192	19.128	50.985	62.559	1.00	19.29	8
ATOM	1334	N	GLY	A	193	21.198	50.136	62.735	1.00	19.36	7
ATOM	1335	CA	GLY	A	193	20.866	49.639	64.090	1.00	21.59	6
ATOM	1336	C	GLY	A	193	21.817	48.449	64.262	1.00	23.46	6
ATOM	1337	O	GLY	A	193	22.550	48.074	63.361	1.00	19.67	8
ATOM	1338	N	PRO	A	194	21.782	47.949	65.484	1.00	25.90	7
ATOM	1339	CG	PRO	A	194	20.970	47.337	67.684	1.00	27.38	6
ATOM	1340	CD	PRO	A	194	20.887	48.403	66.615	1.00	27.18	6
ATOM	1341	CB	PRO	A	194	22.239	46.658	67.360	1.00	22.45	6
ATOM	1342	CA	PRO	A	194	22.600	46.837	65.880	1.00	28.03	6
ATOM	1343	C	PRO	A	194	22.412	45.568	65.036	1.00	21.43	6
ATOM	1344	O	PRO	A	194	23.318	44.731	64.998	1.00	22.19	8
ATOM	1345	N	GLU	A	195	21.274	45.405	64.424	1.00	20.23	7
ATOM	1346	OE2	GLU	A	195	18.569	46.243	65.075	1.00	24.87	8
ATOM	1347	OE1	GLU	A	195	17.965	44.957	66.720	1.00	35.26	8
ATOM	1348	CD	GLU	A	195	18.409	45.076	65.595	1.00	28.08	6
ATOM	1349	CG	GLU	A	195	18.768	43.825	64.864	1.00	21.18	6
ATOM	1350	CB	GLU	A	195	19.456	43.945	63.541	1.00	16.96	6
ATOM	1351	CA	GLU	A	195	20.940	44.207	63.686	1.00	20.96	6
ATOM	1352	C	GLU	A	195	21.528	44.212	62.285	1.00	30.31	6
ATOM	1353	O	GLU	A	195	21.450	43.118	61.697	1.00	23.68	8
ATOM	1354	N	ILE	A	196	22.053	45.362	61.843	1.00	19.60	7
ATOM	1355	CD1	ILE	A	196	20.930	47.604	59.167	1.00	17.78	6
ATOM	1356	CG1	ILE	A	196	22.141	47.811	60.018	1.00	16.83	6
ATOM	1357	CB	ILE	A	196	23.248	46.768	60.069	1.00	20.89	6
ATOM	1358	CG2	ILE	A	196	23.876	46.679	58.658	1.00	16.59	6
ATOM	1359	CA	ILE	A	196	22.643	45.435	60.528	1.00	20.36	6
ATOM	1360	C	ILE	A	196	23.722	44.323	60.503	1.00	23.09	6
ATOM	1361	O	ILE	A	196	24.633	44.261	61.336	1.00	20.15	8
ATOM	1362	N	GLU	A	197	23.649	43.519	59.454	1.00	18.90	7
ATOM	1363	OE2	GLU	A	197	22.575	38.762	60.250	1.00	21.64	8
ATOM	1364	OE1	GLU	A	197	24.285	37.564	59.610	1.00	21.92	8
ATOM	1365	CD	GLU	A	197	23.784	38.629	59.811	1.00	20.50	6
ATOM	1366	CG	GLU	A	197	24.621	39.884	59.573	1.00	24.14	6
ATOM	1367	CB	GLU	A	197	23.810	41.138	59.266	1.00	19.21	6
ATOM	1368	CA	GLU	A	197	24.642	42.460	59.266	1.00	22.15	6
ATOM	1369	C	GLU	A	197	25.599	42.545	58.109	1.00	18.74	6
ATOM	1370	O	GLU	A	197	26.761	42.130	58.148	1.00	17.44	8
ATOM	1371	N	ILE	A	198	25.090	43.096	56.996	1.00	17.50	7
ATOM	1372	CD1	ILE	A	198	28.230	41.260	54.456	1.00	17.58	6
ATOM	1373	CG1	ILE	A	198	26.759	41.350	54.022	1.00	15.01	6
ATOM	1374	CB	ILE	A	198	25.746	41.660	55.141	1.00	17.39	6
ATOM	1375	CG2	ILE	A	198	24.381	41.337	54.553	1.00	14.58	6
ATOM	1376	CA	ILE	A	198	25.916	43.091	55.794	1.00	19.95	6
ATOM	1377	C	ILE	A	198	25.455	44.307	54.934	1.00	16.93	6
ATOM	1378	O	ILE	A	198	24.294	44.655	55.167	1.00	18.25	8
ATOM	1379	N	SER	A	199	26.288	44.736	54.001	1.00	16.29	7
ATOM	1380	OG	SER	A	199	26.677	47.445	54.695	1.00	21.30	8
ATOM	1381	CB	SER	A	199	26.803	47.058	53.330	1.00	20.88	6
ATOM	1382	CA	SER	A	199	25.866	45.811	53.103	1.00	22.96	6
ATOM	1383	C	SER	A	199	26.017	45.418	51.664	1.00	18.35	6
ATOM	1384	O	SER	A	199	26.885	44.606	51.311	1.00	17.27	8
ATOM	1385	N	ALA	A	200	25.292	46.082	50.773	1.00	17.99	7
ATOM	1386	CB	ALA	A	200	24.507	44.703	48.899	1.00	15.76	6
ATOM	1387	CA	ALA	A	200	25.488	45.800	49.315	1.00	14.75	6
ATOM	1388	C	ALA	A	200	25.057	47.101	48.587	1.00	18.96	6
ATOM	1389	O	ALA	A	200	24.393	47.954	49.211	1.00	17.64	8
ATOM	1390	N	PRO	A	201	25.286	47.223	47.306	1.00	20.51	7
ATOM	1391	CG	PRO	A	201	26.661	47.136	45.380	1.00	18.80	6
ATOM	1392	CD	PRO	A	201	26.109	46.242	46.503	1.00	16.13	6
ATOM	1393	CB	PRO	A	201	25.425	47.930	45.033	1.00	17.17	6
ATOM	1394	CA	PRO	A	201	24.903	48.309	46.424	1.00	17.87	6
ATOM	1395	C	PRO	A	201	23.380	48.465	46.492	1.00	19.20	6
ATOM	1396	O	PRO	A	201	22.635	47.530	46.248	1.00	18.47	8
ATOM	1397	N	GLY	A	202	22.926	49.697	46.814	1.00	17.75	7
ATOM	1398	CA	GLY	A	202	21.523	49.979	46.902	1.00	17.51	6

