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## DESCRIPTION

**[0001]** The present disclosure relates to compositions and methods useful for the production of glycoproteins, e.g. recombinant antibodies, having fucosylated N-glycans, in filamentous fungal cells.

### BACKGROUND

**[0002]** Posttranslational modification of eukaryotic proteins, particularly therapeutic proteins such as immunoglobulins, is often necessary for proper protein folding and function. Because standard prokaryotic expression systems lack the proper machinery necessary for such modifications, alternative expression systems have to be used in production of these therapeutic proteins. Yeast and fungi are attractive options for expressing proteins as they can be easily grown at a large scale in simple media, which allows low production costs, and yeast and fungi have posttranslational machinery and chaperones that perform similar functions as found in mammalian cells. Moreover, tools are available to manipulate the relatively simple genetic makeup of yeast and fungal cells as well as more complex eukaryotic cells such as mammalian or insect cells (De Pourcq et al., Appl Microbiol Biotechnol, 87(5):1617-31). Despite these advantages, many therapeutic proteins are still being produced in mammalian cells, which produce therapeutic proteins with posttranslational modifications most resembling the native human proteins, whereas the posttranslational modifications naturally produced by yeast and fungi often differ from those found in mammalian cells.

**[0003]** To address this deficiency, new strains of yeast and fungi are being developed that produce posttranslational modifications that more closely resemble those found in native human proteins. More specifically, new strains of yeast and fungi have been genetically modified so that they express recombinant proteins having N-glycan patterns resembling that of native human proteins. The general strategies include the elimination of endogenous glycosylation enzymes that are involved in producing high mannose N-glycans (such as och1p or Alg3p in yeast), and the introduction of certain glycosyltransferases in order to reproduce the sequential reaction steps of the mammalian glycosylation pathway, including  $\alpha$ 1,2 mannosidase, GnTI, mannosidase II, GnTII, GalT, SiaT enzymes (Wildt and Gerngross, 2005, Nature, 3: 119-127; De Pourcq et al., 2010, Appl Microbiol Biotechnol, 87:1617-1631).

**[0004]** Mammalian and human cells express fucosyltransferase (FucTs) activities and FucTs are therefore one of the enzyme families of interest for remodeling N-glycan patterns on the surface of recombinant glycoproteins produced in yeast or fungi. The presence of fucosylated structures on glycoproteins has indeed been shown to be advantageous in some cases. More specifically, in the production of monoclonal antibodies, immunoglobulin and related glycoproteins comprising Fc fragment, it is well known that the presence of fucosylated N-glycans influence antibody dependent cytotoxicity (ADCC) activity. In some cases, it is desirable to produce antibodies with fucosylated N-glycans, in order to decrease ADCC activity of the resulting protein. It is further well known that the presence of fucosylated N-glycans influence antibody dependent cellular phagocytosis (ADCP) activity (Shibata-Koyama et al. 2009, Exp Hematol 37:309-21). In some cases, it is desirable to produce antibodies with fucosylated N-glycans, in order to decrease ADCP activity of the resulting protein.

**[0005]** WO 2008/112092 reports materials and methods for making lower eukaryotic expression systems that can be used to produce recombinant, fucosylated glycoproteins. The genetic modification of yeast *P. pastoris* strain capable of producing glycoproteins that include fucose is described, including the insertion of polynucleotides encoding human GDP-mannose-4,6 dehydratase (GMD), human GDP-4-keto-6-deoxy-D-mannose-3,5-epimerase/4-reductase (FX) and human  $\alpha$ 1,6 fucosyltransferase (FUT8) proteins in such host cell.

**[0006]** Other reports have suggested genetic modification of yeast strains to produce the GDP-L-fucose, the substrate of fucosyltransferase (Chigira et al. 2008, *Glycobiology* 18 no. 4 pp303-314; Järvinen et al. 2001, *Eur J Biochem* 268, 6458-6464), and/or human-like fucosylated glycoforms (Ma et al., 2006, *Glycobiology* 16(12) pp158-184, US 20050170452, US 2010137565, US2010062485).

**[0007]** Reports of fungal cell expression systems expressing human-like fucosylated N-glycans are lacking. Indeed, due to the industry's focus on mammalian cell culture technology for such a long time, the fungal cell expression systems such as *Trichoderma* are not as well established for therapeutic protein production as mammalian cell culture and therefore suffer from drawbacks when expressing mammalian proteins. In particular, a need remains in the art for improved filamentous fungal cells, such as *Trichoderma* fungus cells, that can stably produce heterologous proteins with mammalian-like N-glycan patterns, preferably at high levels of expression.

**[0008]** The disclosure now provides fungal cell expression system, more specifically *Trichoderma* cells, having reduced protease activity and capable of expressing fucosylated glycoproteins, for example with mammalian-like complex fucosylated N-glycans.

## SUMMARY

**[0009]** Described herein are compositions including filamentous fungal cells, such as *Trichoderma* fungal cells, expressing fucosylation pathway. More specifically, described herein are compositions including filamentous fungal cells with reduced protease activity and expressing fucosylation pathway. Further described herein are methods for producing a glycoprotein, e.g. an antibody, having fucosylated N-glycan, using genetically modified filamentous fungal cells, for example, *Trichoderma* fungal cells, as the expression system. Thus, a particular aspect of the disclosure includes filamentous fungal cells, such as *Trichoderma* fungal cells, comprising at least a mutation that reduces an endogenous protease activity compared to a parental filamentous fungal cell which does not have such mutation and comprising a polynucleotide encoding a polypeptide having fucosyltransferase activity. In certain embodiments, the filamentous fungal cell is selected from the group consisting of *Trichoderma*, *Neurospora*, *Myceliophthora*, *Fusarium*, *Aspergillus*, *Penicillium* and *Chrysosporium* cell.

**[0010]** In certain embodiments, said mutation is a deletion or a disruption of the gene encoding said endogenous protease activity. In certain embodiments, the expression level of at least two, or at least three proteases is reduced. In certain embodiments that may be combined with the preceding embodiments, the cell has a mutation in one or more proteases selected from the group consisting of pep1, pep2, pep3, pep4, pep5, pep7, pep8, pep11, pep12, tpp1, tsp1, slp1, slp2, slp3, slp5, slp6, slp7, slp8, gap1 and gap2. In one embodiment that may be combined with the preceding embodiments, said cell comprises mutations that reduce or eliminate the activity of

1. a) the three endogenous proteases pep1, tsp1 and slp1,
2. b) the three endogenous proteases gap1, slp1 and pep1,
3. c) three endogenous proteases selected from the group consisting of pep1, pep2, pep3, pep4, pep5, pep8, pep11, pep12, tsp1, slp1, slp2, slp3, slp7, gap1 and gap2,
4. d) three to six proteases selected from the group consisting of pep1, pep2, pep3, pep4, pep5, tsp1, slp1, slp2, slp3, gap1 and gap2,
5. e) seven to ten proteases selected from the group consisting of pep1, pep2, pep3, pep4, pep5, pep7, pep8, tsp1, slp1, slp2, slp3, slp5, slp6, slp7, slp8, tpp1, gap1 and gap2.

**[0011]** In certain embodiments that may be combined with the preceding embodiments, said



fucosyltransferase activity (FucT) is selected from the group consisting of:  $\alpha$ 1,2 FucT,  $\alpha$ 1,3/ $\alpha$ 1,4,  $\alpha$ 1,6 FucT and O-FucTs. An example of a polynucleotide encoding a polypeptide having fucosyltransferase activity comprises either the polynucleotide of SEQ ID NO:1, or a functional variant polynucleotide encoding a polypeptide having at least 50%, at least 60%, at least 70%, at least 90%, or at least 95% identity with SEQ ID NO:6, said polypeptide has  $\alpha$ 1,6 fucosyltransferase activity.

**[0012]** In certain embodiments that may be combined with the preceding embodiments, the filamentous fungal cell further comprises one or more polynucleotides encoding a polypeptide having GDP-fucose synthesis activity and, optionally, GDP-fucose transporter activity. In certain embodiments, said one or more polynucleotides encoding a polypeptide having GDP-fucose synthesis activity comprises

1. a) GMD polynucleotide or a functional variant polynucleotide encoding a polypeptide having GDP-mannose-dehydratase activity; and,
2. b) FX polynucleotide or a functional variant polynucleotide encoding a polypeptide having both GDP-keto-deoxy-mannose-epimerase and GDP-keto-deoxy-galactose-reductase activities.

**[0013]** An example of said one or more polynucleotides encoding the polypeptide encoding GDP-fucose synthesis activity comprises

1. a) *C. elegans* GMD polynucleotide of SEQ ID NO:2 or a functional variant polynucleotide encoding a polypeptide having at least 50%, at least 60%, at least 70%, at least 90%, or at least 95% identity with SEQ ID NO:7, wherein said polypeptide has GDP-mannose-dehydratase activity; and,
2. b) *C. elegans* FX polynucleotide of SEQ ID NO:3 or a functional variant polynucleotide encoding a polypeptide having at least 50%, at least 60%, at least 70%, at least 90%, or at least 95% identity with SEQ ID NO:8, wherein said polypeptide has both GDP-keto-deoxy-mannose-epimerase and GDP-keto-deoxy-galactose-reductase activities.

**[0014]** In certain embodiments that may be combined with the preceding embodiments, the filamentous fungal cell further comprises a polynucleotide of SEQ ID NO:4 encoding *C. elegans* GDP-fucose transporter or a functional variant polynucleotide encoding a polypeptide having at least 50%, at least 60%, at least 70%, at least 90%, or at least 95% identity with SEQ ID NO:9 and encoding GDP fucose transporter.

**[0015]** In certain embodiments that may be combined with the preceding embodiments, said polynucleotide encoding a polypeptide having fucosyltransferase activity further comprises a Golgi targeting sequence for targeting expression of said polypeptide in the ER/Golgi compartment of said filamentous fungal cell. An example of said Golgi targeting sequence comprises a polynucleotide sequence encoding a N-terminal portion of the polypeptide of SEQ ID NO:10, or a functional variant polynucleotide suitable for targeting said fucosyltransferase activity in the Golgi compartment of said filamentous fungal cell.

**[0016]** In certain embodiments that may be combined with the preceding embodiments, the filamentous fungal cell is genetically modified to produce a complex N-glycan as an acceptor substrate for said fucosyltransferase activity.

**[0017]** In certain embodiments that may be combined with the preceding embodiments, the filamentous fungal cell has a mutation that reduces the level of expression of an ALG3 gene compared to the level of expression in a parent cell which does not have such mutation. In certain embodiments that may be combined with the preceding embodiment, the filamentous fungal cell further comprises a first polynucleotide encoding N-acetylglucosaminyltransferase I catalytic domain and a second polynucleotide encoding N-acetylglucosaminyltransferase II catalytic domain.

**[0018]** In certain embodiments that may be combined with the preceding embodiments, the filamentous fungal cell further comprises one or more polynucleotides selected from the group consisting of:

1. i. a polynucleotide encoding  $\alpha$ 1,2 mannosidase,
2. ii. a polynucleotide encoding N-acetylglucosaminyltransferase I catalytic domain,
3. iii. a polynucleotide encoding  $\alpha$  mannosidase II,
4. iv. a polynucleotide encoding N-acetylglucosaminyltransferase II catalytic domain.

**[0019]** In certain embodiments that may be combined with the preceding embodiments, the filamentous fungal cell further comprises a polynucleotide encoding  $\beta$ 1,4 galactosyltransferase.

**[0020]** In certain embodiments that may be combined with the preceding embodiments, the filamentous fungal cell further comprises one or more polynucleotides selected from the group consisting of:

1. i. a polynucleotide encoding glucosamine UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase,
2. ii. a polynucleotide encoding N-acetylneuraminic acid synthase,
3. iii. a polynucleotide encoding N-acetylneuraminic acid phosphatase,
4. iv. a polynucleotide encoding cytidine monophosphate N-acetylneuraminic acid synthetase,
5. v. a polynucleotide encoding CMP-sialic acid transporter, and
6. vi. a polynucleotide encoding sialyltransferase.

**[0021]** In certain embodiments that may be combined with the preceding embodiments, the filamentous fungal cell further comprises mutations in one or more genes encoding glycosyl hydrolases, wherein said mutation eliminates or reduces activity of the corresponding hydrolases, and wherein said hydrolases are selected from the group consisting xylanase, cellobiohydrolase, and endoglucanase.

**[0022]** Another aspect includes a method for producing a glycoprotein, e.g. an antibody, having fucosylated N-glycan, comprising:

1. a) providing a filamentous fungal cell as defined above, and comprising a polynucleotide encoding a glycoprotein,
2. b) culturing the filamentous fungal cell to produce said glycoprotein having fucosylated N-glycan.

**[0023]** In certain embodiments that may be combined with the preceding embodiments, the fucose of the N-glycan is in an  $\alpha$ 1,6 linkage. For example, said fucosylated N-glycan is selected from the group consisting of  $\text{Man}_3\text{GlcNAc}_2(\text{Fuc})$ ,  $\text{GlcNAcMan}_3\text{GlcNAc}_2(\text{Fuc})$ ,  $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2(\text{Fuc})$ ,  $\text{Gal}_1\text{-}_2\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2(\text{Fuc})$ ,  $\text{Neu5Ac}_1\text{-}_2\text{Gal}_1\text{-}_2\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2(\text{Fuc})$ . In certain embodiments, at least 5mol%, at least 10mol% or at least 15mol% of the total secreted neutral N-glycans consist of  $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2(\text{Fuc})$  glycoform.

**[0024]** In certain embodiments that may be combined with the preceding embodiments, said polynucleotide encoding a glycoprotein, e.g. an antibody, is a recombinant polynucleotide encoding a heterologous glycoprotein. For example, said heterologous glycoprotein is a mammalian glycoprotein selected from the group consisting of an antibody, an immunoglobulin, a single chain antibody, a monomeric or multimeric single domain antibody, a FAb-fragment, a FAb2-fragment, their antigen-binding fragments or a protein fusion

comprising Fc fragment of an immunoglobulin.

**[0025]** In one embodiment, said polynucleotide encoding said glycoprotein further comprises a polynucleotide encoding CBH1 catalytic domain and linker as a carrier protein and/or *cbh1* promoter.

**[0026]** In an embodiment that may be combined with one or more of the preceding embodiments less than 0.1%, 0.01 %, 0.001 % or 0 % of the N-glycans of the glycoprotein (or secreted glycoprotein) comprises Neu5Gc and/or Gala- structure. In an embodiment that may be combined with the preceding embodiments, less than 0.1%, 0.01 %, 0.001 % or 0 % of the N-glycans of the antibody (or secreted antibody) comprises Neu5Gc and/or Gal $\alpha$ -structure.

**[0027]** In an embodiment that may be combined with one or more of the preceding embodiments, less than 1.0%, 0.5%, 0.1%, 0.01 %, 0.001 %, or 0 % of the glycoprotein (or secreted glycoprotein) comprises glycation structures. In an embodiment that may be combined with the preceding embodiments, less than 1.0%, 0.5%, 0.1%, 0.01 %, 0.001 %, or 0 % of the antibody (or secreted antibody) comprises glycation structures.

## DESCRIPTION OF THE FIGURES

**[0028]**

**Figure 1.** Schematic expression cassette design for plasmids pTTv224, pTTv225 and pTTv226.

**Figure 2.** Neutral N-glycans from day 3 (A) and day 5 (B) supernatant proteins of *T. reesei* fucosylation transformants (pTTv224 + pTTv225) and parental strain M289.

**Figure 3.** Neutral N-glycans of day 5 supernatant proteins from *T. reesei* fucosylation transformant 43A.

**Figure 4.** Protein specific N-glycosylation (70 kDa supernatant protein).

**Figure 5.** Fragmentation analysis of m/z 1485 (FG0)

**Figure 6.** Neutral N-glycans of day 7 supernatant proteins from fermentation of *T. reesei* fucosylation transformant 43A (M525).

**Figure 7.** Neutral N-glycans of day 5 supernatant proteins from fermentation of *T. reesei* fucosylation transformant 12D with GDP fucose transporter

**Figure 8.** Alignment of FX proteins of different species

**Figure 9.** Alignment of GMD proteins of different species

**Figure 10.** Alignment of FUT8 proteins of different species

**Figure 11.** Alignment of fucose transporter proteins of different species

**Figure 12.** Normalised protease activity of day 5 supernatants taken from shake flask cultures done with the M289 parent strain, four pTTv224 + pTTv225 transformants 40A, 43A, 7G and 51E and two pTTv224 + pTTv226 transformants 4B and 12D. Fluorescent casein was incubated with the diluted supernatants (1:4) in citrate buffer pH 4.5 to detect protease activity.

**Figure 13.** Schematic expression cassette design for plasmids pTTv434

**Figure 14** graphically depicts normalized protease activity data from culture supernatants from each of the protease deletion supernatants and the parent strain M124. Protease activity was measured at pH 5.5 in first 5 strains and at pH 4.5 in the last three deletion strains. Protease activity is against green fluorescent casein.

The six protease deletion strain has only 6% of the wild type parent strain and the 7 protease deletion strain protease activity was about 40% less than the 6 protease deletion strain activity.

## DETAILED DESCRIPTION

**[0029]** The present disclosure relates to improved methods for producing glycoproteins with fucosylated N-glycans, and more specifically, fucosylated glycoproteins, such as antibodies or related immunoglobulins or protein fusion comprising Fc fragments.

**[0030]** The present disclosure is based in part on the surprising discovery that filamentous fungal cells, such as *Trichoderma* cells, can be genetically modified to produce fucosylated glycoproteins, and in particular fucosylated glycoproteins with complex fucosylated N-glycans, at a high yield.

**[0031]** In one aspect, the disclosure relates to a fungal cell, including a filamentous fungal cell that produces core fucosylated N-glycan. In one embodiment, the cell comprises reduced or deleted alg3 enzyme activity. In other embodiments, the core fucosylated N-glycans are produced on secreted glycoproteins. In other embodiments, the secreted glycoproteins are heterologous and/or homologous glycoproteins. In other embodiments, the cell is a fungal cell or filamentous fungal cell.

**[0032]** A particular aspect of the disclosure relates to a filamentous fungal cell, comprising at least a mutation that reduces an endogenous protease activity compared to a parental filamentous fungal cell which does not have such mutation and comprising a polynucleotide encoding fucosyltransferase activity, and, optionally, other polynucleotides encoding GDP fucose synthesis and/or GDP-fucose transporter.

**[0033]** Such filamentous fungal cells are useful as an expression system for the production of heterologous glycoproteins, preferably heterologous mammalian glycoproteins, such as an immunoglobulin, an antibody, a single chain antibody, a monomeric or multimeric single domain antibody, a Fab fragment, a Fab2 fragment or a protein fusion comprising an Fc fragment of an immunoglobulin or their antigen-binding fragment.

**[0034]** Typically, the method for producing fucosylated glycoproteins comprises the steps of:

1. a) providing a filamentous fungal cell genetically modified as defined below, and comprising a polynucleotide encoding a glycoprotein, for example encoding an immunoglobulin, an antibody, a single chain antibody, a monomeric or multimeric single domain antibody, a Fab fragment, a Fab2 fragment or a protein fusion comprising an Fc fragment of an immunoglobulin or their antigen-binding fragment, and
2. b) culturing the filamentous fungal cell to produce said glycoprotein having fucosylated N-glycan
3. c) optionally, purifying said fucosylated glycoprotein.

## Definitions

**[0035]** As used herein, an "expression system" or a "host cell" refers to the cell that is genetically modified to enable the transcription, translation and proper folding of a polypeptide or a protein of interest, typically a mammalian protein.

**[0036]** As used herein, a "polynucleotide" or "oligonucleotide" or "nucleic acid" are used interchangeably and refers to a polymer of at least two nucleotides joined together by a phosphodiester bond and may consist of

either ribonucleotides or deoxynucleotides or their derivatives that can be introduced into a host cell for genetic modification of such host cell. For example, a polynucleotide may encode a coding sequence of a protein, and/or comprise control or regulatory sequences of a coding sequence of a protein, such as enhancer or promoter sequences or terminator. A polynucleotide may for example comprise native coding sequence of a gene or their fragments, or variant sequences that have been optimized for optimal gene expression in a specific host cell (for example to take into account codon bias).

**[0037]** As used herein, the term, "optimized" with reference to a polynucleotide means that a polynucleotide has been altered to encode an amino acid sequence using codons that are preferred in the production cell or organism, for example, a filamentous fungal cell such as a *Trichoderma* cell. The optimized nucleotide sequence is typically modified to retain completely or as much as possible the amino acid sequence originally encoded by the starting nucleotide sequence, which is also known as the "parental" sequence. The optimized sequences herein have been engineered to have codons that are preferred in the corresponding production cell or organism, for example the filamentous fungal cell. The amino acid sequences encoded by optimized nucleotide sequences may also be referred to as optimized.

**[0038]** As used herein, a "peptide" or a "polypeptide" is an amino acid sequence including a plurality of consecutive polymerized amino acid residues. The peptide or polypeptide may include modified amino acid residues, naturally occurring amino acid residues not encoded by a codon, and non-naturally occurring amino acid residues. As used herein, a "protein" may refer to a peptide or a polypeptide or a combination of more than one peptide or polypeptide assembled together by covalent or non-covalent bonds. Unless specified, the term "protein" may encompass one or more amino acid sequences with their post-translation modifications, and in particular with N-glycan modifications. As used herein, the term "glycoprotein" refers to a protein which comprises at least one N-linked glycan attached to at least one asparagine residue of a protein.

**[0039]** As used herein, "glycan" refers to an oligosaccharide chain that can be linked to a carrier such as an amino acid, peptide, polypeptide, lipid or a reducing end conjugate. In certain embodiments, the disclosure relates to N-linked glycans ("N-glycan") conjugated to a polypeptide N-glycosylation site such as -Asn-Xxx-Ser/Thr- by N-linkage to side-chain amide nitrogen of asparagine residue (Asn), where Xxx is any amino acid residue except Pro. The disclosure may further relate to glycans as part of dolichol-phospho-oligosaccharide (Dol-P-P-OS) precursor lipid structures, which are precursors of N-linked glycans in the endoplasmic reticulum of eukaryotic cells. The precursor oligosaccharides are linked from their reducing end to two phosphate residues on the dolichol lipid. For example,  $\alpha$ 3-mannosyltransferase Alg3 modifies the Dol-P-P-oligosaccharide precursor of N-glycans. Generally, the glycan structures described herein are terminal glycan structures, where the non-reducing residues are not modified by other monosaccharide residue or residues.

**[0040]** As used throughout the present disclosure, glycolipid and carbohydrate nomenclature is essentially according to recommendations by the IUPAC-IUB Commission on Biochemical Nomenclature (e.g. Carbohydrate Res. 1998, 312, 167; Carbohydrate Res. 1997, 297, 1; Eur. J. Biochem. 1998, 257, 29). It is assumed that Gal (galactose), Glc (glucose), GlcNAc (N-acetylglucosamine), GalNAc (N-acetylgalactosamine), Man (mannose), and Neu5Ac are of the D-configuration, Fuc of the L-configuration, and all the monosaccharide units in the pyranose form (D-Galp, D-Glcp, D-GlcpNAc, D-GalpNAc, D-Manp, L-Fucp, D-Neup5Ac). The amine group is as defined for natural galactose and glucosamines on the 2-position of GalNAc or GlcNAc. Glycosidic linkages are shown partly in shorter and partly in longer nomenclature, the linkages of the sialic acid SA/Neu5X-residues  $\alpha$ 3 and  $\alpha$ 6 mean the same as  $\alpha$ 2-3 and  $\alpha$ 2-6, respectively, and for hexose monosaccharide residues  $\alpha$ 1-3,  $\alpha$ 1-6,  $\beta$ 1-2,  $\beta$ 1-3,  $\beta$ 1-4, and  $\beta$ 1-6 can be shortened as  $\alpha$ 3,  $\alpha$ 6,  $\beta$ 2,  $\beta$ 3,  $\beta$ 4, and  $\beta$ 6, respectively. Lactosamine refers to type II N-acetylglucosamine, Gal $\beta$ 4GlcNAc, and/or type I N-acetylglucosamine. Gal $\beta$ 3GlcNAc and sialic acid (SA) refer to N-acetylneuraminic acid (Neu5Ac), N-glycolylneuraminic acid (Neu5Gc), or any other natural sialic acid including derivatives of Neu5X. Sialic acid is referred to as NeuNX or Neu5X, where preferably X is Ac or Gc. Occasionally Neu5Ac/Gc/X may be referred to as NeuNAc/NeuNGc/NeuNX.

**[0041]** The sugars typically constituting N-glycans found in mammalian glycoprotein, include, without limitation, N-acetylglucosamine (abbreviated hereafter as "GlcNAc"), mannose (abbreviated hereafter as "Man"), glucose (abbreviated hereafter as "Glc"), galactose (abbreviated hereafter as "Gal"), and sialic acid (abbreviated hereafter as "Neu5Ac"). N-glycans share a common pentasaccharide referred as the "core" structure  $\text{Man}_3\text{GlcNAc}_2$ . When a fucose is attached to the core structure, the N-glycan may be represented as  $\text{Man}_3\text{GlcNAc}_2(\text{Fuc})$ . A "complex N-glycan" refers to a N-glycan which has one GlcNAc residue on terminal 1,3 mannose arm of the core structure and one GlcNAc residue on terminal 1,6 mannose arm of the core structure. Such complex N-glycans include  $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2$  (also referred as G0 glycoform),  $\text{Gal}_1\text{-}_2\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2$  (also referred as G1 glycoform), and  $\text{Neu5Ac}_1\text{-}_2\text{Gal}_1\text{-}_2\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2$  (also referred as G2 glycoform), and their core fucosylated glycoforms FG0, FG1 and FG2, respectively  $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2(\text{Fuc})$ ,  $\text{Gal}_1\text{-}_2\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2(\text{Fuc})$ , and  $\text{Neu5Ac}_1\text{-}_2\text{Gal}_1\text{-}_2\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2(\text{Fuc})$ .

**[0042]** "Increased" or "Reduced activity of an endogenous enzyme": The filamentous fungal cell may have increased or reduced levels of activity of various endogenous enzymes. A reduced level of activity may be provided by inhibiting the activity of the endogenous enzyme with an inhibitor, an antibody, or the like. In certain embodiments, the filamentous fungal cell is genetically modified in ways to increase or reduce activity of various endogenous enzymes. "Genetically modified" refers to any recombinant DNA or RNA method used to create a prokaryotic or eukaryotic host cell that expresses a polypeptide at elevated levels, at lowered levels, or in a mutated form. In other words, the host cell has been transfected, transformed, or transduced with a recombinant polynucleotide molecule, and thereby been altered so as to cause the cell to alter expression of a desired protein.

**[0043]** "Genetic modifications" which result in a decrease in gene expression, in the function of the gene, or in the function of the gene product (i.e., the protein encoded by the gene) can be referred to as inactivation (complete or partial), deletion, disruption, interruption, blockage, silencing, or down-regulation, or attenuation of expression of a gene. For example, a genetic modification in a gene which results in a decrease in the function of the protein encoded by such gene, can be the result of a complete deletion of the gene (i.e., the gene does not exist, and therefore the protein does not exist), a mutation in the gene which results in incomplete (disruption) or no translation of the protein (e.g., the protein is not expressed), or a mutation in the gene which decreases or abolishes the natural function of the protein (e.g., a protein is expressed which has decreased or no enzymatic activity or action). More specifically, reference to decreasing the action of proteins discussed herein generally refers to any genetic modification in the host cell in question, which results in decreased expression and/or functionality (biological activity) of the proteins and includes decreased activity of the proteins (e.g., decreased catalysis), increased inhibition or degradation of the proteins as well as a reduction or elimination of expression of the proteins. For example, the action or activity of a protein can be decreased by blocking or reducing the production of the protein, reducing protein action, or inhibiting the action of the protein. Combinations of some of these modifications are also possible. Blocking or reducing the production of a protein can include placing the gene encoding the protein under the control of a promoter that requires the presence of an inducing compound in the growth medium. By establishing conditions such that the inducer becomes depleted from the medium, the expression of the gene encoding the protein (and therefore, of protein synthesis) could be turned off. Blocking or reducing the action of a protein could also include using an excision technology approach similar to that described in U.S. Pat. No. 4,743,546. To use this approach, the gene encoding the protein of interest is cloned between specific genetic sequences that allow specific, controlled excision of the gene from the genome. Excision could be prompted by, for example, a shift in the cultivation temperature of the culture, as in U.S. Pat. No. 4,743,546, or by some other physical or nutritional signal.

**[0044]** In general, according to the present disclosure, an increase or a decrease in a given characteristic of a mutant or modified protein (e.g., enzyme activity) is made with reference to the same characteristic of a parent

(i.e., normal, not modified) protein that is derived from the same organism (from the same source or parent sequence), which is measured or established under the same or equivalent conditions. Similarly, an increase or decrease in a characteristic of a genetically modified host cell (e.g., expression and/or biological activity of a protein, or production of a product) is made with reference to the same characteristic of a wild-type host cell of the same species, and preferably the same strain, under the same or equivalent conditions. Such conditions include the assay or culture conditions (e.g., medium components, temperature, pH, etc.) under which the activity of the protein (e.g., expression or biological activity) or other characteristic of the host cell is measured, as well as the type of assay used, the host cell that is evaluated, etc. As discussed above, equivalent conditions are conditions (e.g., culture conditions) which are similar, but not necessarily identical (e.g., some conservative changes in conditions can be tolerated), and which do not substantially change the effect on cell growth or enzyme expression or biological activity as compared to a comparison made under the same conditions.

**[0045]** Preferably, a genetically modified host cell that has a genetic modification that increases or decreases the activity of a given protein (e.g., an enzyme) has an increase or decrease, respectively, in the activity or action (e.g., expression, production and/or biological activity) of the protein, as compared to the activity of the protein in a parent host cell (which does not have such genetic modification), of at least about 5%, and more preferably at least about 10%, and more preferably at least about 15%, and more preferably at least about 20%, and more preferably at least about 25%, and more preferably at least about 30%, and more preferably at least about 35%, and more preferably at least about 40%, and more preferably at least about 45%, and more preferably at least about 50%, and more preferably at least about 55%, and more preferably at least about 60%, and more preferably at least about 65%, and more preferably at least about 70%, and more preferably at least about 75%, and more preferably at least about 80%, and more preferably at least about 85%, and more preferably at least about 90%, and more preferably at least about 95%, or any percentage, in whole integers between 5% and 100% (e.g., 6%, 7%, 8%, etc.). The same differences are certain when comparing an isolated modified nucleic acid molecule or protein directly to the isolated wild-type nucleic acid molecule or protein (e.g., if the comparison is done in vitro as compared to in vivo).

**[0046]** In another aspect of the disclosure, a genetically modified host cell that has a genetic modification that increases or decreases the activity of a given protein (e.g., an enzyme) has an increase or decrease, respectively, in the activity or action (e.g., expression, production and/or biological activity) of the protein, as compared to the activity of the wild-type protein in a parent host cell, of at least about 2-fold, and more preferably at least about 5-fold, and more preferably at least about 10-fold, and more preferably about 20-fold, and more preferably at least about 30-fold, and more preferably at least about 40-fold, and more preferably at least about 50-fold, and more preferably at least about 75-fold, and more preferably at least about 100-fold, and more preferably at least about 125-fold, and more preferably at least about 150-fold, or any whole integer increment starting from at least about 2-fold (e.g., 3-fold, 4-fold, 5-fold, 6-fold, etc.).

**[0047]** As used herein, the terms "identical" or percent "identity," in the context of two or more nucleic acid or amino acid sequences, refers to two or more sequences or subsequences that are the same. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (i.e., 29% identity, optionally 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% identity over a specified region, or, when not specified, over the entire sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200, or more amino acids) in length.

**[0048]** For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are

entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters. When comparing two sequences for identity, it is not necessary that the sequences be contiguous, but any gap would carry with it a penalty that would reduce the overall percent identity. For blastn, the default parameters are Gap opening penalty=5 and Gap extension penalty=2. For blastp, the default parameters are Gap opening penalty=11 and Gap extension penalty=1.

**[0049]** A "comparison window," as used herein, includes reference to a segment of any one of the number of contiguous positions including, but not limited to from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman (1981), by the homology alignment algorithm of Needleman and Wunsch (1970) *J Mol Biol* 48(3):443-453, by the search for similarity method of Pearson and Lipman (1988) *Proc Natl Acad Sci USA* 85(8):2444-2448, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection [see, e.g., Brent et al., (2003) *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (Ringbou Ed)].

**[0050]** Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1997) *Nucleic Acids Res* 25(17):3389-3402 and Altschul et al. (1990) *J. Mol Biol* 215(3)-403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) or 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix [see Henikoff and Henikoff, (1992) *Proc Natl Acad Sci USA* 89(22):10915-10919] alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

**[0051]** The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin and Altschul, (1993) *Proc Natl Acad Sci USA* 90(12):5873-5877). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

**[0052]** "Functional variant" as used herein refers to a coding sequence or a protein having sequence similarity with a reference sequence, typically, at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% identity with the reference coding sequence or protein, and retaining substantially the same function as said reference coding sequence or protein. A functional variant may retain the same function but with reduced or increased activity. Functional variants include natural variants, for example, homologs from different species or artificial variants, resulting from the introduction of a mutation in the coding sequence. Functional variant may be a variant with only conservatively modified mutations.

**[0053]** "Conservatively modified mutations" as used herein include individual substitutions, deletions or additions to an encoded amino acid sequence which result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic



variants, interspecies homologs, and alleles of the disclosure. The following eight groups contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, *Proteins* (1984)).

#### Filamentous fungal cells

**[0054]** As used herein, "filamentous fungal cells" include cells from all filamentous forms of the subdivision Eumycota and Oomycota (as defined by Hawksworth et al., In, Ainsworth and Bisby's Dictionary of The Fungi, 8th edition, 1995, CAB International, University Press, Cambridge, UK). Filamentous fungal cells are generally characterized by a mycelial wall 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.

**[0055]** Preferably, the filamentous fungal cell is not adversely affected by the transduction of the necessary nucleic acid sequences, the subsequent expression of the proteins (e.g., mammalian proteins), or the resulting intermediates. General methods to disrupt genes of and cultivate filamentous fungal cells are disclosed, for example, for *Penicillium*, in Kopke et al. (2010) *Appl Environ Microbiol.* 76(14):4664-74. doi: 10.1128/AEM.00670-10, for *Aspergillus*, in Maruyama and Kitamoto (2011), *Methods in Molecular Biology*, vol. 765, DOI10.1007/978-1-61779-197-0\_27; for *Neurospora*, in Collopy et al. (2010) *Methods Mol Biol.* 2010;638:33-40. doi: 10.1007/978-1-60761-611-5\_3; and for *Myceliophthora* or *Chrysosporium* PCT/NL2010/000045 and PCT/EP98/06496.

**[0056]** Examples of suitable filamentous fungal cells include, without limitation, cells from an *Acremonium*, *Aspergillus*, *Fusarium*, *Humicola*, *Mucor*, *Myceliophthora*, *Neurospora*, *Penicillium*, *Scytalidium*, *Thielavia*, *Tolypocladium*, or *Trichoderma* strain. In certain embodiments, the filamentous fungal cell is from a *Trichoderma* sp., *Acremonium*, *Aspergillus*, *Aureobasidium*, *Cryptococcus*, *Chrysosporium*, *Chrysosporium lucknowense*, *Filibasidium*, *Fusarium*, *Gibberella*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Myrothecium*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Piromyces*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, or *Tolypocladium* strain.

**[0057]** In some embodiments, the filamentous fungal cell is a *Trichoderma* cell or related species such as *Myceliophthora* or *Chrysosporium*, *Fusarium*, *Neurospora*, *Penicillium*, or *Aspergillus* cell.

**[0058]** *Aspergillus* fungal cells of the present disclosure may include, without limitation, *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus clavatus*, *Aspergillus flavus*, *Aspergillus foetidus*, *Aspergillus fumigatus*, *Aspergillus japonicus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, or *Aspergillus terreus*.

**[0059]** *Neurospora* fungal cells of the present disclosure may include, without limitation, *Neurospora crassa*. *Myceliophthora* fungal cells of the present disclosure may include, without limitation, *Myceliophthora thermophila*.

**[0060]** In a preferred embodiment, the filamentous fungal cell is a *Trichoderma* fungal cell. *Trichoderma* fungal cells of the present disclosure may be derived from a wild-type *Trichoderma* strain or a mutant thereof. Examples of suitable *Trichoderma* fungal cells include, without limitation, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, *Trichoderma atroviride*, *Trichoderma virens*, *Trichoderma viride*; and alternative sexual form thereof (i.e., *Hypocrea*).

[0061] In a more preferred embodiment, the filamentous fungal cell is a *Trichoderma reesei*, and for example, strains derived from ATCC 13631 (QM 6a), ATCC 24449 (radiation mutant 207 of QM 6a), ATCC 26921 (QM 9414; mutant of ATCC 24449), VTT-D-00775 (Selinheimo et al., FEBS J., 2006, 273: 4322-4335), Rut-C30 (ATCC 56765), RL-P37 (NRRL 15709) or *T. harzianum* isolate T3 (Wolffhechel, H., 1989).

#### **Proteases with reduced activity**

[0062] The disclosure described herein relates to filamentous fungal cells, such as *Trichoderma* fungal cells, that have reduced activity in at least one endogenous protease and comprises at least a polynucleotide encoding fucosyltransferase, for use in the production of glycoproteins with fucosylated N-glycans.

[0063] It has been found that reducing protease activity enables to increase substantially the production of heterologous mammalian glycoprotein. Indeed, such proteases found in filamentous fungal cells that express a heterologous polypeptide normally catalyze significant degradation of the expressed recombinant glycoprotein. Thus, by reducing the activity of proteases in filamentous fungal cells that express a heterologous glycoprotein, the stability of the expressed glycoprotein is increased, resulting in an increased level of production of the fucosylated glycoprotein, and in some circumstances, improved quality of the produced fucosylated glycoprotein (e.g., full-length instead of degraded).

[0064] Proteases include, without limitation, aspartic proteases, trypsin-like serine proteases, subtilisin proteases, glutamic proteases, and sedolisin proteases. Such proteases may be identified and isolated from filamentous fungal cells and tested to determine whether reduction in their activity affects the production of a recombinant polypeptide from the filamentous fungal cell. Methods for identifying and isolating proteases are well known in the art, and include, without limitation, affinity chromatography, zymogram assays, and gel electrophoresis. An identified protease may then be tested by deleting the gene encoding the identified protease from a filamentous fungal cell that expresses a recombinant polypeptide, such a heterologous or mammalian polypeptide, and determining whether the deletion results in a decrease in total protease activity of the cell, and an increase in the level of production of the expressed recombinant polypeptide. Methods for deleting genes, measuring total protease activity, and measuring levels of produced protein are well known in the art and include the methods described herein.

#### **Aspartic Proteases**

[0065] Aspartic proteases are enzymes that use an aspartate residue for hydrolysis of the peptide bonds in polypeptides and proteins. Typically, aspartic proteases contain two highly-conserved aspartate residues in their active site which are optimally active at acidic pH. Aspartic proteases from eukaryotic organisms such as *Trichoderma* fungi include pepsins, cathepsins, and renins. Such aspartic proteases have a two-domain structure, which is thought to arise from an ancestral gene duplication. Consistent with such a duplication event, the overall fold of each domain is similar, though the sequences of the two domains have begun to diverge. Each domain contributes one of the catalytic aspartate residues. The active site is in a cleft formed by the two domains of the aspartic proteases. Eukaryotic aspartic proteases further include conserved disulfide bridges, which can assist in identification of the polypeptides as being aspartic acid proteases.

[0066] Nine aspartic proteases have been identified in *Trichoderma reesei* fungal cells: *pep1* (tre74156); *pep2* (tre53961); *pep3* (tre121133); *pep4* (tre77579), *pep5* (tre81004), and *pep7* (tre58669), *pep8* (EGR48424), *pep11* (EGR49498) and *pep12* (EGR52517).

[0067] Examples of suitable aspartic proteases include, without limitation, *Trichoderma reesei pep1* (SEQ ID NO: 17), *Trichoderma reesei pep2* (SEQ ID NO: 18), *Trichoderma reesei pep3* (SEQ ID NO: 19); *pep4* (SEQ ID

NO: 20), *Trichoderma reesei* pep5 (SEQ ID NO: 21), *Trichoderma reesei* pep7 (SEQ ID NO:23), *Trichoderma reesei* pep8 (SEQ ID NO:410), *Trichoderma reesei* pep11 (SEQ ID NO:411) and *Trichoderma reesei* pep12 (SEQ ID NO:412) and homologs thereof. Examples of homologs of pep1, pep2, pep3, pep4, pep5, pep7, pep8, pep11 or pep12 proteases identified in other organisms are also described in US provisional application 61/583,559 or PCT/EP2013/050126, the content of which being incorporated by reference.

### ***Trypsin-Like Serine Proteases***

[0068] Trypsin-like serine proteases are enzymes with substrate specificity similar to that of trypsin. Trypsin-like serine proteases use a serine residue for hydrolysis of the peptide bonds in polypeptides and proteins. Typically, trypsin-like serine proteases cleave peptide bonds following a positively-charged amino acid residue. Trypsin-like serine proteases from eukaryotic organisms such as *Trichoderma* fungi include trypsin 1, trypsin 2, and mesotrypsin. Such trypsin-like serine proteases generally contain a catalytic triad of three amino acid residues (such as histidine, aspartate, and serine) that form a charge relay that serves to make the active site serine nucleophilic. Eukaryotic trypsin-like serine proteases further include an "oxyanion hole" formed by the backbone amide hydrogen atoms of glycine and serine, which can assist in identification of the polypeptides as being trypsin-like serine proteases.

[0069] One trypsin-like serine protease has been identified in *Trichoderma* fungal cells: tsp1 (tre73897). As discussed below, tsp1 has been demonstrated to have a significant impact on expression of recombinant glycoproteins, such as immunoglobulins.

[0070] Examples of suitable tsp1 proteases include, without limitation, *Trichoderma reesei* tsp1 (SEQ ID NO: 24) and homologs thereof. Examples of homologs of tsp1 proteases identified in other organisms are described in US provisional application 61/583,559 or PCT/EP2013/050126.

### ***Subtilisin Proteases***

[0071] Subtilisin proteases are enzymes with substrate specificity similar to that of subtilisin. Subtilisin proteases use a serine residue for hydrolysis of the peptide bonds in polypeptides and proteins. Generally, subtilisin proteases are serine proteases that contain a catalytic triad of the three amino acids aspartate, histidine, and serine. The arrangement of these catalytic residues is shared with the prototypical subtilisin from *Bacillus licheniformis*. Subtilisin proteases from eukaryotic organisms such as *Trichoderma* fungi include furin, MBTPS1, and TPP2. Eukaryotic trypsin-like serine proteases further include an aspartic acid residue in the oxyanion hole.

[0072] Seven subtilisin proteases have been identified in *Trichoderma* fungal cells: slp1 (tre51365); slp2 (tre123244); slp3 (tre123234); slp5 (tre64719), slp6 (tre121495), slp7 (tre123865), and slp8 (tre58698).

[0073] Examples of suitable sip proteases include, without limitation, *Trichoderma reesei* slp1 (SEQ ID NO: 25), slp2 (SEQ ID NO: 26); slp3 (SEQ ID NO: 27); slp5 (SEQ ID NO: 28), slp6 (SEQ ID NO: 29), slp7 (SEQ ID NO: 30), and slp8 (SEQ ID NO: 31), and homologs thereof. Examples of homologs of slp proteases identified in other organisms are described in US provisional application 61/583,559 or PCT/EP2013/050126.

### ***Glutamic Proteases***

[0074] Glutamic proteases are enzymes that hydrolyze the peptide bonds in polypeptides and proteins.

Glutamic proteases are insensitive to pepstatin A, and so are sometimes referred to as pepstatin insensitive acid proteases. While glutamic proteases were previously grouped with the aspartic proteases and often jointly referred to as acid proteases, it has been recently found that glutamic proteases have very different active site residues than aspartic proteases.

**[0075]** Two glutamic proteases have been identified in *Trichoderma* fungal cells: gap1 (tre69555) and gap2 (tre106661).

**[0076]** Examples of suitable gap proteases include, without limitation, *Trichoderma reesei* gap1 (SEQ ID NO: 32), *Trichoderma reesei* gap2 (SEQ ID NO: 33), and homologs thereof. Examples of homologs of gap proteases identified in other organisms are described in US provisional application 61/583,559 or PCT/EP2013/050126.

#### **Sedolisin Proteases and homologs of proteases**

**[0077]** Sedolisin proteases are enzymes that use a serine residue for hydrolysis of the peptide bonds in polypeptides and proteins. Sedolisin proteases generally contain a unique catalytic triad of serine, glutamate, and aspartate. Sedolisin proteases also contain an aspartate residue in the oxyanion hole. Sedolisin proteases from eukaryotic organisms such as *Trichoderma* fungi include tripeptidyl peptidase.

**[0078]** Examples of suitable tpp1 proteases include, without limitation, *Trichoderma reesei* tpp1 (SEQ ID NO: 34) and homologs thereof. Examples of homologs of tpp1 proteases identified in other organisms are described in US provisional application 61/583,559 or PCT/EP2013/050126.

**[0079]** As used in reference to protease, the term "homolog" refers to a protein which has protease activity and exhibit sequence similarity with a known (reference) protease sequence. Homologs may be identified by any method known in the art, preferably, by using the BLAST tool to compare a reference sequence to a single second sequence or fragment of a sequence or to a database of sequences. As described in the "Definitions" section, BLAST will compare sequences based upon percent identity and similarity.

**[0080]** Preferably, a homologous protease has at least 30% identity with (optionally 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% identity over a specified region, or, when not specified, over the entire sequence), when compared to one of the protease sequences listed above, including *T. reesei* pep1, pep2, pep3, pep4, pep5, pep7, pep8, pep11, pep12, tsp1, slp1, slp2, slp3, slp5, slp6, slp7, slp8, tpp1, gap1 and gap2.

#### **Reducing the Activity of Proteases in the filamentous fungal cell of the disclosure**

**[0081]** The filamentous fungal cells according to the disclosure have reduced activity of at least one endogenous protease, typically 2, 3, 4, 5 or more, in order to improve the stability and production of the glycoprotein with fucosylated N-glycans in said filamentous fungal cell.

**[0082]** The activity of proteases found in filamentous fungal cells can be reduced by any method known to those of skill in the art. In some embodiments reduced activity of proteases is achieved by reducing the expression of the protease, for example, by promoter modification or RNAi.

**[0083]** In other embodiments, reduced activity of proteases is achieved by modifying the gene encoding the protease. Examples of such modifications include, without limitation, a mutation, such as a deletion or disruption of the gene encoding said endogenous protease activity.

**[0084]** Accordingly, the disclosure relates to a filamentous fungal cell, such as a *Trichoderma* cell, which has a mutation that reduces at least one endogenous protease activity compared to a parental filamentous fungal cell which does not have such mutation, said filamentous fungal cell further comprising a polynucleotide encoding fucosyltransferase activity.

**[0085]** Deletion or disruption mutation includes without limitation knock-out mutation, a truncation mutation, a point mutation, a missense mutation, a substitution mutation, a frameshift mutation, an insertion mutation, a duplication mutation, an amplification mutation, a translocation mutation, or an inversion mutation, and that results in a reduction in the corresponding protease activity. Methods of generating at least one mutation in a protease encoding gene of interest are well known in the art and include, without limitation, random mutagenesis and screening, site-directed mutagenesis, PCR mutagenesis, insertional mutagenesis, chemical mutagenesis, and irradiation.

**[0086]** In certain embodiments, a portion of the protease encoding gene is modified, such as the region encoding the catalytic domain, the coding region, or a control sequence required for expression of the coding region. Such a control sequence of the gene may be a promoter sequence or a functional part thereof, i.e., a part that is sufficient for affecting expression of the gene. For example, a promoter sequence may be inactivated resulting in no expression or a weaker promoter may be substituted for the native promoter sequence to reduce expression of the coding sequence. Other control sequences for possible modification include, without limitation, a leader sequence, a propeptide sequence, a signal sequence, a transcription terminator, and a transcriptional activator.

**[0087]** Protease encoding genes that are present in filamentous fungal cells may also be modified by utilizing gene deletion techniques to eliminate or reduce expression of the gene. Gene deletion techniques enable the partial or complete removal of the gene thereby eliminating their expression. In such methods, deletion of the gene may be accomplished by homologous recombination using a plasmid that has been constructed to contiguously contain the 5' and 3' regions flanking the gene.

**[0088]** The protease encoding genes that are present in filamentous fungal cells may also be modified by introducing, substituting, and/or removing one or more nucleotides in the gene, or a control sequence thereof required for the transcription or translation of the gene. For example, nucleotides may be inserted or removed for the introduction of a stop codon, the removal of the start codon, or a frame-shift of the open reading frame. Such a modification may be accomplished by methods known in the art, including without limitation, site-directed mutagenesis and PCR generated mutagenesis (see, for example, Botstein and Shortie, 1985, Science 229: 4719; Lo et al., 1985, Proceedings of the National Academy of Sciences USA 81: 2285; Higuchi et al., 1988, Nucleic Acids Research 16: 7351; Shimada, 1996, Meth. Mol. Biol. 57: 157; Ho et al., 1989, Gene 77: 61; Horton et al., 1989, Gene 77: 61; and Sarkar and Sommer, 1990, BioTechniques 8: 404).

**[0089]** Additionally, protease encoding genes that are present in filamentous fungal cells may be modified by gene disruption techniques by inserting into the gene a disruptive nucleic acid construct containing a nucleic acid fragment homologous to the gene that will create a duplication of the region of homology and incorporate construct nucleic acid between the duplicated regions. Such a gene disruption can eliminate gene expression if the inserted construct separates the promoter of the gene from the coding region or interrupts the coding sequence such that a nonfunctional gene product results. A disrupting construct may be simply a selectable marker gene accompanied by 5' and 3' regions homologous to the gene. The selectable marker enables identification of transformants containing the disrupted gene.

**[0090]** The disruptive nucleic acid construct may comprise one or more polynucleotides encoding fucosylation pathway proteins, a polynucleotide encoding GMD activity, a polynucleotide encoding FX activity, a polynucleotide encoding GDP-fucose transporter, and a polynucleotide encoding  $\alpha$ 1,6 fucosyltransferase activity. Further, the disruptive nucleic acid construct may comprise one or more polynucleotides encoding an

$\alpha$ -1,2 mannosidase, an N-acetylglucosaminyltransferase I catalytic domain, an  $\alpha$  mannosidase II, an N-acetylglucosaminyltransferase II catalytic domain, a  $\beta$ 1,4 galactosyltransferase, a glucosamine UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase, an N-acetylneuraminic acid synthase, an N-acetylneuraminic acid phosphatase, a cytidine monophosphate N-acetylneuraminic acid synthetase, a CMP-sialic acid transporter, and/or a sialyltransferase.

**[0091]** Protease encoding genes that are present in filamentous fungal cells may also be modified by the process of gene conversion (see, for example, Iglesias and Trautner, 1983, Molecular General Genetics 189:5 73-76). For example, in the gene conversion a nucleotide sequence corresponding to the gene is mutagenized in vitro to produce a defective nucleotide sequence, which is then transformed into a *Trichoderma* strain to produce a defective gene. By homologous recombination, the defective nucleotide sequence replaces the endogenous gene. It may be desirable that the defective nucleotide sequence also contains a marker for selection of transformants containing the defective gene.

**[0092]** Protease encoding genes of the present disclosure that are present in filamentous fungal cells that express a recombinant polypeptide may also be modified by established anti-sense techniques using a nucleotide sequence complementary to the nucleotide sequence of the gene (see, for example, Parish and Stoker, 1997, FEMS Microbiology Letters 154: 151-157). In particular, expression of the gene by filamentous fungal cells may be reduced or inactivated by introducing a nucleotide sequence complementary to the nucleotide sequence of the gene, which may be transcribed in the strain and is capable of hybridizing to the mRNA produced in the cells. Under conditions allowing the complementary anti-sense nucleotide sequence to hybridize to the mRNA, the amount of protein translated is thus reduced or eliminated.

**[0093]** Protease encoding genes that are present in filamentous fungal cells may also be modified by random or specific mutagenesis using methods well known in the art, including without limitation, chemical mutagenesis (see, for example, Hopwood, The Isolation of Mutants in Methods in Microbiology (J.R. Norris and D.W. Ribbons, eds.) pp. 363-433, Academic Press, New York, 25 1970). Modification of the gene may be performed by subjecting filamentous fungal cells to mutagenesis and screening for mutant cells in which expression of the gene has been reduced or inactivated. The mutagenesis, which may be specific or random, may be performed, for example, by use of a suitable physical or chemical mutagenizing agent, use of a suitable oligonucleotide, subjecting the DNA sequence to PCR generated mutagenesis, or any combination thereof. Examples of physical and chemical mutagenizing agents include, without limitation, ultraviolet (UV) irradiation, hydroxylamine, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), N-methyl-N'-nitrosoguanidine (NTG) O-methyl hydroxylamine, nitrous acid, ethyl methane sulphonate (EMS), sodium bisulphite, formic acid, and nucleotide analogues. When such agents are used, the mutagenesis is typically performed by incubating the *Trichoderma* cells to be mutagenized in the presence of the mutagenizing agent of choice under suitable conditions, and then selecting for mutants exhibiting reduced or no expression of the gene.

**[0094]** In certain embodiments, the at least one mutation or modification in a protease encoding gene of the present disclosure results in a modified protease that has no detectable protease activity. In other embodiments, the at least one modification in a protease encoding gene of the present disclosure results in a modified protease that has at least 25% less, at least 50% less, at least 75% less, at least 90%, at least 95%, or a higher percentage less protease activity compared to a corresponding non-modified protease.

**[0095]** In a preferred embodiment, a filamentous fungal cell according to the disclosure is a *Trichoderma* cell which has a deletion or disruption in at least 3 endogenous proteases, resulting in no detectable activity for such deleted or disrupted endogenous proteases and further comprising at least one or more polynucleotides encoding fucosylation pathway. In one embodiment, that may be combined with the preceding embodiments, said cell may comprise mutations that reduce or eliminate the activity of

1. a) the three endogenous proteases pep1, tsp1 and slp1,
2. b) the three endogenous proteases gap1, slp1 and pep1,

3. c) three endogenous proteases selected from the group consisting of pep1, pep2, pep3, pep4, pep5, pep8, pep11, pep12, tsp1, slp1, slp2, slp3, slp7, gap1 and gap2,
4. d) three to six proteases selected from the group consisting of pep1, pep2, pep3, pep4, pep5, tsp1, slp1, slp2, slp3, gap1 and gap2, or
5. e) seven to ten proteases selected from the group consisting of pep1, pep2, pep3, pep4, pep5, pep7, pep8, tsp1, slp1, slp2, slp3, slp5, slp6, slp7, slp8, tpp1, gap1 and gap2.

**[0096]** In certain embodiments, the filamentous fungal cell or *Trichoderma* cell, has reduced or no detectable protease activity in pep1, tsp1, and slp1. Advantageously, in such triple deletion mutant, the protease activity may be reduced by more than 3 fold. In certain embodiments, the filamentous fungal cell or *Trichoderma* cell, has reduced or no detectable protease activity in gap1, slp1, and pep1. In certain embodiments, the filamentous fungal cell or *Trichoderma* cell, has reduced or no detectable protease activity in slp2, pep1 and gap1. In certain embodiments, the filamentous fungal cell or *Trichoderma* cell, has reduced or no detectable protease activity in pep1, tsp1, slp1, and gap1. Advantageously, in such quadruple deletion mutant, the protease activity may be reduced by more than 7 fold. In certain embodiments, the filamentous fungal cell or *Trichoderma* cell, has reduced or no detectable protease activity in pep1, tsp1, slp1, gap1, and gap2. Advantageously, in such 5-fold deletion mutant, the protease activity may be reduced by more than 10 fold. In certain embodiments, the filamentous fungal cell or *Trichoderma* cell, has reduced or no detectable protease activity in pep1, tsp1, slp1, gap1, gap2, and pep4. Advantageously, in such 6-fold deletion mutant, the protease activity may be reduced by more than 15 fold. In certain embodiments, the filamentous fungal cell or *Trichoderma* cell, has reduced or no detectable protease activity in pep1, tsp1, slp1, gap1, gap2, pep4, and pep3. Advantageously, in such 7-fold deletion mutant, the protease activity may be reduced by more than 18 fold. In certain embodiments, the filamentous fungal cell or *Trichoderma* cell, has reduced or no detectable protease activity in pep1, tsp1, slp1, gap1, gap2, pep4, pep3, and pep5. In certain embodiments, the filamentous fungal cell or *Trichoderma* cell, has reduced or no detectable protease activity in pep1, tsp1, slp1, gap1, gap2, pep4, pep3, pep5, and pep2. In certain embodiments, the filamentous fungal cell or *Trichoderma* cell, has reduced or no detectable protease activity in pep1, tsp1, slp1, gap1, gap2, pep4, pep3, pep5, pep2, and pep11. In certain embodiments, the filamentous fungal cell or *Trichoderma* cell, has reduced or no detectable protease activity in pep1, tsp1, slp1, gap1, gap2, pep4, pep3, and slp2. In certain embodiments, the filamentous fungal cell or *Trichoderma* cell, has reduced or no detectable protease activity in pep1, tsp1, slp1, gap1, gap2, pep4, pep3, pep5, and pep12.

#### **Polynucleotides encoding fucosylation pathway**

**[0097]** Genes and proteins involved in the fucosylation pathways of prokaryotes and eukaryotes have been identified and characterized in the art (see for a review, Ma et al, 2006, *Glycobiology*, 16(12) 158-144).

**[0098]** As used herein the term "fucosylation pathway" relates to the sequential enzymatic steps required for in vivo fucosylation of a glycoprotein. There is no fucosylation pathway in filamentous fungal cells, such as *Trichoderma* cells. One of the major goals of the present disclosure is to provide tools and materials for the production of glycoproteins with fucosylated N-glycans, for example of fucosylated G0 glycoform, in a filamentous fungal cell.

**[0099]** In vivo fucosylation requires at least expression of one enzyme of the fucosyltransferase family. Accordingly, a filamentous fungal cell with reduced protease activity according to the disclosure comprises at least one polynucleotide encoding fucosyltransferase activity.

**[0100]** If GDP-fucose is not provided in the medium or naturally synthesized in the filamentous fungal cell, the

filamentous fungal cell according to the disclosure may advantageously contain one or more polynucleotides encoding GDP-fucose synthesis and, optionally, GDP-fucose transporter.

**[0101]** Depending on the structure of the fucosylated N-glycan that is desired to be produced by the filamentous fungal cell according to the disclosure, the skilled person will select the appropriate sequences encoding polypeptides with fucosyltransferase activity.

**[0102]** Various fucosyltransferase enzymes and their coding sequences have been identified in the art. Fucosyltransferase (FucTs) are indeed widely expressed in vertebrates such as mammalian and human cells, invertebrates, plants and bacteria. FucT belong to the glycosyltransferase superfamily (EC 2.4.1.x.y) which is defined in the category of Carbohydrate-Active enzymes (CAZY) available on the internet.

**[0103]** More specifically, as use herein, the term "fucosyltransferase" or "FucTs" refers to the enzyme catalysing the reaction that transfers the donor guanosine-diphosphate fucose (GDP-Fuc) to an acceptor glycoprotein.

**[0104]** FucTs thus include enzymes with  $\alpha$ 1,2 fucosyltransferase activity (encoded for example by human FUT1 and FUT2 genes),  $\alpha$ 1,3/ $\alpha$ 1,4 fucosyltransferase activity (encoded for example by human FUT9 and FUT5 genes), O-FucTs (encoded for example by plant O-FUT1 and 2) and  $\alpha$ 1,6 fucosyltransferase activity (encoded for example by human FUT8 gene), which is further described in detail below.

**[0105]** In a preferred embodiment, the filamentous fungal cell according to the disclosure comprises a polynucleotide encoding a polypeptide having  $\alpha$ 1,6 fucosyltransferase activity.  $\alpha$ 1,6 FucT adds fucose to the innermost GlcNAc moiety of the chitobiose unit of the core Asn-linked glycans at an  $\alpha$ 1,6 linkage. In mammals,  $\alpha$ 1,6 fucosyltransferase acting at late Golgi cisternae requires an unsubstituted  $\beta$ 1,2 linked GlcNAc on the  $\alpha$ 1,3 mannose arm of the core N-glycan.  $\alpha$ 1,6 fucosyltransferase activity is useful in particular in methods for producing fucosylated complex N-glycans such as the FG0, FG1 or FG2 glycoforms.

**[0106]** Human  $\alpha$ 1,6 FucT encoded by FUT8 gene is widely expressed in human tissues. Polynucleotide sequences encoding  $\alpha$ 1,6 FucT that may be used in the present disclosure includes without limitation the human FUT8 coding sequence of SEQ ID NO:1, FUT8 isoforms or other homologous FUT8 coding sequences from mammalian species, including without limitation any one of SEQ ID NOs 142-149.

**[0107]** In one embodiment, said filamentous fungal cell of the disclosure comprises a polynucleotide of human FUT8 coding sequence (SEQ ID NO:1), or a functional variant polynucleotide encoding a polypeptide having at least 50%, at least 60%, at least 70%, at least 90%, or at least 95% identity with SEQ ID NO:6, said functional variant encoding  $\alpha$ 1,6 fucosyltransferase activity.

**[0108]** Expression of  $\alpha$ 1,6 fucosyltransferase activity in a filamentous fungal cell of the disclosure may be determined by structural analysis of N-glycans produced by such filamentous fungal cell, as described in the Examples below.

**[0109]** The substrate of fucosyltransferase is GDP-fucose. In order to obtain in vivo fucosylation, it is therefore advantageous to provide filamentous fungal cells which further comprise enzymes required for GDP-fucose synthesis and its transport into the ER/Golgi compartment where fucosyltransferase reaction occurs. Accordingly, the filamentous fungal cell may advantageously further comprise one or more polynucleotides encoding GDP-fucose synthesis and, optionally, GDP-fucose transporter.

**[0110]** In eukaryote, GDP-fucose synthesis can be synthesized either by the de novo pathway or the minor salvage pathway. The de novo pathway starts from GDP-D-mannose which is dehydrated by GDP-mannose-4,6 dehydratase (hereafter referred as "GMD"). This leads to the formation of an unstable GDP-4-keto-6-



deoxy-D-mannose, which undergoes a subsequent 3,5 epimerization and then a NADPH-dependent reduction with the consequent formation of GDP-L-fucose. These two last steps are catalysed by GDP-4-keto-6-deoxy-D-mannose-3,5-epimerase/4-reductase (hereafter referred as "FX").

**[0111]** Accordingly, in a specific embodiment, the filamentous fungal cell of the disclosure, for example *Trichoderma* cell further comprises one or more polynucleotides encoding a polypeptide having GDP-fucose synthesis activity, selected from the group consisting of:

1. i. GMD polynucleotide or a functional variant polynucleotide encoding a polypeptide having GDP-mannose-dehydratase activity; and,
2. ii. FX polynucleotide or a functional variant polynucleotide encoding a polypeptide having both GDP-keto-deoxy-mannose-epimerase and GDP-keto-deoxy-galactose-reductase activities.

**[0112]** GMD encoding polynucleotide sequences have been described in the art and include without limitation *C. elegans* GMD optimized polynucleotide of SEQ ID NO:2, *H. pylori* GMD optimized polynucleotide of SEQ ID NO:16, or polynucleotides encoding homologous eukaryotic proteins of any one of SEQ ID NOs:124-138 or polynucleotides encoding homologous prokaryotic proteins of any one of SEQ ID NOs: 139-141, or their functional variant polynucleotide encoding polypeptides having at least 50%, at least 60%, at least 70%, at least 90%, or at least 95% identity with said any one of SEQ ID NO:7, SEQ ID NO:15 or SEQ ID NOs: 124-141, and having GDP-mannose-dehydratase activity (see also Mattila et al., 2000, Glycobiology 10(10) pp 1041-1047 and Järvinen et al, 2001, Eur J Biochem 268, 6458-6464).

**[0113]** FX encoding polynucleotide sequences have also been described in the art and include without limitation *C. elegans* FX polynucleotide of SEQ ID NO: 3, *H. pylori* FX polynucleotide of SEQ ID NO: 14 or a homologous FX polynucleotide encoding any one of SEQ ID NOs 112-123, or their functional variant polynucleotide encoding a polypeptide having at least 50%, at least 60%, at least 70%, at least 90%, or at least 95% identity with any one of said polynucleotide sequences of SEQ ID NO:8, SEQ ID NO:13 or SEQ ID NOs: 112-123 and having both GDP-keto-deoxy-mannose-epimerase and GDP-keto-deoxy-galactose-reductase activities (see also Mattila et al., 2000, Glycobiology 10(10) pp 1041-1047 and Järvinen et al, 2001, Eur J Biochem 268, 6458-6464).

**[0114]** In one specific embodiment, the filamentous fungal cell of the disclosure, such as a *Trichoderma* cell, further comprises said one or more polynucleotides encoding polypeptides with GDP-fucose synthesis activity comprising

1. i. *C. elegans* GMD polynucleotide of SEQ ID NO:2 or a functional variant polynucleotide encoding a polypeptide having at least 50%, at least 60%, at least 70%, at least 90%, or at least 95% identity with SEQ ID NO:7 and having GDP-mannose-dehydratase activity; and,
2. ii. *C. elegans* FX polynucleotide of SEQ ID NO:3 or a functional variant polynucleotide encoding a polypeptide having at least 50%, at least 60%, at least 70%, at least 90%, or at least 95% identity with SEQ ID NO:8 and having both GDP-keto-deoxy-mannose-epimerase and GDP-keto-deoxy-galactose-reductase activities.

**[0115]** GDP-fucose synthesis may be detected in vivo for example by purification and MALDI-TOF MS analysis of GDP-L-fucose as described in Mattila et al 2000, supra.

**[0116]** GDP-fucose synthesis takes place in the cytosol whereas fucosyltransferase activity occurs in vivo in the Golgi compartment. Therefore, it may be advantageous to further introduce into the filamentous fungal cell of the disclosure a polynucleotide encoding GDP fucose transporter (hereafter referred as "GFTTr").

**[0117]** GDP fucose transporter encoding genes have been cloned and characterized from various organisms. GDP fucose transporter encoding polynucleotide includes without limitation *C. elegans* GDP fucose transporter polynucleotide of SEQ ID NO: 4, a homologous FX polynucleotide encoding any one of SEQ ID NOs: 150-162, or their functional variant polynucleotide encoding a polypeptide at least 50%, at least 60%, at least 70%, at least 90%, or at least 95% identity with any one of SEQ ID NO:9, or SEQ ID NOs: 150-162 and having GDP fucose transporter.

**[0118]** In one specific embodiment, the filamentous fungal cell of the disclosure, such as a *Trichoderma* cell, further comprises a GDP-fucose transporter *C. elegans* GFTr polynucleotide of SEQ ID NO:4 or a functional variant polynucleotide encoding a polypeptide having at least 50%, at least 60%, at least 70%, at least 90%, or at least 95% identity with SEQ ID NO:9 and having GDP fucose transporter.

**[0119]** To increase Golgi targeting of fucosyltransferase, it may be required to include Golgi targeting sequence in the polynucleotide encoding fucosyltransferase activity that is introduced in the filamentous fungal cell according to the disclosure.

**[0120]** Accordingly, the filamentous fungal cell of the disclosure comprises a polynucleotide encoding fucosyltransferase linked to a Golgi targeting sequence for targeting expression of said fucosyltransferase activity in the Golgi compartment.

**[0121]** In specific embodiments, the filamentous fungal cell of the disclosure further comprises a polynucleotide encoding GnTI, GnTII, GalT, or sialyltransferase linked to a Golgi targeting sequence for targeting expression of said GnTI, GnTII, GalT, or sialyltransferase activity in the Golgi compartment.

**[0122]** To increase Golgi targeting of GnTI, GnTII, GalT, or sialyltransferase, the Golgi targeting sequence can be linked to the polynucleotide encoding GnTI, GnTII, GalT, or sialyltransferase activity that is introduced in the filamentous fungal cell of the disclosure, such that the targeting sequence and the GnTI, GnTII, GalT, or sialyltransferase is expressed as a single polypeptide.

**[0123]** Examples of Golgi targeting polynucleotide sequences that may be used for targeting fucosyltransferase, GnTI, GnTII, GalT, or sialyltransferase in the Golgi compartment are described in PCT/EP2011/070956 and include without limitation, N-terminal portion of SEQ ID NO: 5. Other targeting sequences that may be used are described more in details in the next section.

**[0124]** In a specific embodiment, a filamentous fungal cell according to the disclosure, such as *Trichoderma* cell, further comprises a polynucleotide encoding the N-terminal portion of Golgi targeting sequence of SEQ ID NO:10, or a functional variant polynucleotide having at least 50%, at least 60%, at least 70%, at least 90%, or at least 95% identity with SEQ ID NO:5 linked to the polynucleotide sequence encoding fucosyltransferase activity, such as SEQ ID NO:1. In such embodiment, a preferred filamentous fungal cell is a *Trichoderma reesei* cell.

**[0125]** In a specific embodiment, a filamentous fungal cell of the disclosure, preferably a *Trichoderma* cell, and more preferably a *Trichoderma reesei* cell, may advantageously comprise the following features:

1. a) a mutation in at least one endogenous protease that reduces the activity of said endogenous protease, preferably the protease activity of two or three or more endogenous proteases is reduced, in order to improve production or stability of the glycoprotein with fucosylated N-glycans to be produced,
2. b) a polynucleotide encoding a glycoprotein, preferably a heterologous glycoprotein, such as an immunoglobulin, an antibody, or a protein fusion comprising Fc fragment of an immunoglobulin.
3. c) a polynucleotide encoding GMD and FX activities for GDP-fucose synthesis,
4. d) a polynucleotide encoding GDP-fucose transporter, for transporting GDP-fucose transporter in the

- Golgi compartment where fucosyltransferase activity occurs in vivo,
5. e) a polynucleotide encoding  $\alpha$ 1,6 fucosyltransferase activity linked with a Golgi targeting sequence for targeting said  $\alpha$ 1,6 fucosyltransferase activity to the Golgi compartment.

### Targeting Sequences

**[0126]** In certain embodiments, recombinant enzymes, such as  $\alpha$ 1,6 fucosyltransferase, or other glycosyltransferases introduced into the filamentous fungal cells, include a targeting peptide linked to the catalytic domains. The term "linked" as used herein means that two polymers of amino acid residues in the case of a polypeptide or two polymers of nucleotides in the case of a polynucleotide are either coupled directly adjacent to each other or are within the same polypeptide or polynucleotide but are separated by intervening amino acid residues or nucleotides. A "targeting peptide", as used herein, refers to any number of consecutive amino acid residues of the recombinant protein that are capable of localizing the recombinant protein to the endoplasmic reticulum (ER) or Golgi apparatus (Golgi) within the host cell. The targeting peptide may be N-terminal or C-terminal to the catalytic domains. In certain embodiments, the targeting peptide is N-terminal to the catalytic domains. In certain embodiments, the targeting peptide provides binding to an ER or Golgi component, such as to a mannosidase II enzyme. In other embodiments, the targeting peptide provides direct binding to the ER or Golgi membrane.