APPENDIX D-continued

3D Structure of Esperase

ATOM	1399	C	GLY	A	202	21.097	51.274	46.221	1.00	14.82	6
ATOM	1400	O	GLY	A	202	19.959	51.700	46.457	1.00	18.85	8
ATOM	1401	N	VAL	A	203	21.915	51.907	45.439	1.00	16.17	7
ATOM	1402	CG2	VAL	A	203	22.372	54.486	47.007	1.00	17.72	6
ATOM	1403	CG1	VAL	A	203	22.264	55.632	44.833	1.00	25.37	6
ATOM	1404	CB	VAL	A	203	22.496	54.306	45.506	1.00	22.60	6
ATOM	1405	CA	VAL	A	203	21.601	53.222	44.828	1.00	17.78	6
ATOM	1406	C	VAL	A	203	21.846	53.134	43.307	1.00	16.42	6
ATOM	1407	O	VAL	A	203	22.908	52.700	42.814	1.00	17.23	8
ATOM	1408	N	ASN	A	204	20.759	53.554	42.619	1.00	16.98	7
ATOM	1409	ND2	ASN	A	204	22.049	56.884	41.402	1.00	24.32	7
ATOM	1410	OD1	ASN	A	204	19.987	56.330	40.912	1.00	24.35	8
ATOM	1411	CG	ASN	A	204	21.182	56.036	40.900	1.00	23.87	6
ATOM	1412	CB	ASN	A	204	21.683	54.651	40.519	1.00	18.76	6
ATOM	1413	CA	ASN	A	204	20.810	53.543	41.142	1.00	20.24	6
ATOM	1414	C	ASN	A	204	21.115	52.155	40.598	1.00	19.44	6
ATOM	1415	O	ASN	A	204	22.059	52.014	39.793	1.00	19.05	8
ATOM	1416	N	VAL	A	205	20.304	51.197	41.050	1.00	16.97	7
ATOM	1417	CG2	VAL	A	205	21.243	49.080	42.914	1.00	19.48	6
ATOM	1418	CG1	VAL	A	205	20.212	47.386	41.427	1.00	16.95	6
ATOM	1419	CB	VAL	A	205	20.268	48.874	41.764	1.00	19.38	6
ATOM	1420	CA	VAL	A	205	20.599	49.801	40.597	1.00	16.59	6
ATOM	1421	C	VAL	A	205	19.701	49.489	39.385	1.00	15.34	6
ATOM	1422	O	VAL	A	205	18.461	49.433	39.519	1.00	16.76	8
ATOM	1423	N	ASN	A	206	20.246	49.285	38.208	1.00	15.47	7
ATOM	1424	ND2	ASN	A	206	18.309	49.679	34.515	1.00	17.91	7
ATOM	1425	OD1	ASN	A	206	20.019	48.800	33.506	1.00	25.78	8
ATOM	1426	CG	ASN	A	206	19.539	49.313	34.549	1.00	20.79	6
ATOM	1427	CB	ASN	A	206	20.396	49.386	35.803	1.00	18.19	6
ATOM	1428	CA	ASN	A	206	19.494	48.977	37.009	1.00	15.81	6
ATOM	1429	C	ASN	A	206	19.179	47.474	37.041	1.00	18.98	6
ATOM	1430	O	ASN	A	206	20.072	46.681	37.314	1.00	15.12	8
ATOM	1431	N	SER	A	207	17.979	47.102	36.724	1.00	16.57	7
ATOM	1432	OG	SER	A	207	17.057	43.866	37.998	1.00	15.36	8
ATOM	1433	CB	SER	A	207	17.276	45.255	38.130	1.00	20.35	6
ATOM	1434	CA	SER	A	207	17.570	45.714	36.707	1.00	19.00	6
ATOM	1435	C	SER	A	207	16.343	45.545	35.805	1.00	22.26	6
ATOM	1436	O	SER	A	207	15.858	46.526	35.217	1.00	19.33	8
ATOM	1437	N	THR	A	208	15.892	44.328	35.624	1.00	16.17	7
ATOM	1438	CG2	THR	A	208	15.719	41.808	33.875	1.00	16.30	6
ATOM	1439	OG1	THR	A	208	14.725	41.939	36.073	1.00	18.37	8
ATOM	1440	CB	THR	A	208	14.653	42.429	34.738	1.00	20.01	6
ATOM	1441	CA	THR	A	208	14.750	43.997	34.777	1.00	18.19	6
ATOM	1442	C	THR	A	208	13.490	44.637	35.310	1.00	16.06	6
ATOM	1443	O	THR	A	208	13.445	44.830	36.515	1.00	18.68	8
ATOM	1444	N	TYR	A	209	12.439	44.866	34.532	1.00	15.66	7
ATOM	1445	OH	TYR	A	209	8.061	49.995	38.353	1.00	24.32	8
ATOM	1446	CD2	TYR	A	209	9.608	48.724	35.372	1.00	21.41	6
ATOM	1447	CE2	TYR	A	209	8.725	49.455	36.172	1.00	18.95	6
ATOM	1448	CZ	TYR	A	209	8.877	49.300	37.523	1.00	21.61	6
ATOM	1449	CE1	TYR	A	209	9.825	48.409	38.105	1.00	20.67	6
ATOM	1450	CD1	TYR	A	209	10.663	47.694	37.280	1.00	16.67	6
ATOM	1451	CG	TYR	A	209	10.576	47.859	35.859	1.00	20.84	6
ATOM	1452	CB	TYR	A	209	11.535	47.084	34.944	1.00	14.85	6
ATOM	1453	CA	TYR	A	209	11.262	45.514	35.051	1.00	17.74	6
ATOM	1454	C	TYR	A	209	10.037	45.095	34.241	1.00	18.77	6
ATOM	1455	O	TYR	A	209	10.306	44.671	33.159	1.00	17.94	8
ATOM	1456	N	THR	A	210	8.808	45.263	34.610	1.00	17.67	7
ATOM	1457	CG2	THR	A	210	6.190	44.160	35.943	1.00	18.91	6
ATOM	1458	OG1	THR	A	210	6.688	46.353	35.498	1.00	22.54	8
ATOM	1459	CB	THR	A	210	6.354	45.166	34.830	1.00	22.53	6
ATOM	1460	CA	THR	A	210	7.576	44.936	33.961	1.00	15.65	6
ATOM	1461	C	THR	A	210	7.530	45.630	32.615	1.00	21.08	6
ATOM	1462	O	THR	A	210	8.245	46.596	32.337	1.00	21.96	8
ATOM	1463	N	GLY	A	211	6.772	45.091	31.686	1.00	21.88	7
ATOM	1464	CA	GLY	A	211	6.639	45.433	30.294	1.00	16.41	6
ATOM	1465	C	GLY	A	211	7.894	45.195	29.496	1.00	20.65	6
ATOM	1466	O	GLY	A	211	8.073	45.931	28.520	1.00	21.25	8
ATOM	1467	N	ASN	A	212	8.774	44.261	29.787	1.00	18.14	7
ATOM	1468	ND2	ASN	A	212	10.850	42.997	25.498	1.00	19.71	7
ATOM	1469	OD1	ASN	A	212	12.024	42.844	27.473	1.00	22.97	8
ATOM	1470	CG	ASN	A	212	10.949	43.075	26.839	1.00	24.04	6
ATOM	1471	CB	ASN	A	212	9.727	43.459	27.633	1.00	20.82	6
ATOM	1472	CA	ASN	A	212	9.992	44.039	29.021	1.00	18.32	6
ATOM	1473	C	ASN	A	212	10.824	45.313	29.009	1.00	21.79	6