**[0127]** Components of the targeting peptide may come from any enzyme that normally resides in the ER or Golgi apparatus. Such enzymes include mannosidases, mannosyltransferases, glycosyltransferases, Type 2 Golgi proteins, and MNN2, MNN4, MNN6, MNN9, MNN10, MNS1, KRE2, VAN1, and OCH1 enzymes. Such enzymes may come from a yeast or fungal species such as those of *Acremonium*, *Aspergillus*, *Aureobasidium*, *Cryptococcus*, *Chrysosporium*, *Chrysosporium lucknowense*, *Filobasidium*, *Fusarium*, *Gibberella*, *Humicola*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Myrothecium*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Piromyces*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, *Tolypocladium*, and *Trichoderma*. Sequences for such enzymes can be found in the GenBank sequence database.

**[0128]** In certain embodiments the targeting peptide comes from the same enzyme and organism as one of the catalytic domains of the recombinant protein. For example, if the recombinant protein includes a human GnTII catalytic domain, the targeting peptide of the recombinant protein is from the human GnTII enzyme. In other embodiments, the targeting peptide may come from a different enzyme and/or organism as the catalytic domains of the recombinant protein.

**[0129]** Examples of various targeting peptides for use in targeting proteins to the ER or Golgi that may be used for targeting the recombinant enzymes, such as  $\alpha$ 1,6 fucosyltransferase or other glycosyltransferases, include: Kre2/Mnt1 N-terminal peptide fused to galactosyltransferase (Schwientek, JBC 1996, 3398), HDEL for localization of mannosidase to ER of yeast cells to produce Man5 (Chiba, JBC 1998, 26298-304; Callewaert, FEBS Lett 2001, 173-178), OCH1 targeting peptide fused to GnTI catalytic domain (Yoshida et al, Glycobiology 1999, 53-8), yeast N-terminal peptide of Mns1 fused to  $\alpha$ 2-mannosidase (Martinet et al, Biotech Lett 1998, 1171), N-terminal portion of Kre2 linked to catalytic domain of GnTI or  $\beta$ 4GalT (Vervecken, Appl. Environ Microb 2004, 2639-46), various approaches reviewed in Wildt and Gerngross (Nature Rev Biotech 2005, 119), full-length GnTI in *Aspergillus nidulans* (Kalsner et al, Glycocon. J 1995, 360-370), full-length GnTI in *Aspergillus oryzae* (Kasajima et al, Biosci Biotech Biochem 2006, 2662-8), portion of yeast Sec12 localization structure fused to *C. elegans* GnTI in *Aspergillus* (Kainz et al 2008), N-terminal portion of yeast Mnn9 fused to human GnTI in *Aspergillus* (Kainz et al 2008), N-terminal portion of *Aspergillus* Mnn10 fused to human GnTI (Kainz et al, Appl. Environ Microb 2008, 1076-86), and full-length human GnTI in *T. reesei* (Maras et al, FEBS Lett 1999, 365-70).

[0130] In certain embodiments the targeting peptide is an N-terminal portion of the Mnt1 targeting peptide having the amino acid sequence of SEQ ID NO: 10 (for example encoded by the polynucleotide of SEQ ID NO:5).

[0131] Further examples of sequences that may be used for targeting peptides include the sequences listed in Table 1 below.

**Table 1: Targeting peptides. Putative transmembrane domains are underlined. In KRE2/MNT1, the stem domain enabling Golgi localization is underlined and double-underlined. Other01 and Other02 are putative mannosylation-related proteins.**

| Homologous to      | Cytoplasmic    | Transmembrane  | Luminal        |
|--------------------|----------------|----------------|----------------|
| KRE2               | SEQ ID NO: 171 | SEQ ID NO: 172 | SEQ ID NO: 173 |
| KRE2 alternative1  | SEQ ID NO: 174 | SEQ ID NO: 175 | SEQ ID NO: 176 |
| OCH1               | SEQ ID NO: 177 | SEQ ID NO: 178 | SEQ ID NO: 179 |
| OCH1 alternative1  | SEQ ID NO: 180 | SEQ ID NO: 181 | SEQ ID NO: 182 |
| MNN9               | SEQ ID NO: 183 | SEQ ID NO: 184 | SEQ ID NO: 185 |
| MNN9 alternative1  | SEQ ID NO: 186 | SEQ ID NO: 187 | SEQ ID NO: 188 |
| MNN9 alternative2  | SEQ ID NO: 189 | SEQ ID NO: 190 | SEQ ID NO: 191 |
| MNN10              | SEQ ID NO: 192 | SEQ ID NO: 193 | SEQ ID NO: 194 |
| MNN10 alternative1 | SEQ ID NO: 195 | SEQ ID NO: 196 | SEQ ID NO: 197 |
| MNS1               | SEQ ID NO: 198 | SEQ ID NO: 199 | SEQ ID NO: 200 |
| MNS1 alternative1  | SEQ ID NO: 201 | SEQ ID NO: 202 | SEQ ID NO: 203 |
| MNS1 alternative2  | SEQ ID NO: 204 | SEQ ID NO: 205 | SEQ ID NO: 206 |
| MNS1 alternative3  | SEQ ID NO: 207 | SEQ ID NO: 208 | SEQ ID NO: 209 |
| MNS1 alternative4  | -              | SEQ ID NO: 210 | SEQ ID NO: 211 |
| VAN1               | SEQ ID NO: 212 | SEQ ID NO: 213 | SEQ ID NO: 214 |
| VAN1 alternative1  | SEQ ID NO: 215 | SEQ ID NO: 216 | SEQ ID NO: 217 |
| VAN1 alternative2  | SEQ ID NO: 218 | SEQ ID NO: 219 | SEQ ID NO: 220 |
| Other01            | SEQ ID NO: 221 | SEQ ID NO: 222 | SEQ ID NO: 223 |
| Other02            | SEQ ID NO: 224 | SEQ ID NO: 225 | SEQ ID NO: 226 |

[0132] Uncharacterized sequences may be tested for use as targeting peptides by expressing enzymes of the glycosylation pathway in a host cell, where one of the enzymes contains the uncharacterized sequence as the sole targeting peptide, and measuring the glycans produced in view of the cytoplasmic localization of glycan biosynthesis (e.g. as in Schwientek JBC 1996 3398), or by expressing a fluorescent reporter protein fused with the targeting peptide, and analyzing the localization of the protein in the Golgi by immunofluorescence or by fractionating the cytoplasmic membranes of the Golgi and measuring the location of the protein.

#### **Filamentous fungal cell for producing glycoproteins with complex fucosylated N-glycans**

[0133] The filamentous fungal cells according to the present disclosure may be useful in particular for producing glycoproteins with mammalian-like fucosylated N-glycan, such as complex fucosylated N-glycans.

[0134] Accordingly, in one aspect, the filamentous fungal cell is genetically modified to produce a complex N-glycan as an acceptor substrate for the fucosyltransferase activity, thereby enabling in vivo production of

glycoprotein with complex fucosylated N-glycans. In certain embodiments, this aspect includes methods of producing glycoproteins with human-like fucosylated N-glycans in a *Trichoderma* cell or related species such as *Neurospora*, *Myceliophthora*, *Fusarium*, *Aspergillus*, *Penicillium* and *Chrysosporium* species.

**[0135]** In certain embodiment, the complex fucosylated N-glycan includes any glycan having the formula  $\text{[GlcNAc}\beta\text{2]}_z\text{Man}\alpha\text{3}(\text{[GlcNAc}\beta\text{2]}_w\text{Man}\alpha\text{6})\text{Man}\{\beta\text{4GlcNAc}\beta\text{4}(\text{Fuc}\alpha\text{x})\text{4GlcNAc}\}$ , where x is 3 or 6, where ( ) defines a branch in the structure, where [ ] or { } define a part of the glycan structure either present or absent in a linear sequence, and where z and w are 0 or 1. Preferably w and z are 1.

**[0136]** In certain embodiments, the complex fucosylated N-glycan includes  $\text{GlcNAc}\beta\text{2Man}\alpha\text{3}(\text{GlcNAc}\beta\text{2Man}\alpha\text{6})\text{Man}\beta\text{4GlcNAc}\beta\text{4}(\text{Fuc}\alpha\text{6})\text{GlcNAc}$ ,  $\text{GlcNAc}\beta\text{2Man}\alpha\text{3}(\text{Man}\alpha\text{6})\text{Man}\beta\text{4GlcNAc}\beta\text{4}(\text{Fuc}\alpha\text{6})\text{GlcNAc}$ , and  $\text{Man}\alpha\text{3}(\text{Man}\alpha\text{6})\text{Man}\beta\text{4GlcNAc}\beta\text{4}(\text{Fuc}\alpha\text{6})\text{GlcNAc}$ .

**[0137]** In certain embodiments, the filamentous fungal cell generates a mixture of different N-glycans. The secreted complex fucosylated neutral N-glycans may constitute at least 1%, at least 3%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, or at least 75% or more of total secreted (mol%) neutral N-glycans of the filamentous fungal cells. In certain embodiment, the filamentous fungal cell generates core fucosylated FG0 N-glycan structure  $\text{GlcNAc}\beta\text{2Man}\alpha\text{3}[\text{GlcNAc}\beta\text{2Man}\alpha\text{6}]\text{Man}\beta\text{4GlcNAc}\beta\text{4}(\text{Fuc}\alpha\text{6})\text{GlcNAc}$ . In specific embodiments, the filamentous fungal cell generates the trimannosyl N-glycan structure  $\text{Man}\alpha\text{3}[\text{Man}\alpha\text{6}]\text{Man}\beta\text{4GlcNAc}\beta\text{4GlcNAc}$ . In certain embodiments, total secreted N-glycans comprises less than 60%, 50%, 40%, 30%, or less than 20% of the non-fucosylated  $\text{Man}\alpha\text{3}[\text{Man}\alpha\text{6}]\text{Man}\beta\text{4GlcNAc}\beta\text{4GlcNAc}$  structure.

**[0138]** In other embodiments, the filamentous fungal cell generates the G0 N-glycan structure  $\text{GlcNAc}\beta\text{2Man}\alpha\text{3}[\text{GlcNAc}\beta\text{2Man}\alpha\text{6}]\text{Man}\beta\text{4GlcNAc}\beta\text{4GlcNAc}$ .

**[0139]** In certain embodiments, total secreted N-glycans comprises less than 60%, 50%, 40%, 30%, or less than 20% of the non-fucosylated G0 glycans. In other embodiments, less than 0.5%, 0.1%, 0.05%, or less than 0.01% of the N-glycan comprises galactose. In certain embodiments, none of the secreted N-glycans comprise galactose.

**[0140]** In certain embodiments, the glycoprotein comprises the complex fucosylated N-glycan, as a major fucosylated glycoform,  $\text{GlcNAc}\beta\text{2Man}\alpha\text{3}(\text{GlcNAc}\beta\text{2Man}\alpha\text{6})\text{Man}\beta\text{4GlcNAc}\beta\text{4}(\text{Fuc}\alpha\text{6})\text{GlcNAc}$ . In an embodiment the glycoform is the major glycoform of the neutral complex type glycoforms.

**[0141]** In certain embodiments, the glycoprotein comprises the complex fucosylated N-glycan, as a major fucosylated glycoform,  $\text{Gal}\beta\text{4GlcNAc}\beta\text{2Man}\alpha\text{3}(\text{Gal}\beta\text{4GlcNAc}\beta\text{2Man}\alpha\text{6})\text{Man}\beta\text{4GlcNAc}\beta\text{4}(\text{Fuc}\alpha\text{6})\text{GlcNAc}$ . In an embodiment the glycoform is the major glycoform of the neutral complex type glycoforms

**[0142]** In certain embodiments, the glycoprotein comprises the complex fucosylated N-glycan, as a major fucosylated glycoform,  $\text{GlcNAc}\beta\text{2Man}\alpha\text{3}(\text{Gal}\beta\text{4GlcNAc}\beta\text{2Man}\alpha\text{6})\text{Man}\beta\text{4GlcNAc}\beta\text{4}(\text{Fuc}\alpha\text{6})\text{GlcNAc}$  or  $\text{Gal}\beta\text{4GlcNAc}\beta\text{2Man}\alpha\text{3}(\text{GlcNAc}\beta\text{2Man}\alpha\text{6})\text{Man}\beta\text{4GlcNAc}\beta\text{4}(\text{Fuc}\alpha\text{6})\text{GlcNAc}$ . In an embodiment the glycoform is the major glycoform of the neutral complex type glycoforms.

**[0143]** In certain embodiments, the glycoprotein comprises the complex fucosylated N-glycan, as a major fucosylated glycoform,  $\text{GlcNAc}\beta\text{2Man}\alpha\text{3}(\text{Man}\alpha\text{6})\text{Man}\beta\text{4GlcNAc}\beta\text{4}(\text{Fuc}\alpha\text{6})\text{GlcNAc}$ . In an embodiment the glycoform is the major glycoform of the neutral complex type glycoforms.

**[0144]** In certain embodiments, the glycoprotein comprises the complex fucosylated N-glycan, as a major fucosylated glycoform,  $\text{Man}\alpha\text{3}(\text{Man}\alpha\text{6})\text{Man}\beta\text{4GlcNAc}\beta\text{4}(\text{Fuc}\alpha\text{6})\text{GlcNAc}$ .

**[0145]** In certain embodiments, the filamentous fungal cell of the disclosure produces glycoprotein composition with a mixture of different fucosylated N-glycans.

**[0146]** In some embodiments, GlcNAc $\beta$ 2Man $\alpha$ 3(GlcNAc $\beta$ 2Man $\alpha$ 6)Man $\beta$ 4GlcNAc $\beta$ 4(Fuc $\alpha$ 6)GlcNAc represents at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 20%, at least at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or more of total (mol%) neutral or complex type N-glycans of a heterologous glycoprotein, as expressed in a filamentous fungal cells of the disclosure.

**[0147]** In other embodiments, GlcNAc $\beta$ 2Man $\alpha$ 3(Man $\alpha$ 6)Man $\beta$ 4GlcNAc $\beta$ 4(Fuc $\alpha$ 6)GlcNAc represents at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 20%, at least at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or more of total (mol%) neutral or complex type N-glycans of a heterologous glycoprotein, as expressed in a filamentous fungal cells of the disclosure.

**[0148]** In other embodiments, Man $\alpha$ 3(Man $\alpha$ 6)Man $\beta$ 4GlcNAc $\beta$ 4(Fuc $\alpha$ 6)GlcNAc represents at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 20%, at least at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or more of total (mol%) neutral N-glycans of a heterologous glycoprotein, as expressed in a filamentous fungal cells of the disclosure.

**[0149]** In some embodiments, less than 0.5%, 0.1%, 0.05%, or less than 0.01% of the fucosylated N-glycan of the glycoprotein produced by the host cell of the disclosure, comprises galactose. In certain embodiments, none of fucosylated N-glycans comprise galactose.

**[0150]** The Neu5Gc and Gala- (non-reducing end terminal Gal $\alpha$ 3Gal $\beta$ 4GlcNAc) structures are known xenoantigenic (animal derived) modifications of heterologous proteins such as antibodies which are produced in animal cells such as CHO cells. The structures may be antigenic and, thus, harmful even at low concentrations. The filamentous fungi of the present disclosure lack biosynthetic pathways to produce the terminal Neu5Gc and Gal $\alpha$ -structures. In an embodiment that may be combined with the preceding embodiments less than 0.1%, 0.01 %, 0.001 % or 0 % of the fucosylated N-glycans of the glycoprotein comprises Neu5Gc and/or Gala- structure. In an embodiment that may be combined with the preceding embodiments, less than 0.1%, 0.01 %, 0.001 % or 0 % of the fucosylated N-glycans of the antibody comprises Neu5Gc and/or Gala- structure.

**[0151]** The terminal Gal $\beta$ 4GlcNAc structure of N-glycan of mammalian cell produced glycans affects bioactivity of antibodies and Gal $\beta$ 3GlcNAc may be xenoantigenic structure. In an embodiment that may be combined with one or more of the preceding embodiments, less than 0.1%, 0.01 %, 0.001 %, or 0 % of fucosylated N-glycan of the glycoprotein comprises terminal galactose epitopes Gal $\beta$ 3/4GlcNAc. In an embodiment that may be combined with one or more of the preceding embodiments, less than 0.1%, 0.01 %, 0.001 %, or 0 % of the fucosylated N-glycan of the antibody comprises terminal galactose epitopes Gal $\beta$ 3/4GlcNAc.

**[0152]** Glycation is a common post-translational modification of proteins, resulting from the chemical reaction between reducing sugars such as glucose and the primary amino groups on protein. Glycation occurs typically in neutral or slightly alkaline pH in cell cultures conditions, for example, when producing antibodies in CHO cells and analysing them (see, for example, Zhang et al. (2008) Unveiling a glycation hot spot in a recombinant humanized monoclonal antibody. Anal Chem. 80(7):2379-2390). As filamentous fungi of the present disclosure are typically cultured in acidic pH, occurrence of glycation is reduced. In an embodiment that may be combined with the preceding embodiments, less than 1.0%, 0.5%, 0.1%, 0.01 %, 0.001 %, or 0 % of the fucosylated glycoprotein comprises glycation structures. In an embodiment that may be combined with the preceding embodiments, less than 1.0%, 0.5%, 0.1%, 0.01 %, 0.001 %, or 0 % of the antibody comprises glycation

structures.

**[0153]** Fucosylated structures and their quantitation may also be determined as mole % of fucosylated (non-fucosylated) per total polypeptide as produced by the host cell of the disclosure. Analytical methods, such as MALDI TOF MS analysis may be used to determine fucosylation level are described in detail in the Examples below. In brief, a polypeptide as produced by a filamentous fungal cell is purified to determine its fucosylation level. Non-fucosylated and fucosylated structure of the polypeptide are separated and quantified by MALDI-TOF MS analysis. For example, the quantification of fucosylation level may be performed by determining area values or intensity of the different peaks of MALDI-TOF MS spectrum.

**[0154]** The complex fucosylated N-glycan is attached to a molecule such as an amino acid, a peptide, or a polypeptide. The asparagine residue is in aminoglycosidic linkage from the side-chain amide (a biologic mammalian polypeptide N-glycan linkage structure) and may be part of a peptide chain such as a dipeptide, an oligopeptide, or a polypeptide. The glycan may be a reducing end derivative such as an N-, O-, or C-linked, preferably glycosidic, derivative of the reducing GlcNAc or Man, such as a spacer or terminal organic residue with a certain glycan linked structure selected from the group of an amino acid, alkyl, heteroalkyl, acyl, alkyloxy, aryl, arylalkyl, and heteroarylalkyl. The spacer may be further linked to a polyvalent carrier or a solid phase. In certain embodiments, alkyl-containing structures include methyl, ethyl, propyl, and C4-C26 alkyls, lipids such as glycerolipids, phospholipids, dolichol-phospholipids and ceramides and derivatives. The reducing end may also be derivatized by reductive amination to a secondary amine linkage or a derivative structure. Certain carriers include biopoly- or oligomers such as (poly)peptides, poly(saccharides) such as dextran, cellulose, amylose, or glycosaminoglycans, and other organic polymers or oligomers such as plastics including polyethylene, polypropylene, polyamides (e.g., nylon or polystyrene), polyacrylamide, and polylactic acids, dendrimers such as PAMAM, Starburst or Starfish dendrimers, or polylysine, and polyalkylglycols such as polyethylene glycol (PEG). Solid phases may include microtiter wells, silica particles, glass, metal (including steel, gold and silver), polymer beads such as polystyrene or resin beads, polylactic acid beads, polysaccharide beads or organic spacers containing magnetic beads.

**[0155]** In certain embodiments, the complex fucosylated N-glycan is attached to a heterologous polypeptide. In certain embodiments, the heterologous polypeptide is a therapeutic protein. Therapeutic proteins may include immunoglobulin, or a protein fusion comprising a Fc fragment or other therapeutic glycoproteins, such as antibodies, erythropoietins, interferons, growth hormones, enzymes, or blood-clotting factors and may be useful in the treatment of humans or animals. For example, the glycoproteins with complex fucosylated N-glycan as produced by the filamentous fungal cell may be a therapeutic glycoprotein such as rituximab. In an embodiment, the heterologous protein or heterologous glycoprotein is selected from the group consisting of: an immunoglobulin, such as IgG, a light chain or heavy chain of an immunoglobulin, a heavy chain or a light chain of an antibody, a single chain antibody, a monomeric or multimeric single domain antibody, a FAb-fragment, a FAb2-fragment, and, their antigen-binding fragments.

**[0156]** Methods for generating complex N-glycans as acceptor substrate for fucosyltransferase are described for example in PCT/EP2011/070956 which content is incorporated by reference.

**[0157]** In one aspect, the filamentous fungal cell according to the disclosure as described above, is further genetically modified to mimic the traditional pathway of mammalian cells, starting from Man5 N-glycans as acceptor substrate for GnTI, and followed sequentially by GnT1, mannosidase II and GnTII reaction steps (hereafter referred as the "traditional pathway" for producing G0 glycoforms). In one variant, a single recombinant enzyme comprising the catalytic domains of GnTI and GnTII, is used.

**[0158]** Alternatively, in a second aspect, the filamentous fungal cell according to the disclosure as described above is further genetically modified to have *alg3* reduced expression, allowing the production of core Man<sub>3</sub>GlcNAc<sub>2</sub> N-glycans, as acceptor substrate for GnTI and GnTII subsequent reactions and bypassing the

need for mannosidase  $\alpha$ 1,2 or mannosidase II enzymes (the reduced "alg3" pathway). In one variant, a single recombinant enzyme comprising the catalytic domains of GnTI and GnTII, is used.

**[0159]** In such embodiments for mimicking the traditional pathway for producing glycoproteins with complex fucosylated N-glycans, a Man<sub>5</sub> expressing filamentous fungal cell, such as *T. reesei* strain, may be transformed with a GnTI or a GnTII/GnTI fusion enzyme using random integration or by targeted integration to a known site known not to affect Man<sub>5</sub> glycosylation. Strains that produce GlcNAcMan<sub>5</sub> are selected. The selected strains are further transformed with a catalytic domain of a mannosidase II-type mannosidase capable of cleaving Man<sub>5</sub> structures to generate GlcNAcMan<sub>3</sub>. In certain embodiments mannosidase II-type enzymes belong to glycoside hydrolase family 38 ([cazy.org/GH38\\_all.html](http://cazy.org/GH38_all.html)). Characterized enzymes include enzymes listed in [cazy.org/GH38\\_characterized.html](http://cazy.org/GH38_characterized.html). Especially useful enzymes are Golgi-type enzymes that cleaving glycoproteins, such as those of subfamily  $\alpha$ -mannosidase II (Man2A1;ManA2). Examples of such enzymes include human enzyme AAC50302, *D. melanogaster* enzyme (Van den Elsen J.M. et al (2001) EMBO J. 20: 3008-3017), those with the 3D structure according to PDB-reference 1HTY, and others referenced with the catalytic domain in PDB. For cytoplasmic expression, the catalytic domain of the mannosidase is typically fused with an N-terminal targeting peptide (for example as disclosed in the above Section) or expressed with endogenous animal or plant Golgi targeting structures of animal or plant mannosidase II enzymes. After transformation with the catalytic domain of a mannosidase II-type mannosidase, strains are selected that produce GlcNAcMan<sub>3</sub> (if GnTI is expressed) or strains are selected that effectively produce GlcNAc<sub>2</sub>Man<sub>3</sub> (if a fusion of GnTI and GnTII is expressed). For strains producing GlcNAcMan<sub>3</sub>, such strains are further transformed with a polynucleotide encoding a catalytic domain of GnTII and transformant strains that are capable of producing GlcNAc<sub>2</sub>Man<sub>3</sub>GlcNAc<sub>2</sub> are selected.

**[0160]** In embodiments using the reduced alg3 pathway, the filamentous fungal cell, such as a *Trichoderma* cell, has a reduced level of activity of a dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase compared to the level of activity in a parent host cell. Dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase (EC 2.4.1.130) transfers an  $\alpha$ -D-mannosyl residue from dolichyl-phosphate D-mannose into a membrane lipid-linked oligosaccharide. Typically, the dolichyl-P-Man:Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase enzyme is encoded by an *alg3* gene. In certain embodiments, the filamentous fungal cell for producing glycoproteins with complex fucosylated N-glycans has a reduced level of expression of an *alg3* gene compared to the level of expression in a parent strain.

**[0161]** More preferably, the filamentous fungal cell comprises a mutation of *alg3*. The ALG3 gene may be mutated by any means known in the art, such as point mutations or deletion of the entire *alg3* gene. For example, the function of the *alg3* protein is reduced or eliminated by the mutation of *alg3*. In certain embodiments, the *alg3* gene is disrupted or deleted from the filamentous fungal cell, such as *Trichoderma* cell. In certain embodiments, the filamentous fungal cell is a *T. reesei* cell. SEQ ID NOs: 163 and 164 provide the nucleic acid and amino acid sequences of the *alg3* gene in *T. reesei*, respectively.

**[0162]** In certain embodiments, the filamentous fungal cell has a reduced level of activity of a  $\alpha$ -1,6-mannosyltransferase compared to the level of activity in a parent strain.  $\alpha$ -1,6-mannosyltransferase (EC 2.4.1.232) transfers an  $\alpha$ -D-mannosyl residue from GDP-mannose into a protein-linked oligosaccharide, forming an elongation initiating  $\alpha$ -(1 $\rightarrow$ 6)-D-mannosyl-D-mannose linkage in the Golgi apparatus. Typically, the  $\alpha$ -1,6-mannosyltransferase enzyme is encoded by an *och1* gene. In certain embodiments, the filamentous fungal cell has a reduced level of expression of an *och1* gene compared to the level of expression in a parent filamentous fungal cell. In certain embodiments, the *och1* gene is deleted from the filamentous fungal cell.

**[0163]** The filamentous fungal cells used in the methods of producing glycoprotein with complex fucosylated N-glycans may further contain a polynucleotide encoding an N-acetylglucosaminyltransferase I catalytic domain (GnTI) that catalyzes the transfer of N-acetylglucosamine to a terminal Man $\alpha$ 3 and a polynucleotide



encoding an N-acetylglucosaminyltransferase II catalytic domain (GnTII), that catalyses N-acetylglucosamine to a terminal Man $\alpha$ 6 residue of an acceptor glycan to produce a complex N-glycan. In one embodiment, said polynucleotides encoding GnTI and GnTII are linked so as to produce a single protein fusion comprising both catalytic domains of GnTI and GnTII.

**[0164]** As disclosed herein, N-acetylglucosaminyltransferase I (GlcNAc-TI; GnTI; EC 2.4.1.101) catalyzes the reaction  $\text{UDP-N-acetyl-D-glucosamine} + 3\text{-(}\alpha\text{-D-mannosyl)-}\beta\text{-D-mannosyl-R} \rightleftharpoons \text{UDP} + 3\text{-(2-(N-acetyl-beta-D-glucosaminyl)-}\alpha\text{-D-mannosyl)-}\beta\text{-D-mannosyl-R}$ , where R represents the remainder of the N-linked oligosaccharide in the glycan acceptor. An N-acetylglucosaminyltransferase I catalytic domain is any portion of an N-acetylglucosaminyltransferase I enzyme that is capable of catalyzing this reaction. GnTI enzymes are listed in the CAZy database in the glycosyltransferase family 13 ([cazy.org/GT13\\_all](http://cazy.org/GT13_all)). Enzymatically characterized species includes *A. thaliana* AAR78757.1 (US6 653 459), *C. elegans* AAD03023.1 (Chen S. et al J. Biol.Chem 1999;274(1):288-97), *D. melanogaster* AAF57454.1 (Sarkar & Schachter Biol Chem. 2001 Feb;382(2):209-17); *C. griseus* AAC52872.1 (Puthalakath H. et al J. Biol.Chem 1996 271(44):27818-22); *H. sapiens* AAA52563.1 (Kumar R. et al Proc Natl Acad Sci U S A. 1990 Dec;87(24):9948-52); *M. auratus* AAD04130.1 (Opat As et al Biochem J. 1998 Dec 15;336 (Pt 3):593-8), (including an example of deactivating mutant), Rabbit, *O. cuniculus* AAA31493.1 (Sarkar M et al. Proc Natl Acad Sci U S A. 1991 Jan 1;88(1):234-8). Amino acid sequences for N-acetylglucosaminyltransferase I enzymes from various organisms are described for example in PCT/EP2011/070956. Additional examples of characterized active enzymes can be found at [cazy.org/GT13\\_characterized](http://cazy.org/GT13_characterized). The 3D structure of the catalytic domain of rabbit GnTI was defined by X-ray crystallography in Unligil UM et al. EMBO J. 2000 Oct 16;19(20):5269-80. The Protein Data Bank (PDB) structures for GnTI are 1FO8, 1FO9, 1FOA, 2AM3, 2AM4, 2AM5, and 2APC. In certain embodiments, the N-acetylglucosaminyltransferase I catalytic domain is from the human N-acetylglucosaminyltransferase I enzyme (SEQ ID NO: 165), or variants thereof. In certain embodiments, the N-acetylglucosaminyltransferase I catalytic domain contains a sequence that is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to amino acid residues 84-445 of SEQ ID NO: 165. In some embodiments, a shorter sequence can be used as a catalytic domain (e.g. amino acid residues 105-445 of the human enzyme or amino acid residues 107-447 of the rabbit enzyme; Sarkar et al. (1998) Glycoconjugate J 15:193-197). Additional sequences that can be used as the GnTI catalytic domain include amino acid residues from about amino acid 30 to 445 of the human enzyme or any C-terminal stem domain starting between amino acid residue 30 to 105 and continuing to about amino acid 445 of the human enzyme, or corresponding homologous sequence of another GnTI or a catalytically active variant or mutant thereof. The catalytic domain may include N-terminal parts of the enzyme such as all or part of the stem domain, the transmembrane domain, or the cytoplasmic domain.

**[0165]** As disclosed herein, N-acetylglucosaminyltransferase II (GlcNAc-TII; GnTII; EC 2.4.1.143) catalyzes the reaction  $\text{UDP-N-acetyl-D-glucosamine} + 6\text{-(}\alpha\text{-D-mannosyl)-}\beta\text{-D-mannosyl-R} \rightleftharpoons \text{UDP} + 6\text{-(2-(N-acetyl-beta-D-glucosaminyl)-}\alpha\text{-D-mannosyl)-}\beta\text{-D-mannosyl-R}$ , where R represents the remainder of the N-linked oligosaccharide in the glycan acceptor. An N-acetylglucosaminyltransferase II catalytic domain is any portion of an N-acetylglucosaminyltransferase II enzyme that is capable of catalyzing this reaction. Amino acid sequences for N-acetylglucosaminyltransferase II enzymes from various organisms are listed in PCT/EP2011/070956. In certain embodiments, the N-acetylglucosaminyltransferase II catalytic domain is from the human N-acetylglucosaminyltransferase II enzyme (SEQ ID NO: 166), or variants thereof. Additional GnTII species are listed in the CAZy database in the glycosyltransferase family 16 ([cazy.org/GT16\\_all](http://cazy.org/GT16_all)). Enzymatically characterized species include GnTII of *C. elegans*, *D. melanogaster*, *Homo sapiens*, *Rattus norvegicus*, *Sus scrofa* ([cazy.org/GT16\\_characterized](http://cazy.org/GT16_characterized)). In certain embodiments, the N-acetylglucosaminyltransferase II catalytic domain contains a sequence that is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to amino acid residues from about 30 to about 447 of SEQ ID NO: 166. The catalytic domain may include N-terminal parts of the enzyme such as all or part of the stem domain, the transmembrane domain, or the cytoplasmic domain.

**[0166]** In embodiments where the filamentous fungal cell contains a fusion protein of the disclosure, the fusion protein may further contain a spacer in between the N-acetylglucosaminyltransferase I catalytic domain and the N-acetylglucosaminyltransferase II catalytic domain. In certain preferred embodiments, the spacer is an EGIV spacer, a 2xG4S spacer, a 3xG4S spacer, or a CBHI spacer. In other embodiments, the spacer contains a sequence from a stem domain.

**[0167]** For ER/Golgi expression the N-acetylglucosaminyltransferase I and/or N-acetylglucosaminyltransferase II catalytic domain is typically fused with a targeting peptide or a part of an ER or early Golgi protein, or expressed with an endogenous ER targeting structures of an animal or plant N-acetylglucosaminyltransferase enzyme. In certain preferred embodiments, the N-acetylglucosaminyltransferase I and/or N-acetylglucosaminyltransferase II catalytic domain contains any of the targeting peptides of the disclosure as described in the section entitled "Targeting sequences." Preferably, the targeting peptide is linked to the N-terminal end of the catalytic domain. In some embodiments, the targeting peptide contains any of the stem domains of the disclosure as described in the section entitled "Targeting sequences." In certain preferred embodiments, the targeting peptide is a Kre2/Mnt1 targeting peptide. In other embodiments, the targeting peptide further contains a transmembrane domain linked to the N-terminal end of the stem domain or a cytoplasmic domain linked to the N-terminal end of the stem domain. In embodiments where the targeting peptide further contains a transmembrane domain, the targeting peptide may further contain a cytoplasmic domain linked to the N-terminal end of the transmembrane domain. Further examples of sequences that may be used for targeting peptides include the targeting sequences as described in WO2012/069593 or PCT/EP2013/050126.

**[0168]** The filamentous fungal cells may also contain a polynucleotide encoding a UDP-GlcNAc transporter. The polynucleotide encoding the UDP-GlcNAc transporter may be endogenous (i.e., naturally present) in the host cell, or it may be heterologous to the filamentous fungal cell.

**[0169]** In certain embodiments, the filamentous fungal cell may further contain a polynucleotide encoding a  $\alpha$ -1,2-mannosidase. The polynucleotide encoding the  $\alpha$ -1,2-mannosidase may be endogenous in the host cell, or it may be heterologous to the host cell. Heterologous polynucleotides are especially useful for a host cell expressing high-mannose glycans transferred from the Golgi to the ER without effective exo- $\alpha$ -2-mannosidase cleavage. The  $\alpha$ -1,2-mannosidase may be a mannosidase I type enzyme belonging to the glycoside hydrolase family 47 ([cazy.org/GH47\\_all.html](http://cazy.org/GH47_all.html)). In certain embodiments the  $\alpha$ -1,2-mannosidase is an enzyme listed at [cazy.org/GH47\\_characterized.html](http://cazy.org/GH47_characterized.html). In particular, the  $\alpha$ -1,2-mannosidase may be an ER-type enzyme that cleaves glycoproteins such as enzymes in the subfamily of ER  $\alpha$ -mannosidase I EC 3.2.1.113 enzymes. Examples of such enzymes include human  $\alpha$ -2-mannosidase 1B (AAC26169), a combination of mammalian ER mannosidases, or a filamentous fungal enzyme such as  $\alpha$ -1,2-mannosidase (MDS1) (*T. reesei* AAF34579; Maras M et al J Biotech. 77, 2000, 255). For cytoplasmic expression the catalytic domain of the mannosidase is typically fused with a targeting peptide, such as HDEL, KDEL, or part of an ER or early Golgi protein, or expressed with an endogenous ER targeting structures of an animal or plant mannosidase I enzyme.

**[0170]** In certain embodiments, the filamentous fungal cell may also further contain a polynucleotide encoding a galactosyltransferase. Galactosyltransferases transfer  $\beta$ -linked galactosyl residues to terminal N-acetylglucosaminyl residue. In certain embodiments the galactosyltransferase is a  $\beta$ -1,4-galactosyltransferase. Generally,  $\beta$ -1,4-galactosyltransferases belong to the CAZy glycosyltransferase family 7 ([cazy.org/GT7\\_all.html](http://cazy.org/GT7_all.html)) and include  $\beta$ -N-acetylglucosaminyl-glycopeptide  $\beta$ -1,4-galactosyltransferase (EC 2.4.1.38), which is also known as N-acetylglucosamine synthase (EC 2.4.1.90). Useful subfamilies include  $\beta$ 4-GalT1,  $\beta$ 4-GalT-II, -III, -IV, -V, and -VI, such as mammalian or human  $\beta$ 4-GalTI or  $\beta$ 4GalT-II, -III, -IV, -V, and -VI or any combinations thereof.  $\beta$ 4-GalT1,  $\beta$ 4-GalTII, or  $\beta$ 4-GalTIII are especially useful for galactosylation of terminal GlcNAc $\beta$ 2-structures on N-glycans such as GlcNAcMan3, GlcNAc2Man3, or GlcNAcMan5 (Guo S. et al. Glycobiology 2001, 11:813-20). The three-dimensional structure of the catalytic region is known (e.g. (2006) J.Mol.Biol. 357: 1619-1633), and the structure has been represented in the PDB database with code 2FYD.

The CAZy database includes examples of certain enzymes. Characterized enzymes are also listed in the CAZy database at [cazy.org/GT7\\_characterized.html](http://cazy.org/GT7_characterized.html). Examples of useful  $\beta$  4GalT enzymes include  $\beta$ 4GalT1, e.g. bovine *Bos taurus* enzyme AAA30534.1 (Shaper N.L. et al Proc. Natl. Acad. Sci. U.S.A. 83 (6), 1573-1577 (1986)), human enzyme (Guo S. et al. Glycobiology 2001, 11:813-20), and *Mus musculus* enzyme AAA37297 (Shaper, N.L. et al. 1998 J. Biol. Chem. 263 (21), 10420-10428);  $\beta$ 4GalTII enzymes such as human  $\beta$  4GalTII BAA75819.1, Chinese hamster *Cricetulus griseus* AAM77195, *Mus musculus* enzyme BAA34385, and Japanese Medaka fish *Oryzias latipes* BAH36754; and  $\beta$ 4GalTIII enzymes such as human  $\beta$ 4GalTIII BAA75820.1, Chinese hamster *Cricetulus griseus* AAM77196 and *Mus musculus* enzyme AAF22221.

**[0171]** The galactosyltransferase may be expressed in the cytoplasm of the host cell. A heterologous targeting peptide, such as a Kre2 peptide described in Schwientek J.Biol. Chem 1996 3398, may be used. Promoters that may be used for expression of the galactosyltransferase include constitutive promoters such as *gpd*, promoters of endogenous glycosylation enzymes and glycosyltransferases such as mannosyltransferases that synthesize N-glycans in the Golgi or ER, and inducible promoters of high-yield endogenous proteins such as the *cbh1* promoter.

**[0172]** In certain embodiments of the disclosure where the filamentous fungal cell contains a polynucleotide encoding a galactosyltransferase, the filamentous fungal cell also contains a polynucleotide encoding a UDP-Gal 4 epimerase and/or UDP-Gal transporter. In certain embodiments of the disclosure where the filamentous fungal cell contains a polynucleotide encoding a galactosyltransferase, lactose may be used as the carbon source instead of glucose when culturing the host cell. The culture medium may be between pH 4.5 and 7.0 or between 5.0 and 6.5. In certain embodiments of the disclosure where the filamentous fungal cell contains a polynucleotide encoding a galactosyltransferase and a polynucleotide encoding a UDP-Gal 4 epimerase and/or UDP-Gal transporter, a divalent cation such as  $Mn^{2+}$ ,  $Ca^{2+}$  or  $Mg^{2+}$  may be added to the cell culture medium.

**[0173]** In certain embodiments, the filamentous fungal cell contains a polynucleotide encoding a sialyltransferase. A sialyltransferase transfers  $\alpha$ 3- or  $\alpha$ 6-linked sialic acid, such as Neu5Ac, to the terminal Gal of galactosylated complex glycans. Examples of suitable sialyltransferases can be found in the glycosylation protein family 29 ([cazy.org/GT29.html](http://cazy.org/GT29.html)). Useful  $\alpha$ 3- or  $\alpha$ 6-sialyltransferases include  $\beta$ -galactoside  $\alpha$ -2,6-sialyltransferase (EC 2.4.99.1) with a certain subfamily ST6Gal-I, and N-acetyllactosaminide  $\alpha$ -2,3-sialyltransferase (EC 2.4.99.6) with possible cross-reactivity with  $\beta$ -galactoside  $\alpha$ -2,3-sialyltransferase (EC 2.4.99.4). Useful subtypes of  $\alpha$ 3-sialyltransferases include ST3Gal-III and ST3Gal-IV. Certain enzymatically characterized species of these are listed as characterized in the CAZy database of carbohydrate active enzymes ([cazy.org/GT29\\_characterized.html](http://cazy.org/GT29_characterized.html)). The polynucleotide encoding the  $\alpha$ 3- or  $\alpha$ 6-linked sialyltransferase may be endogenous to the host cell, or it may be heterologous to the host cell. Sialylation in the host cell may require expression of enzymes synthesizing the donor CMP-sialic acid (CMP-Sia) such as CMP-Neu5Ac, especially in fungal, plant, nematode/parasite, or insect cells.

**[0174]** Enzymes involved in sialylation pathway result a cellular pool of CMP-Sia in the filamentous fungal cell which can be utilized in the production of sialylated glycans on glycoproteins of interest.

**[0175]** The synthesis of the CMP-Sia donor molecule in e.g. mammals is a multiple reaction process starting with the substrate UDP-GlcNAc and resulting in CMP-Sia. The process initiates in the cytoplasm producing sialic acid which is then converted to CMP-Sia by CMP-sialic acid synthase (NANS). Subsequently, CMP-Sia is then transported into the Golgi where sialyltransferases catalyze the transfer of sialic acid onto the acceptor glycan on a glycoprotein.

**[0176]** Using standard techniques known to those skilled in the art, nucleic acid molecules encoding one or more enzymes (or catalytically active fragments thereof) involved in the sialylation pathway, i.e. GNE, NANS, NANP, CMAS, SLC35A1, and a sialyltransferase (see Example 9) inserted into appropriate expression vectors under the transcriptional control of promoters and/or other expression control sequences capable of driving

transcription in a filamentous fungal cell of the disclosure. The functional expression of such enzymes in the filamentous fungal cell of the disclosure can be detected using e.g. by measuring the intermediates formed by the enzymes or detaching and analyzing the glycans on glycoproteins using the methods described in the Examples.

**[0177]** Accordingly, in certain embodiments, the filamentous fungal cell of the disclosure, for example, selected among *Neurospora*, *Trichoderma*, *Fusarium*, *Aspergillus*, *Penicillium*, *Myceliophthora*, or *Chrysosporium* cell, and more preferably a *Trichoderma* cell and even more preferably *Trichoderma reesei* cell, may comprise the following features:

1. a) a mutation in at least one endogenous protease that reduces the activity of said endogenous protease, for example, pep4 protease, preferably the protease activity of two or three or more endogenous proteases is reduced, in order to improve production or stability of the glycoprotein with fucosylated N-glycans to be produced,
2. b) a polynucleotide encoding a glycoprotein, preferably a heterologous glycoprotein, such as an immunoglobulin, an antibody, or a protein fusion comprising Fc fragment of an immunoglobulin.
3. c) one or more polynucleotides encoding polypeptides with GMD and FX activities for GDP-fucose synthesis,
4. d) a polynucleotide encoding GDP-fucose transporter, for transporting GDP-fucose transporter in the Golgi compartment where fucosyltransferase activity occurs in vivo,
5. e) a polynucleotide encoding  $\alpha$ 1,6 fucosyltransferase activity linked with a Golgi targeting sequence for targeting said  $\alpha$ 1,6 fucosyltransferase activity to the Golgi compartment,
6. f) a deletion or disruption of the alg3 gene,
7. g) a polynucleotide encoding N-acetylglucosaminyltransferase I catalytic domain and a polynucleotide encoding N-acetylglucosaminyltransferase II catalytic domain,
8. h) optionally, a polynucleotide encoding  $\beta$ 1,4 galactosyltransferase,
9. i) optionally, a polynucleotide or polynucleotides encoding UDP-Gal 4 epimerase and/or transporter,
10. j) optionally, a polynucleotide or polynucleotides encoding sialylation pathway enzymes and transporter.

**[0178]** In certain embodiments, said polynucleotides encoding sialylation pathway enzymes and transporter include one or more of the polynucleotides selected from the group consisting of:

1. i) a polynucleotide encoding glucosamine UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase,
2. ii) a polynucleotide encoding N-acetylneuraminic acid synthase,
3. iii) a polynucleotide encoding N-acetylneuraminic acid phosphatase,
4. iv) a polynucleotide encoding cytidine monophosphate N-acetylneuraminic acid synthetase,
5. v) a polynucleotide encoding CMP-sialic acid transporter, and
6. vi) a polynucleotide encoding sialyltransferase.

#### **Methods for producing a glycoprotein having fucosylated N-glycan**

**[0179]** The filamentous fungal cells as described above are useful in methods for producing a glycoprotein, e.g., an antibody, having fucosylated N-glycan.

**[0180]** Accordingly, in another aspect, the disclosure relates to a method for producing a glycoprotein, e.g., an antibody, having fucosylated N-glycan, comprising:

1. a. providing a filamentous fungal cell according to the disclosure as described above, and comprising a polynucleotide encoding a glycoprotein,
2. b. culturing the filamentous fungal cell to produce said glycoprotein having fucosylated N-glycan.

**[0181]** In methods of the disclosure, typically, cells are grown at 35°C in appropriate media. Certain growth media in the present disclosure include, for example, common commercially-prepared media such as Luria-Bertani (LB) broth, Sabouraud Dextrose (SD) broth or Yeast medium (YM) broth. Other defined or synthetic growth media may also be used and the appropriate medium for growth of the particular host cell will be known by someone skilled in the art of microbiology or fermentation science. Temperature ranges and other conditions suitable for growth are known in the art (see, e.g., Bailey and Ollis 1986). In certain embodiments the pH of cell culture is between 3.5 and 7.5, between 4.0 and 7.0, between 4.5 and 6.5, between 5 and 5.5, or at 5.5.

**[0182]** In some embodiments, the glycoprotein is a heterologous glycoprotein, preferably a mammalian glycoprotein. In other embodiments, the heterologous glycoprotein is a non-mammalian glycoprotein.

**[0183]** In certain embodiments, the mammalian glycoprotein is selected from an immunoglobulin, immunoglobulin heavy chain, an immunoglobulin light chain, a monoclonal antibody, a Fab fragment, a single chain antibody, a hybrid antibody, an F(ab')<sub>2</sub> antibody fragment, a monomeric or multimeric single domain antibody, a functional antibody fragment comprising a Fc fragment of an immunoglobulin, an immunoadhesin, a protein fusion comprising a Fc fragment of an immunoglobulin, or their antigen-binding fragments. A fragment of a protein, as used herein, consists of at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 consecutive amino acids of a reference protein.

**[0184]** As used herein, an "immunoglobulin" refers to a multimeric protein containing a heavy chain and a light chain covalently coupled together and capable of specifically combining with antigen. Immunoglobulin molecules are a large family of molecules that include several types of molecules such as IgM, IgD, IgG, IgA, and IgE.

**[0185]** As used herein, an "antibody" refers to intact immunoglobulin molecules, as well as fragments thereof which are capable of binding an antigen. These include hybrid (chimeric) antibody molecules (see, e.g., Winter et al. Nature 349:293-99225, 1991; and U.S. Pat No. 4,816,567 226); F(ab')<sub>2</sub> molecules; non-covalent heterodimers [227, 228]; dimeric and trimeric antibody fragment constructs; humanized antibody molecules (see e.g., Riechmann et al. Nature 332, 323-27, 1988; Verhoeyan et al. Science 239, 1534-36, 1988; and GB 2,276,169); and any functional fragments obtained from such molecules, as well as antibodies obtained through non-conventional processes such as phage display or transgenic mice. Preferably, the antibodies are classical antibodies with Fc region. Methods of manufacturing antibodies are well known in the art.

**[0186]** As used herein the term "Fc", "Fc region" or "Fc fragment" refers to the constant region of an immunoglobulin. An Fc fragment comprises at least the CH<sub>2</sub> and CH<sub>3</sub> domain, optionally, the hinge region which is located between the heavy chain CH<sub>1</sub> domain and CH<sub>2</sub>. Fc fragments could be obtained for example by papain digestion of an immunoglobulin. Fc fragment include at least one asparagine residue in the hinge region which is linked with N-glycan. As used herein, the term Fc fragment further include Fc variants of native Fc domain into which a substitution, deletion or insertion of at least one amino acid has been introduced. In one embodiment, the hinge region is modified such that the number of cysteine residues in the hinge region is altered, e.g., increased or decreased. This approach is described further in U.S. Patent No. 5,677,425 by Bodmer et al. The number of cysteine residues in the hinge region of CH<sub>1</sub> is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the fusion protein. In another embodiment, the Fc region is modified to increase its biological half-life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in

U.S. Patent No. 6,277,375 to Ward. In yet other embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector functions of the Fc portion. For example, one or more amino acids can be replaced with a different amino acid residue such that the Fc portion has an altered affinity for an effector ligand. This approach is described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter et al. In yet another embodiment, the Fc region is modified to increase or decrease the ability of the fusion polypeptide to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase or decrease the affinity of the Fc region for an Fc receptor by modifying one or more amino acids. This approach is described further in PCT Publication WO 00742072 by Presta. Moreover, the binding sites on human IgG1 for FcγRI, FcγRII, FcγRIII and FcRn have been mapped and variants with improved or reduced binding have been described (see Shields, R L. et al. 2001 J. Biol. Chem. 276:6591-6604). In one embodiment, the Fc domain is of human origin and may be from any of the immunoglobulin classes, such as IgG or IgA and from any subtype such as human IgG1, IgG2, IgG3 and IgG4. In other embodiments the Fc domain is from a non-human animal, for example, but not limited to, a mouse, rat, rabbit, camel, shark, non-human primate or hamster. In certain embodiments, the Fc domain of IgG1 isotype is used. In some specific embodiments, a mutant variant of IgG1 Fc fragment is used, e.g. a mutant IgG1 Fc which reduces or eliminates the ability of the fusion protein to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to bind to an Fcγ receptor. An example of an IgG1 isotype silent mutant, is a so-called LALA mutant, wherein Leucine residue is replaced by Alanine residue at amino acid positions 234 and 235 as described in J. Virol 2001 by Hezareh et al.

**[0187]** In further embodiments, the yield of the mammalian glycoprotein is at least 0.5, at least 1, at least 2, at least 3, at least 4, or at least 5 grams per liter. In certain embodiments, the mammalian glycoprotein is an antibody, optionally, IgG1, IgG2, IgG3, or IgG4. In further embodiments, the yield of the antibody is at least 0.5, at least 1, at least 2, at least 3, at least 4, or at least 5 grams per liter. In further embodiments, the mammalian glycoprotein is an antibody, and the antibody contains at least 70 %, at least 80 %, at least 90 %, at least 95 %, or at least 98 % of a natural antibody C-terminus and N-terminus without additional amino acid residues. In other embodiments, the mammalian glycoprotein is an antibody, and the antibody contains at least 70 %, at least 80 %, at least 90 %, at least 95 %, or at least 98 % of a natural antibody C-terminus and N-terminus that do not lack any C-terminal or N-terminal amino acid residues.

**[0188]** In certain embodiments where the mammalian glycoprotein is purified from cell culture, the culture containing the mammalian glycoprotein contains polypeptide fragments that make up a mass percentage that is less than 50%, less than 40%, less than 30%, less than 20%, or less than 10% of the mass of the produced polypeptides. In certain preferred embodiments, the mammalian glycoprotein is an antibody, and the polypeptide fragments are heavy chain fragments and/or light chain fragments. In other embodiments, where the mammalian glycoprotein is an antibody and the antibody purified from cell culture, the culture containing the antibody contains free heavy chains and/or free light chains that make up a mass percentage that is less than 50%, less than 40%, less than 30%, less than 20%, or less than 10% of the mass of the produced antibody. Methods of determining the mass percentage of polypeptide fragments are well known in the art and include, measuring signal intensity from an SDS-gel.

**[0189]** In certain embodiments, where the mammalian glycoprotein is purified from cell culture, the culture contains at least 5%, 10%, 15%, 20%, 25%, 30% of secreted complex fucosylated neutral N-glycans (mol%) compared to total secreted neutral N-glycans (as measured for example as described in the Examples). In certain embodiments where the mammalian glycoprotein is purified from cell culture, and where the strain is a *Trichoderma* cell genetically engineered to produce complex N-glycans as acceptor substrate for α1,6 fucosyltransferase activity, the culture comprises at least 5%, 10%, 15%, 20%, 25%, 30% of secreted complex fucosylated neutral N-glycans (mol%) compared to total secreted neutral N-glycans (as measured for example as described in the Examples). In certain embodiments, the purified mammalian glycoprotein comprises the core fucosylated FG0 N-glycan structure GlcNAcβ2Manα3[GlcNAcβ2Manα6]Manβ4GlcNAcβ4(Fuca6)GlcNAc. In some embodiments, GlcNAcβ2Manα3(GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fuca6)GlcNAc represents at least

2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or more of total (mol%) neutral or complex type N-glycans of a glycoprotein. In some embodiments,  $\text{Gal}\beta 4\text{GlcNAc}\beta 2\text{Man}\alpha 3(\text{Gal}\beta 4\text{GlcNAc}\beta 2\text{Man}\alpha 6)\text{Man}\beta 4\text{GlcNAc}\beta 4(\text{Fuc}\alpha 6)\text{GlcNAc}$  represents at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or more of total (mol%) neutral or complex type N-glycans of a glycoprotein. In some embodiments,  $\text{GlcNAc}\beta 2\text{Man}\alpha 3(\text{Gal}\beta 4\text{GlcNAc}\beta 2\text{Man}\alpha 6)\text{Man}\beta 4\text{GlcNAc}\beta 4(\text{Fuc}\alpha 6)\text{GlcNAc}$  represents at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or more of total (mol%) neutral or complex type N-glycans of a glycoprotein. In some embodiments,  $\text{Gal}\beta 4\text{GlcNAc}\beta 2\text{Man}\alpha 3(\text{GlcNAc}\beta 2\text{Man}\alpha 6)\text{Man}\beta 4\text{GlcNAc}\beta 4(\text{Fuc}\alpha 6)\text{GlcNAc}$  represents at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or more of total (mol%) neutral or complex type N-glycans of a glycoprotein.

**[0190]** In other embodiments, the culture comprises the trimannosyl N-glycan structure  $\text{Man}\alpha 3[\text{Man}\alpha 6]\text{Man}\beta 4\text{GlcNAc}\beta 4\text{GlcNAc}$ . In other embodiments, the culture comprises less than 60%, 50%, 40%, 30%, 20% of the  $\text{Man}\alpha 3[\text{Man}\alpha 6]\text{Man}\beta 4\text{GlcNAc}\beta 4\text{GlcNAc}$  structure compared to the amount (mol%) of total secreted N-glycans or the amount (mol%) of G0 glycans. In other embodiments, the culture comprises the G0 N-glycan structure  $\text{GlcNAc}\beta 2\text{Man}\alpha 3[\text{GlcNAc}\beta 2\text{Man}\alpha 6]\text{Man}\beta 4\text{GlcNAc}\beta 4\text{GlcNAc}$ . In other embodiments, the culture comprises less than 60%, 50%, 40%, 30%, 20% of the non-fucosylated G0 glycoform compared to the amount (mol%) of total secreted N-glycans or the amount of secreted fucosylated FG0 N-glycans. In other embodiments, the culture comprises less than 0.5%, 0.1%, 0.05%, 0.01% galactosylated N-glycans. In certain embodiments, the culture comprises no galactosylated N-glycans. In some embodiments,  $\text{Man}\alpha 3(\text{Man}\alpha 6)\text{Man}\beta 4\text{GlcNAc}\beta 4(\text{Fuc}\alpha 6)\text{GlcNAc}$  represents at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or more of total (mol%) neutral N-glycans of a glycoprotein.

**[0191]** In some embodiments,  $\text{GlcNAc}\beta 2\text{Man}\alpha 3(\text{GlcNAc}\beta 2\text{Man}\alpha 6)\text{Man}\beta 4\text{GlcNAc}\beta 4(\text{Fuc}\alpha 6)\text{GlcNAc}$  and  $\text{GlcNAc}\beta 2\text{Man}\alpha 3(\text{GlcNAc}\beta 2\text{Man}\alpha 6)\text{Man}\beta 4\text{GlcNAc}\beta 4\text{GlcNAc}$  represents at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% (mol%) of total complex type neutral N-glycans of a glycoprotein. In certain embodiments, at least 40 %, 50 % or 55 % of complex type glycans are fucosylated.

**[0192]** In some embodiments,  $\text{Gal}\beta 4\text{GlcNAc}\beta 2\text{Man}\alpha 3(\text{Gal}\beta 4\text{GlcNAc}\beta 2\text{Man}\alpha 6)\text{Man}\beta 4\text{GlcNAc}\beta 4(\text{Fuc}\alpha 6)\text{GlcNAc}$  and  $\text{Gal}\beta 4\text{GlcNAc}\beta 2\text{Man}\alpha 3(\text{Gal}\beta 4\text{GlcNAc}\beta 2\text{Man}\alpha 6)\text{Man}\beta 4\text{GlcNAc}\beta 4\text{GlcNAc}$  represents at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% (mol%) of total complex type neutral N-glycans of a glycoprotein. In certain embodiments, at least 40 %, 50 % or 55 % of complex type glycans are fucosylated.

**[0193]** In present disclosure the "complex type N-glycan" comprises at least core structure  $\text{GlcNAc}\beta 2\text{Man}\alpha 3(\text{Man}\alpha 6)\text{Man}\beta 4\text{GlcNAc}\beta 4\text{GlcNAc}$ , which may be elongated to  $\text{GlcNAc}\beta 2\text{Man}\alpha 3(\text{GlcNAc}\beta 2\text{Man}\alpha 6)\text{Man}\beta 4\text{GlcNAc}\beta 4\text{GlcNAc}$  and to galactosylated and/or fucosylated variants  $[\text{Gal}\beta 4]_a\text{GlcNAc}\beta 2\text{Man}\alpha 3([\text{Gal}\beta 4]_b\text{GlcNAc}\beta 2\text{Man}\alpha 6)\text{Man}\beta 4\text{GlcNAc}\beta 4(\text{Fuc}\alpha 6)_c\text{GlcNAc}$ , wherein a, b and c are integers 0 or 1, independently.

**[0194]** In certain embodiments of any of the disclosed methods, the method includes the further step of providing one or more, two or more, three or more, four or more, or five or more protease inhibitors. In certain embodiments, the protease inhibitors are peptides that are co-expressed with the mammalian polypeptide. In other embodiments, the inhibitors inhibit at least two, at least three, or at least four proteases from a protease family selected from aspartic proteases, trypsin-like serine proteases, subtilisin proteases, and glutamic proteases.

**[0195]** In certain embodiments of any of the disclosed methods, the filamentous fungal cell or *Trichoderma* fungal cell also contains a carrier protein. As used herein, a "carrier protein" is portion of a protein that is endogenous to and highly secreted by a filamentous fungal cell or *Trichoderma* fungal cell. Suitable carrier proteins include, without limitation, those of *T. reesei* mannanase I (Man5A, or MANI), *T. reesei* cellobiohydrolase II (Cel6A, or CBHII) (see, e.g., Paloheimo et al Appl. Environ. Microbiol. 2003 December; 69(12): 7073-7082) or *T. reesei* cellobiohydrolase I (CBHI). In some embodiments, the carrier protein is CBH1. In other embodiments, the carrier protein is a truncated *T. reesei* CBH1 protein that includes the CBH1 core region and part of the CBH1 linker region. In some embodiments, a carrier such as a cellobiohydrolase or its fragment is fused to a glycoprotein, for example, an antibody light chain and/or an antibody heavy chain. In some embodiments, a carrier-antibody fusion polypeptide comprises a Kex2 cleavage site. In certain embodiments, Kex2, or other carrier cleaving enzyme, is endogenous to a filamentous fungal cell. In certain embodiments, carrier cleaving protease is heterologous to the filamentous fungal cell, for example, another Kex2 protein derived from yeast or a TEV protease. In certain embodiments, carrier cleaving enzyme is overexpressed. In certain embodiments, the carrier consists of about 469 to 478 amino acids of N-terminal part of the *T. reesei* CBH1 protein GenBank accession No. EGR44817.1.

**[0196]** In certain embodiments, the filamentous fungal cell of the disclosure overexpress KEX2 protease. In an embodiment the heterologous protein is expressed as fusion construct comprising an endogenous fungal polypeptide, a protease site such as a Kex2 cleavage site, and the heterologous protein such as an antibody heavy and/or light chain. Useful 2-7 amino acids combinations preceding Kex2 cleavage site have been described, for example, in Mikosch et al. (1996) J. Biotechnol. 52:97-106; Goller et al. (1998) Appl Environ Microbiol. 64:3202-3208; Spencer et al. (1998) Eur. J. Biochem. 258:107-112; Jalving et al. (2000) Appl. Environ. Microbiol. 66:363-368; Ward et al. (2004) Appl. Environ. Microbiol. 70:2567-2576; Ahn et al. (2004) Appl. Microbiol. Biotechnol. 64:833-839; Paloheimo et al. (2007) Appl Environ Microbiol. 73:3215-3224; Paloheimo et al. (2003) Appl Environ Microbiol. 69:7073-7082; and Margolles-Clark et al. (1996) Eur J Biochem. 237:553-560.

**[0197]** The disclosure further relates to the glycoprotein composition, for example the antibody composition, obtainable or obtained by the method as disclosed above.

**[0198]** In specific embodiment, such antibody composition obtainable or obtained by the methods of the disclosure, comprises at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% of the antibodies that are fucosylated (mol%, as determined for example by MALDI TOF MS analysis, and measuring area or intensity of peaks as described in Examples). In other specific embodiments, such antibody composition further comprises at least 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60 %, 70 % or 80 % (mole% neutral N-glycan) or more, of the following glycoform:

1. (i) Man $\alpha$ 3(Man $\alpha$ 6)Man $\beta$ 4GlcNA $\beta$ 4(Fuca6)GlcNAc;
2. (ii) Gal $\beta$ 4GlcNAc $\beta$ 2Man $\alpha$ 3(Gal $\beta$ 4GlcNAc $\beta$ 2Man $\alpha$ 6)Man $\beta$ 4GlcNAc $\beta$ 4(Fuca6)GlcNAc (FG2);
3. (iii) GlcNAc $\beta$ 2Man $\alpha$ 3(Gal $\beta$ 4GlcNAc $\beta$ 2Man $\alpha$ 6)Man $\beta$ 4GlcNAc $\beta$ 4(Fuca6)GlcNAc (FG1),
4. (iv) Gal $\beta$ 4GlcNAc $\beta$ 2Man $\alpha$ 3(GlcNAc $\beta$ 2Man $\alpha$ 6)Man $\beta$ 4GlcNAc $\beta$ 4(Fuca6)GlcNAc (FG1);
5. (v) GlcNAc $\beta$ 2Man $\alpha$ 3(GlcNAc $\beta$ 2Man $\alpha$ 6)Man $\beta$ 4GlcNAc $\beta$ 4(Fuca6)GlcNAc (FG0);
6. (vi) GlcNAc $\beta$ 2Man $\alpha$ 3(Man $\alpha$ 6)Man $\beta$ 4GlcNAc $\beta$ 4(Fuca6)GlcNAc.



**[0199]** In some embodiments the N-glycan composition according to i-v comprises less than 15 %, 10 %, 7 %, 5 %, 3 %, 1 % or 0.5 % or is devoid of Man5 glycan (Man $\alpha$ 3[Man $\alpha$ 6(Man $\alpha$ 3)Man $\alpha$ 6]Man $\beta$ 4GlcNA $\beta$ 4GlcNAc) and/or Man3 glycan (Man $\alpha$ 3(Man $\alpha$ 6)Man $\beta$ 4GlcNA $\beta$ 4GlcNAc).

**Pharmaceutical Compositions Containing Glycoprotein with Complex Fucosylated N-Glycans Produced by the Methods of the Disclosure**

**[0200]** In another aspect, the present disclosure provides a composition, e.g., a pharmaceutical composition, containing glycoproteins with complex fucosylated N-glycans produced by the methods of the disclosure, formulated together with a pharmaceutically acceptable carrier. Pharmaceutical compositions of the disclosure also can be administered in combination therapy, i.e., combined with other agents. For example, the combination therapy can include a glycoprotein with fucosylated N-glycans (e.g. complex fucosylated N-glycans) according to the present disclosure combined with at least one other therapeutic agent.

**[0201]** As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion). Depending on the route of administration, the active compound, i.e., the fucosylated N-glycan attached to a heterologous molecule according to the disclosure, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

**[0202]** The pharmaceutical compositions of the disclosure may include one or more pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see e.g., Berge, S.M., et al. (1977) J. Pharm. Sci. 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanic acids, hydroxy alkanic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chlorprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

**[0203]** A pharmaceutical composition of the disclosure also may include a pharmaceutically acceptable antioxidant. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

**[0204]** Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the disclosure include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

**[0205]** These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization

procedures, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

**[0206]** Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the disclosure is contemplated. Supplementary active compounds can also be incorporated into the compositions.

**[0207]** Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

**[0208]** Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the certain methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

**[0209]** The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 0.01 percent to about ninety-nine percent of active ingredient, preferably from about 0.1 percent to about 70 percent, most preferably from about 1 percent to about 30 percent of active ingredient in combination with a pharmaceutically acceptable carrier.

**[0210]** Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

**[0211]** For administration of the glycoprotein with fucosylated N-glycans, in particular where such glycoprotein is an antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example, dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months. Certain dosage regimens for antibodies with fucosylated N-glycan include 1 mg/kg body weight or 3 mg/kg body weight via intravenous administration, with the antibody being given using one of the following dosing schedules: (i) every four weeks for six dosages, then every three months; (ii) every three weeks; (iii) 3 mg/kg body weight once followed by 1 mg/kg body weight every three weeks.

**[0212]** Alternatively a glycoprotein with fucosylated N-glycan according to the disclosure can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the administered substance in the patient. In general, human antibodies show the longest half life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies.

**[0213]** The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

**[0214]** Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present disclosure may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present disclosure employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

**[0215]** A "therapeutically effective dosage" of a glycoprotein of the disclosure preferably results in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. For example, for the treatment of tumors, a "therapeutically effective dosage" preferably inhibits cell growth or tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit tumor growth can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition in vitro by assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

**[0216]** A composition of the present disclosure can be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Certain routes of

administration for binding moieties of the disclosure include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

**[0217]** Alternatively, a glycoprotein with fucosylated N-glycan according to the disclosure can be administered via a nonparenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

**[0218]** The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

**[0219]** Therapeutic compositions can be administered with medical devices known in the art. For example, in a certain embodiment, a therapeutic composition of the disclosure can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Patent Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; or 4,596,556. Examples of well-known implants and modules useful in the present disclosure include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multichamber compartments; and U.S. Patent No. 4,475,196, which discloses an osmotic drug delivery system.

**[0220]** In certain embodiments, the use of the glycoprotein with fucosylated N-glycan according to the disclosure is for the treatment of any disease that may be treated with therapeutic antibodies, more specifically silent therapeutic antibodies, with low or no ADCC activity, including without limitation antibodies for use in treating autoimmune and inflammatory disorders, and/or to prevent from graft rejection.

**[0221]** It is to be understood that, while the disclosure has been described in conjunction with the certain specific embodiments thereof, the foregoing description is intended to illustrate and not limit the scope of the disclosure.

## EXAMPLES

### EXAMPLE 1

#### *Cloning and deletion of *T. reesei* *alg3* and generation of *GnTIII* fusion protein*

[0222] The gene encoding the ALG3 mannosyltransferase was identified in the *Trichoderma reesei* genome sequence. A disruption construct was designed to insert the acetamidase selection marker between 1000 bp 5' and 3' flanking region fragments of the *alg3* gene. The flanking region fragments were amplified by PCR, and the construct was made by homologous recombination cloning in *Saccharomyces cerevisiae*. The disruption cassette was released from its backbone vector by digestion and transformed into the *T. reesei* strain M124 (mus53 deletion of M44 (VTT-D-00775; Selinheimo et al., FEBS J. 2006, 273(18): 4322-35). Transformants were selected on acetamidase medium and screened by PCR with a forward primer outside the 5' flanking region fragment of the construct and the reverse primer inside the AmdS selection marker.

[0223] A vector having the chimeric GnTII/GnTI sequence (SEQ ID NO: 240) under the control of the *cbh1* promoter was constructed with a *pyr4* gene loopout marker and subcloned into a backbone vector between *alg3* flanking region fragments for targeted integration, resulting in plasmid pTTv110. A PmeI-digested expression cassette was transformed into *T. reesei* strain M127 (*pyr4*- strain of M124). After plate selection, the clones were PCR-screened and purified through single spores. Five PCR positive transformants indicating correct integration to the *alg3* locus in the M127 transformation were cultivated in a 300 ml volume for seven days at +28°C in a media containing TrMM, pH 5.5, supplemented with 40 g/l lactose, 20 g/l spent grain extract, and 100 mM PIPPS. To avoid bacterial contamination, 100 mg/l ampicillin was added into the flasks at the time of inoculation.

## EXAMPLE 2

### *Cloning of C. elegans GMD, FX, GDP fucose transporter and human FUT8*

[0224] The coding sequences of the *Ceanorhabditis elegans* GMD, FX, GDP-fucose transporter and human FUT8 transferase were optimized for *Trichoderma reesei* expression. The GMD and Fut8 genes were cloned into a *T. reesei* expression vector between the *gpdA* promoter and *TrpC* terminator, and the FX and GDP-fucose transporter were cloned into an *E. coli* cloning plasmid.

[0225] A plasmid containing expression cassettes for both *C. elegans* GMD and FX was generated from the optimized sequences. The plasmid was cloned using yeast homologous recombination and, as vector backbone, the yeast vector pRS426, EcoRI-XhoI digested, was used (Colot et al., PNAS 2006, 103(27):10352-7). The GMD expression cassette was digested with NotI-HindIII, resulting in a 4.3 kb fragment, containing the *gpdA* promoter and *trpC* terminator flanking the ORF. The FX ORF was digested with *KpnI* and *SacI*, and the *tef1* promoter and *egl2* terminator for the FX expression were created by PCR from genomic DNA from parent strain M124. The *pep4* 5' integration flank and the first half of the *pyr4* marker were obtained by PCR, using a *pep4* deletion plasmid (pTTv181) with *pyr4* marker as a template (see *pep4* deletion plasmid construction below). The primers used are listed in Table 2. The digested fragments and PCR products were separated with agarose gel electrophoresis and the correct fragments were isolated from the gel with a gel extraction kit (Qiagen) according to manufacturer's protocol. The plasmid was constructed using the yeast homologous recombination method, using overlapping oligonucleotides for the recombination of the GMD fragment. The plasmid DNA was rescued from yeast and transformed into electro competent TOP10 *E. coli* that were plated on ampicillin (100 µg/ml) selection plates. Miniprep plasmid preparations were made from several colonies. The presence of the GMD and FX genes was confirmed by digesting the prepared plasmids with *SacI*-*SacII* and two positive clones were sequenced to verify the sequence. One correct clone was chosen to be the final vector pTTv224.