APPENDIX D-continued

3D Structure of Esperase

ATOM	1474	O	ASN	A	212	11.338	45.759	27.979	1.00	19.34	8
ATOM	1475	N	ARG	A	213	11.135	45.871	30.138	1.00	22.53	7
ATOM	1476	NH2	ARG	A	213	9.855	52.374	32.384	1.00	51.03	7
ATOM	1477	NH1	ARG	A	213	7.807	51.315	33.080	1.00	51.09	7
ATOM	1478	CZ	ARG	A	213	8.906	51.367	32.264	1.00	58.97	6
ATOM	1479	NE	ARG	A	213	9.162	50.491	31.270	1.00	46.66	7
ATOM	1480	CD	ARG	A	213	8.664	49.317	30.665	1.00	34.39	6
ATOM	1481	CG	ARG	A	213	9.943	48.779	30.110	1.00	26.79	6
ATOM	1482	CB	ARG	A	213	11.019	48.187	30.997	1.00	18.34	6
ATOM	1483	CA	ARG	A	213	11.923	47.139	30.309	1.00	21.06	6
ATOM	1484	C	ARG	A	213	13.028	46.814	31.301	1.00	20.29	6
ATOM	1485	O	ARG	A	213	13.179	45.698	31.856	1.00	22.59	8
ATOM	1486	N	TYR	A	214	13.880	47.783	31.513	1.00	19.32	7
ATOM	1487	OH	TYR	A	214	16.343	42.867	28.955	1.00	22.20	8
ATOM	1488	CD2	TYR	A	214	16.835	45.207	31.687	1.00	21.30	6
ATOM	1489	CE2	TYR	A	214	16.827	44.005	30.925	1.00	25.13	6
ATOM	1490	CZ	TYR	A	214	16.378	44.008	29.622	1.00	22.68	6
ATOM	1491	CE1	TYR	A	214	15.989	45.194	29.017	1.00	22.65	6
ATOM	1492	CD1	TYR	A	214	15.994	46.390	29.760	1.00	27.11	6
ATOM	1493	CG	TYR	A	214	16.429	46.385	31.106	1.00	20.60	6
ATOM	1494	CB	TYR	A	214	16.401	47.666	31.882	1.00	20.69	6
ATOM	1495	CA	TYR	A	214	15.005	47.781	32.462	1.00	19.05	6
ATOM	1496	C	TYR	A	214	14.859	49.127	33.171	1.00	27.90	6
ATOM	1497	O	TYR	A	214	14.650	50.072	32.408	1.00	24.71	8
ATOM	1498	N	VAL	A	215	14.933	49.316	34.454	1.00	20.15	7
ATOM	1499	CG2	VAL	A	215	13.057	52.183	35.930	1.00	35.02	6
ATOM	1500	CG1	VAL	A	215	12.963	50.184	36.901	1.00	21.12	6
ATOM	1501	CB	VAL	A	215	13.309	50.726	35.561	1.00	22.47	6
ATOM	1502	CA	VAL	A	215	14.790	50.566	35.197	1.00	19.28	6
ATOM	1503	C	VAL	A	215	15.780	50.613	36.352	1.00	26.25	6
ATOM	1504	O	VAL	A	215	16.115	49.538	36.921	1.00	18.11	8
ATOM	1505	N	SER	A	216	16.242	51.836	36.638	1.00	18.21	7
ATOM	1506	OG	SER	A	216	18.922	53.199	38.291	1.00	28.38	8
ATOM	1507	CB	SER	A	216	18.437	52.619	37.132	1.00	19.54	6
ATOM	1508	CA	SER	A	216	17.173	52.022	37.788	1.00	14.76	6
ATOM	1509	C	SER	A	216	16.379	52.452	38.994	1.00	15.93	6
ATOM	1510	O	SER	A	216	15.417	53.260	38.998	1.00	17.76	8
ATOM	1511	N	LEU	A	217	16.536	51.851	40.157	1.00	15.32	7
ATOM	1512	CD2	LEU	A	217	12.758	52.145	40.632	1.00	14.71	6
ATOM	1513	CD1	LEU	A	217	12.750	49.735	41.258	1.00	16.65	6
ATOM	1514	CG	LEU	A	217	13.614	50.916	40.808	1.00	16.11	6
ATOM	1515	CB	LEU	A	217	14.725	51.092	41.795	1.00	14.31	6
ATOM	1516	CA	LEU	A	217	15.935	51.919	41.450	1.00	15.26	6
ATOM	1517	C	LEU	A	217	16.939	51.939	42.603	1.00	15.62	6
ATOM	1518	O	LEU	A	217	18.064	51.549	42.450	1.00	16.30	8
ATOM	1519	N	SER	A	218	16.586	52.646	43.680	1.00	20.18	7
ATOM	1520	OG	SER	A	218	18.487	54.649	44.162	1.00	18.87	8
ATOM	1521	CB	SER	A	218	17.616	54.260	45.170	1.00	14.18	6
ATOM	1522	CA	SER	A	218	17.407	52.767	44.891	1.00	15.36	6
ATOM	1523	C	SER	A	218	16.603	52.333	46.074	1.00	11.18	6
ATOM	1524	O	SER	A	218	15.384	52.612	46.252	1.00	15.32	8
ATOM	1525	N	GLY	A	219	17.294	51.647	46.946	1.00	13.73	7
ATOM	1526	CA	GLY	A	219	16.541	51.213	48.130	1.00	14.10	6
ATOM	1527	C	GLY	A	219	17.263	50.056	48.790	1.00	13.62	6
ATOM	1528	O	GLY	A	219	18.107	49.446	48.142	1.00	15.45	8
ATOM	1529	N	THR	A	220	16.951	49.763	50.039	1.00	17.44	7
ATOM	1530	CG2	THR	A	220	18.258	49.511	52.936	1.00	16.33	6
ATOM	1531	OG1	THR	A	220	15.916	48.713	52.516	1.00	15.80	8
ATOM	1532	CB	THR	A	220	17.286	48.535	52.245	1.00	14.42	6
ATOM	1533	CA	THR	A	220	17.461	48.580	50.735	1.00	17.50	6
ATOM	1534	C	THR	A	220	16.870	47.306	50.022	1.00	19.97	6
ATOM	1535	O	THR	A	220	17.485	46.256	50.007	1.00	15.81	8
ATOM	1536	N	SER	A	221	15.767	47.403	49.310	1.00	20.24	7
ATOM	1537	OG	SER	A	221	12.959	47.113	48.642	1.00	16.51	8
ATOM	1538	CB	SER	A	221	13.930	46.838	47.667	1.00	14.33	6
ATOM	1539	CA	SER	A	221	15.123	46.390	48.506	1.00	12.90	6
ATOM	1540	C	SER	A	221	16.074	46.003	47.362	1.00	15.12	6
ATOM	1541	O	SER	A	221	15.824	44.880	46.946	1.00	17.53	8
ATOM	1542	N	MET	A	222	16.866	46.875	46.831	1.00	17.58	7
ATOM	1543	CE	MET	A	222	14.201	47.976	44.365	1.00	20.40	6
ATOM	1544	SD	MET	A	222	15.531	48.968	44.957	1.00	19.45	16
ATOM	1545	CG	MET	A	222	17.005	48.396	44.101	1.00	13.71	6
ATOM	1546	CB	MET	A	222	18.168	47.953	44.968	1.00	15.78	6
ATOM	1547	CA	MET	A	222	17.828	46.631	45.753	1.00	16.66	6
ATOM	1548	C	MET	A	222	19.114	46.047	46.344	1.00	18.62	6

APPENDIX D-continued

3D Structure of Esperase											
ATOM	1549	O	MET	A	222	19.914	45.476	45.641	1.00	18.19	8
ATOM	1550	N	ALA	A	223	19.567	46.403	47.559	1.00	18.17	7
ATOM	1551	CB	ALA	A	223	21.100	46.725	49.390	1.00	15.52	6
ATOM	1552	CA	ALA	A	223	20.798	45.907	48.119	1.00	17.73	6
ATOM	1553	C	ALA	A	223	20.550	44.390	48.476	1.00	18.17	6
ATOM	1554	O	ALA	A	223	21.442	43.582	48.237	1.00	15.32	8
ATOM	1555	N	THR	A	224	19.505	43.993	49.096	1.00	15.05	7
ATOM	1556	CG2	THR	A	224	17.181	41.475	50.592	1.00	17.28	6
ATOM	1557	OG1	THR	A	224	17.567	43.643	51.132	1.00	18.23	8
ATOM	1558	CB	THR	A	224	17.580	42.815	49.991	1.00	19.73	6
ATOM	1559	CA	THR	A	224	19.045	42.695	49.562	1.00	16.32	6
ATOM	1560	C	THR	A	224	19.361	41.608	48.529	1.00	18.10	6
ATOM	1561	O	THR	A	224	20.139	40.681	48.831	1.00	18.64	8
ATOM	1562	N	PRO	A	225	18.887	41.707	47.295	1.00	19.05	7
ATOM	1563	CG	PRO	A	225	18.136	42.513	45.242	1.00	14.92	6
ATOM	1564	CD	PRO	A	225	17.891	42.669	46.729	1.00	13.28	6
ATOM	1565	CB	PRO	A	225	18.243	40.998	45.078	1.00	16.78	6
ATOM	1566	CA	PRO	A	225	19.095	40.659	46.305	1.00	16.58	6
ATOM	1567	C	PRO	A	225	20.555	40.511	46.005	1.00	18.27	6
ATOM	1568	O	PRO	A	225	20.931	39.450	45.449	1.00	18.34	8
ATOM	1569	N	HIS	A	226	21.430	41.465	46.154	1.00	14.72	7
ATOM	1570	CD2	HIS	A	226	24.294	43.788	43.752	1.00	18.07	6
ATOM	1571	NE2	HIS	A	226	23.748	44.863	43.075	1.00	19.52	7
ATOM	1572	CE1	HIS	A	226	22.668	45.150	43.774	1.00	14.51	6
ATOM	1573	ND1	HIS	A	226	22.493	44.451	44.863	1.00	17.68	7
ATOM	1574	CG	HIS	A	226	23.536	43.522	44.843	1.00	17.79	6
ATOM	1575	CB	HIS	A	226	23.792	42.501	45.921	1.00	16.33	6
ATOM	1576	CA	HIS	A	226	22.850	41.289	45.803	1.00	15.23	6
ATOM	1577	C	HIS	A	226	23.338	40.212	46.774	1.00	16.97	6
ATOM	1578	O	HIS	A	226	24.229	39.428	46.452	1.00	18.46	8
ATOM	1579	N	VAL	A	227	22.891	40.288	48.000	1.00	16.78	7
ATOM	1580	CG2	VAL	A	227	23.890	41.135	50.680	1.00	14.40	6
ATOM	1581	CG1	VAL	A	227	23.403	38.803	51.556	1.00	16.55	6
ATOM	1582	CB	VAL	A	227	23.078	39.851	50.480	1.00	16.86	6
ATOM	1583	CA	VAL	A	227	23.317	39.328	49.058	1.00	19.40	6
ATOM	1584	C	VAL	A	227	22.622	37.966	48.813	1.00	18.81	6
ATOM	1585	O	VAL	A	227	23.389	37.026	48.945	1.00	18.80	8
ATOM	1586	N	ALA	A	228	21.341	37.929	48.499	1.00	16.53	7
ATOM	1587	CB	ALA	A	228	19.234	36.911	47.825	1.00	14.14	6
ATOM	1588	CA	ALA	A	228	20.698	36.697	48.134	1.00	15.92	6
ATOM	1589	C	ALA	A	228	21.468	36.063	46.986	1.00	18.89	6
ATOM	1590	O	ALA	A	228	21.717	34.844	46.986	1.00	18.57	8
ATOM	1591	N	GLY	A	229	21.867	36.825	45.976	1.00	17.16	7
ATOM	1592	CA	GLY	A	229	22.612	36.385	44.821	1.00	18.29	6
ATOM	1593	C	GLY	A	229	23.921	35.754	45.298	1.00	20.93	6
ATOM	1594	O	GLY	A	229	24.368	34.727	44.804	1.00	18.60	8
ATOM	1595	N	VAL	A	230	24.721	36.337	46.178	1.00	19.63	7
ATOM	1596	CG2	VAL	A	230	27.344	37.967	46.499	1.00	16.69	6
ATOM	1597	CG1	VAL	A	230	28.071	36.209	48.063	1.00	17.82	6
ATOM	1598	CB	VAL	A	230	26.831	36.870	47.428	1.00	18.91	6
ATOM	1599	CA	VAL	A	230	25.995	35.834	46.650	1.00	21.31	6
ATOM	1600	C	VAL	A	230	25.729	34.506	47.398	1.00	20.36	6
ATOM	1601	O	VAL	A	230	26.608	33.630	47.327	1.00	18.14	8
ATOM	1602	N	ALA	A	231	24.704	34.423	48.186	1.00	16.19	7
ATOM	1603	CB	ALA	A	231	23.099	33.453	49.852	1.00	15.64	6
ATOM	1604	CA	ALA	A	231	24.303	33.272	48.943	1.00	19.23	6
ATOM	1605	C	ALA	A	231	24.106	32.150	47.878	1.00	26.28	6
ATOM	1606	O	ALA	A	231	24.646	31.063	48.051	1.00	18.81	8
ATOM	1607	N	ALA	A	232	23.425	32.341	46.769	1.00	23.31	7
ATOM	1608	CB	ALA	A	232	22.170	31.917	44.677	1.00	17.17	6
ATOM	1609	CA	ALA	A	232	23.190	31.406	45.678	1.00	19.51	6
ATOM	1610	C	ALA	A	232	24.513	30.938	45.055	1.00	22.03	6
ATOM	1611	O	ALA	A	232	24.669	29.709	44.797	1.00	21.60	8
ATOM	1612	N	LEU	A	233	25.450	31.831	44.890	1.00	18.00	7
ATOM	1613	CD2	LEU	A	233	27.058	32.978	41.722	1.00	19.56	6
ATOM	1614	CD1	LEU	A	233	28.229	34.741	42.822	1.00	22.23	6
ATOM	1615	CG	LEU	A	233	27.261	33.626	43.063	1.00	25.68	6
ATOM	1616	CB	LEU	A	233	27.734	32.638	44.100	1.00	19.00	6
ATOM	1617	CA	LEU	A	233	26.758	31.512	44.380	1.00	19.18	6
ATOM	1618	C	LEU	A	233	27.478	30.583	45.399	1.00	32.14	6
ATOM	1619	O	LEU	A	233	28.163	29.617	44.985	1.00	26.65	8
ATOM	1620	N	VAL	A	234	27.417	30.811	46.694	1.00	23.95	7
ATOM	1621	CG2	VAL	A	234	28.911	31.915	49.153	1.00	20.13	6
ATOM	1622	CG1	VAL	A	234	28.484	29.847	50.295	1.00	18.16	6
ATOM	1623	CB	VAL	A	234	28.054	30.627	49.104	1.00	20.46	6

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3D Structure of Esperase

ATOM	1624	CA	VAL	A	234	28.187	30.033	47.683	1.00	20.36	6
ATOM	1625	C	VAL	A	234	27.586	28.631	47.676	1.00	21.66	6
ATOM	1626	O	VAL	A	234	28.344	27.700	47.665	1.00	22.83	8
ATOM	1627	N	LYS	A	235	26.274	28.546	47.694	1.00	21.98	7
ATOM	1628	NZ	LYS	A	235	22.620	23.743	50.078	1.00	29.40	7
ATOM	1629	CE	LYS	A	235	22.462	24.483	48.842	1.00	25.24	6
ATOM	1630	CD	LYS	A	235	23.510	25.553	48.797	1.00	30.16	6
ATOM	1631	CG	LYS	A	235	23.079	26.379	47.585	1.00	26.63	6
ATOM	1632	CB	LYS	A	235	23.988	27.625	47.594	1.00	22.69	6
ATOM	1633	CA	LYS	A	235	25.469	27.337	47.688	1.00	27.46	6
ATOM	1634	C	LYS	A	235	25.907	26.501	46.462	1.00	34.52	6
ATOM	1635	O	LYS	A	235	26.029	25.292	46.590	1.00	26.32	8
ATOM	1636	N	SER	A	236	26.101	27.082	45.314	1.00	23.34	7
ATOM	1637	OG	SER	A	236	27.255	28.235	42.597	1.00	24.48	8
ATOM	1638	CB	SER	A	236	26.224	27.305	42.840	1.00	20.87	6
ATOM	1639	CA	SER	A	236	26.457	26.441	44.069	1.00	27.78	6
ATOM	1640	C	SER	A	236	27.893	25.932	44.239	1.00	32.81	6
ATOM	1641	O	SER	A	236	28.289	24.881	43.697	1.00	33.79	8
ATOM	1642	N	ARG	A	237	28.779	26.633	44.889	1.00	27.94	7
ATOM	1643	NH2	ARG	A	237	36.693	26.015	46.199	1.00	43.63	7
ATOM	1644	NH1	ARG	A	237	34.671	24.734	46.068	1.00	50.62	7
ATOM	1645	CZ	ARG	A	237	35.394	25.866	45.921	1.00	55.63	6
ATOM	1646	NE	ARG	A	237	34.768	26.943	45.423	1.00	45.03	7
ATOM	1647	CD	ARG	A	237	33.356	26.880	44.981	1.00	35.54	6
ATOM	1648	CG	ARG	A	237	32.431	27.220	46.107	1.00	36.78	6
ATOM	1649	CB	ARG	A	237	31.048	27.417	45.451	1.00	36.35	6
ATOM	1650	CA	ARG	A	237	30.183	26.229	45.057	1.00	30.62	6
ATOM	1651	C	ARG	A	237	30.294	25.177	46.187	1.00	37.26	6
ATOM	1652	O	ARG	A	237	31.226	24.364	46.081	1.00	32.10	8
ATOM	1653	N	TYR	A	238	29.478	25.193	47.202	1.00	25.70	7
ATOM	1654	OH	TYR	A	238	35.377	26.896	48.995	1.00	38.64	8
ATOM	1655	CD2	TYR	A	238	31.736	26.903	49.223	1.00	26.69	6
ATOM	1656	CE2	TYR	A	238	33.029	27.369	49.095	1.00	30.27	6
ATOM	1657	CZ	TYR	A	238	34.086	26.481	49.141	1.00	38.00	6
ATOM	1658	CE1	TYR	A	238	33.828	25.135	49.328	1.00	33.49	6
ATOM	1659	CD1	TYR	A	238	32.531	24.676	49.487	1.00	30.85	6
ATOM	1660	CG	TYR	A	238	31.457	25.546	49.441	1.00	33.19	6
ATOM	1661	CB	TYR	A	238	30.081	24.961	49.606	1.00	24.64	6
ATOM	1662	CA	TYR	A	238	29.529	24.325	48.331	1.00	23.05	6
ATOM	1663	C	TYR	A	238	28.122	23.867	48.656	1.00	25.25	6
ATOM	1664	O	TYR	A	238	27.514	24.266	49.659	1.00	30.61	8
ATOM	1665	N	PRO	A	239	27.688	22.920	47.848	1.00	27.20	7
ATOM	1666	CG	PRO	A	239	27.396	21.618	45.894	1.00	27.10	6
ATOM	1667	CD	PRO	A	239	28.420	22.386	46.677	1.00	28.97	6
ATOM	1668	CB	PRO	A	239	26.237	21.401	46.789	1.00	27.07	6
ATOM	1669	CA	PRO	A	239	26.374	22.336	47.936	1.00	24.32	6
ATOM	1670	C	PRO	A	239	26.018	21.775	49.271	1.00	27.11	6
ATOM	1671	O	PRO	A	239	24.832	21.805	49.646	1.00	34.83	8
ATOM	1672	N	SER	A	240	27.032	21.338	49.983	1.00	28.71	7
ATOM	1673	OG	SER	A	240	28.905	20.696	51.933	1.00	44.71	8
ATOM	1674	CB	SER	A	240	27.802	19.807	51.651	1.00	32.98	6
ATOM	1675	CA	SER	A	240	26.658	20.772	51.295	1.00	31.35	6
ATOM	1676	C	SER	A	240	26.514	21.852	52.339	1.00	35.23	6
ATOM	1677	O	SER	A	240	26.021	21.373	53.361	1.00	33.72	8
ATOM	1678	N	TYR	A	241	26.917	23.099	52.126	1.00	33.49	7
ATOM	1679	OH	TYR	A	241	32.514	26.940	53.424	1.00	39.30	8
ATOM	1680	CD2	TYR	A	241	28.974	26.952	52.686	1.00	28.15	6
ATOM	1681	CE2	TYR	A	241	30.301	27.321	52.920	1.00	31.52	6
ATOM	1682	CZ	TYR	A	241	31.258	26.429	53.256	1.00	27.23	6
ATOM	1683	CE1	TYR	A	241	30.883	25.121	53.346	1.00	31.25	6
ATOM	1684	CD1	TYR	A	241	29.567	24.730	53.129	1.00	37.65	6
ATOM	1685	CG	TYR	A	241	28.574	25.641	52.769	1.00	33.89	6
ATOM	1686	CB	TYR	A	241	27.141	25.321	52.486	1.00	29.46	6
ATOM	1687	CA	TYR	A	241	26.737	24.060	53.228	1.00	26.63	6
ATOM	1688	C	TYR	A	241	25.346	24.320	53.736	1.00	25.38	6
ATOM	1689	O	TYR	A	241	24.430	24.339	52.874	1.00	29.17	8
ATOM	1690	N	THR	A	242	25.146	24.489	55.044	1.00	24.62	7
ATOM	1691	CG2	THR	A	242	23.731	22.950	57.120	1.00	42.00	6
ATOM	1692	OG1	THR	A	242	24.567	25.108	57.591	1.00	31.68	8
ATOM	1693	CB	THR	A	242	23.519	24.442	56.951	1.00	33.79	6
ATOM	1694	CA	THR	A	242	23.802	24.846	55.488	1.00	26.63	6
ATOM	1695	C	THR	A	242	23.625	26.399	55.366	1.00	31.00	6
ATOM	1696	O	THR	A	242	24.567	27.112	55.026	1.00	24.59	8
ATOM	1697	N	ASN	A	243	22.455	26.868	55.672	1.00	23.08	7
ATOM	1698	ND2	ASN	A	243	19.284	28.150	58.303	1.00	25.05	7