[0226] A vector was constructed by yeast cloning using the *EcoRI*-*XhoI* pRS426 as backbone, and the human optimized FUT8 sequence. For targeting of the FUT8 to Golgi, the transmembrane region of a *T. reesei* native mannosyltransferase MNT1 was used. The first 85 amino acids of the MNT1 were fused to human FUT8

without the transmembrane domain (amino acids 1-31). The *mnt1* localisation fragment was generated by PCR from a vector containing genomic DNA of the *mnt1*, and the *FUT8* fragment was generated by PCR from the optimized sequence. The *cdna1* promoter and *cbh2* terminator were chosen for the *FUT8* expression and these were generated by PCR from vectors containing *T. reesei* *cdna1* promoter, and *cbh2* terminator, respectively. The *pep4* 3' integration flank and the second half of the *pyr4* marker were obtained by PCR, using the *pep4* deletion plasmid pTTv181 as a template. There is an overlap of 900 bp between the two parts of the *pyr4* marker, which enables efficient recombination of the two different fragments in *T. reesei*. The 300 bp *egl2* terminator repetitive sequence was created by PCR from genomic DNA from the strain M124. The cloning was performed as described above, but the presence of the human *FUT8* gene was confirmed by digestion with *NcoI*-*PvuI*. One correct clone was chosen to be the final vector pTTv225.

[0227] The pTTv225 vector was linearised with *SgfI*, and the *C. elegans* GDP-fucose transporter, together with the *tef2* promoter and *xyn1* terminator, was inserted by yeast recombination. The optimized transporter coding sequence was digested with *KpnI* and *SacI*, and the *tef2* promoter and *xyn1* terminator were created by PCR from genomic DNA from strain M124. The cloning was performed as described for pTTv224, but the presence of the transporter gene was checked by digestion with *XhoI*. One correct clone was chosen to be the final vectors pTTv226. The primers used for sequencing the vectors are listed in Table 3.

**Table 2. List of primers used for cloning vectors pTTv224, pTTv225 and pTTv226**

| Fragment                      | Primer          | Primer sequence                                                                                                        |
|-------------------------------|-----------------|------------------------------------------------------------------------------------------------------------------------|
| <i>pep4</i> 5'flank           | T298_77579_5f   | GTAACGCCAGGGTTTTCCAGTCACGAC<br>GGTTTAACTCAGGTCAACCACCGAGGA<br>C (SEQ ID NO: 35)                                        |
|                               | T_758_pTTv224_1 | TTCTTCTTATTGATTGAGCCTGTGTGTAG<br>AGATACAAGGATTTAAATTGAATGGGATG<br>GTTTCGATTGCT (SEQ ID NO: 36)                         |
| GMD 5' overlappin g<br>oligos | T729_pTTv227_3  | AAGTTCCTTCCTCTGGCAGCAATCGAAC<br>CATCCCATTC AATTTAAATCCTTGATCTC<br>TACACACAGGCTCAAATCAATAAGAAGAA<br><br>(SEQ ID NO: 37) |
|                               | T730_pTTv227_4  | TTCTTCTTATTGATTGAGCCTGTGTGTAG<br>AGATACAAGGATTTAAATTGAATGGGATG<br>GTTTCGATTGCTGCCAGAGGAAGGGAAC<br>T (SEQ ID NO: 38)    |
| GMD 3' overlappin g<br>oligos | T731_pTTv227_5  | AAGCGCCCACTCCACATCTCCACTCGAC<br>CTGCAGGCATGCGGCGCGCCACTGGGA<br>GCTGTGCCGAGTTTGCTGGCTACTTACCT<br>AGTC (SEQ ID NO: 39)   |
|                               | T732_pTTv227_6  | GACTAGGTAAGTAGCCAGCAAACCTCGGC<br>ACAGCTCCCACTGGCGCGCCCATGCCT<br>GCAGGTCGAGTGGAGATGTGGAGTGGGC<br>GCTT (SEQ ID NO: 40)   |
| <i>tef1</i> promoter          | T733_pTTv227_7  | AAGCGCCCACTCCACATCTCCACTCGAC<br>CTGCAGGCATGCGGCGCGCCACTGGGA<br>GCTGTGCCGAGTTTG (SEQ ID NO: 41)                         |

| Fragment                          | Primer         | Primer sequence                                                                                 |
|-----------------------------------|----------------|-------------------------------------------------------------------------------------------------|
|                                   | T734_pTTv227_8 | CCACCAGACCAGTGCCGCCCGTGACGAG<br>GATGGTTTTTCATTTTGACGGTTTGTGTGAT<br>GTAGCGT (SEQ ID NO: 42)      |
|                                   |                |                                                                                                 |
| <i>egl2</i> terminator            | T735_pTTv227_9 | CCAGTGGTTCGTTGACAACTACGAAACCG<br>CCCGGAAGTAACACTCTGAGCTGAATGC<br>AGAAGC (SEQ ID NO: 43)         |
|                                   | T759_pTTv224_2 | TACAATAACACAGATCTTTTATGACGG (SEQ ID NO: 44)                                                     |
| First part of <i>pyr4</i> marker  | T760_pTTv224_3 | TTGTAATGTTCTACCGTCATAAAAGATCTG<br>TGTTATTGTAGCGATCGCCTAGCATCGAC<br>TACTGCTGCTCT (SEQ ID NO: 45) |
|                                   | T761_pTTv224_4 | GCGGATAACAATTTACACAGGAAACAGC<br>GTTTAAACCTCCACCGACCGATCCGTTGG<br>(SEQ ID NO: 46)                |
| Second part of <i>pyr4</i> marker | T762_pTTv225_1 | GTAACGCCAGGGTTTTCCAGTCACGAC<br>GGTTTAACTCAAGCTCATGGACCTCAAG<br>GC (SEQ ID NO: 47)               |
|                                   | T763_pTTv225_2 | CCATGCAAAGATACACATCAATCG (SEQ ID NO: 48)                                                        |
| <i>egl2</i> terminator loop-out   | T764_pTTv225_3 | GATTGTACCCAGCTGCGATTGATGTGTA<br>TCTTTCATGGGGCATCCGTAGTTGTCGC<br>AAGAA (SEQ ID NO: 49)           |
|                                   | T759_pTTv224_2 | TACAATAACACAGATCTTTTATGACGG (SEQ ID NO: 50)                                                     |
| <i>cDNA1</i> promoter             | T765_pTTv225_4 | TTGTAATGTTCTACCGTCATAAAAGATCTG<br>TGTTATTGTAGGTCTGAAGGACGTGGAAT<br>GATG (SEQ ID NO: 51)         |
|                                   | T738_pTTv228_2 | GTTGAGAGAAGTTGTTGGATTGATCA<br>(SEQ ID NO: 52)                                                   |
| <i>mnt1</i> 1-85                  | T739_pTTv228_3 | AACCAAAGACTTTTTGATCAATCCAACAA<br>CTTCTCTCACTTAATTAATGGCGTCAAC<br>AAATGCGCGCTAT (SEQ ID NO: 53)  |
|                                   | T740_pTTv228_4 | GTTCAATTCGAGGGCCGGGA (SEQ ID NO: 54)                                                            |
| <i>FUT8</i> 32-575                | T741_pTTv228_5 | CAACGACCTCGTCGGCATCGCTCCCGGC<br>CCTCGAATGAACGACAACGACCACCCTG<br>ATCATTC (SEQ ID NO: 55)         |

| Fragment               | Primer         | Primer sequence                                                                                  |
|------------------------|----------------|--------------------------------------------------------------------------------------------------|
|                        | T742_pTTv228_6 | TTACTTCTCGGCCTCGGGGTAG (SEQ ID NO: 56)                                                           |
| <i>cbh2</i> terminator | T743_pTTv228_7 | GACTGTTAAGTACCCGACCTACCCCGAG<br>GCCGAGAAGTAAGGCCGGCCGCTTTTCG<br>TGACCGGGCTTCAAA (SEQ ID NO: 57)  |
|                        | T744_pTTv228_8 | GTATCAGTCAGCGAGCAAGCCATT (SEQ NO: 58)                                                            |
| <i>pep4</i> 5'flank    | T766_pTTv225_5 | ATGATGCCTTTGCAGAAATGGCTTGCTCG<br>CTGACTGATACGCGATCGCAGGTAGACG<br>CTTTGCGAGTGTG (SEQ ID NO: 59)   |
|                        | T301_77579_3r  | GCGGATAACAATTTACACAGGAAACAGC<br>GTTTAACTGAACTGACGCGGACTGA<br>(SEQ ID NO: 60)                     |
| <i>tef2</i> promoter   | T780_pTTv226_1 | GCCTTTGCAGAAATGGCTTGCTCGCTGAC<br>TGATACGCGATCGCTTGGTGCTCGTATTA<br>GTGCCAATG (SEQ ID NO: 61)      |
|                        | T781_pTTv226_2 | CCTCGAACAGGGCCTTGTTGTTTTCTCG<br>TGGAGCTTCATATTTAAATCTTGCGCGTA<br>TTGCGGCTCGG (SEQ ID NO: 62)     |
| <i>xyn1</i> terminator | T782_pTTv226_3 | CCTGGGCAGAGATGGTAACGCCGCCGAG<br>GAATCCGTTTAAGGCCGCCGCTTCTGTTG<br>ATGTTGACTTGGAGT (SEQ ID NO: 63) |
|                        | T783_pTTv226_4 | CTTCTTAGATACACACACTCGCAAAGC<br>GTCTACCTGCGATCGCTGGGGGCGGATA<br>GAGGAGCAG (SEQ ID NO: 64)         |

Table 3. List of primers used for sequencing vectors pTTv224, pTTv225 and pTTv226

| Primer                   | Sequence                                 |
|--------------------------|------------------------------------------|
| T023_pRS426_5.1sekv      | GCGGAAAGGGGGATGTGCTG (SEQ ID 65)         |
| T228_pRS426_3.1sekv      | CCCAGGCTTTACACTTTATG (SEQ ID 66)         |
| T094_pyr4_F              | TAGCATCGACTACTGCTGC (SEQ ID NO: 67)      |
| T770_alg3_3pr_int_pyr4_F | CAGCCTCTCTCAGCCTCATC (SEQ ID NO: 68)     |
| T785                     | GCCAAAGCACCCAAGTACC (SEQ ID NO: 69)      |
| T786                     | GACGAGCCCGACATTAAAGC (SEQ ID NO: 70)     |
| T787                     | GCACGGCTTCCTCATCTTCG (SEQ ID NO: 71)     |
| T790                     | GAAGTAATCTCTGCAGATCTTTCG (SEQ ID NO: 72) |
| T791                     | ATTTGCTTTCCAGGCTGAG (SEQ ID NO: 73)      |



| Primer | Sequence                                  |
|--------|-------------------------------------------|
| T792   | CCCTACAACGACCATCAAAGTC (SEQ ID NO: 74)    |
| T793   | GAGAATATGGAGCTTCATCGA (SEQ ID NO: 75)     |
| T794   | CAGTATATTCATCTTCCCATCC (SEQ ID NO: 76)    |
| T795   | TTCTCCCTCCACTACGG (SEQ ID NO: 77)         |
| T796   | CGAGTACGTCGAGGCTATGTG (SEQ ID NO: 78)     |
| T797   | CAAGCAGCAAAGAGTGC (SEQ ID NO: 79)         |
| T798   | TTTACAACCTCTCCTATGAGTCG (SEQ ID NO: 80)   |
| T799   | CAATCGGAAGGGTGTCG (SEQ ID NO: 81)         |
| T800   | AGCCACTGGCACTTGCA (SEQ ID NO: 82)         |
| T801   | CCGGTGAAGTCGATTGC (SEQ ID NO: 83)         |
| T802   | CAGTGCGAGAACGTTGTC (SEQ ID NO: 84)        |
| T803   | GGCCTCTTCCACAACCT (SEQ ID NO: 85)         |
| T804   | CTCAGCGTGAACGAGTC (SEQ ID NO: 86)         |
| T805   | TGTAACCTCAGGTTAATTGTTGGGC (SEQ ID NO: 87) |
| T809   | TGAAGGACGTGGAATGATGG (SEQ ID NO: 88)      |
| T810   | AAACAAGCAACCTTGAACC (SEQ ID NO: 89)       |
| T811   | ACACAGATAAACCACCAACTC (SEQ ID NO: 90)     |
| T812   | CGATTGACCAAGGCCCA (SEQ ID NO: 91)         |
| T813   | ACGCTGATCTTGGAGTC (SEQ ID NO: 92)         |
| T814   | CCTCGCTGCTCAAAGAG (SEQ ID NO: 93)         |
| T815   | CCGGGCTTCAAACAATGATGTG (SEQ ID NO: 94)    |
| T816   | GGAGCATGAGCCTATGG (SEQ ID NO: 95)         |
| T817   | CTGAGGACGGGCAATTCAAGTC (SEQ ID NO: 96)    |
| T818   | CACATCAACCGTTGACAAGG (SEQ ID NO: 97)      |
| T819   | TTTCTTCCTCCTACACCAC (SEQ ID NO: 98)       |
| T820   | ACGTCGTCTGCACCTACCT (SEQ ID NO: 99)       |
| T821   | CTGTTGTGGTGGACGTC (SEQ ID NO: 100)        |
| T824   | CTGCGAGGTCAAGACGT (SEQ ID NO: 101)        |
| T825   | CAGGGCCAGCAGTACAACAC (SEQ ID NO: 102)     |

[0228] The pTTv224 vector contains the first part of the *pyr4* marker; the *pyr4* promoter and *pyr4* ORF nucleotides 1-979. The pTTv225 and pTTv226 vectors contain the second part of the marker, the *pyr4* ORF nucleotides 81-1146 and the *pyr4* terminator. The 300 bp *egl2* terminator fragment after the *pyr4* marker enables loopout of the *pyr4* marker. The constructs were targeted to the aspartic protease locus *pep4* (tre77579) using the *pep4* sequence from the 5' and 3' flanks of the gene (see above sequences). These listed sequences were included in the cassette to allow the cassette to integrate into the *pep4* locus via homologous recombination.

#### **Transformation into G0 *T. reesei* strain**

[0229] To prepare the vectors for transformation, the vectors were cut with *PmeI* to release the expression cassettes (Figure 1). The fragments were separated with agarose gel electrophoresis and the correct fragment was isolated from the gel with a gel extraction kit (Qiagen) according to manufacturer's protocol. The purified expression cassette DNA (5 µg) was then co-transformed into protoplasts of the *Trichoderma reesei* M289 G0 strain expressing fusion protein GNT2/1 in the *alg3* locus (obtained by transforming *pyr4*- strain of M124 with GntII/I to *alg3* locus in Example 1), the ALG3 deficient strains expressing GntII and GntI as fusion protein GNT2/1 are also described in International Patent Application No. PCT/EP2011/070956. Preparation of protoplasts and transformation were carried out essentially according to methods in Penttilä et al. (1987, Gene 61:155-164) and Gruber et al (1990, Curr. Genet. 18:71-76) for *pyr4* selection. The transformed protoplasts were plated onto *Trichoderma* minimal media (TrMM) plates containing sorbitol.

[0230] Transformants were then streaked onto TrMM plates with 0.1% TritonX-100. Transformants growing fast as selective streaks were screened by PCR using the primers listed in Table 4. DNA from mycelia was purified and analyzed by PCR to look at the integration of the 5' and 3' flanks of cassette and the existence of the *pep4* ORF. The cassette was targeted into the *pep4* locus; therefore the open reading frame was not present in the positively integrated transformants, purified to single cell clones. To screen for 5' integration, sequence outside of the 5' integration flank was used to create a forward primer that would amplify genomic DNA flanking *pep4* and the reverse primer was made from sequence in the *gpdA* promoter of the cassette. To check for proper integration of the cassette in the 3' flank, a reverse primer was made from sequence outside of the 3' integration flank that would amplify genomic DNA flanking *pep4* and the forward primer was made from sequence in the *cbh2* terminator for pTTv225 and GDP-fucose transporter for pTTv226. Thus, one primer would amplify sequence from genomic DNA outside of the cassette and the other would amplify sequence from DNA in the cassette. The transformation efficiency and integration of the split marker fragments was comparable to that of one expression construct carrying the full *pyr4* marker.

**Table 4. List of primers used for PCR screening of *T. reesei* transformants**

|                                      |                                       |
|--------------------------------------|---------------------------------------|
| 5' flank screening primers:          | 1361 bp product                       |
| T302_77579_5int                      | GATTCATCACAGGGGCAGTC (SEQ ID NO: 103) |
| T018_pgpdA_5rev                      | GAGCAGGCTCGACGTATTTC (SEQ ID NO: 104) |
| 3' flank screening primers, pTTv225: | 1318 bp product                       |
| T816                                 | GGAGCATGAGCCTATGG (SEQ ID NO: 105)    |
| T415_77579_3screen                   | ACGCCGTTGCTGAGCCTTG (SEQ ID NO: 106)  |
| 3' flank screening primers, pTTv226: | 1666 bp product                       |
| T821                                 | CTGTTGTGGTGGACGTC (SEQ ID NO: 107)    |
| T415_77579_3screen                   | ACGCCGTTGCTGAGCCTTG (SEQ ID NO: 106)  |
| pep4 ORF primers:                    | 1347 bp product                       |
| T416_77579_probeF                    | GAGCCCATCATCAACACCTC (SEQ ID NO: 108) |
| T417_77579_probeR                    | TGCCAAGGTCGTAGACGGA (SEQ ID NO: 109)  |

#### **Fermentation of strain M525, clone 43A**

[0231] *T. reesei* strain M525 (pTTv224 + pTTv226 transformant 43A) was grown on lactose - whole spent grain in 2 l batch mode fermentation (Sartorius, Biostat B plus) with 1 litre working volume for 9 days. 100 µl of M525 spores were cultivated in 100 ml of 30 g/l glucose, 15 g/l whole spent grain, 5 g/l KH<sub>2</sub>PO<sub>4</sub>, 5 g/l (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> -medium, pH 5.5 at +30°C with 200 rpm for 4 days. To avoid contaminations 100 µg/ml ampicillin was added in the inoculum medium. 90 ml of seed was used as the fermenter inoculum.

**[0232]** Fermentation was performed on 60 g/l lactose, 40 g/l whole spent grain, 40 g/l cellobiose, 20 g/l glucose, 1 ml/l 1000x TrTES, (Ilmén et al, 1997, AEM 63:1298-1306), 5 g/l KH<sub>2</sub>PO<sub>4</sub>, 5 g/l (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> - medium, pH 5.5. 1.5 ml of Struktol J633 antifoam (Schill-Seilacher) was added in the medium. Medium was sterilized by autoclaving at +121 °C for 40 min. 2.4 ml of 1M MgSO<sub>4</sub> and 4.1 ml of 1M CaCl<sub>2</sub> were added in the medium after autoclave. Fermentation was conducted at +28°C and agitation speed was 715 rpm (tip speed 2.05 m/s) with aerating rate of 0.4 vvm. The pH of the medium was adjusted with 5% NH<sub>3</sub> and with 15% H<sub>3</sub>PO<sub>4</sub>. Struktol J633 diluted in RO-water (2:1) was used as an antifoam agent. Sampling was performed on days 0, 2, 3, 4, 7 and 9 and samples were filtered through the GF/A filters (Millipore, cat no APFA04700).

### ***N-glycan analysis***

**[0233]** Six *T. reesei* fucosylation transformants containing *C. elegans* GMD, FX and human FUT8 (pTTv224 + pTTv225; clones 40A, 40D, 43A, 43D, 57A, 57D), seven transformants containing *C. elegans* GMD, FX, human FUT8 and *C. elegans* GDP-Fuc transporter (pTTv224 + pTTv226; clones 2B, 3B, 4B, 5B, 5D, 12C, 12D) and parental M289 G0 strain were cultivated in shake flasks in TrMM, 40 g/l lactose, 20 g/l spent grain extract, pH 5.0, at +28°C. The protein concentrations of the day 5 and 3 or 7 culture supernatants were measured by Bradford based assay (BioRad Quickstart Bradford Protein Assay) using BSA as a standard, and the N-glycans were analysed in triplicate from 10 µg of EtOH precipitated and SDS denatured supernatant proteins using 0,625 mU PNGase F (Europa Bioproducts) in 20 mM sodium phosphate buffer, pH 7.3, in overnight reaction at +37°C. The released N-glycans were purified with Hypersep C-18 and Hypersep Hypercarb (Thermo Scientific) and analysed with MALDI-TOF MS. Native fragmentation analysis was performed on signal m/z 1485, 1485 [M+Na]<sup>+</sup> corresponding to FG0.

**[0234]** For protein specific N-glycan analysis 80 µl of culture supernatant (~15 µg of protein) of fucosylation clone 40A was run in reducing SDS-PAGE and blotted to PVDF membrane, where from ~70 kDa and ~55 kDa bands were excised. The N-glycans were released from the membrane with 2.5 mU PNGase F (Europa Bioproducts) in 20 mM sodium phosphate buffer, pH 7.3, in overnight reaction at +37°C. The released N-glycans were purified with Hypersep C-18 and Hypersep Hypercarb (Thermo Scientific) and analysed with MALDI-TOF MS.

### **Results**

**[0235]** The N-glycan analysis revealed that all the fucosylation transformants containing *C. elegans* GMD, FX and human FUT8 produced 6-15% of fucosylated G0 (FG0) on supernatants proteins in three days of cultivation in shake flasks (Fig. 2A, Table 5) and 8-19% in five days (Fig. 2B, Table 6). The best clones were 40A, 43A (Fig. 3) and 57A. All the fucosylation transformants with GDP-Fuc transporter produced over 11% of FG0 on supernatant proteins in five days of cultivation in shake flasks (Table 4), the best clone being 12D with 25% of FG0 (Fig. 7).

**[0236]** The protein specific N-glycan analysis showed that there is FG0 both in ~70 kDa and ~55 kDa supernatant proteins (Fig. 4) ruling out the possibility that it would originate from the medium.

**[0237]** The fragmentation analysis of signal m/z 1485 [M+Na]<sup>+</sup> in its native form brought further proof that this signal is FG0 (Fig. 5). The signal m/z 1339 demonstrates the loss of fucose and the signal m/z 593 proves that the fucose is in N-glycan core.

**[0238]** In fermentation of the fucosylation transformant clone 43A (strain M525) the amount of FG0 increased until day 7 when there was 28% of FG0 (Table 7, Fig. 6).

Table 5. Relative proportions of neutral N-glycans from day 3 supernatant proteins of *T. reesei* fucosylation transformants (pTTv224 + pTTv225) and parental strain M289.

|                 |       |         | day 3 |       |       |       |       |       |       |
|-----------------|-------|---------|-------|-------|-------|-------|-------|-------|-------|
|                 |       |         | M289  | #40A  | #40D  | #43A  | #43D  | #57A  | #57D  |
| Composition     | Short | m/z     | %     | %     | %     | %     | %     | %     | %     |
| Hex3HexNAc2     | Man3  | 933.31  | 0.00  | 26.31 | 18.08 | 18.47 | 14.89 | 18.70 | 15.68 |
| Hex4HexNAc2     | Man4  | 1095.37 | 0.00  | 2.12  | 1.37  | 2.56  | 2.72  | 2.45  | 2.38  |
| Hex3HexNAc3     | H3N3  | 1136.40 | 0.00  | 1.78  | 1.12  | 1.56  | 1.09  | 1.74  | 1.64  |
| Hex5HexNAc2     | Man5  | 1257.42 | 35.32 | 3.92  | 4.10  | 5.04  | 4.55  | 4.07  | 4.03  |
| Hex3HexNAc4     | G0    | 1339.48 | 0.84  | 31.28 | 40.02 | 30.22 | 44.59 | 30.58 | 42.92 |
| Hex6HexNAc2     | Hex6  | 1419.48 | 28.46 | 16.48 | 21.72 | 26.30 | 25.20 | 26.07 | 24.88 |
| Hex3HexNAc4dHex | FG0   | 1485.53 | 0.00  | 15.12 | 11.00 | 13.31 | 5.52  | 13.78 | 6.36  |
| Hex4HexNAc4     | H4N4  | 1501.53 | 0.00  | 2.41  | 1.72  | 1.24  | 0.66  | 1.31  | 0.81  |
| Hex7HexNAc2     | Hex7  | 1581.53 | 8.78  | 0.58  | 0.52  | 0.85  | 0.61  | 0.90  | 0.85  |
| Hex8HexNAc2     | Hex8  | 1743.58 | 15.77 | 0.00  | 0.26  | 0.30  | 0.18  | 0.31  | 0.37  |
| Hex9HexNAc2     | Hex9  | 1905.63 | 10.84 | 0.00  | 0.00  | 0.14  | 0.00  | 0.00  | 0.00  |
| Hex10HexNAc2    | Hex10 | 2067.69 | 0.00  | 0.00  | 0.10  | 0.00  | 0.00  | 0.10  | 0.08  |

Table 6. Relative proportions of neutral N-glycans from day 5 supernatant proteins of *T. reesei* fucosylation transformants (pTTv224 + pTTv225) and parental strain M289.

|                 |       |         | day 5 |       |       |       |       |       |       |
|-----------------|-------|---------|-------|-------|-------|-------|-------|-------|-------|
|                 |       |         | M289  | #40A  | #40D  | #43A  | #43D  | #57A  | #57D  |
| Composition     | Short | m/z     | %     | %     | %     | %     | %     | %     | %     |
| Hex3HexNAc2     | Man3  | 933.31  | 12.53 | 27.43 | 15.03 | 16.11 | 17.63 | 19.30 | 18.28 |
| Hex4HexNAc2     | Man4  | 1095.37 | 2.01  | 2.60  | 3.26  | 3.74  | 3.41  | 3.75  | 2.78  |
| Hex3HexNAc3     | H3N3  | 1136.40 | 1.90  | 1.22  | 1.25  | 1.29  | 1.47  | 1.48  | 1.56  |
| Hex5HexNAc2     | Man5  | 1257.42 | 4.31  | 4.12  | 5.28  | 5.67  | 5.01  | 5.13  | 4.44  |
| Hex3HexNAc4     | G0    | 1339.48 | 61.17 | 28.65 | 35.89 | 25.43 | 36.03 | 24.59 | 39.55 |
| Hex6HexNAc2     | Hex6  | 1419.48 | 16.08 | 16.89 | 24.99 | 27.35 | 26.47 | 28.00 | 23.56 |
| Hex3HexNAc4dHex | FG0   | 1485.53 | 0.00  | 17.40 | 12.70 | 18.75 | 7.95  | 15.23 | 7.98  |
| Hex4HexNAc4     | H4N4  | 1501.53 | 0.73  | 1.04  | 0.80  | 0.83  | 0.73  | 1.26  | 0.96  |
| Hex7HexNAc2     | Hex7  | 1581.53 | 0.89  | 0.57  | 0.80  | 0.83  | 0.78  | 0.93  | 0.89  |
| Hex8HexNAc2     | Hex8  | 1743.58 | 0.37  | 0.00  | 0.00  | 0.00  | 0.35  | 0.31  | 0.00  |
| Hex9HexNAc2     | Hex9  | 1905.63 | 0.00  | 0.08  | 0.00  | 0.00  | 0.17  | 0.00  | 0.00  |
| Hex10HexNAc2    | Hex10 | 2067.69 | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  |

Table 7. Relative proportions of neutral N-glycans from supernatant proteins of *T. reesei* strain M525 (pTTv224 + pTTv225 transformant clone 43A) fermented for 3, 4, 7 and 9 days.

|             |        |         | M525  |       |       |       |
|-------------|--------|---------|-------|-------|-------|-------|
|             |        |         | d3    | d4    | d7    | d9    |
| Composition | Short  | m/z     | %     | %     | %     | %     |
| Hex3HexNAc2 | Man3   | 933.31  | 11.96 | 19.40 | 30.87 | 38.24 |
| Hex4HexNAc2 | Man4   | 1095.37 | 5.35  | 3.68  | 4.09  | 3.70  |
| Hex3HexNAc3 | GnMan3 | 1136.40 | 0.00  | 1.16  | 0.96  | 1.07  |
| Hex5HexNAc2 | Man5   | 1257.42 | 7.56  | 4.10  | 3.25  | 3.13  |

|                 |         |         | M525  |       |       |       |
|-----------------|---------|---------|-------|-------|-------|-------|
|                 |         |         | d3    | d4    | d7    | d9    |
| Composition     | Short   | m/z     | %     | %     | %     | %     |
| Hex3HexNAc3dHex | GnMan3F | 1282.45 | 0.00  | 0.94  | 0.78  | 0.89  |
| Hex3HexNAc4     | G0      | 1339.48 | 8.25  | 10.86 | 11.80 | 11.75 |
| Hex6HexNAc2     | Hex6    | 1419.48 | 54.34 | 35.42 | 14.74 | 12.64 |
| Hex3HexNAc4dHex | FG0     | 1485.53 | 6.45  | 19.62 | 27.93 | 20.88 |
| Hex4HexNAc4     | H4N4    | 1501.53 | 0.13  | 1.06  | 1.24  | 1.18  |
| Hex7HexNAc2     | Hex7    | 1581.53 | 5.30  | 3.19  | 3.11  | 3.42  |
| Hex8HexNAc2     | Hex8    | 1743.58 | 0.68  | 0.37  | 0.62  | 1.49  |
| Hex9HexNAc2     | Hex9    | 1905.63 | 0.00  | 0.14  | 0.38  | 1.02  |
| Hex10HexNAc2    | Hex10   | 2067.69 | 0.00  | 0.05  | 0.22  | 0.57  |

Table 8. Relative proportions of neutral N-glycans from day 5 supernatant proteins of *T. reesei* fucosylation transformants with GDP-Fuc transporter (pTTv224 + pTTv226).

|                 |          |         | d5    |       |       |       |       |       |       |       |
|-----------------|----------|---------|-------|-------|-------|-------|-------|-------|-------|-------|
|                 |          |         | M289  | #2B   | #3B   | #4B   | #5B   | #5D   | #12C  | #12D  |
| Composition     | Short    | m/z     | %     | %     | %     | %     | %     | %     | %     | %     |
| Hex3HexNAc2     | Man3     | 933.31  | 11.10 | 12.17 | 10.82 | 8.48  | 9.54  | 8.68  | 9.78  | 9.53  |
| Hex4HexNAc2     | Man4     | 1095.37 | 4.72  | 5.55  | 5.45  | 4.73  | 6.31  | 6.20  | 4.57  | 3.83  |
| Hex3HexNAc3     | GnMan3   | 1136.40 | 1.48  | 1.10  | 1.39  | 1.26  | 1.25  | 2.02  | 1.57  | 1.49  |
| Hex5HexNAc2     | Man5     | 1257.42 | 10.29 | 11.37 | 9.81  | 10.22 | 12.23 | 11.95 | 9.27  | 9.46  |
| Hex3HexNAc3dHex | GnMan3F  | 1282.45 | 0.00  | 0.68  | 0.00  | 0.64  | 0.00  | 0.00  | 0.00  | 0.57  |
| Hex3HexNAc4     | G0       | 1339.48 | 40.88 | 20.12 | 25.76 | 16.66 | 12.88 | 17.64 | 29.43 | 14.29 |
| Hex6HexNAc2     | Hex6     | 1419.48 | 28.06 | 26.32 | 32.20 | 32.31 | 39.34 | 40.03 | 28.98 | 33.66 |
| Hex3HexNAc4dHex | FG0      | 1485.53 | 0.00  | 20.67 | 12.91 | 23.88 | 16.81 | 11.74 | 14.58 | 25.13 |
| Hex4HexNAc4     | G0 + Hex | 1501.53 | 0.61  | 0.58  | 0.31  | 0.28  | 0.22  | 0.24  | 0.73  | 0.32  |
| Hex7HexNAc2     | Hex7     | 1581.53 | 1.48  | 0.87  | 1.12  | 0.87  | 1.04  | 1.07  | 0.94  | 1.11  |
| Hex8HexNAc2     | Hex8     | 1743.58 | 0.69  | 0.33  | 0.23  | 0.44  | 0.38  | 0.16  | 0.14  | 0.60  |
| Hex9HexNAc2     | Hex9     | 1905.63 | 0.69  | 0.26  | 0.00  | 0.24  | 0.00  | 0.28  | 0.00  | 0.00  |

### Protease assay

[0239] Total protease activity was measured with BODIPY casein FL (enzCheck protease assay kit #E6638, Molecular Probes) according to the manufacturer's protocol. The day 5 shake flask culture supernatants were analysed and the protease activity was normalised to the protein concentration (Figure 12). All transformants showed decrease in protease activity by 40-55%, when compared to the parent strain M289.

### EXAMPLE 3

*Cloning of H. pylori GMD and FX and transformation into T. reesei and production of GDP-fucose*

[0240] The coding sequences of the *Helicobacter pylori* GMD and FX were codon optimized for *Trichoderma reesei* expression. The GMD and FX genes were cloned into a *T. reesei* expression vector between the *gpdA* promoter and *trpC* terminator. The GMD and FX expression plasmids were introduced into the *T. reesei* M124 strain by co-transformation with hygromycin resistance gene as the selective marker. 5 µg of circular plasmids were used for all three plasmids. The hygromycin marker gene is under the *gpdA* promoter and *trpC* terminator in the (pBluekan) plasmid used. Preparation of protoplasts and transformation were carried out essentially according to methods in Penttilä et al. (1987, Gene 61:155-164) and Gruber et al (1990, Curr. Genet. 18:71-76) for *pyr4* selection. The transformed protoplasts were plated onto *Trichoderma* minimal media (TrMM) plates containing sorbitol and 150 µg/ml Hygromycin B.

[0241] Transformants were then streaked onto TrMM plates with 0.1% TritonX-100 and 125 µg/ml Hygromycin B for two successive rounds. Transformants growing fast as second selective streaks were screened by PCR using the primers listed in Table 9. DNA from mycelia was purified and analyzed by PCR to confirm the presence of the GMD, FX and hygromycin marker in the transformants. The genes are not targeted to specific loci in the genome and therefore ectopic integration will occur at random sites in the genome.

**Table 9. List of primers used for PCR screening of *T. reesei* transformants**

|                               |                                          |
|-------------------------------|------------------------------------------|
| GMD screening primers:        | 664 bp product                           |
| Hp GMD forw                   | AAGATCGCCCTCATTACCGGCGT (SEQ ID NO: 110) |
| Hp GMD rev                    | TCCGAAGGGTATATCCGCCGT (SEQ ID NO: 111)   |
| FX screening primers:         | 1123 bp product                          |
| Hp FX forw                    | AGCGAGCTGTGCCTCTTGGA (SEQ ID NO: 167)    |
| Hp FX rev                     | AGTCGAGCACTTGCGCGACCT (SEQ ID NO: 168)   |
| Hygromycin resistance marker: | 1666 bp product                          |
| Ann 79 hph                    | TTTGTGTCATATTTTCCTGC (SEQ ID NO: 169)    |
| Hph-gene 3.1 PCR              | TTGCCAGTGATACACATGGG (SEQ ID NO: 170)    |

[0242] Nine transformants out of forty-two screened, were PCR positive for the presence of the GMD, FX and hygromycin marker. These transformants were purified to single cell clones and cultivated in shake flask cultures.

#### **Quantification of relative amounts of GDP-Fuc from *T. reesei* strains expressing *H. pylori* GMD and FX**

[0243] *Purification of nucleotide sugars from T.reesei.* 80 ml of *T. reesei* cell culture medium was pelleted and boiled (diluted in 10 mM ammonium bicarbonate) for 5 minutes prior to homogenization with glass beads. Parallel 15 ml samples were collected for dry weight determination. The samples were stored at -20°C. The purification and analysis method has been described earlier in Răbină et al, 2001, Glycoconjugate Journal, 18:799-805. The pellet from cell lysate was discarded and the solution was applied in carbograph column (sample equivalent to 9.6 mg of dry weight; Extract Clean Carbo 150 mg/4 ml column from Alltech; the column was equilibrated first with 4 ml of methanol and then with 8 ml of MQ-H<sub>2</sub>O). The column was washed with 1) 2 ml of MQ-H<sub>2</sub>O, 2) 2 ml of 25% ACN (acetonitrile), and 3) 2 ml of 50 mM TEAA (triethylammonium acetate) buffer, pH 7.0. Nucleotide sugars were eluted with 2 ml of 25% ACN in 50 mM TEAA buffer.