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3D Structure of Esperase

ATOM	1699	OD1	ASN	A	243	21.147	26.869	58.079	1.00	29.80	8
ATOM	1700	CG	ASN	A	243	20.365	27.718	57.665	1.00	31.10	6
ATOM	1701	CB	ASN	A	243	20.705	28.307	56.289	1.00	26.51	6
ATOM	1702	CA	ASN	A	243	22.106	28.250	55.754	1.00	21.33	6
ATOM	1703	C	ASN	A	243	23.148	28.899	56.695	1.00	27.65	6
ATOM	1704	O	ASN	A	243	23.802	29.886	56.362	1.00	25.83	8
ATOM	1705	N	ASN	A	244	23.468	28.330	57.867	1.00	26.03	7
ATOM	1706	ND2	ASN	A	244	22.587	29.649	60.811	1.00	34.79	7
ATOM	1707	OD1	ASN	A	244	22.802	27.357	61.068	1.00	44.84	8
ATOM	1708	CG	ASN	A	244	23.191	28.490	60.756	1.00	35.31	6
ATOM	1709	CB	ASN	A	244	24.543	28.310	60.131	1.00	23.50	6
ATOM	1710	CA	ASN	A	244	24.468	28.913	58.741	1.00	23.28	6
ATOM	1711	C	ASN	A	244	25.852	29.042	58.177	1.00	20.60	6
ATOM	1712	O	ASN	A	244	26.588	29.986	58.528	1.00	25.11	8
ATOM	1713	N	GLN	A	245	26.288	28.065	57.405	1.00	26.52	7
ATOM	1714	NE2	GLN	A	245	29.731	23.917	56.910	1.00	46.58	7
ATOM	1715	OE1	GLN	A	245	27.592	23.848	56.288	1.00	31.92	8
ATOM	1716	CD	GLN	A	245	28.495	24.413	56.857	1.00	31.91	6
ATOM	1717	CG	GLN	A	245	28.158	25.769	57.424	1.00	31.13	6
ATOM	1718	CB	GLN	A	245	28.079	26.789	56.257	1.00	22.63	6
ATOM	1719	CA	GLN	A	245	27.641	28.159	56.822	1.00	25.01	6
ATOM	1720	C	GLN	A	245	27.700	29.217	55.724	1.00	22.64	6
ATOM	1721	O	GLN	A	245	28.812	29.747	55.628	1.00	22.33	8
ATOM	1722	N	ILE	A	246	26.579	29.386	55.029	1.00	19.17	7
ATOM	1723	CD1	ILE	A	246	24.121	28.540	51.765	1.00	24.28	6
ATOM	1724	CG1	ILE	A	246	25.491	28.913	52.305	1.00	22.92	6
ATOM	1725	CB	ILE	A	246	25.388	30.250	53.066	1.00	24.08	6
ATOM	1726	CG2	ILE	A	246	25.359	31.365	52.019	1.00	15.42	6
ATOM	1727	CA	ILE	A	246	26.626	30.376	53.946	1.00	20.73	6
ATOM	1728	C	ILE	A	246	26.625	31.770	54.595	1.00	21.00	6
ATOM	1729	O	ILE	A	246	27.450	32.600	54.231	1.00	21.98	8
ATOM	1730	N	ARG	A	247	25.815	31.946	55.595	1.00	17.95	7
ATOM	1731	NH2	ARG	A	247	21.172	36.496	61.002	1.00	23.24	7
ATOM	1732	NH1	ARG	A	247	20.813	34.285	60.509	1.00	25.64	7
ATOM	1733	CZ	ARG	A	247	21.541	35.380	60.384	1.00	22.42	6
ATOM	1734	NE	ARG	A	247	22.621	35.221	59.659	1.00	20.46	7
ATOM	1735	CD	ARG	A	247	23.075	33.985	59.041	1.00	23.20	6
ATOM	1736	CG	ARG	A	247	24.278	34.245	58.197	1.00	24.44	6
ATOM	1737	CB	ARG	A	247	24.599	32.992	57.408	1.00	19.57	6
ATOM	1738	CA	ARG	A	247	25.664	33.174	56.359	1.00	19.19	6
ATOM	1739	C	ARG	A	247	27.002	33.597	56.927	1.00	25.09	6
ATOM	1740	O	ARG	A	247	27.519	34.707	56.756	1.00	22.02	8
ATOM	1741	N	GLN	A	248	27.650	32.632	57.527	1.00	20.56	7
ATOM	1742	NE2	GLN	A	248	31.226	29.317	59.418	1.00	42.74	7
ATOM	1743	OE1	GLN	A	248	30.871	30.465	61.389	1.00	46.93	8
ATOM	1744	CD	GLN	A	248	30.990	30.383	60.165	1.00	51.60	6
ATOM	1745	CG	GLN	A	248	30.736	31.700	59.458	1.00	35.08	6
ATOM	1746	CB	GLN	A	248	29.288	31.684	59.012	1.00	25.69	6
ATOM	1747	CA	GLN	A	248	28.981	32.908	58.114	1.00	22.60	6
ATOM	1748	C	GLN	A	248	30.017	33.161	57.069	1.00	21.32	6
ATOM	1749	O	GLN	A	248	30.901	33.970	57.349	1.00	21.82	8
ATOM	1750	N	ARG	A	249	29.967	32.465	55.934	1.00	19.01	7
ATOM	1751	NH2	ARG	A	249	35.824	29.779	51.206	1.00	40.53	7
ATOM	1752	NH1	ARG	A	249	34.826	29.545	53.357	1.00	37.14	7
ATOM	1753	CZ	ARG	A	249	34.844	29.899	52.078	1.00	40.51	6
ATOM	1754	NE	ARG	A	249	33.791	30.659	51.779	1.00	39.30	7
ATOM	1755	CD	ARG	A	249	33.221	31.268	52.999	1.00	30.94	6
ATOM	1756	CG	ARG	A	249	31.842	31.741	52.706	1.00	25.20	6
ATOM	1757	CB	ARG	A	249	30.911	31.639	53.899	1.00	22.51	6
ATOM	1758	CA	ARG	A	249	31.014	32.723	54.960	1.00	22.86	6
ATOM	1759	C	ARG	A	249	30.910	34.140	54.373	1.00	17.79	6
ATOM	1760	O	ARG	A	249	31.962	34.746	54.144	1.00	19.55	8
ATOM	1761	N	ILE	A	250	29.677	34.545	54.142	1.00	18.51	7
ATOM	1762	CD1	ILE	A	250	26.140	35.220	51.913	1.00	18.42	6
ATOM	1763	CG1	ILE	A	250	27.639	35.352	52.018	1.00	18.91	6
ATOM	1764	CB	ILE	A	250	27.933	36.102	53.316	1.00	23.88	6
ATOM	1765	CG2	ILE	A	250	27.571	37.586	53.219	1.00	20.31	6
ATOM	1766	CA	ILE	A	250	29.420	35.892	53.596	1.00	22.42	6
ATOM	1767	C	ILE	A	250	29.936	36.914	54.662	1.00	20.70	6
ATOM	1768	O	ILE	A	250	30.697	37.770	54.268	1.00	20.41	8
ATOM	1769	N	ASN	A	251	29.611	36.778	55.909	1.00	16.56	7
ATOM	1770	ND2	ASN	A	251	27.085	37.213	59.132	1.00	21.27	7
ATOM	1771	OD1	ASN	A	251	27.518	38.556	57.396	1.00	20.03	8
ATOM	1772	CG	ASN	A	251	27.884	37.722	58.234	1.00	20.12	6
ATOM	1773	CB	ASN	A	251	29.340	37.365	58.291	1.00	14.58	6

APPENDIX D-continued

3D Structure of Esperase											
ATOM	1774	CA	ASN	A	251	30.053	37.650	56.988	1.00	19.60	6
ATOM	1775	C	ASN	A	251	31.548	37.703	57.148	1.00	22.53	6
ATOM	1776	O	ASN	A	251	32.201	38.759	57.273	1.00	20.64	8
ATOM	1777	N	GLN	A	252	32.182	36.536	57.064	1.00	22.96	7
ATOM	1778	NE2	GLN	A	252	33.954	32.143	57.495	1.00	26.44	7
ATOM	1779	OE1	GLN	A	252	34.257	32.144	59.601	1.00	34.65	8
ATOM	1780	CD	GLN	A	252	33.983	32.794	58.626	1.00	33.52	6
ATOM	1781	CG	GLN	A	252	33.666	34.300	58.676	1.00	32.35	6
ATOM	1782	CB	GLN	A	252	34.161	35.012	57.438	1.00	19.97	6
ATOM	1783	CA	GLN	A	252	33.609	36.444	57.294	1.00	22.53	6
ATOM	1784	C	GLN	A	252	34.428	37.094	56.208	1.00	21.65	6
ATOM	1785	O	GLN	A	252	35.605	37.391	56.464	1.00	22.97	8
ATOM	1786	N	THR	A	253	33.896	37.119	55.011	1.00	18.20	7
ATOM	1787	CG2	THR	A	253	35.122	35.288	53.155	1.00	26.77	6
ATOM	1788	OG1	THR	A	253	33.244	36.457	52.335	1.00	20.36	8
ATOM	1789	CB	THR	A	253	34.578	36.633	52.726	1.00	22.10	6
ATOM	1790	CA	THR	A	253	34.689	37.647	53.898	1.00	19.73	6
ATOM	1791	C	THR	A	253	34.340	39.071	53.463	1.00	19.95	6
ATOM	1792	O	THR	A	253	34.913	39.503	52.482	1.00	20.25	8
ATOM	1793	N	ALA	A	254	33.429	39.726	54.117	1.00	19.64	7
ATOM	1794	CB	ALA	A	254	31.740	41.369	54.617	1.00	18.36	6
ATOM	1795	CA	ALA	A	254	32.987	41.091	53.782	1.00	24.84	6
ATOM	1796	C	ALA	A	254	34.120	42.087	53.921	1.00	21.63	6
ATOM	1797	O	ALA	A	254	35.058	41.906	54.708	1.00	20.05	8
ATOM	1798	N	THR	A	255	34.176	43.140	53.147	1.00	21.26	7
ATOM	1799	CG2	THR	A	255	36.230	46.013	52.142	1.00	25.89	6
ATOM	1800	OG1	THR	A	255	35.698	44.059	51.035	1.00	26.17	8
ATOM	1801	CB	THR	A	255	35.139	44.994	51.925	1.00	22.57	6
ATOM	1802	CA	THR	A	255	35.193	44.192	53.240	1.00	21.57	6
ATOM	1803	C	THR	A	255	34.718	45.197	54.248	1.00	19.15	6
ATOM	1804	O	THR	A	255	33.550	45.592	54.161	1.00	19.14	8
ATOM	1805	N	TYR	A	256	35.458	45.555	55.262	1.00	21.54	7
ATOM	1806	OH	TYR	A	256	35.344	50.333	61.399	1.00	27.67	8
ATOM	1807	CD2	TYR	A	256	35.133	47.291	59.487	1.00	18.22	6
ATOM	1808	CE2	TYR	A	256	34.941	48.186	60.527	1.00	19.33	6
ATOM	1809	CZ	TYR	A	256	35.581	49.413	60.435	1.00	23.39	6
ATOM	1810	CE1	TYR	A	256	36.360	49.758	59.359	1.00	21.50	6
ATOM	1811	CD1	TYR	A	256	36.542	48.786	58.359	1.00	24.73	6
ATOM	1812	CG	TYR	A	256	35.930	47.528	58.414	1.00	18.54	6
ATOM	1813	CB	TYR	A	256	36.204	46.511	57.365	1.00	19.73	6
ATOM	1814	CA	TYR	A	256	35.044	46.469	56.350	1.00	22.62	6
ATOM	1815	C	TYR	A	256	34.821	47.867	55.744	1.00	21.73	6
ATOM	1816	O	TYR	A	256	35.663	48.297	54.920	1.00	21.87	8
ATOM	1817	N	LEU	A	257	33.684	48.448	56.082	1.00	19.62	7
ATOM	1818	CD2	LEU	A	257	32.720	49.475	52.464	1.00	18.99	6
ATOM	1819	CD1	LEU	A	257	30.367	48.966	53.151	1.00	21.76	6
ATOM	1820	CG	LEU	A	257	31.817	48.960	53.516	1.00	20.48	6
ATOM	1821	CB	LEU	A	257	31.922	49.666	54.836	1.00	19.04	6
ATOM	1822	CA	LEU	A	257	33.313	49.753	55.519	1.00	27.37	6
ATOM	1823	C	LEU	A	257	33.263	50.866	56.576	1.00	27.50	6
ATOM	1824	O	LEU	A	257	33.107	52.015	56.207	1.00	25.78	8
ATOM	1825	N	GLY	A	258	33.152	50.534	57.828	1.00	22.89	7
ATOM	1826	CA	GLY	A	258	33.057	51.513	58.894	1.00	21.99	6
ATOM	1827	C	GLY	A	258	32.163	50.880	59.937	1.00	24.86	6
ATOM	1828	O	GLY	A	258	31.926	49.672	60.084	1.00	24.18	8
ATOM	1829	N	SER	A	259	31.569	51.743	60.724	1.00	20.88	7
ATOM	1830	OG	SER	A	259	29.158	52.213	63.426	1.00	31.61	8
ATOM	1831	CB	SER	A	259	29.974	52.583	62.307	1.00	24.36	6
ATOM	1832	CA	SER	A	259	30.733	51.337	61.822	1.00	24.45	6
ATOM	1833	C	SER	A	259	29.770	50.171	61.540	1.00	27.60	6
ATOM	1834	O	SER	A	259	28.843	50.318	60.730	1.00	22.13	8
ATOM	1835	N	PRO	A	260	29.842	49.141	62.343	1.00	21.74	7
ATOM	1836	CG	PRO	A	260	31.036	47.393	63.408	1.00	24.82	6
ATOM	1837	CD	PRO	A	260	30.994	48.911	63.310	1.00	25.73	6
ATOM	1838	CB	PRO	A	260	29.514	47.117	63.404	1.00	21.61	6
ATOM	1839	CA	PRO	A	260	29.031	47.947	62.217	1.00	19.23	6
ATOM	1840	C	PRO	A	260	27.609	48.328	62.386	1.00	21.40	6
ATOM	1841	O	PRO	A	260	26.757	47.607	61.855	1.00	21.68	8
ATOM	1842	N	SER	A	261	27.313	49.416	63.117	1.00	24.57	7
ATOM	1843	OG	SER	A	261	26.184	51.724	64.185	1.00	39.92	8
ATOM	1844	CB	SER	A	261	25.584	50.471	64.588	1.00	26.32	6
ATOM	1845	CA	SER	A	261	25.846	49.736	63.266	1.00	21.73	6
ATOM	1846	C	SER	A	261	25.265	50.281	61.945	1.00	22.12	6
ATOM	1847	O	SER	A	261	24.035	50.276	61.642	1.00	24.01	8
ATOM	1848	N	LEU	A	262	26.160	50.717	61.066	1.00	16.86	7