[0244] The sample was treated with 0.25 U of alkaline phosphatase (Shrimp alkaline phosphatase, Fermentas) in 50 µl of 10 mM Tris-HCl pH 7.5, 10 mM MgCl<sub>2</sub>, 0.1 mg/ml BSA at 37°C for 4 h. The sample (dissolved in 10 mM NH<sub>4</sub>HCO<sub>3</sub>) was purified in DEAE Sepharose Fast Flow -column (1.3 ml, GE Healthcare,

equilibrated with 10 mM  $\text{NH}_4\text{HCO}_3$ ) by washing with 5 ml of 10 mM  $\text{NH}_4\text{HCO}_3$  and eluting with 5 ml 250 mM  $\text{NH}_4\text{HCO}_3$ . Finally, prior to HPLC, the sample was purified with carbograph column as described above and dried. The sample was diluted in 15  $\mu\text{l}$  of 20 mM TEAA buffer (pH 6.0), 5  $\mu\text{l}$  was analyzed with ion-pair reversed phase UPLC.

**[0245]** *Ion-pair reversed phase UPLC.* For screening the transfected strains of *T. reesei*, nucleotide sugars were separated with ion-pair reversed phase UPLC using Acquity UPLC BEH C18 -column (2.1x100 mm, 1.7  $\mu\text{m}$ , Waters) and following gradient: Isocratic 20 mM TEAA buffer (pH 6.0) for 2.2 min, then linear gradient of 10% ACN in 20 mM TEAA buffer (pH 6.0) up to 20% over 2.8 min. Finally, the column was washed rising to 40% of 10% ACN in 20 mM TEAA buffer (pH 6.0) in 0.3 min and keeping there for 1 min. The amounts of nucleotide sugars were quantified integrating peak areas and comparing them to external standards.

## Results

**[0246]** Nucleotide sugars from wild type (WT M124) and *T. reesei* strains transfected with *H. pylori* (pTTv19+21 51a) GDP-fucose synthesizing enzymes were quantified. A sample equal to 9.6 mg (cell pellet dry weight) was purified and one third was analyzed with ion-pair reversed phase UPLC. Samples from two culture media (with either lactose or glucose) and three time points were analyzed (days 3, 5, and 7). Quantification was performed by comparing to external nucleotide sugar standards. The relative amounts of GDP-fucose from transfected strains cultured in lactose containing medium were 18, 6, and 4 pmol/mg at days 3, 5, and 7, respectively, and in glucose containing medium 5, 4, and 4 pmol/mg at days 3, 5, and 7, respectively. No GDP-fucose was detected in wild type strain.

## EXAMPLE 4

### Generation of single, double, triple, 4-, 5-, 6-, and 7-fold protease deletion strains

**[0247]**

Table 10. Overview of generation of the single, double, triple, 4, 5, 6, 7, 8, 9, and 10-fold deletion strains.

| Strain | Vector        | Clone      | Strain transformed | Locus        | Proteases k/o       |
|--------|---------------|------------|--------------------|--------------|---------------------|
| M127   | 5-FOA of M124 |            |                    | pyr4 mutant  | None                |
| M195   | pTTv71        | 9-35A-1    | M127               | K/o pep1     | pep1                |
| M196   | 5-FOA of M195 | 9-35A-1-1A | pyr4-of M195       | pyr4 loopout | pep1                |
| M219   | pTTv72        | 16-5AA     | M196               | K/o tsp1     | pep1 tsp1           |
| M228   | 5-FOA of M219 | 16-5AA-1AA | pyr4-of M219       | pyr4 loopout | pep1 tsp1           |
| M277   | pTTv126       | 18-5A      | M228               | K/o slp1     | pep1 tsp1 slp1      |
| M306   | 5-FOA of M277 | 2A         | pyr4-of M277       | pyr4 loopout | pep1 tsp1 slp1      |
| M307   | pTTv117       | 117-37A    | M306               | K/o gap1     | pep1 tsp1 slp1 gap1 |
| M321   | 5-FOA of M307 | 9A         | pyr4-of M307       | pyr4 loopout | pep1 tsp1 slp1 gap1 |

| Strain | Vector        | Clone      | Strain transformed | Locus             | Proteases k/o                                      |
|--------|---------------|------------|--------------------|-------------------|----------------------------------------------------|
| M369   | pTTv145       | 7-30A      | M321               | K/o gap2          | pep1 tsp1 slp1 gap1 gap2                           |
| M381   | 5-FOA of M369 | 14         | pyr4-of M369       | pyr4 loopout      | pep1 tsp1 slp1 gap1 gap2                           |
| M396   | pTTv181       | 25-120A    | M381               | K/o pep4          | pep1 tsp1 slp1 gap1 gap2 pep4                      |
| M402   | 5-FOA of M396 | 25-120A-62 | pyr4-of M396       | pyr4 loopout      | pep1 tsp1 slp1 gap1 gap2 pep4                      |
| M486   | pTTv205       | 34-14A-a   | M402               | pep3              | pep1 tsp1 slp1 gap1 gap2 pep4 pep3                 |
| M496   | 5-FOA of M486 | 2A-a       | pyr4-of M486       | pyr4 loopout      | pep1 tsp1 slp1 gap1 gap2 pep4 pep3                 |
| M504   | pTTv229       | 38-48A     | M496               | pep5 (Trire81004) | pep1 tsp1 slp1 gap1 gap2 pep4 pep3 pep5            |
| M521   | 5-FOA of M504 | 1a-1       | pyr4-of M504       | pyr4 loopout      | pep1 tsp1 slp1 gap1 gap2 pep4 pep3 pep5            |
| M575   | pTTv245       | 42-45B     | M521               | pep12 (tre119876) | pep1 tsp1 slp1 gap1 gap2 pep4 pep3 pep5 pep12      |
| M574   | pTTv246       | 41-45G     | M521               | pep2 (tre53961)   | pep1 tsp1 slp1 gap1 gap2 pep4 pep3 pep5 pep2       |
| M597   | 5-FOA of M574 | 1AA        | pyr4-of M574       | pyr4 loopout      | pep1 tsp1 slp1 gap1 gap2 pep4 pep3 pep5 pep2       |
| M658   | pTTv312       | 47-62B-1   | M597               | pep11             | pep1 tsp1 slp1 gap1 gap2 pep4 pep3 pep5 pep2 pep11 |

#### ***Deletion of pep 1***

**[0248]** 1066 bp of 5' flanking region and 1037 bp of 3' flanking region were selected as the basis of the *pep1* deletion plasmid. Fragments were produced by PCR. Products were separated with agarose gel electrophoresis and correct fragments were isolated from the gel with a gel extraction kit (Qiagen) using standard laboratory methods. Template DNA used in the amplification of the flanking regions was from the *T. reesei* wild type strain QM6a (ATCC13631).

**[0249]** For the yeast homologous recombination system used in cloning, overlapping sequences for the vector and the selection marker were placed to the appropriate PCR-primers. To enable marker switch in the construct, NotI restriction sites were introduced between the flanking regions and the selection marker. PmeI restriction sites were placed between the vector and the flanking regions for removal of vector sequence prior to transformation into *T. reesei*. Vector backbone pRS426 was digested with restriction enzymes (EcoRI and XhoI). The restriction fragments were then separated with agarose gel electrophoresis, and the correct fragments were isolated from the gel with a gel extraction kit (Qiagen) using standard laboratory methods.

**[0250]** To construct the deletion plasmid, the vector backbone and the appropriate marker and flanking region



fragments were transformed into *Saccharomyces cerevisiae* (strain H3488/FY834). The yeast transformation protocol was based on the method for homologous yeast recombination described in the *Neurospora* knockouts workshop material of Colot and Colopy, (Dartmouth *Neurospora* genome protocols website), and the Gietz laboratory protocol (University of Manitoba, Gietz laboratory website). The plasmid DNA from the yeast transformants was rescued by transformation into *Escherichia coli*. A few clones were cultivated, plasmid DNA was isolated and digested to screen for correct recombination using standard laboratory methods. A few clones with correct insert sizes were sequenced and stored.

**[0251]** The first deletion plasmid for *pep1* (plasmid pTTv41, Table 11) used another selection marker, bar. The flanking region and marker fragments were produced by PCR and assembled to a plasmid using the yeast recombination method described above. To clone the second *pep1* deletion plasmid (pTTv71, Table 11), the bar marker was removed from the deletion plasmid pTTv41 with NotI digestion and replaced by the *pyr4* blaster cassette using the yeast homologous recombination system. The *pyr4* blaster cassette contains *T. reesei* *pyr4* gene followed by 310 bp direct repeat from *pyr4* 5' untranslated region. The direct repeat enables removal of *pyr4* gene under 5-FOA selection pressure via homologous recombination of the sequences and thus recycling of the selection marker. These deletion plasmids for *pep1* (pTTv41 and pTTv71) result in 1874 bp deletion in the *pep1* locus and cover the complete coding sequence of PEP1.

**Table 11: Primers for generating *pep1* deletion plasmids.**

| <b>Deletion plasmid pTTv41 for <i>pep1</i> (TrelD74156), vector backbone pRS426</b> |                                                                                              |
|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| <b>Primer</b>                                                                       | <b>Sequence</b>                                                                              |
| 5flankfw                                                                            | GTAACGCCAGGGTTTTCCAGTCACGACGGTTAAAC<br>GTATTGCGATGAGCAGCAGA (SEQ ID NO: 241)                 |
| 5flankrev                                                                           | ATCCACTTAACGTTACTGAAATCTGGTCTCCTAACCCA<br>CCAAG (SEQ ID NO: 242)                             |
| 3flankfw                                                                            | CTCCTTCAATATCATCTTCTGTCTGTGAAATGAGGTCC<br>CTTCC (SEQ ID NO: 243)                             |
| 3flankrev                                                                           | GCGGATAACAATTTACACAGGAAACAGCGTTTAAAC<br>CAAACGCAGCAGAAACCATA (SEQ ID NO: 244)                |
| PTfwd                                                                               | GATTTTCAGTAACGTTAAGTGGATGCGGCCGCGACAGA<br>AGATGATATTGAAG (SEQ ID NO: 245)                    |
| PTrev                                                                               | GACAGAAGATGATATTGAAGGAGCGGCCGCTTAAG<br>TGGATCCCGGTGAC (SEQ ID NO: 246)                       |
| <b>Deletion plasmid pTTv71 for <i>pep1</i> (TrelD74156), vector backbone pTTv41</b> |                                                                                              |
| <b>Primer</b>                                                                       | <b>Sequence</b>                                                                              |
| T315_pyr4_for                                                                       | GGTGGGTTAGGAGACCAGATTTTCAGTAACGTTAAGTG<br>GATGCGGCCGCTAGCATCGACTACTGCTGC (SEQ ID<br>NO: 247) |
| T316_pyr4_rev                                                                       | GCAGCAGTAGTCGATGCTAGGCGGCCATGCAAAGA<br>TACACATCAA (SEQ ID NO: 248)                           |

| Deletion plasmid pTTv71 for <i>pep1</i> (TrelD74156), vector backbone pTTv41 |                                                                                            |
|------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| Primer                                                                       | Sequence                                                                                   |
| T317_pyr4_loop_for                                                           | TTGATGTGTATCTTTGCATGGCGCGCCTAGCATCGAC<br>TACTGCTGC (SEQ ID NO: 249)                        |
| T318_pyr4_loop_rev                                                           | AGGGACCTCATTTACAGACAGAAGATGATATTGAAG<br>GAGGCGGCCGCGCTGATGAGGCTGAGAGAG (SEQ<br>ID NO: 250) |

[0252] To enable recycling of the selection marker and allow rapid deletion of subsequent protease genes, *pep1* was deleted from M127 (*pyr4*<sup>-</sup> mutant of the basic strain M124) using the *pyr4* blaster cassette described above. To remove the vector sequence, plasmid pTTv71 ( $\Delta pep1$ -*pyr4*) was digested with *PmeI* and the correct fragment was purified from an agarose gel using QIAquick Gel Extraction Kit (Qiagen). Approximately 5  $\mu$ g of the *pep1* deletion cassette was used to transform strain M127. Preparation of protoplasts and transformation for *pyr4* selection were carried out essentially according to methods in Penttilä et al. (1987, Gene 61:155-164) and Gruber et al (1990, Curr. Genet. 18:71-76).

[0253] 200 clones were picked as selective streaks and 24 transformants growing fast as selective streaks were screened by PCR using the primers listed in Table 12 for the correct integration using standard laboratory methods. Seven putative disruptants were purified to single cell clones. Deletion of *pep1* was verified by Southern analyses from these clones. Southern analyses also verified that four of the clones were single integrants. Three clones indicated multiple or inaccurate integration of the deletion cassette and were discarded. Two pure clones were designated with strain numbers M181 (9-20A-1) and M195 (9-35A-1).

**Table 12: Primers for screening integration of *pep1* deletion constructs**

| For screening integration of pTTv41       |                                       |
|-------------------------------------------|---------------------------------------|
| Primer                                    | Sequence                              |
| T075_74156_5int                           | TCGCTGTAACGAACTTCTGT (SEQ ID NO: 251) |
| T032_Bar_end_for                          | CATTGTTGACCTCCACTAGC (SEQ ID NO: 252) |
| T076_74156_3int                           | GCTGCTGATCGGACATTTTT (SEQ ID NO: 253) |
| T031_Bar_begin_rev2                       | GTTTCTGGCAGCTGGACT (SEQ ID NO: 254)   |
|                                           |                                       |
| For screening integration of pTTv71       |                                       |
| Primer                                    | Sequence                              |
| T075_74156_5int                           | TCGCTGTAACGAACTTCTGT (SEQ ID NO: 255) |
| T027_Pyr4_orf_start_re v                  | TGCGTCGCCGTCTCGCTCCT (SEQ ID NO: 256) |
|                                           |                                       |
| For screening deletion of <i>pep1</i> ORF |                                       |
| Primer                                    | Sequence                              |
| T077_74156_50rf_pcr                       | CGACGATCTACAGCCATCTG (SEQ ID NO: 257) |
| T078_74156_30rf_pcr                       | ACCCAAAGCGTCCTTCATTA (SEQ ID NO: 22)  |

**Generation of *pep1tsp1* double deletion strain M219**

[0254] The deletion plasmids for the trypsin-like serine protease *tsp1* (TreID71322/TreID73897) were constructed essentially as described for the *pep1* deletion plasmids. 953 bp of 5' flanking region and 926 bp of 3' flanking region were selected as the basis of the *tsp1* deletion plasmids. As for *pep1*, the first deletion plasmid for *tsp1* (pTTv42) used bar as the selection marker. The flanking region fragments were produced by PCR using the primers listed in Table 13. The products were separated with agarose gel electrophoresis and the correct fragments were isolated from the gel with gel extraction kit (Qiagen). Template DNA used in the PCR of the flanking regions was from the *T. reesei* wild type strain QM6a. The bar marker was obtained from pTTv41 with NotI digestion. The vector backbone was EcoRI/XhoI digested pRS426 as above. The plasmid was constructed using the yeast homologous recombination method as described above.

[0255] To clone the second *tsp1* deletion plasmid (pTTv72), the bar marker was removed from the deletion plasmid pTTv42 with NotI digestion. The *pyr4* blaster cassette was obtained from pTTv71 with NotI digestion, ligated to NotI cut pTTv42 and transformed into *E. coli*. A few transformants were cultivated, plasmid DNA isolated and digested to screen for correct ligation and orientation of the *pyr4* blaster cassette. One clone with correct insert size and orientation was sequenced and stored. These deletion plasmids for *tsp1* (pTTv42 and pTTv72) result in a 1252 bp deletion in the *tsp1* locus and cover the complete coding sequence of TSP1.

**Table 13: Primers for generating *tsp1* deletion plasmids.**

| <b>Deletion plasmid pTTv42 for <i>tsp1</i> (TreID71322/TreID73897), vector backbone pRS426</b>       |                                                                                            |
|------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| Primer                                                                                               | Sequence                                                                                   |
| T303_71322_5f                                                                                        | GTAACGCCAGGGTTTTCCAGTCACGACGGTTAACTGC<br>TGTTGCTGTTTGTGATG (SEQ ID NO:259)                 |
| T304_71322_5r_pt                                                                                     | CCCGTCACCGAGATCTGATCCGTCACCGGGATCCACTTAA<br>GCGGCCGCCTGTGGTGAGATCTCCAGACG (SEQ ID NO:260)  |
| T305_71322_3f_pt                                                                                     | GCCAAGCCCCAAAAGTGCTCCTTCAATATCATCTTCTGTCTG<br>CGGCCGCACTGTGCCCAACAATAAGCAG (SEQ ID NO:261) |
| T306_71322_3r                                                                                        | GCGGATAACAATTTACACAGGAAACAGCGTTTAAACCCAA<br>GGCGCTGGCTGTTA (SEQ ID NO:262)                 |
| <b>Deletion plasmid pTTv72 for <i>tsp1</i> (TreID71322/TreID73897), vector backbone pTTv42</b>       |                                                                                            |
| Primer                                                                                               | Sequence                                                                                   |
| no new primers, pTTv42 pTTv71 digested with NotI and ligated with <i>pyr4</i> -loopout fragment from |                                                                                            |

[0256] To reuse *pyr4* as the selection marker, removal of the *pyr4* blaster cassette from the *pep1* deletion strain M195 was carried out. Spores were spread onto minimal medium plates containing 20 g/l glucose, 2 g/l proteose peptone, 1 ml/l Triton X-100, 5 mM uridine and 1.5 g/l 5-FOA, pH 4.8. 5-FOA resistant colonies were picked after 5-7 days to 0.9% NaCl, suspended thoroughly by vortexing and filtrated through a cotton-filled pipette tip. To purify clones to single cell clones, filtrates were spread again onto plates described above. Purified clones were sporulated on plates containing 39 g/l potato dextrose agarose.

[0257] These clones were tested for uridine auxotrophy by plating spores onto minimal medium plates (20 g/l

glucose, 1 ml/l Triton X-100) where no growth was observed, indicating that the selected clones were *pyr4*<sup>-</sup>. All clones were further tested by PCR (using the primers listed in Table 14) for the removal of the blaster cassette and were shown to be correct. The clone (9-35A-1A-a) used to generate the double protease deletion strain (M219) was designated with strain number M196 (*Δpep1, pyr4*<sup>-</sup>).

**[0258]** To remove vector sequence, plasmid pTTv72 (*Δtsp1-pyr4* loopout) was digested with PmeI and the correct fragment was purified from an agarose gel. Approximately 5 μg of the *tsp1* deletion cassette was used to transform M196 (*Δpep1, pyr4*<sup>-</sup>). Preparation of protoplasts and transformation were carried out using *pyr4* selection essentially as described for the *pep1* deletion strains M181 and M195 as described above.

**[0259]** Over 100 colonies were picked and 48 were screened by PCR using the primers listed in Table 14 for the correct integration of the deletion cassette and also for the deletion of the *tsp1* ORF. Four putative *Δtsp1* clones were purified to single cell clones. Deletion of *tsp1* was verified by Southern analyses. Southern analyses indicated that four transformants (clones 16-5AA, 16-5BA, 16-11AA, 16-11BA) were single integrants. The other clones were determined to carry additional copies somewhere else in the genome and were discarded. The clone (16-5AA) used in removal of the *pyr4* blaster cassette (and to generate the triple deletion strain M277) was designated with strain number M219 (*Δpep1Δtsp1*).

**Table 14: Primers for screening removal of *pyr4* blaster cassette and for screening *tsp1* integration and strain purity.**

| For screening removal of <i>pyr4</i> blaster cassette from M195 |                                       |
|-----------------------------------------------------------------|---------------------------------------|
| T083_74156_5a_seq                                               | GATCGACAAAGGTTCCAGCG (SEQ ID NO:263)  |
| T084_74156_3a_seq                                               | AATTGTATCATTCCGAGGCT (SEQ ID NO:264)  |
| For screening integration of pTTv72                             |                                       |
| Primer                                                          | Sequence                              |
| T307_71322_5int                                                 | CTGTTTGGCCCTCGAAACT (SEQ ID NO:265)   |
| T027_Pyr4_orf_start_rev                                         | TGCGTCGCCGTCTCGCTCCT (SEQ ID NO:266)  |
| T308_71322_3int                                                 | TTCGCCATCCAAATTTCTTC (SEQ ID NO:267)  |
| T028_Pyr4_flank_rev                                             | CATCCTCAAGGCCTCAGAC (SEQ ID NO: 268)  |
| For screening deletion of <i>tsp1</i> ORF                       |                                       |
| T309_71322_5orfpcr                                              | CCCAAGTCGTCTCAGCTCTC (SEQ ID NO:269)  |
| T310_71322_3orfpcr                                              | TCGAAGGCTTCAGTGAGGTAA (SEQ ID NO:270) |

#### **Generation of *pep1 tsp1 slp1* triple deletion strain M277**

**[0260]** The deletion plasmid for the subtilisin-like protease *slp1* (TreID51365) was constructed essentially as described for *pep1* deletion plasmid pTTv41. 1094 bp of 5' flanking regions and 1247 bp of 3' flanking region were selected as the basis of the *slp1* deletion plasmid. Fragments were produced by PCR using the primers listed in Table 15. The products were separated with agarose gel electrophoresis and the correct fragments were isolated from the gel with gel extraction kit (Qiagen) using standard laboratory methods. Template used in the PCR of the flanking regions was from the *T. reesei* wild type strain QM6a. The *pyr4* blaster cassette was

obtained from pTTv71 with NotI digestion. The vector backbone was EcoRI/XhoI digested pRS426 and the plasmid was constructed using the yeast homologous recombination method as described above. This deletion plasmid for *slp1* (pTTv126) results in 2951 bp deletion in the *slp1* locus and covers the complete coding sequence of SLP1.

**Table 15: Primers for generating *slp1* deletion plasmid.**

| Deletion plasmid pTTv126 for <i>slp1</i> (TreID51365), vector backbone pRS426 |                                                                                          |
|-------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| Primer                                                                        | Sequence                                                                                 |
| 5flankfw_vect                                                                 | GTAACGCCAGGGTTTTCCAGTCACGACGGTTTAAACAT<br>CTCGGAGTGATGCTTCCT (SEQ ID NO:271)             |
| slp1_5flankrev_pyr4Prom                                                       | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCT<br>AGGCGGCCGCATCAGACGAAACCAGACGAG (SEQ ID NO:272) |
| slp1_3flankfw_pyr4Term                                                        | CAACCAGCCGCAGCCTCAGCCTCTCTCAGCCTCATCAG<br>CCGCGGCCGCAGGAATCGAGTTGATGATTC (SEQ ID NO:273) |
| 3flankrev_vect                                                                | GCGGATAACAATTTACACAGGAAACAGCGTTTAAACCT<br>GGTTGGGATCTGACCACT (SEQ ID NO:274)             |

**[0261]** To generate a marker-free triple protease deletion strain, the looping out of the *pyr4* marker was applied to strain M219 essentially as described above for looping out *pyr4* from the single protease deletion strain  $\Delta pep1$ . Three consecutive 5-FOA selection steps were carried out to ensure that the clones selected were originating from single cells. Final clones were verified for the looping out of *pyr4* by PCR (using the primers listed in Table 16); no specific signals were seen with primers annealing with the looped out part of the *pyr4*. The looping out was further verified by plating the clones onto minimal medium plates with or without 5 mM uridine. The clone used to generate the triple protease deletion strain was designated with strain number M228 ( $\Delta pep1\Delta tsp1$ , *pyr4*<sup>-</sup>).

**[0262]** To remove vector sequence, plasmid pTTv126 ( $\Delta slp1$ -*pyr4* loopout) was digested with PmeI and the correct fragment purified from an agarose gel using QIAquick Gel Extraction Kit (Qiagen). Approximately 5  $\mu$ g of the *slp1* deletion cassette was used to transform M228 ( $\Delta pep1\Delta tsp1$ , *pyr4*<sup>-</sup>) above. Preparation of protoplasts and transformation were carried out essentially as described above for the strains M181 and M195 using *pyr4* selection.

**[0263]** 200 clones were picked as first streaks and 48 of these streaks were screened by PCR using the primers listed in Table 16 for the correct integration. Five putative triple protease disruptants were purified to single cell clones. Deletion of *slp1* was verified by Southern analyses of the five clones and three of the clones were single integrants. The clone used in removal of the *pyr4* blaster cassette (and to generate the quadruple protease deletion strain M307 below) was designated with strain number M277 ( $\Delta pep1\Delta tsp1\Delta slp1$ ).

**Table 16: Primers for screening removal of *pyr4* blaster cassette and for screening *slp1* integration and strain purity.**

| For screening removal of <i>pyr4</i> blaster cassette from M219 |                                      |
|-----------------------------------------------------------------|--------------------------------------|
| Primer                                                          | Sequence                             |
| T307_71322_5int                                                 | CTGTTTGGCCCTCGAACT (SEQ ID NO:275)   |
| T026_Pyr4_orf_5rev2                                             | CCATGAGCTTGAACAGGTAA (SEQ ID NO:276) |

| For screening removal of <i>pyr4</i> blaster cassette from M219 |                                       |
|-----------------------------------------------------------------|---------------------------------------|
| Primer                                                          | Sequence                              |
|                                                                 | NO:276)                               |
| T308_71322_3int                                                 | TTCGCCATCCAAATTTCTTC (SEQ ID NO:277)  |
| T028_Pyr4_flank_rev                                             | CATCCTCAAGGCCTCAGAC (SEQ ID NO:278)   |
|                                                                 |                                       |
| For screening integration of pTTv126                            |                                       |
| Primer                                                          | Sequence                              |
| T079_slp1_scrn_5forw                                            | GCAGACAAACAGAGCAACGA (SEQ ID NO:279)  |
| T026_Pyr4_orf_5rev2                                             | CCATGAGCTTGAACAGGTAA (SEQ ID NO:280)  |
| T080_slp1_scrn_3rev                                             | TAGAGGGTGTGCGATGGAAGC (SEQ ID NO:281) |
| T028_Pyr4_flank_rev                                             | CATCCTCAAGGCCTCAGAC (SEQ ID NO:282)   |
|                                                                 |                                       |
| For screening deletion of <i>slp1</i> ORF                       |                                       |
| T081_slp1_orf_fw                                                | GGTCTCTTCTTTGCCAGCAC (SEQ ID NO:283)  |
| T082_slp1_orf_rev                                               | TGTCGCTGAACTGAATTTGC (SEQ ID NO:284)  |

#### Generation of quadruple protease deletion strain M307

[0264] To generate a marker-free triple protease deletion strain, removal of the *pyr4* blaster cassette was applied to strain M277 essentially as described above. Three consecutive 5-FOA selection steps were carried out to ensure that the clones selected were originating from single cells. Final clones were verified for the removal of the blaster cassette by PCR using the primers listed in Table 18 and by plating the clones onto minimal medium plates with or without 5 mM uridine. The clone used to generate the quadruple protease deletion strain was designated with strain number M306 ( $\Delta pep1 \Delta tsp1 \Delta slp1$ , *pyr4*-).

[0265] The deletion plasmid pTTv117 for the glutamic protease *gap1* (TrelD69555) was constructed essentially as described for *pep1* deletion plasmid pTTv41. 1000 bp of 5' flanking region and 1100 bp of 3' flanking region were selected as the basis of the *gap1* deletion plasmid. Flanking region fragments were produced by PCR using the primers listed in Table 17. The products were separated with agarose gel electrophoresis and the correct fragments were isolated from the gel with gel extraction kit (Qiagen). Template DNA used in the PCR of the flanking regions was from the *T. reesei* wild type strain QM6a. The *pyr4* blaster cassette was obtained from pTTv71 with NotI digestion. The vector backbone was EcoRI/XhoI digested pRS426 and the plasmid was constructed using the yeast homologous recombination method as described above. This deletion plasmid for *gap1* (pTTv117) resulted in a 1037 bp deletion in the *gap1* locus and covers the complete coding sequence of GAP1.

**Table 17: Primers for generating *gap1* deletion plasmid.**

| Deletion plasmid pTTv117 for <i>gap1</i> (TreID69555), vector backbone pRS426 |                                                                                           |
|-------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| Primer                                                                        | Sequence                                                                                  |
| JJ-045 primer                                                                 | GATTAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGACG<br>GTTTAAACACCTCATGAGGGACTATGG (SEQ ID NO:285)    |
| JJ-046 primer                                                                 | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTAG<br>GCGGCCGCCAAGAAGAGGCAGAGGGTAAT (SEQ ID NO:286) |
| JJ-047 primer                                                                 | CAACCAGCCGCAGCCTCAGCCTCTCTCAGCCTCATCAGCC<br>GCGGCCGCCTATACATACTGATGATACA (SEQ ID NO:287)  |
| JJ-048 primer                                                                 | TGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCG<br>TTTAAACGCCCCATGTATGGACTCTAG (SEQ ID NO:288)   |

[0266] To remove vector sequence, plasmid pTTv117 was digested with PmeI and the correct fragment purified from an agarose gel using QIAquick Gel Extraction Kit (Qiagen). Approximately 5 µg of the *gap1* deletion cassette was used to transform M306 ( $\Delta pep1\Delta tsp1\Delta slp1$ , *pyr4*<sup>-</sup>) above. Preparation of protoplasts and transformation were carried out essentially as described for the strains M181 and M195 using *pyr4* selection.

[0267] 150 clones were picked as first streaks and 48 of these streaks were screened by PCR using the primers listed in Table 18 for the correct integration. Eight putative quadruple protease disruptants were purified to single cell clones. Deletion of *gap1* was verified by Southern analyses of the eight clones and it verified that three of the clones were single integrants. The clone used in removal of the *pyr4* blaster cassette (and to generate the quintuple protease deletion strain M369 below) was designated with strain number M307 ( $\Delta pep1\Delta tsp1\Delta slp1\Delta gap1$ ).

**Table 18: Primers for screening removal of *pyr4* blaster cassette and for screening *gap1* integration and strain purity.**

| For screening removal of <i>pyr4</i> blaster cassette from M277 |                                         |
|-----------------------------------------------------------------|-----------------------------------------|
| Primer                                                          | Sequence                                |
| T079_slp1_scrn_5forw                                            | GCAGACAAACAGAGCAACGA (SEQ ID NO:289)    |
| T026_Pyr4_orf_5rev2                                             | CCATGAGCTTGAACAGGTAA (SEQ ID NO:290)    |
| T080_slp1_scrn_3rev                                             | TAGAGGGTGTTCGATGGAAGC (SEQ ID NO:291)   |
| T028_Pyr4_flank_rev                                             | CATCCTCAAGGCCTCAGAC (SEQ ID NO:292)     |
|                                                                 |                                         |
| For screening integration of pTTv117                            |                                         |
| Primer                                                          | Sequence                                |
| T052_gap1_5screen_F                                             | CTCAGAAAGGTTGTAGTTGTGA (SEQ ID NO:293)  |
| T026_Pyr4_orf_5rev2                                             | CCATGAGCTTGAACAGGTAA (SEQ ID NO:294)    |
| T053_gap1_3screen_R                                             | GATGTTGTGTTTTTCAGTCTGCA (SEQ ID NO:295) |
| T028_Pyr4_flank_rev                                             | CATCCTCAAGGCCTCAGAC (SEQ ID NO:296)     |
|                                                                 |                                         |

| For screening deletion of <i>gap1</i> ORF |                                       |
|-------------------------------------------|---------------------------------------|
| T109_gap1_ORF_F                           | ATGTTTCATCGCTGGCGTCTG (SEQ ID NO:297) |
| T110_gap1_ORF_R                           | CTAAACGTAAGAGCAGGTCAA (SEQ ID NO:298) |

#### Generation of quintuple protease deletion strain M369

[0268] To generate a marker-free quadruple protease deletion strain, removal of the *pyr4* blaster cassette was applied to strain M307 essentially as described above. Three consecutive 5-FOA selection steps were carried out and the final clones were verified for the removal of the blaster cassette by PCR using the primers listed in Table 20 and by plating the clones onto minimal medium plates with or without 5 mM uridine. The clone used to generate the quintuple protease deletion strain was designated with strain number M321 ( $\Delta pep1\Delta tsp1\Delta slp1\Delta gap1, pyr4^-$ ).

[0269] The pTTv145 deletion plasmid for the glutamic protease *gap2* (TreID106661) was constructed essentially as described for *pep1* deletion plasmid pTTv41. 1021 bp of 5' flanking region and 1010 bp of 3' flanking region were selected as the basis of the *gap2* deletion plasmid. In this plasmid the direct repeat fragment of the *pyr4* blaster cassette was changed from *pyr4* 5'UTR to 320 bp direct repeat from the end of *gap2* 5' flanking region and no *Ascl* site was added between the *pyr4* and the 5' direct repeat. Fragments were produced by PCR using the primers listed in Table 19 and the products were separated with agarose gel electrophoresis and the correct fragments were isolated from the gel with gel extraction kit (Qiagen). Template DNA used in the PCR of the flanking regions was the *T. reesei* wild type strain QM6a. The *pyr4* marker gene was obtained from pHHO5 with *NotI* digestion and the vector backbone was *EcoRI/XhoI* digested pRS426.

[0270] The plasmid was constructed using the yeast homologous recombination method as described above. This deletion plasmid for *gap2* (pTTv145) results in a 944 bp deletion in the *gap2* locus and covers the complete coding sequence of GAP2.

**Table 19: Primers for generating *gap2* deletion plasmid.**

| Deletion plasmid pTTv145 for <i>gap2</i> (TreID106661), vector backbone pRS426 |                                                                                               |
|--------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Primer                                                                         | Sequence                                                                                      |
| T101_gap2_5flank_F_pRS426                                                      | GATTAAGTTGGGTAAAGCCAGGGTTTTCCAGTCA<br>CGACGGTTTAAACGCTACTACGCGAGCAAGTG<br>(SEQ ID NO: 299)    |
| T102_gap2_5flank_R_pyr4                                                        | GGAAGTGTGCGCGATTGGGAGAATTCGTGCGAT<br>CGCGGCGGCCGCCGATGAAGATGTGCAGTTG<br>(SEQ ID NO: 300)      |
| T103gap2-loop_F_pyr4                                                           | AGGGAACATATCACCTCGGGCATTTTTCATTTGG<br>TAGGCGGCGCGCTAAGATATCTTCAAGCTTATGCG<br>(SEQ ID NO: 301) |
| T104gap2-loop_R                                                                | CGGATGAAGATGTGCAGTTG (SEQ ID NO: 302)                                                         |
| T105gap2_3flank_F_loop                                                         | TGTCTCACTTCCACCCATCTCAACTGCACATCTTC<br>ATCCGAGCAACAACATGAGGTTGAA (SEQ ID NO: 303)             |
| T106_gap2_3flank_R_pRS426                                                      |                                                                                               |



| Deletion plasmid pTTv145 for <i>gap2</i> (TreID106661), vector backbone pRS426 |                                                                                               |
|--------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Primer                                                                         | Sequence                                                                                      |
|                                                                                | CCTATGTTGTGTGGAATTGTGAGCGGATAACAATT<br>TCACAGTTTAAACACAACGCATGTCCAGCTTTTG<br>(SEQ ID NO: 304) |

[0271] To remove vector sequence, plasmid pTTv145 ( $\Delta gap2$ -*pyr4* loopout) was digested with PmeI and the correct fragment purified from an agarose gel using QIAquick Gel Extraction Kit (Qiagen). Approximately 5  $\mu$ g of the *gap2* deletion cassette was used to transform M321. Preparation of protoplasts and transformation were carried out essentially as described above.

[0272] 100 clones were picked as first streaks and all 20 growing streaks were screened by PCR using the primers listed in Table 20 for the correct integration. 10 putative quintuple protease disruptants ( $\Delta pep1\Delta tsp1\Delta slp1\Delta gap1\Delta gap2$ ) were purified to single cell clones and rescreened by PCR and one purified clone was negative for the *gap2* ORF. The *gap2* deletion was verified by Southern analyses of the clone. The clone 7-30A was designated with strain number M369 ( $\Delta pep1\Delta tsp1\Delta slp1\Delta gap1\Delta gap2$ ) and it was used for removal of the *pyr4* blaster cassette and to generate the 6-fold protease deletion strain M396.