APPENDIX D-continued

3D Structure of Esperase

ATOM	1849	CD2	LEU	A	262	25.190	53.985	60.792	1.00	19.12	6
ATOM	1850	CD1	LEU	A	262	27.301	54.691	59.591	1.00	24.54	6
ATOM	1851	CG	LEU	A	262	26.558	53.641	60.336	1.00	21.01	6
ATOM	1852	CB	LEU	A	262	26.462	52.472	59.338	1.00	17.76	6
ATOM	1853	CA	LEU	A	262	25.690	51.214	59.777	1.00	20.61	6
ATOM	1854	C	LEU	A	262	25.743	50.137	58.665	1.00	22.18	6
ATOM	1855	O	LEU	A	262	24.898	50.044	57.784	1.00	20.95	8
ATOM	1856	N	TYR	A	263	26.839	49.424	58.640	1.00	20.86	7
ATOM	1857	OH	TYR	A	263	29.102	54.204	55.461	1.00	26.47	8
ATOM	1858	CD2	TYR	A	263	29.566	51.211	57.467	1.00	26.53	6
ATOM	1859	CE2	TYR	A	263	29.687	52.535	57.088	1.00	20.10	6
ATOM	1860	CZ	TYR	A	263	28.983	52.914	55.953	1.00	29.82	6
ATOM	1861	CE1	TYR	A	263	28.242	51.962	55.229	1.00	23.04	6
ATOM	1862	CD1	TYR	A	263	28.099	50.658	55.660	1.00	21.97	6
ATOM	1863	CG	TYR	A	263	28.770	50.273	56.804	1.00	22.44	6
ATOM	1864	CB	TYR	A	263	28.675	48.901	57.334	1.00	18.72	6
ATOM	1865	CA	TYR	A	263	27.257	48.431	57.689	1.00	19.34	6
ATOM	1866	C	TYR	A	263	27.356	46.941	58.112	1.00	20.55	6
ATOM	1867	O	TYR	A	263	27.557	46.151	57.208	1.00	20.61	8
ATOM	1868	N	GLY	A	264	27.252	46.559	59.371	1.00	23.63	7
ATOM	1869	CA	GLY	A	264	27.399	45.182	59.846	1.00	23.19	6
ATOM	1870	C	GLY	A	264	28.879	44.821	59.611	1.00	20.89	6
ATOM	1871	O	GLY	A	264	29.792	45.612	59.912	1.00	22.16	8
ATOM	1872	N	ASN	A	265	29.016	43.657	58.986	1.00	20.54	7
ATOM	1873	ND2	ASN	A	265	28.705	40.460	59.762	1.00	18.28	7
ATOM	1874	OD1	ASN	A	265	31.001	40.510	60.158	1.00	22.08	8
ATOM	1875	CG	ASN	A	265	29.953	40.799	59.474	1.00	23.12	6
ATOM	1876	CB	ASN	A	265	30.177	41.671	58.249	1.00	22.83	6
ATOM	1877	CA	ASN	A	265	30.354	43.162	58.629	1.00	18.37	6
ATOM	1878	C	ASN	A	265	30.933	43.918	57.463	1.00	17.82	6
ATOM	1879	O	ASN	A	265	32.101	43.734	57.184	1.00	19.89	8
ATOM	1880	N	GLY	A	266	30.149	44.653	56.673	1.00	18.61	7
ATOM	1881	CA	GLY	A	266	30.810	45.365	55.570	1.00	16.52	6
ATOM	1882	C	GLY	A	266	30.147	44.955	54.258	1.00	14.39	6
ATOM	1883	O	GLY	A	266	29.012	44.489	54.261	1.00	17.41	8
ATOM	1884	N	LEU	A	267	30.938	45.180	53.248	1.00	17.00	7
ATOM	1885	CD2	LEU	A	267	31.818	46.464	48.528	1.00	20.10	6
ATOM	1886	CD1	LEU	A	267	29.447	46.337	49.267	1.00	17.92	6
ATOM	1887	CG	LEU	A	267	30.836	45.825	49.468	1.00	21.20	6
ATOM	1888	CB	LEU	A	267	31.195	45.897	50.957	1.00	17.31	6
ATOM	1889	CA	LEU	A	267	30.473	44.933	51.911	1.00	19.77	6
ATOM	1890	C	LEU	A	267	30.613	43.483	51.457	1.00	19.17	6
ATOM	1891	O	LEU	A	267	31.713	43.027	51.499	1.00	18.24	8
ATOM	1892	N	VAL	A	268	29.515	42.890	51.007	1.00	18.79	7
ATOM	1893	CG2	VAL	A	268	28.108	39.597	49.871	1.00	21.18	6
ATOM	1894	CG1	VAL	A	268	27.562	41.922	48.977	1.00	17.72	6
ATOM	1895	CB	VAL	A	268	28.154	41.080	50.102	1.00	18.91	6
ATOM	1896	CA	VAL	A	268	29.593	41.487	50.497	1.00	19.33	6
ATOM	1897	C	VAL	A	268	30.656	41.429	49.439	1.00	21.97	6
ATOM	1898	O	VAL	A	268	30.848	42.376	48.631	1.00	22.07	8
ATOM	1899	N	HIS	A	269	31.459	40.358	49.345	1.00	18.59	7
ATOM	1900	CD2	HIS	A	269	36.030	41.419	48.127	1.00	21.57	6
ATOM	1901	NE2	HIS	A	269	36.783	41.004	47.080	1.00	22.77	7
ATOM	1902	CE1	HIS	A	269	36.264	39.941	46.468	1.00	21.29	6
ATOM	1903	ND1	HIS	A	269	35.180	39.655	47.082	1.00	20.84	7
ATOM	1904	CG	HIS	A	269	35.025	40.514	48.132	1.00	18.43	6
ATOM	1905	CB	HIS	A	269	33.878	40.370	49.071	1.00	17.66	6
ATOM	1906	CA	HIS	A	269	32.544	40.254	48.361	1.00	19.72	6
ATOM	1907	C	HIS	A	269	32.331	38.894	47.726	1.00	22.36	6
ATOM	1908	O	HIS	A	269	32.629	37.899	48.397	1.00	20.96	8
ATOM	1909	N	ALA	A	270	31.766	38.818	46.559	1.00	22.28	7
ATOM	1910	CB	ALA	A	270	30.573	37.918	44.601	1.00	17.20	6
ATOM	1911	CA	ALA	A	270	31.431	37.593	45.842	1.00	21.32	6
ATOM	1912	C	ALA	A	270	32.677	36.745	45.532	1.00	26.89	6
ATOM	1913	O	ALA	A	270	32.564	35.516	45.514	1.00	23.48	8
ATOM	1914	N	GLY	A	271	33.851	37.281	45.257	1.00	20.68	7
ATOM	1915	CA	GLY	A	271	35.107	36.638	44.880	1.00	24.34	6
ATOM	1916	C	GLY	A	271	35.612	35.980	46.150	1.00	30.38	6
ATOM	1917	O	GLY	A	271	35.866	34.786	46.145	1.00	29.87	8
ATOM	1918	N	ARG	A	272	35.718	36.672	47.271	1.00	25.63	7
ATOM	1919	NH2	ARG	A	272	39.216	41.988	51.543	1.00	39.62	7
ATOM	1920	NH1	ARG	A	272	37.245	41.084	52.031	1.00	33.73	7
ATOM	1921	CZ	ARG	A	272	38.322	41.035	51.261	1.00	29.01	6
ATOM	1922	NE	ARG	A	272	38.462	40.006	50.408	1.00	27.85	7
ATOM	1923	CD	ARG	A	272	37.427	38.979	50.545	1.00	24.30	6

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3D Structure of Esperase

ATOM	1924	CG	ARG	A	272	37.529	37.929	49.449	1.00	24.96	6
ATOM	1925	CB	ARG	A	272	36.387	36.959	49.653	1.00	24.60	6
ATOM	1926	CA	ARG	A	272	36.154	35.998	48.480	1.00	24.91	6
ATOM	1927	C	ARG	A	272	35.202	34.911	48.922	1.00	26.40	6
ATOM	1928	O	ARG	A	272	35.641	33.851	49.431	1.00	28.24	8
ATOM	1929	N	ALA	A	273	33.914	35.188	48.929	1.00	19.69	7
ATOM	1930	CB	ALA	A	273	31.517	34.902	49.536	1.00	20.87	6
ATOM	1931	CA	ALA	A	273	32.936	34.244	49.474	1.00	24.30	6
ATOM	1932	C	ALA	A	273	32.968	32.852	48.766	1.00	27.05	6
ATOM	1933	O	ALA	A	273	32.536	31.854	49.362	1.00	24.22	8
ATOM	1934	N	THR	A	274	33.319	32.767	47.501	1.00	24.53	7
ATOM	1935	CG2	THR	A	274	31.085	32.479	45.548	1.00	21.97	6
ATOM	1936	OG1	THR	A	274	33.334	32.912	44.673	1.00	21.52	8
ATOM	1937	CB	THR	A	274	32.493	32.003	45.307	1.00	23.92	6
ATOM	1938	CA	THR	A	274	33.266	31.637	46.614	1.00	27.06	6
ATOM	1939	C	THR	A	274	34.616	30.968	46.450	1.00	23.60	6
ATOM	1940	O	THR	A	274	34.742	30.024	45.712	1.00	26.35	8
ATOM	1941	N	GLN	A	275	35.613	31.466	47.075	1.00	24.38	7
ATOM	1942	NE2	GLN	A	275	38.108	33.169	50.922	1.00	25.89	7
ATOM	1943	OE1	GLN	A	275	39.935	31.618	50.540	1.00	44.50	8
ATOM	1944	CD	GLN	A	275	38.904	32.283	50.229	1.00	56.87	6
ATOM	1945	CG	GLN	A	275	38.801	31.879	48.740	1.00	54.09	6
ATOM	1946	CB	GLN	A	275	37.513	31.251	48.481	1.00	27.55	6
ATOM	1947	CA	GLN	A	275	36.966	30.920	47.124	1.00	31.54	6
ATOM	1948	C	GLN	A	275	36.688	29.412	47.422	1.00	38.56	6
ATOM	1949	O	GLN	A	275	37.587	28.549	47.205	1.00	37.01	8
ATOM	1950	OE	GLN	A	275	36.105	29.125	48.479	1.00	31.65	8

SEQUENCE LISTING

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<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
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
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                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

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
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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


<210> SEQ ID NO 3
<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
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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
    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
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<210> SEQ ID NO 4
<211> LENGTH: 213
<212> TYPE: PRT

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<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


<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
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        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

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
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<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
            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
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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

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

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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

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
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<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
            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
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<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
    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
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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

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
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<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

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
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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


<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
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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

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
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<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

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
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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

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
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                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
                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 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
                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
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<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
                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
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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

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
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```
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

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
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```
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

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
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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 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
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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

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
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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
            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
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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.

<400> SEQUENCE: 37

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
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    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
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
<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
```

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<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

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
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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

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
```

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```
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

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
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<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
                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
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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
            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
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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

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
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            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

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
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        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
<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
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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
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

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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

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
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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

Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
            260                 265
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<210> SEQ ID NO 58
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Bacillus

<400> SEQUENCE: 58

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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
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                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
<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
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```
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


<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
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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


<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


<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
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<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
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
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<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

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
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-continued

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<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

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
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<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

<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
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<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

<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
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<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
<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
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<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
<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
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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
<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

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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


<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
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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


<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
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<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


<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
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<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
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
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<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

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
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<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
<213> ORGANISM: Myceliophthora

<400> SEQUENCE: 177

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
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<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

<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
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<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
<212> TYPE: PRT
<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

<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
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<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
<213> ORGANISM: Betula

<400> SEQUENCE: 198

Ala Gly Ile Leu
1


<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
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<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
1


<210> SEQ ID NO 205
<211> LENGTH: 6
<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
<213> ORGANISM: Betula

<400> SEQUENCE: 206

Ser Thr Ala Asn
1


<210> SEQ ID NO 207
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 207

Ala Ile Leu Val
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1


<210> SEQ ID NO 208
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Betula

<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
1


<210> SEQ ID NO 210
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 210

Asp Glu Cys Thr
1


<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
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 212

Leu Arg Trp Ala
1


<210> SEQ ID NO 213
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 213

Arg Lys Gln Thr
1


<210> SEQ ID NO 214
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 214
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Gln Arg Ser Trp
1


<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
<213> ORGANISM: Betula

<400> SEQUENCE: 216

Ala Glu His Asn Pro Thr
1               5


<210> SEQ ID NO 217
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 217

Ala Gly Leu Lys Met
1               5


<210> SEQ ID NO 218
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 218

Glu Asp Lys Trp
1


<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
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Ser Tyr Leu Asn
1


<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
1


<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


<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
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<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
1


<210> SEQ ID NO 232
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 232

Thr Tyr Ala Pro
1


<210> SEQ ID NO 233
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 233

Ile Leu Val Ser
1


<210> SEQ ID NO 234
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 234

His Asn Gln Gly Cys
1               5


<210> SEQ ID NO 235
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Betula
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<400> SEQUENCE: 235

Ala Val Leu Cys Tyr
1               5


<210> SEQ ID NO 236
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 236

Leu Phe Gln Ala
1


<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
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
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<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
1               5


<210> SEQ ID NO 246
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Betula

<400> SEQUENCE: 246

Asp Glu Lys Gln His Thr
1               5


<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;

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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;

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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;

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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;

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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;

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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;

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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;

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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,

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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,

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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,

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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 any of the amino acid sequence of SEQ ID NO: 24, 26, 27, 28, 29, 30, 31, 32, 34, 35.

144. 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.

145. The savinase-like subtilisin of claim **144**, wherein the subtilisin has any of the amino acid sequence of SEQ ID NO: 24, 26, 27, 28, 29, 30, 31, 32, 34, 35.

146. 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.

147. 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.

148. 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.

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: 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,

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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,

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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.

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: 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,

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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.

151. A composition comprising the protein variant of claim **135**.

152. A DNA construct comprising a DNA sequence encoding a protein variant of claim **135**.

153. A host cell which is capable of expressing a polypeptide and comprising a DNA construct of claim **152**.

154. A method of producing a protein variant having reduced immunogenicity as compared to the parent protein, comprising:

(a) culturing the host cell of claim **153** 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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