**Table 20: Primers for screening removal of *pyr4* blaster cassette and for screening *gap2* integration and strain purity.**

| For screening removal of <i>pyr4</i> blaster cassette from M307 |                                         |
|-----------------------------------------------------------------|-----------------------------------------|
| Primer                                                          | Sequence                                |
| T052_gap1_5screen_F                                             | CTCAGAAAGGTTGTAGTTGTGA (SEQ ID NO:305)  |
| T026_Pyr4_orf_5rev2                                             | CCATGAGCTTGAACAGGTAA (SEQ ID NO:306)    |
| T053_gap1_3screen_R                                             | GATGTTGTGTTTTTCAGTCTGCA (SEQ ID NO:307) |
| T028_Pyr4_flank_rev                                             | CATCCTCAAGGCCTCAGAC (SEQ ID NO:308)     |
|                                                                 |                                         |
| For screening integration of pTTv145                            |                                         |
| Primer                                                          | Sequence                                |
| T048_gap2_5screen_F                                             | GCTTGGCATCACGGAAGCT (SEQ ID NO:309)     |
| T026_Pyr4_orf_5rev2                                             | CCATGAGCTTGAACAGGTAA (SEQ ID NO:310)    |
| T049_gap2_3screen_R                                             | TTGACAAGAAAGGTCCGGTTG (SEQ ID NO:311)   |
| T028_Pyr4_flank_rev                                             | CATCCTCAAGGCCTCAGAC (SEQ ID NO:312)     |
|                                                                 |                                         |
| For screening deletion of <i>gap2</i> ORF                       |                                         |
| T107_gap2_ORF_F                                                 | ATGGATGCTATCCGAGCCAG (SEQ ID NO:313)    |
| T108_gap2_ORF_R                                                 | CTATTCATACTCAACAGTCACA (SEQ ID NO:314)  |

#### **Generation of 6-fold protease deletion strains M396**

[0273] To generate a marker-free quintuple protease deletion strain, removal of the *pyr4* marker was applied

to strain M369 essentially as described above. Three consecutive 5-FOA selection steps were carried out to ensure that the clones selected were originating from single cells and the final clones were verified by PCR using the primers listed in Table 22. Removal was further verified by plating the clones onto minimal medium plates with or without 5 mM uridine and by Southern analyses. The clone used to generate the 6-fold protease deletion strain was designated with strain number M381 ( $\Delta pep1\Delta tsp1\Delta slp1\Delta gap1\Delta gap2$ , *pyr4*-).

**[0274]** The deletion plasmid pTTv181 for the sixth protease gene, aspartic protease *pep4* (TreID77579) was constructed essentially as described above for the pTTv71. 959 bp of 5' flanking region and 992 bp of 3' flanking region were selected as the basis of the *pep4* deletion plasmid. As for *pep1*, the first deletion plasmid for *pep4* (pTTv43, Table 21) carried another selection marker, *bar*, which was replaced with the *pyr4* blaster cassette. The blaster cassette was obtained from pTTv71 with NotI digestion, ligated to NotI cut pTTv43, and then transformed into *E. coli*. A few transformants were cultivated, plasmid DNA isolated and digested to screen for correct ligation and orientation of the *pyr4* blaster cassette. One clone with correct insert size and orientation was sequenced and stored (pTTv73, Table 21). The blaster cassette was changed: the direct repeat fragment used in removal of *pyr4* was changed from 308 bp of *pyr4* 5'UTR to 300 bp direct repeat from the end of *pep4* 5' flanking region (as in pTTv145, *gap2-pyr4*). This was made by removing the existing *pyr4* blaster cassette from pTTv73 with NotI digestion. The *pyr4* gene was amplified by PCR using pTTv73 as a template using the primers in Table 21. For the yeast homologous recombination system used in cloning, overlapping sequences for the vector were placed to the appropriate PCR-primers. To enable marker switch in the construct, NotI restriction sites were introduced on both sides of the *pyr4* selection marker and for additional cloning steps an Ascl site was introduced between the *pep4* 5'direct repeat and 3' flank. This type of blaster cassette should not leave any additional sequence to the locus of the deleted gene after excision. The 300 bp *pep4* 5'direct repeat was amplified by PCR using the *T. reesei* wild type strain QM6a as a template. Products were separated with agarose gel electrophoresis and the correct fragments were isolated from the gel with gel extraction kit (Qiagen). A few of the clones obtained from the recombination were cultivated, and plasmid DNA was isolated and digested to screen for correct recombination. These deletion plasmids for *pep4* (pTTv43, pTTv73 and pTTv181, Table 21) result in a 1413 bp deletion in the *pep4* locus and cover the complete coding sequence of PEP4.

**Table 21: Primers for generating *pep4* deletion plasmids.**

| <b>Deletion plasmid pTTv43 for <i>pep4</i> (TreID77579), vector backbone pRS426</b>                  |                                                                                                                                                                                                                                                                                                               |
|------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Primer                                                                                               | Sequence                                                                                                                                                                                                                                                                                                      |
| T298_77579_5f                                                                                        |                                                                                                                                                                                                                                                                                                               |
| T299_77579_5r_pt                                                                                     | GTAACGCCAGGGTTTTCCAGTCACGACGGTTTAA<br>CTCAGGTCAACCAACGAGGAC (SEQ ID NO: 35)<br>CCGTCACCGAGATCTGATCCGTCACCGGATCCAC<br>TTAAGCGGCCGCTGAATGGGATGGTTCGATTG<br>GCCAAGCCCAAAAAGTGCTCCTTCAATATCATCTTCT<br>GTCGCGGCCGAGGTAGACGCTTTGCGAGTG<br>GCGGATAACAATTTCACACAGGAACAGCGTTTAAAC<br>TGAAGTACGCGGACTGA (SEQ ID NO: 60) |
| T300_77579_3f_pt                                                                                     |                                                                                                                                                                                                                                                                                                               |
| T301_77579_3r                                                                                        |                                                                                                                                                                                                                                                                                                               |
| <b>Deletion plasmid pTTv73 for <i>pep4</i> (TreID77579), vector backbone pTTv43</b>                  |                                                                                                                                                                                                                                                                                                               |
| Primer                                                                                               | Sequence                                                                                                                                                                                                                                                                                                      |
| no new primers, pTTv43 digested with NotI and ligated with <i>pyr4</i> -loopout fragment from pTTv71 |                                                                                                                                                                                                                                                                                                               |

| Deletion plasmid pTTv181 for <i>pep4</i> (TrelD77579), vector backbone pTTv73 |                                                                                                                                                                                            |
|-------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Primer                                                                        | Sequence                                                                                                                                                                                   |
| T209_pyr4_f_recpep4_5f                                                        | AAGTTCCCTTCCTCTGGCAGCAATCGAACCATCCCAT<br>TCAGCGGCCGCTAGCATCGACTACTGCTGC (SEQ<br>ID NO:315)                                                                                                 |
| T210_pyr4_r                                                                   | CATGCAAAGATACACATCAA (SEQ ID NO:                                                                                                                                                           |
| T211_pep4_loop_f_recpyr4                                                      | TGATTGTACCCAGCTGCGATTGATGTGTATCTTTGC<br>ATGGCGGCCGCTCAATGTTGACTGCCCCAGG (SEQ<br>ID NO:316)<br>GCACCTTCTTAGATACACACACACTCGCAAAGCGTCTA<br>CCTGGCGCGCCTGAATGGGATGGTTCGATTG (SEQ<br>ID NO:317) |
| T212_pep4_loop_r_recpep4_3f                                                   |                                                                                                                                                                                            |

[0275] To remove vector sequence, plasmid pTTv181 ( $\Delta pep4$ -*pyr4* loopout) was digested with PmeI and the correct fragment purified from an agarose gel using QIAquick Gel Extraction Kit (Qiagen). Approximately 5  $\mu$ g of the *pep4* deletion cassette was used to transform M381. Preparation of protoplasts and transformation were carried out essentially as described above.

[0276] Over 200 transformants were picked as first streaks and 32 growing streaks were screened by PCR using the primers listed in Table 22 for correct integration. Seven clones gave the expected signals and were purified to single cell clones and rescreened by PCR using the primers of Table 22. Deletion of *pep4* was verified also by Southern analyses from five clones. Southern analyses also indicated that all transformants were single integrants. Clone 25-120A used for removal of the *pyr4* blaster cassette (and in generation of the 7-fold protease deletion strain) was designated with strain number M396.

**Table 22: Primers for screening removal of *pyr4* blaster cassette from M369 and for screening *pep4* integration and strain purity.**

| For screening removal of <i>pyr4</i> blaster cassette from M369 |                                           |
|-----------------------------------------------------------------|-------------------------------------------|
| Primer                                                          | Sequence                                  |
| T222_gap2_5f_f2                                                 | GGCAGGTTCGCAGAGCAAGACA (SEQ ID<br>NO:318) |
| T049_gap2_3screen_R                                             | TTGACAAGAAAGGTCCGGTTG (SEQ ID<br>NO:319)  |
|                                                                 |                                           |
| For screening integration of pTTv181                            |                                           |
| Primer                                                          | Sequence                                  |
| T302_77579_5int                                                 | GATTCATCACAGGGGCAGTC (SEQ ID<br>NO:320)   |
| T027_Pyr4_orf_start_rev                                         | TGCGTCGCCGTCTCGCTCCT (SEQ ID<br>NO:321)   |
| T415_77579_3screen                                              | ACGCCGTTGCTGAGCCTTG (SEQ ID NO:322)       |
| T061_pyr4_orf_screen_2F                                         | TTAGGCGACCTCTTTTCCA (SEQ ID NO:323)       |
|                                                                 |                                           |

| For screening deletion of <i>pep4</i> ORF |                                      |
|-------------------------------------------|--------------------------------------|
| T416_77579_probeF                         | GAGCCCATCATCAACACCTC (SEQ ID NO:324) |
| T417_77579_probeR                         | TGCCAAGGTCGTAGACGGA (SEQ ID NO:325)  |

#### Generation of 7-Fold deletion strain M486

**[0277]** The first deletion plasmid pTTv188 for the seventh protease gene, aspartic protease *pep3* (TreID121133) was constructed essentially as described for  $\Delta pep1$  plasmid pTTv41 above. 1215 bp of 5' flanking region and 1082 bp of 3' flanking region were selected as the basis of the *pep3* deletion plasmid. In this plasmid the direct repeat fragment is a 300 bp stretch from the end of *pep3* 5' flanking region. Fragments were produced by PCR using the primers listed in Table 23. NotI restriction sites were introduced on both sides of the *pyr4* selection marker and for additional cloning steps and Ascl site was introduced between the *pep3* 5'direct repeat and 3' flank. The products were separated with agarose gel electrophoresis and the correct fragments were isolated from the gel with gel extraction kit (Qiagen). Template used in the PCR of the flanking regions was the *T. reesei* wild type strain QM6a. The *pyr4* marker gene was obtained from pTTv181 with NotI digestion. The vector backbone was EcoRI/XhoI digested pRS426 and the plasmid was constructed using the yeast homologous recombination method as described above.

**[0278]** The second deletion plasmid for the aspartic protease *pep3* (TreID121133), pTTv192, was constructed using the plasmid pTTv188 above as the backbone. This second plasmid carries a native KEX2 (TreID123156) overexpression cassette and uses acetamidase (*AmdS*) gene from *Aspergillus nidulans* as the selection marker. The *pyr4* blaster cassette was removed from pTTv188 with NotI-Ascl double digestion. The fragments for *cDNA1* promoter (template: pTHN3 plasmid DNA), native *kex2* (template: *T. reesei* QM6a genomic DNA), *trpC* terminator (template: pHHO2 plasmid DNA) and *AmdS* marker (template: pHHO1 plasmid DNA) were produced by PCR using the primers listed in Table 23. As for pTTv188 above, to enable marker switch in the construct, NotI restriction sites were introduced on both sides of the *AmdS* selection marker. The products were separated with agarose gel electrophoresis and the correct fragments were isolated from the gel with gel extraction kit (Qiagen) and the plasmid was constructed using the yeast homologous recombination method as described above.

**[0279]** The third deletion plasmid for the aspartic protease *pep3* (TreID121133), pTTv205, was constructed using the plasmid pTTv192 above as the backbone. The *AmdS* marker was removed from pTTv192 with NotI digestion. Fragments for a new *pyr4* blaster cassette (located after the KEX2 overexpression cassette) were produced by PCR using the primers listed in Table 23. In this blaster cassette, the direct repeat is a 300 bp stretch from the beginning of the *pep3* 3' flanking region and located before the *pyr4* gene. As for pTTv192 above, to enable marker switch in the construct, NotI restriction sites were introduced on both sides of the *pyr4* blaster cassette. The products were separated with agarose gel electrophoresis and the correct fragments were isolated from the gel with gel extraction kit (Qiagen) and the plasmid was constructed using the yeast homologous recombination method as described above.

**[0280]** These deletion plasmids for *pep3* (pTTv188, pTTv192 and pTTv205, Table 23) result in a 2590 bp deletion in the *pep3* locus and cover the complete coding sequence of PEP3.

**Table 23: Primers for generating *pep3* deletion plasmids.**

| Deletion plasmid pTTv188 for <i>pep3</i> (TreID121133), vector backbone pRS426  |                                                                                       |
|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Primer                                                                          | Sequence                                                                              |
| T346_pep3_5f_for                                                                | GGTAACGCCAGGGTTTTCCAGTCACGACGGTTTAAACGTGAGCCCCCTGGACACCT (SEQ ID NO:326)              |
| T347_pep3_5f_rev                                                                | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTAGGCGGCCGCCATCGCCGTCGCGGACATGA (SEQ ID NO:327)  |
| T348_pep3_loop_for                                                              | TGATTGTACCCCAGCTGCGATTGATGTGTATCTTTGCATGGCGGCCGCTCGACGTTGTATCTGCACTC (SEQ ID NO:328)  |
| T349_pep3_loop_rev                                                              | GTACGTTCTGATTGCCAACTACGGACCAGACCAGGGCTCCGGCGCGCCCATCGCCGTCGCGGACATGA (SEQ ID NO:329)  |
| T350_pep3_3f_for                                                                | GGAGCCCTGGTCTGGTCCGT (SEQ ID NO:330)                                                  |
| T351_pep3_3f_rev                                                                | AGCGGATAACAATTTACACAGGAAACAGCGTTTAAACACGGCGTTCAACATGCCCA (SEQ ID NO:331)              |
|                                                                                 |                                                                                       |
| Deletion plasmid pTTv192 for <i>pep3</i> (TreID121133), vector backbone pTTv188 |                                                                                       |
| Primer                                                                          | Sequence                                                                              |
| T389_cDNApromoter_pep3fl ank                                                    | GCTGGCGCTGGGAATAGCGTCATGTCCGCGACGGCGATGGAATTCGGTCTGAAGGACGT (SEQ ID NO:332)           |
| T138_cDNA1_Rev                                                                  | GTTGAGAGAAGTTGTTGGATTG (SEQ ID NO:333)                                                |
| T139_123561 For_cDNA1                                                           | AACCAAAGACTTTTTGATCAATCCAACAATTCTCTCAACATGAAGATTCCTCGATCCTTG (SEQ ID NO:334)          |
| 123561Rev                                                                       | TCAGCGCCGTAACCTCTGC (SEQ ID NO:335)                                                   |
| trpCterm For_123561                                                             | TGATGGTGATGAGGCGGAAAAGCAGAGGTTACGGCGCTGAGGATCCACTTAACGTTACTGA (SEQ ID NO:336)         |
| T390_trpCtermR_AmdS                                                             | TCTCTCAAAGGAAGAATCCCTTCAGGGTTGCGTTTCCAGTGCGGCCGCTGCTCTTAGAAAGAAGGATTA (SEQ ID NO:337) |
| T391_AmdS_endR                                                                  | ACTGGAAACGCAACCCTGAA (SEQ ID NO:338)                                                  |
| T390_trpCtermR_AmdS                                                             | TCTGATTGCCAACTACGGACCAGACCAGGGCTCCGGCGCGGCCGCGCTAGATCTACG (SEQ ID NO:339)             |
|                                                                                 |                                                                                       |

| Deletion plasmid pTTv205 for <i>pep3</i> (TreID121133), vector backbone pTTv192 |                                                                                               |
|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Primer                                                                          | Sequence                                                                                      |
| T428_peg3_3flankDR_F-trpCterm                                                   | GTACACTTGTTTAGAGGTAATCCTTCTTTCTAGAAGGA<br>GAGCGGCCGCGGAGCCCTGGTCTGGTCC (SEQ ID NO:340)        |
| T429_peg3_3flankDR_R-pyr4                                                       | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATG<br>CTAGAAGCTGACGGGCGTCAACG (SEQ ID NO:341)               |
| T094_pyr4_F                                                                     | TAGCATCGACTACTGCTGC (SEQ ID NO:342)                                                           |
| T430_pyr4_R-peg3_3flank                                                         | GTACGTTCTGATTGCCAACTACGGACCAGACCAGGG<br>CTCCGCGGCCGCCATGCAAAGATACACATCAATC<br>(SEQ ID NO:343) |

[0281] To generate a marker-free 6-fold protease deletion strain, removal of the *pyr4* marker was applied to the 6-fold deletion strain M396 essentially as described above. Four consecutive 5-FOA selection steps were carried out to ensure that the clones selected were originating from single cells.

[0282] Final clones were verified by PCR using the primers listed in Table 24 and removal of the blaster cassette was further verified by plating the clones onto minimal medium plates with or without 5 mM uridine and by Southern analyses. The clone (25-120A-62) used to generate the 7-fold protease deletion strain was designated with strain number M402.

[0283] Transformation was carried out with pTTv205 (KEX2 overexpression included). To remove vector sequence, plasmid pTTv205 was digested with PmeI and the correct fragment purified from agarose gel using QIAquick Gel Extraction Kit (Qiagen). Approximately 5 µg of the deletion cassette was used to transform M402 ( $\Delta pep1\Delta tsp1\Delta slp1\Delta gap1\Delta gap2\Delta pep4$ , *pyr4*<sup>-</sup>). Preparation of protoplasts and transformation were carried out essentially as described above.

[0284] Transformants were picked as first streaks and growing streaks were screened by PCR (using the primers listed in Table 24) for correct integration. Clones giving the expected signals were purified to single cell clones and rescreened by PCR using the primers listed in Table 24. Deletion of *pep3* was verified by Southern analyses from selected clones.

**Table 24: Primers for screening removal of *pyr4* blaster cassette from M396 and for screening *pep3* integration and strain purity**

| For screening removal of <i>pyr4</i> blaster cassette from M396 |                                           |
|-----------------------------------------------------------------|-------------------------------------------|
| Primer                                                          | Sequence                                  |
| T302_77579_5int                                                 | GATTCATCACAGGGGCAGTC (SEQ ID NO:344)      |
| T214_peg4_3f_seq_r1                                             | CCGCTCTCAAACCTGCCCAAA (SEQ ID NO:345)     |
|                                                                 |                                           |
| For screening integration of pTTv205                            |                                           |
| T625_peg3_5int_new                                              | ACGTGAAGTTGCCCATCAA (SEQ ID NO:346)       |
| T140_cDNA1 promoter_seqR1                                       | TAACTTGTACGCTCTCAGTTTCGAG (SEQ ID NO:347) |
| T626_peg3_3int_new                                              | GACCAATGGCTTCACGAAGT (SEQ ID NO:348)      |
| T061_pyr4_orf_screen_2F                                         | TTAGGCGACCTCTTTTCCA (SEQ ID NO:349)       |

|                                                  |                                      |
|--------------------------------------------------|--------------------------------------|
| <b>For screening integration of pTTv205</b>      |                                      |
|                                                  |                                      |
| <b>For screening deletion of <i>pep3</i> ORF</b> |                                      |
| T352_peg3_orf_for                                | CAGCAGCACCGCATCCACCA (SEQ ID NO:350) |
| T353_peg3_orf_rev                                | GCCGAATCGCTGGTTGCCCT (SEQ ID NO:351) |

#### **Generation of 8, 9, and 10-Fold Deletion Strains**

**[0285]** Generation of 8, 9, and 10-Fold deletion strains are described in the International Patent Application PCT/EP2013/050126.

**[0286]** Briefly, to generate an 8-fold protease deletion strain, removal of the *pyr4* marker was applied to the 7-fold deletion strain M486 essentially as described above. Four consecutive 5-FOA selection steps were carried out to ensure that the clones selected were originating from single cells. Final clones were verified by PCR using the primers listed in Table 24b, removal of the blaster cassette was further verified by plating the clones onto minimal medium plates with or without 5 mM uridine, and with Southern analyses. A *pyr4*<sup>-</sup> clone was designated as M496.

**[0287]** To remove vector sequence, plasmid pTTv229 (Example 5) was digested with PmeI + XbaI and the correct fragment purified from an agarose gel using a QIAquick Gel Extraction Kit (Qiagen). Approximately 5 µg of the deletion cassette was used to transform protoplasts of M496. Transformants were picked as first streaks, growing streaks were screened by PCR (using the primers listed in Table 24b) for correct integration and clones giving the expected signals were purified to single cell clones and rescreened by PCR using the primers listed in Table 24b. Deletion of *pep5* was verified by Southern analyses. An 8-fold deletion strain clone was designated as M504.

**Table 24b: Primers for screening removal of *pyr4* blaster cassette from 7-fold strain and for screening integration of *pep5* deletion plasmid pTTv229 integration and strain purity.**

|                                                                                          |                                        |
|------------------------------------------------------------------------------------------|----------------------------------------|
| <b>For screening removal of <i>pyr4</i> blaster cassette from M486 and strain purity</b> |                                        |
| Primer                                                                                   | Sequence                               |
| T047_trpC_term_end_F                                                                     | CCTATGAGTCGTTTACCCAGA (SEQ ID NO: 413) |
| T854_peg3_3f_r2                                                                          | TGGCCGAGTCTATGCGTA (SEQ ID NO: 414)    |
| T488_pyr4_5utr_rev                                                                       | GGAGTTGCTTTAATGTCGGG (SEQ ID NO: 415)  |
| T061_pyr4_orf_screen_2F                                                                  | TTAGGCGACCTCTTTTTCCA (SEQ ID NO: 416)  |
| T855_peg3_orf_f3                                                                         | GTAAGACGCCCGTCTC (SEQ ID NO: 417)      |
| T754_peg3_orf_rev2                                                                       | TGGATCATGTTGGCGACG (SEQ ID NO: 418)    |
|                                                                                          |                                        |
| <b>For screening integration of pTTv229</b>                                              |                                        |
| Primer                                                                                   | Sequence                               |
| T627_peg5_5int_new                                                                       | GTCGAAGATGTCCTCGAGAT (SEQ ID NO: 419)  |
| T488_pyr4_5utr_rev                                                                       | GGAGTTGCTTTAATGTCGGG (SEQ ID NO: 420)  |
| T061_pyr4_orf_screen_2F                                                                  | TTAGGCGACCTCTTTTTCCA (SEQ ID NO: 421)  |
| T628_peg5_3int_new                                                                       | TAGTCCATGCCGAAGTGC (SEQ ID NO: 422)    |
|                                                                                          |                                        |

| For screening deletion of pep5 ORF |                                       |
|------------------------------------|---------------------------------------|
| Primer                             | Sequence                              |
| T418_pep5_orf_for                  | CCGGACCTGCACCGCAAGTT (SEQ ID NO: 423) |
| T419_pep5_orf_rev                  | AGGGCAATGTCGCCCAGCAC (SEQ ID NO: 424) |
| T859_pep5_orf_f2                   | GACCTGCACCGCAAGTT (SEQ ID NO: 425)    |
| T860_pep5_orf_f3                   | GTCGAGCGTCTGATATTCAC (SEQ ID NO: 426) |
| T861_pep5_orf_r2                   | GACGGAGACCTCCCACA (SEQ ID NO: 427)    |

| Generation | of    | 9-fold | Protease | Deletion | Strain | Having | Deletions |
|------------|-------|--------|----------|----------|--------|--------|-----------|
| Apep1      | Δtsp1 | Δslp1  | Δgap1    | Δgap2    | Δapep4 | Δapep3 | Δapep5    |
| Apep12     |       |        |          |          |        |        |           |

### Generation of pep12 deletion plasmids

**[0288]** The first deletion plasmid, pTTv209, for the aspartic protease *pep12*(tre119876) was constructed essentially as described for pTTv41 above but a second selection marker cassette (*bar*) of *Streptomyces* ssp., was placed after the *pyr4* gene creating a deletion plasmid with a double selection marker blaster cassette. The second deletion plasmid for the aspartic protease *pep12* (pTTv245) was constructed using the plasmid pTTv209 above as the backbone. The *pyr4-bar* double marker was removed from pTTv209 with *NotI* digestion and the new *pyr4* marker gene was obtained from pTTv181 with *NotI* digestion. 1019 bp of 5' flanking region and 895 bp of 3' flanking region were selected as the basis of the *pep12* deletion plasmids. A 300 bp stretch from the end of *pep12* 5' flank was used as the direct repeat fragment. These fragments were amplified by PCR using the primers listed in Table 24c. The double marker (*pyr4-bar*) was digested from pTTv202 ( $\Delta$ *pep5-pyr4-bar*) with *NotI*. To enable removal of the complete double marker cassette, *NotI* restriction sites were introduced on both sides of the double marker cassette. *AscI* site was introduced between the *pep12* 5'direct repeat and 3' flank. Vector backbone was *EcoRI/XhoI* digested pRS426. The plasmid pTTv209 was constructed using the yeast homologous recombination method as described. These deletion plasmids for *pep12* (pTTv209 and pTTv245, Table 24c) result in a 2198 bp deletion in the *pep12* locus and cover the complete coding sequence of PEP12.

**Table 24c: Primers for generating *pep12* deletion plasmids.**

| Deletion plasmid pTTv209 ( $\Delta$ pep12-pyr4-bar), vector backbone pRS426 |                                                                                            |
|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| Primer                                                                      | Sequence                                                                                   |
| T477_pep12_5f_for                                                           | GGTAACGCCAGGGTTTTCCAGTCACGACGGTTTAAAC<br>CGACAGCACGTTGTGTGCTCC (SEQ ID NO: 428)            |
| T478_pep12_5f_rev                                                           | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTA<br>GGCGGCCCGCTGGAGACCCAGCAGCCAGCA (SEQ ID NO: 429) |
| T479_pep12_DR_for                                                           | CCCGTCACCGAGATCTGATCCGTCACCGGGATCCACTTAA<br>GCGGCCGCTCAGAGGGAGGCTGCCCAAC (SEQ ID NO: 430)  |
| T480_pep12_DR R_rev                                                         |                                                                                            |



| Deletion plasmid pTTv209 ( $\Delta pep12$ -pyr4-bar), vector backbone pRS426                  |                                                                                              |
|-----------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| Primer                                                                                        | Sequence                                                                                     |
|                                                                                               | GAGACTCGAACAAGACATCTTTGGGACCTCGTCCAC<br><br>GGCGGCGCGCCTGGAGACCCAGCAGCCAGCA (SEQ ID NO: 431) |
| T481_pep12_3f_for                                                                             | GCCGTGGACGAGGTCGCAAA (SEQ ID NO: 432)                                                        |
| T482_pep12_3f_rev                                                                             | AGCGGATAACAATTTACACAGGAAACAGCGTTTAAAC<br><br>CCCTGCGCCCTCTTCTGCAC (SEQ ID NO: 433)           |
| Deletion plasmid pTTv245 ( $\Delta pep12$ -pyr4)                                              |                                                                                              |
| Primer                                                                                        | Sequence                                                                                     |
| no new primers, pTTv209 digested with <i>NotI</i> and ligated with pyr4 fragment from pTTv181 |                                                                                              |

#### Generation of 9-fold protease deletion strain with *pep12* (*tre119876*); M575

[0289] To generate a 9-fold protease deletion strain, removal of the *pyr4* marker was applied to the 8-fold deletion strain M504 essentially as described above using primers listed in Table 24d and resulting in a *pyr4*-clone designated as M521. To remove vector sequence, plasmid pTTv245 was digested with *MssI* and approximately 5  $\mu$ g of the deletion cassette was used to transform M521.

[0290] Transformants were picked as first streaks and growing streaks were screened by PCR using the primers listed in Table 24d for correct integration. Clones giving the expected signals were purified to single cell clones and rescreened by PCR using the primers listed in Table 24d. Deletion of *pep12* was verified by Southern analyses from selected clones. Clone 42-45B was designated with strain number M575.

**Table 24d: Primers for screening removal of *pyr4* blaster cassette from 8-fold protease deletion strain and for screening pTTv245/ $\Delta pep12$ -pyr4 integration and strain purity.**

| For screening removal of <i>pyr4</i> blaster cassette from M504 and strain purity |                                        |
|-----------------------------------------------------------------------------------|----------------------------------------|
| Primer                                                                            | Sequence                               |
| T858_pep5_5f_f3                                                                   | GGAATCGTCACCAAGGAG (SEQ ID NO: 434)    |
| T755_pep5_3f_rev3                                                                 | CTTCTGGTGACATTCCGAC (SEQ ID NO: 435)   |
| T627_pep5_5int_new                                                                | GTCTGAAGATGTCCTCGAGAT (SEQ ID NO: 436) |
| T488_pyr4_5utr_rev                                                                | GGAGTTGCTTTAATGTCGGG (SEQ ID NO: 437)  |
| T860_pep5_orf_f3                                                                  | GTCGAGCGTCTGATATTCAC (SEQ ID NO: 438)  |
| T861_pep5_orf_r2                                                                  | GACGGAGACCTCCCACA (SEQ ID NO: 439)     |
| For screening integration of pTTv245 ( $\Delta pep12$ -pyr4)                      |                                        |
| Primer                                                                            | Sequence                               |
| T517_pep12_5int                                                                   | AGCAGTCCACCTGCTCAAAA (SEQ ID NO: 440)  |
| T026_Pyr4_orf_5rev2                                                               | CCATGAGCTTGAACAGGTAA (SEQ ID NO: 441)  |
| T061_pyr4_orf_screen_2F                                                           | TTAGGCGACCTCTTTTCCA (SEQ ID NO: 442)   |
| T518_pep12_3int                                                                   | GATTCACACCAATGAGTCGG (SEQ ID NO: 443)  |

| For screening deletion of <i>pep12</i> (tre119876) ORF |                                       |
|--------------------------------------------------------|---------------------------------------|
| T486_peg12_orf_probef                                  | CCCCGACTTTGCCCCGTCAC (SEQ ID NO: 444) |
| T487_peg12_orf_prober                                  | TCGTCAGAGTCGTCGCCCGT (SEQ ID NO: 445) |
| T1057_peg12_orf_probef2                                | GCGCAGCTAATGTCCTCTGT (SEQ ID NO: 446) |
| T1058_peg12_orf_prober2                                | TTGTTGAGCCAGAGTCGAGA (SEQ ID NO: 447) |

**Generation of 9-fold Protease Deletion Strain Having Deletions  
Apep1Δtsp1Δslp1Δgap1Δgap2Δpep4Δpep3Δpep5Δpep2**

**Generation of *pep2* deletion plasmids**

[0291] The first deletion plasmid, pTTv213, for the aspartic protease *pep2* (tre0053961) was constructed essentially as for pTTv41 above but an additional second selection marker cassette carrying hygromycin phosphotransferase gene (*hph*), was placed after the *pyr4* gene creating a deletion plasmid with a double selection marker blaster cassette. In addition to the double marker, the first deletion plasmid contained also an overexpression cassette for native KEX2 (tre123561; promoter *cDNA1*, terminator *cbh2*). The second deletion plasmid for the aspartic protease *pep2* (pTTv232) was constructed using the plasmid pTTv213 above as the backbone. The *kex2* overexpression cassette (*pcDNA1-kex2-tcbh2*) was removed from pTTv213 with *Ascl* digestion. The third deletion plasmid for the aspartic protease *pep2* (pTTv246) was constructed using the plasmid pTTv232 above as the backbone. The *pyr4-hph* double marker was removed from pTTv232 with *NotI* digestion. The *pyr4* marker gene was obtained from pTTv181 (*Δpep4-pyr4* above) with *NotI* digestion.

[0292] 1000 bp of 5' flanking region and 1020 bp of 3' flanking region were selected as the basis of the *pep2* deletion plasmids. A 300 bp stretch from the end of *pep2* 5' flank was used as the direct repeat fragment. These fragments as well as the second selection marker cassette (*hph*), *cDNA1* promoter, native *kex2* gene and *cbh2* terminator were amplified by PCR using the primers listed in Table 24e and cloned. The *pyr4* selection marker was obtained from pTTv181 (*Δpep4-pyr4* above) with *NotI* digestion. To enable removal of the complete double marker cassette in pTTv213, *NotI* restriction sites were introduced on both sides of the double marker cassette, and a *Swal* site between the two selection markers. *Ascl* sites were introduced on both sides of the *kex2* overexpression cassette (between *pep2* 5'direct repeat and 3' flank). Vector backbone was *EcoRI/XhoI* digested pRS426 and the plasmid pTTv213 was constructed using the yeast homologous recombination method described. These deletion plasmids for *pep2* (pTTv213, pTTv232 and pTTv246, Table 24e) result in a 1580 bp deletion in the *pep2* locus and cover the complete coding sequence of PEP2.

**Table 24e: Primers for generating *pep2* deletion plasmids.**

| Deletion plasmid pTTv213, vector backbone pRS426 |                                                                                          |
|--------------------------------------------------|------------------------------------------------------------------------------------------|
| Primer                                           | Sequence                                                                                 |
| T431_peg2-5flankF-pRS426                         | GATTAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGACGG<br>TTTAAACCGGTTGTCCATTTTCCTTC (SEQ ID NO: 448)  |
| T629_peg2_5f_rev_pyr4                            | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTAG<br>GCGGCCGCGGGGAAGCAAGTTTCAAGT (SEQ ID NO: 449) |
| T630_peg2_5DR_for_trpC                           | GTACACTTGTTTAGAGGTAATCCTTCTTTCTAGAAGGAGAGC<br>GGCCGCCTCCAGCTCTTGGCCAC (SEQ ID NO: 450)   |

| Deletion plasmid pTTv213, vector backbone pRS426                                                                   |                                                                                          |
|--------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| Primer                                                                                                             | Sequence                                                                                 |
| T631_pep2_5DR_rev_cDNA1                                                                                            | GTCATTAAGTCCATCATTCCACGTCCTTCAGACCGAATTCGG<br>CGCGCCGGGAAGCAAGTTTCGAAGT (SEQ ID NO: 451) |
| T632_pep2_3f_for_tcbh2                                                                                             | ATGATGCCTTTGCAGAAATGGCTTGCTCGCTGACTGATACG<br>GCGCGCTATCGCGAAAGTAGCCAATA (SEQ ID NO: 452) |
| T633_pep2_3f_rev                                                                                                   | AGCGGATAACAATTTACACAGGAAACAGCGTTTAAACCATC<br>CTTTCTCACCACGA (SEQ ID NO: 453)             |
| T491_hph_recpyr4_for3                                                                                              | TGATTGTACCCAGCTGCGATTGATGTGTATCTTGCATGAT<br>TTAAATTCTCCTTAGCTCTGTACAGT (SEQ ID NO: 454)  |
| T492_hph_rev2                                                                                                      | GCGGCCGCTCTCCTTCTAGAAAGAAGGA (SEQ ID NO: 455)                                            |
| T495_cDNA1_for                                                                                                     | GAATTCGGTCTGAAGGACGT (SEQ ID NO: 456)                                                    |
| T138_cDNA1_Rev                                                                                                     | GTTGAGAGAAGTTGTTGGATTG (SEQ ID NO: 457)                                                  |
| T139_123561 For_cDNA1                                                                                              | AACCAAAGACTTTTTGATCAATCCAACAACTTCTCTCAACAT<br>GAAGATTTCTCGATCCTTG (SEQ ID NO: 458)       |
| T516_123561Rev                                                                                                     | TCAGCGCCGTAACCTCTGC (SEQ ID NO: 459)                                                     |
| T496_tcbh2_for                                                                                                     | TGATGGTGATGAGGCGGAAAAGCAGAGGTTACGGCGCTGA<br>GGCTTTCGTGACCGGGCTTC (SEQ ID NO: 460)        |
| T497_tcbh2_rev                                                                                                     | GTATCAGTCAGCGAGCAAGC (SEQ ID NO: 461)                                                    |
| Deletion plasmid pTTv232                                                                                           |                                                                                          |
| Primer                                                                                                             | Sequence                                                                                 |
| no new primers, pTTv213 digested with <i>Ascl</i> (to remove <i>kex2</i> overexpression cassette) and self-ligated |                                                                                          |
|                                                                                                                    |                                                                                          |
| Deletion plasmid pTTv246                                                                                           |                                                                                          |
| Primer                                                                                                             | Sequence                                                                                 |
| no new primers, pTTv232 digested with <i>NotI</i> and ligated with <i>pyr4/NotI</i> -fragment from pTTv181         |                                                                                          |

**Generation of 9-fold protease deletion strain with *pep2* (*tre53961*); M574**

**[0293]** To generate a 9-fold protease deletion strain, removal of the *pyr4* marker was applied to the 8-fold deletion strain M504 essentially as described above using consecutive 5-FOA selection steps. Clones were verified by PCR using the primers listed in Table 24f and plating the clones onto minimal medium plates with or without 5 mM uridine. The strain used in generation of 9-fold protease deletion strain was designated with strain number M521.

[0294] To remove vector sequence, plasmid pTTv246 ( $\Delta pep2$ - $pyr4$ ) was digested with *MssI*, purified and approximately 5  $\mu$ g of the deletion cassette was used to transform strain M521. Growing streaks were screened by PCR (using the primers listed in Table 24f) for correct integration. Clones giving the expected signals were purified to single cell clones and rescreened by PCR using the primers listed in Table 24f, and deletion of *pep2* was verified by Southern analyses. The clone 41-45G was designated with strain number M574.

**Table 24f: Primers for screening removal of *pyr4* blaster cassette from 8-fold protease deletion strain and for screening pTTv246/ $\Delta pep2$ - $pyr4$  integration and strain purity.**

| For screening removal of <i>pyr4</i> blaster cassette from M504 and strain purity |                                        |
|-----------------------------------------------------------------------------------|----------------------------------------|
| Primer                                                                            | Sequence                               |
| T858_pep5_5f_f3                                                                   | GGAATCGTCACCAAGGAG (SEQ ID NO: 462)    |
| T755_pep5_3f_rev3                                                                 | CTTCTGGTGACATTCCGAC (SEQ ID NO: 463)   |
| T627_pep5_5int_new                                                                | GTCGAAGATGTCCTCGAGAT (SEQ ID NO: 464)  |
| T488_pyr4_5utr_rev                                                                | GGAGTTGCTTTAATGTCGGG (SEQ ID NO: 465)  |
| T860_pep5_orf_f3                                                                  | GTCGAGCGTCTGATATTCAC (SEQ ID NO: 466)  |
| T861_pep5_orf_r2                                                                  | GACGGAGACCTCCACACA (SEQ ID NO: 467)    |
| For screening integration of pTTv246 ( $\Delta pep2$ - $pyr4$ )                   |                                        |
| Primer                                                                            | Sequence                               |
| T596_pep2 fwd 5'flank screen                                                      | CCTCTGCGTTGAGCAACATA (SEQ ID NO: 468)  |
| T026_Pyr4_orf_5rev2                                                               | CCATGAGCTTGAACAGGTAA (SEQ ID NO: 469)  |
| T061_pyr4_orf_screen_2F                                                           | TTAGGCGACCTCTTTTCCA (SEQ ID NO: 470)   |
| T600_pep2 rev 3'flank screen                                                      | CGAAAGCGTGGAGTCTTCTC (SEQ ID NO: 471)  |
| For screening deletion of <i>pep2</i> (tre53961) ORF                              |                                        |
| T601_pep2 fwd                                                                     | GACGTGGTACGACAACATCG (SEQ ID NO: 472)  |
| T623_pep2 rev                                                                     | TATCAAGGTACCGGGGACAG (SEQ ID NO: 473)  |
| T1077_pep2_orf_probef2                                                            | AACAAAGCCTTCACAGGCC (SEQ ID NO: 474)   |
| T1078_pep2_orf_prober2                                                            | TGAGGCTCCTTCCAACCTTTT (SEQ ID NO: 475) |

**Generation of 10-fold Protease Deletion Strain Having Deletions  $\Delta pep1 \Delta tsp1 \Delta slp1 \Delta gap1 \Delta gap2 \Delta pep4 \Delta pep3 \Delta pep5 \Delta pep2 \Delta pep11$**

#### **Generation of *pep11* deletion plasmid**

[0295] The deletion plasmid pTTv312 for the aspartic protease *pep11* (tre121306) was constructed essentially as described above. 956 bp of 5' flanking region and 943 bp of 3' flanking region were selected as the basis of the *pep11* deletion plasmid. A 307 bp stretch from the end of *pep11* 5' flank was used as the direct repeat fragment. These fragments were amplified by PCR using the primers listed in Table 24g and the products were isolated from the gel. The *pyr4* cassette was obtained from pTTv181 ( $\Delta pep4$ - $pyr4$  above) with *NotI* digestion. To enable removal of the marker cassette, *NotI* restriction sites were introduced on both sides of the cassette. *Ascl* site was introduced between the *pep11* 5'direct repeat and 3' flank. Vector backbone was *EcoRI/XhoI*

digested pRS426 and the plasmid was constructed using the yeast homologous recombination method as described. This deletion plasmid for *pep11* (pTTv312, Table 24g) results in 2624 bp deletion in the *pep11* locus and covers the complete coding sequence of PEP11.

**Table 24g: Primers for generating *pep11* deletion plasmids.**

| Deletion plasmid pTTv312 ( $\Delta pep11$ - <i>pyr4</i> ), vector backbone pRS426 |                                                                                           |
|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| Primer                                                                            | Sequence                                                                                  |
| T1009_peg11_5flkw_vector                                                          | GTAACGCCAGGGTTTTCCAGTCACGACGGTTTAAAC<br>ATGAGCGTGATCGACAAGTG (SEQ ID NO: 476)             |
| T1010_peg11_5flkrev_pyr4Pr<br>om                                                  | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTAG<br>GCGGCCGCCCTCTGAGGTCGAGATGGAG (SEQ ID NO: 477) |
| T1144_peg11_5dr_for                                                               | TGATTGTACCCAGCTGCGATTGATGTGTATCTTTGCAT<br>GGCGGCCGCACGACTAATATCCACTGCCG (SEQ ID NO: 478)  |
| T1145_peg11_5dr_rev                                                               | AACCAAAGTGTAATGCTCATCTCGTATTCACATGCAAA<br>GGCGGCCGCCCTCTGAGGTCGAGATGGAG (SEQ ID NO: 479)  |
| T1146_peg11_3f_for                                                                | TTTGCATGTGAATACGAGATGA (SEQ ID NO: 480)                                                   |
| T1012_peg11_3flkrev_vector                                                        | GCGGATAACAATTTACACAGGAAACAGCGTTTAAAC<br>TGCTCGATCCTACTCCAAGG (SEQ ID NO: 481)             |

**Generation of 10-fold protease deletion strain with *pep11* (*tre121306*); M658**

**[0296]** To generate a 10-fold protease deletion strain, removal of the *pyr4* marker was applied to the 9-fold deletion strain M574 essentially as described above using consecutive 5-FOA selection steps. Final clones were verified by PCR using the primers listed in Table 24h and by plating the clones onto minimal medium plates with or without 5 mM uridine. Resulting strain used in generation of 10-fold protease deletion strain was designated with strain number M597.

**[0297]** To remove vector sequence, plasmid pTTv312 ( $\Delta pep11$ -*pyr4*) was digested with *MssI* and approximately 5  $\mu$ g of the deletion cassette was used to transform M597. Transformants were picked as first streaks and growing streaks were screened by PCR (using the primers listed in Table 24h) for correct integration. Clones were purified to single cell clones and rescreened by PCR using the primers listed in Table 24h and deletion of *pep11* was verified by Southern analyses. Clone 47-62B was designated with strain number M632. An additional single cell purification step was applied to strain M632 to obtain 10-fold protease deletion strain M658.

**Table 24h: Primers for screening removal of *pyr4* blaster cassette from 9-fold protease deletion strain and for screening pTTv312/ $\Delta pep11$ -*pyr4* integration and strain purity.**

| For screening removal of <i>pyr4</i> blaster cassette from M574 and strain purity |                                       |
|-----------------------------------------------------------------------------------|---------------------------------------|
| Primer                                                                            | Sequence                              |
| T1162_pep2_5f_f2                                                                  | CTGTAAAGGCAGCATCGG (SEQ ID NO: 482)   |
| T1163_pep2_3f_r2                                                                  | TCAGAACGGCTTCAATCATT (SEQ ID NO: 483) |
| T1162_pep2_5f_f2                                                                  | CTGTAAAGGCAGCATCGG (SEQ ID NO: 484)   |
| T488_pyr4_5utr_rev                                                                | GGAGTTGCTTTAATGTCGGG (SEQ ID NO: 485) |
| T601_pep2 fwd                                                                     | GACGTGGTACGACAACATCG (SEQ ID NO: 486) |
| T623_pep2 rev                                                                     | TATCAAGGTACCGGGGACAG (SEQ ID NO: 487) |
| For screening integration of pTTv312 ( $\Delta$ <i>pep11-pyr4</i> )               |                                       |
| Primer                                                                            | Sequence                              |
| T1013_pep11_screen_5flk_fw d                                                      | TTACGACTCGATCCCTGTCC (SEQ ID NO: 488) |
| T488_pyr4_5utr_rev                                                                | GGAGTTGCTTTAATGTCGGG (SEQ ID NO: 489) |
| T061_pyr4_orf_screen_2F                                                           | TTAGGCGACCTCTTTTTCCA (SEQ ID NO: 490) |
| T1016_pep11_screen_3flk_re v                                                      | GCCGCTAGGATCGTGATAAG (SEQ ID NO: 491) |
| For screening deletion of <i>pep11</i> ORF                                        |                                       |
| T1017_pep11_orf_fwd                                                               | GTGTCCCAGGACGACAACTT (SEQ ID NO: 492) |
| T1018_pep11_orf_rev                                                               | TGAAGGTTGCAGTGATCTCG (SEQ ID NO: 493) |

**EXAMPLE 5*****Generations of deletion plasmids for pep5, pep7, pep8, tpp1, slp2, slp3, slp5, slp6, slp7 and slp8***

[0298] The deletion plasmid for the aspartic protease *pep5* (TrID81004) was constructed essentially as described for the  $\Delta$ *pep1* plasmid pTTv41 but an additional second selection marker cassette, bar, was placed after the *pyr4* gene creating a deletion plasmid with a double selection marker blaster cassette.

[0299] 1348 bp of 5' flanking region and 1164 bp of 3' flanking region were selected as the basis of the *pep5* deletion plasmid. A 300 bp stretch from the end of *pep5* 5' flank was used as the direct repeat fragment. These fragments as well as the second selection marker cassette, bar, were amplified by PCR using the primers listed in Table 25. The products were separated with agarose gel electrophoresis and the correct fragments were isolated from the gel with a gel extraction kit (Qiagen). To enable removal of the complete double marker cassette, *NotI* restriction sites were introduced on both sides of the double marker cassette, and an *AsiSI* site between the two selection markers. An *Ascl* site was introduced between the *pep5* 5'direct repeat and 3' flank. Vector backbone was *EcoRI/XhoI* digested pRS426. The *pyr4* selection marker was obtained from pTTv181 ( $\Delta$ *pep4-pyr* above) with *NotI* digestion. The plasmid was constructed using the yeast homologous recombination method as described. This deletion plasmid for *pep5* (pTTv202, Table 25) results in a 1687 bp deletion in the *pep5* locus and covers the complete coding sequence of PEP5. pTTv229 was cloned by removing *pyr4*-bar double selection marker with *NotI* digestion and ligating *pyr4* marker (*NotI* fragment from pTTv181) into it.

**Table 25: Primers for generating *pep5* deletion plasmid.**

| Deletion plasmid pTTv202 for <i>pep5</i> (TreID81004), vector backbone pRS426          |                                                                                             |
|----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| Primer                                                                                 | Sequence                                                                                    |
| T372_pep5_5f_for                                                                       | GGTAACGCCAGGGTTTTCCAGTCACGACGGTTTAAAC<br>CGGAGGCTGCGACACCGTCTG (SEQ ID NO:352)              |
| T373_pep5_5f_rev                                                                       | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATG<br>CTAGGCGGCCGCCCGGCCTGAAACGACCTCCC (SEQ<br>ID NO:353) |
| T376_pep5_5DR_for                                                                      | CCCGTCACCGAGATCTGATCCGTCACCGGGATCCAC<br>TTAAGCGGCCGCGAGAGAGAAACAAACAGTG (SEQ<br>ID NO:354)  |
| T377_pep5_5DR_rev                                                                      | ACATTCCGACCGTTTACTGATCCAAGCCGTGCAACCG<br>ACTGGCGCGCCCGGCCTGAAACGACCTCCC (SEQ<br>ID NO:355)  |
| T378_pep5_3f_for                                                                       | AGTCGGTTGCACGGCTTGGA (SEQ ID NO:356)                                                        |
| T379_pep5_3f_rev                                                                       | AGCGGATAACAATTTACACAGGAAACAGCGTTTAAAC<br>CGAGACGGACGCCTGCACCAC (SEQ ID NO:357)              |
| T374_bar_recpyr4_for2                                                                  | TGATTGTACCCAGCTGCGATTGATGTGTATCTTTGC<br>ATGGCGATCGCGACAGAAGATGATATTGAAG (SEQ ID<br>NO:358)  |
| T375_bar_rev                                                                           | TTAAGTGGATCCCGGTGACG (SEQ ID NO:359)                                                        |
| Deletion plasmid pTTv229 for <i>pep5</i> (TreID81004), vector backbone pTTv202         |                                                                                             |
| Primer                                                                                 | Sequence                                                                                    |
| no new primers, pTTv202 digested with NotI and ligated with pyr4 fragment from pTTv181 |                                                                                             |

[0300] The deletion plasmid for the aspartic protease *pep7* (TreID58669) is constructed essentially as described for *pep1* deletion plasmid pTTv41. 1062 bp of 5' flanking regions and 1121 bp of 3' flanking region are selected as the basis of the *pep7* deletion plasmid. Fragments are produced by PCR using the primers listed in Table 26. This deletion plasmid for *pep7* results in deletion in the *pep7* locus and covers the complete coding sequence of PEP7

**Table 26: Primers for generating *pep7* deletion plasmids.**

| Deletion plasmid for <i>pep7</i> (TreID58669), vector backbone pRS426 |                                                                                           |
|-----------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| Primer                                                                | Sequence                                                                                  |
| 5flankfw_pRS426                                                       | GTAACGCCAGGGTTTTCCAGTCACGACGGTTTAAACCATAA<br>ACTTGCGCAGTCGAA (SEQ ID NO:360)              |
| 5flankrev_pyr4                                                        | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTAG<br>GCGGCCGCCCTTCTAGGATGGAGCGCTTG (SEQ ID NO:361) |
| 3flankfw_pyr4                                                         | GACGACCGCCGACCGTCACCGTCTCTGACCGTCATGACCGC                                                 |

| Deletion plasmid for <i>pep7</i> (TreID58669), vector backbone pRS426 |                                                                                             |
|-----------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| Primer                                                                | Sequence                                                                                    |
|                                                                       | CAACCCAGCCGCGAGCCTCAGCCTCTCTCAGCCTCATCAGCCG<br>CGGCCGCGAGACGGCTTCTTCCAAAACA (SEQ ID NO:362) |
| 3flankrev_pRS426                                                      | GCGGATAACAATTTACACAGGAAACAGCGTTTAAACCCCA<br>GGGAGGCTATTCTAC (SEQ ID NO:363)                 |
|                                                                       |                                                                                             |
| For screening integration of <i>pep7</i> deletion cassette            |                                                                                             |
| Primer                                                                | Sequence                                                                                    |
| scrn_5forw                                                            | CTTTCCAAGCGTTTGAGTCC (SEQ ID NO:364)                                                        |
| T026_Pyr4_orf_5rev2                                                   | CCATGAGCTTGAACAGGTAA (SEQ ID NO:365)                                                        |
| scrn_3rev                                                             | GCGTGTTTTATCCTGGTGCT (SEQ ID NO:366)                                                        |
| T028_Pyr4_flank_rev                                                   | CATCCTCAAGGCCTCAGAC (SEQ ID NO:367)                                                         |
|                                                                       |                                                                                             |
| For screening deletion of <i>pep7</i> ORF                             |                                                                                             |
| orf_fw                                                                | CACCTCCGTCGATGAGTTTT (SEQ ID NO:368)                                                        |
| orf_rev                                                               | AGAAGAAGGTGGTGGTGGTG (SEQ ID NO:369)                                                        |

[0301] A deletion plasmid pTTv319 for aspartic protease *pep8* (tre122076) was constructed essentially as described above. The second deletion plasmid for *pep8* (pTTv328) was constructed using the plasmid pTTv319 above as the backbone. The *pyr4* marker was removed from pTTv319 with *NotI* digestion. The *pyr4-hph* cassette was obtained from pTTv210 ( $\Delta$ *sep1-pyr4-hph*) with *NotI* digestion. Cloning of the plasmid pTTv328 was done with standard ligation using T4 DNA ligase at room temperature and part of the ligation mixture was transformed into *E. coli* with electroporation. Correct ligation and orientation of the marker was further verified by sequencing.

[0302] 1095 bp of 5' flanking region and 988 bp of 3' flanking region were selected as the basis of the *pep8* deletion plasmids. A 324 bp stretch from the end of *pep8* 5' flank was used as the direct repeat fragment. These fragments were amplified by PCR using the primers listed in Table 26-1. The *pyr4* selection marker used in pTTv319 was obtained from pTTv181. To enable removal of the *pyr4* marker cassette, *NotI* restriction sites were introduced on both sides of the cassette and *Ascl* site was introduced between the *pep8* 5'direct repeat and 3' flank. Vector backbone was *EcoRI/XhoI* digested pRS426. These deletion plasmids for *pep8* (pTTv319 and pTTv328, Table 26-1) result in a 1543 bp deletion in the *pep8* locus and cover the complete coding sequence of PEP8.

**Table 26-1: Primers for generating *pep8* deletion plasmid.**

| Deletion plasmid pTTv319 ( $\Delta$ <i>pep8-pyr4</i> ), vector backbone pRS426 |                                                                                             |
|--------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| Primer                                                                         | Sequence                                                                                    |
| T1019_pep8_5flkw_vector                                                        | GTAACGCCAGGGTTTTCCAGTCACGACGGTTTAAAC<br>AGGTTTGGGTTGTGAGATCG (SEQ ID NO:494)                |
| T1020_pep8_5flkrev_pyr4Pro<br>m                                                | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTA<br>GGCGGCCGCGCGCAAAGCTACTGGGCTAT (SEQ ID<br>NO:495) |



| Deletion plasmid pTTv319 ( $\Delta pep8$ - <i>pyr4</i> ), vector backbone pRS426                           |                                                                                                  |
|------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Primer                                                                                                     | Sequence                                                                                         |
| T1167_peg8_5DR_for                                                                                         | -----<br>TGATTGTACCCAGCTGCGATTGATGTGTATCTTTGCAT<br>GGCGGCCGCTCTGCTCTGCTCTGTTCTGC (SEQ ID NO:496) |
| T1168_peg8_5DR_rev                                                                                         | AAAGTTCGTCAAAGAGCACTCATAGGGCTGAGAAAA<br>GCCAGGCGCGCCGCGCAAAGCTACTGGGCTAT (SEQ ID NO:497)         |
| T1169_peg8_3f_for2                                                                                         | TGGCTTTTCTCAGCCCTATG (SEQ ID NO:498)                                                             |
| T1022_peg8_3flkrev_vector                                                                                  | GCGGATAACAATTTACACAGGAAACAGCGTTTAAAC<br>CAATGTGTGCCTGTTTTTCG (SEQ ID NO:499)                     |
| Deletion plasmid pTTv328 ( $\Delta pep8$ - <i>pyr4</i> - <i>hph</i> )                                      |                                                                                                  |
| Primer                                                                                                     | Sequence                                                                                         |
| no new primers, pTTv319 digested with NotI and ligated with <i>pyr4</i> - <i>hph</i> fragment from pTTv210 |                                                                                                  |

[0303] The third deletion plasmid pTTv266 for *pep8* was constructed essentially as described above. 1095 bp of 5' flanking region and 988 bp of 3' flanking region were selected as the basis. These fragments were amplified by PCR using the primers listed in Table 26-2. The *pyr4*-*hph* selection marker was obtained from pTTv194 ( $\Delta pep4$ -*pyr*-*hph*) with *NotI* digestion. To enable removal of the *pyr4*-*hph* marker cassette, *NotI* restriction sites were introduced on both sides of the cassette. Vector backbone was *EcoRI/XhoI* digested pRS426. The plasmid pTTv266 was constructed with the 5' flank, 3' flank, *pyr4*-*hph* marker, and vector backbone using the yeast homologous recombination method. The deletion plasmids for *pep8* (pTTv266, Table 26-2) result in a 1543 bp deletion in the *pep8* locus and cover the complete coding sequence of PEP8.

**Table 26-2: Primers for generating *pep8* deletion plasmid.**

| Deletion plasmid pTTv266 ( $\Delta pep8$ - <i>pyr4</i> - <i>hph</i> ), vector backbone pRS426 |                                                                                          |
|-----------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| Primer                                                                                        | Sequence                                                                                 |
| T1019_peg8_5flkw_vector                                                                       | GTAACGCCAGGGTTTTCCAGTCACGACGGTTTAAAC<br>AGGTTTGGGTTGTGAGATCG (SEQ ID NO:500)             |
| T1020_peg8_5flkrev_pyr4Prom                                                                   | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATG<br>CTAGGCGGCCGCGCGCAAAGCTACTGGGCTAT (SEQ ID NO:501) |
| T1021_peg8_3flkw_pyr4loop                                                                     | CAACCAGCCGCAGCCTCAGCCTCTCTCAGCCTCATCA<br>GCCGCGGCCGCTGGCTTTTCTCAGCCCTATG (SEQ ID NO:502) |
| T1022_peg8_3flkrev_vector                                                                     | GCGGATAACAATTTACACAGGAAACAGCGTTTAAAC<br>CAATGTGTGCCTGTTTTTCG (SEQ ID NO:503)             |

[0304] The deletion plasmid pTTv331 (2152 bp deletion in the *tpp1* locus and covers the complete coding sequence of TPP1) for tripeptidyl peptidase *tpp1* (tre82623) was constructed essentially as described above

with the marker used for selection was a double marker *pyr4-hph*. 1245 bp of 5' flanking region and 1025 bp of 3' flanking region were selected as the basis. A 311 bp stretch from the end of *tpp1* 5' flank was used as the direct repeat fragment and these fragments were amplified using the primers of Table 26-3. The *pyr4-hph* cassette was obtained from pTTv210 ( $\Delta$ *sep1-pyr4-hph*) with *NotI* digestion. To enable removal of the complete double marker cassette, *NotI* restriction sites were introduced on both sides of the double marker cassette. *AscI* site was introduced between the *tpp1* 5'direct repeat and 3' flank. Vector backbone was *EcoRI/XhoI* digested pRS426.

**Table 26-3: Primers for generating *tpp1* deletion plasmid.**

| Deletion plasmid pTTv331 ( $\Delta$ <i>tpp1-pyr4-hph</i> ), vector backbone pRS426 |                                                                                          |
|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| Primer                                                                             | Sequence                                                                                 |
| T311_82623_5for                                                                    | GTAACGCCAGGGTTTTCCAGTCACGACGGTTTAAAC<br>CGCATTACGAATGCACAAAG (SEQ ID NO:504)             |
| T1190_tpp1_5f_rev2                                                                 | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTA<br>GGCGGCCGCCCATGTCAGCTCAGACCAAT (SEQ ID NO:505) |
| T1191_tpp1_5dr_for                                                                 | GTACACTTGTITAGAGGTAATCCTCTTTCTAGAAGGAGA<br>GCGGCCGCAGGCCCTGGACTGCTAGTTT (SEQ ID NO:506)  |
| T1192_tpp1_5dr_rev                                                                 | CGAGCCATCCGCGCGGCCCTATATTCCACCCGAGTCCT<br>CGGCGCGGCCCATGTCAGCTCAGACCAAT (SEQ ID NO:507)  |
| T1193_tpp1_3f_for2                                                                 | GAGGACTCGGGTGGAATATAGG (SEQ ID NO:508)                                                   |
| T314_82623_3rev                                                                    | GCGGATAACAATTTACACAGGAAACAGCGTTTAAAC<br>TTGGTCTTGAATGAAAGGTGTG (SEQ ID NO:509)           |

**[0305]** The deletion plasmids for the subtilisin-like proteases *slp2* (TrlD123244) and *slp3* (TrlD123234) were constructed essentially as described for *pep1* deletion plasmid pTTv41. 1000 bp of 5' and 1100 bp of 3' flanking regions were selected as the basis of the *slp2* deletion plasmid. For *slp3*, 1000 bp of 5' and 1100 bp of 3' flanking regions were selected. Fragments were produced by PCR using the primers listed in Table 26-4. Template used in the PCR of the flanking regions was from the *T. reesei* wild type strain QM6a. The *pyr4* blaster cassette was obtained from pTTv71 with *NotI* digestion. The vector backbone was *EcoRI/XhoI* digested pRS426 and the plasmids were constructed using the yeast homologous recombination method described above. The deletion plasmid for *slp2* (pTTv115) results in a 2114 bp deletion in the *slp2* locus and covers the complete coding sequence of SLP2. The deletion plasmid for *slp3* (pTTv116) results in a 1597 bp deletion in the *slp3* locus and covers the complete coding sequence of SLP3.

**Table 26-4: Primers used for plasmids.**

| Deletion plasmid pTTv115 for <i>slp2</i> (TreID123244), vector backbone pRS426 |                                                                                              |
|--------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| Primer                                                                         | Sequence                                                                                     |
| JJ-037 primer                                                                  | GATTAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGACGG<br>TTTAAACGCAGTCTATCCCATCCCTG (SEQ ID NO: 510)      |
| JJ-038 primer                                                                  | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTAG<br>GCGGCCGCGCGGATGATGAAGGAAGAAG (SEQ ID NO: 511)    |
| JJ-039 primer                                                                  | CAACCAGCCGCAGCCTCAGCCTCTCTCAGCCTCATCAGCC<br>GCGGCCGCAACAGCTGTTGCGACGCGTG (SEQ ID NO: 512)    |
| JJ-040 primer                                                                  | TGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCG<br>TTTAAACGGCTGGGCATTGGGGCCG (SEQ ID NO: 513)       |
|                                                                                |                                                                                              |
| Deletion plasmid pTTv116 for <i>slp3</i> (TreID123234), vector backbone pRS426 |                                                                                              |
| Primer                                                                         | Sequence                                                                                     |
| JJ-041 primer                                                                  | GATTAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGACGG<br>TTTAAACAAACAAGGCACAAAGGCCTG (SEQ ID NO: 514)     |
| JJ-042 primer                                                                  | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTAG<br>GCGGCCGCATCCAAGGATGAGGAGAAC (SEQ ID NO: 515)     |
| JJ-043 primer                                                                  | CAACCAGCCGCAGCCTCAGCCTCTCTCAGCCTCATCAGCC<br>GCGGCCGCACCTAATGGTTTCTTCGTTTTTC (SEQ ID NO: 516) |
| JJ-044 primer                                                                  | TGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCG<br>TTTAAACCGGTCCGAAGGGTGTGTTTGG (SEQ ID NO: 517)    |

[0306] The deletion plasmid for the aspartic protease *slp5* (TreID64719) is constructed essentially as described above. 1044 bp of 5' flanking regions and 1003 bp of 3' flanking region are selected as the basis of the *slp5* deletion plasmid. Fragments are produced by PCR using the primers listed in Table 27. This deletion plasmid for *slp5* results in deletion in the *slp5* locus and covers the complete coding sequence of SLP5.

**Table 27: Primers for generating *slp5* deletion plasmids.**

| Deletion plasmid for <i>slp5</i> (TreID64719), vector backbone pRS426 |                                                                                         |
|-----------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| Primer                                                                | Sequence                                                                                |
| 5flankfw_pRS426                                                       | GTAACGCCAGGGTTTTCCAGTCACGACGGTTTAAACGTTT<br>GAGCATTCTCCAAGC (SEQ ID NO:370)             |
| 5flankrev_pyr4                                                        | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTAG<br>GCGGCCGCCGCCATTTGAAGAAGATGC (SEQ ID NO:371) |

| Deletion plasmid for <i>slp5</i> (TreID64719), vector backbone pRS426 |                                                                                           |
|-----------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| Primer                                                                | Sequence                                                                                  |
| 3flankfw_pyr4                                                         | CAACCAGCCGCGAGCCTCAGCCTCTCTCAGCCTCATCAGCC<br>GCGGCCGCATGCTCCCTCGTCATTAAGC (SEQ ID NO:372) |
| 3flankrev_pRS426                                                      | GCGGATAACAATTTACACAGGAAACAGCGTTTAAACACAA<br>CACCTTCTCCGACACC (SEQ ID NO:373)              |
|                                                                       |                                                                                           |
| For screening integration of <i>slp5</i> deletion cassette            |                                                                                           |
| Primer                                                                | Sequence                                                                                  |
| scrn_5forw                                                            | ATGCCCAAGTTTCGTACCTG (SEQ ID NO:374)                                                      |
| T026_Pyr4_orf_5rev2                                                   | CCATGAGCTTGAACAGGTAA (SEQ ID NO:375)                                                      |
| scrn_3rev                                                             | GGCGCATTCAGAAGAAGAAC (SEQ ID NO:376)                                                      |
| T028_Pyr4_flank_rev                                                   | CATCCTCAAGGCCTCAGAC (SEQ ID NO:377)                                                       |
|                                                                       |                                                                                           |
| For screening deletion of <i>slp5</i> ORF                             |                                                                                           |
| orf_fw                                                                | CACTTGATGAACGCTGGCTA (SEQ ID NO:378)                                                      |
| orf_rev                                                               | CGTAATGGCGTTGTTGACAG (SEQ ID NO:379)                                                      |

[0307] A deletion plasmid for the aspartic protease *slp6* (TreID121495) is based on 1192 bp of 5' flanking regions and 1114 bp of 3' flanking regions. Fragments are produced by PCR using the primers listed in Table 28. This deletion plasmid for *slp6* results in deletion in the *slp6* locus and covers the complete coding sequence of SLP6.

Table 28: Primers for generating *slp6* deletion plasmids.

| Deletion plasmid for <i>slp6</i> (TreID121495), vector backbone pRS426 |                                                                                           |
|------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| Primer                                                                 | Sequence                                                                                  |
| 5flankfw_pRS426                                                        | GTAACGCCAGGGTTTTCCAGTCACGACGGTTTAAACGAG<br>GCAGCCAAAAAGTGAAG (SEQ ID NO:380)              |
| 5flankrev_pyr4                                                         | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTAG<br>GCGGCCGCTGAAAGAAGGCAGGACCAGT (SEQ ID NO:381)  |
| 3flankfw_pyr4                                                          | CAACCAGCCGCGAGCCTCAGCCTCTCTCAGCCTCATCAGCC<br>GCGGCCGCAAGAGGCTCGGACAAAGACA (SEQ ID NO:382) |
| 3flankrev_pRS426                                                       | GCGGATAACAATTTACACAGGAAACAGCGTTTAAACGATC<br>GTGGTGCACGAGACTA (SEQ ID NO:383)              |
|                                                                        |                                                                                           |
| For screening integration of <i>slp6</i> deletion cassette             |                                                                                           |
| Primer                                                                 | Sequence                                                                                  |
| scrn_5forw                                                             | GCACTGCGTTGCCTTTCTAT (SEQ ID NO:384)                                                      |

| For screening integration of <i>slp6</i> deletion cassette |                                       |
|------------------------------------------------------------|---------------------------------------|
| Primer                                                     | Sequence                              |
| T026_Pyr4_orf_5rev2                                        | CCATGAGCTTGAACAGGTAA (SEQ ID NO:385)  |
| scrn_3rev                                                  | GAAAGCATGGCTCGTTTCTC (SEQ ID NO:386)  |
| T028_Pyr4_flank_rev                                        | CATCCTCAAGGCCTCAGAC (SEQ ID NO:387)   |
|                                                            |                                       |
| For screening deletion of <i>slp6</i> ORF                  |                                       |
| orf_fw                                                     | ACCCGGCTCAACTAGCTACA (SEQ ID NO:388)  |
| orf_rev                                                    | AGCTGGCCTTTTCGTTACAGA (SEQ ID NO:389) |

[0308] The deletion plasmid for the aspartic protease *slp7* (TreID123865) is based on 1134 bp of 5' flanking regions and 1005 bp of 3' flanking regions. Fragments are produced by PCR using the primers listed in Table 29-1. This deletion plasmid for *slp7* results in deletion in the *slp7* locus and covers the complete coding sequence of SLP7. Alternatively, a deletion plasmid pTTv269 for *slp7* (tre123865) was constructed with the marker pyr4-hph from pTTv194. This plasmid has 949 bp of 5' flanking region and 1025 bp of 3' flanking region and the plasmid was constructed using the primers listed in Table 29-2. This deletion plasmid for *slp7* (pTTv269, Table 29-2) results in a 2019 bp deletion in the *slp7* locus and covers the complete coding sequence of SLP7.

**Table 29-1: Primers for generating *slp7* deletion plasmids.**

| Deletion plasmid for <i>slp7</i> (TreID123865), vector backbone pRS426 |                                                                                           |
|------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| Primer                                                                 | Sequence                                                                                  |
| 5flankfw_pRS426                                                        | GTAACGCCAGGGTTTTCCAGTCACGACGGTTTAACTTGG<br>TTTGAACAGCTGCAAG (SEQ ID NO:390)               |
| 5flankrev_pyr4                                                         | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTAG<br>GCGGCCGCTTTGCAGCAAGATGTCGTTTC (SEQ ID NO:391) |
| 3flankfw_pyr4                                                          | CAACCAGCCGCGAGCCTCAGCCTCTCTCAGCCTCATCAGCC<br>GCGGCCGCGCTGTGAAGACGGGCTTATC (SEQ ID NO:392) |
| 3flankrev_pRS426                                                       | GCGGATAACAATTTACACAGGAAACAGCGTTTAAACCAAG<br>AACAGCATCGAGGACA (SEQ ID NO:393)              |
| For screening integration of <i>slp7</i> deletion cassette             |                                                                                           |
| Primer                                                                 | Sequence                                                                                  |
| scrn_5forw                                                             | GGGCGACGACGAGTTTTAT (SEQ ID NO:394)                                                       |
| T026_Pyr4_orf_5rev2                                                    | CCATGAGCTTGAACAGGTAA (SEQ ID NO:395)                                                      |
| scrn_3rev                                                              | GAATGGATCAAGTCGCTGCT (SEQ ID NO:396)                                                      |
| T028_Pyr4_flank_rev                                                    | CATCCTCAAGGCCTCAGAC (SEQ ID NO:397)                                                       |
|                                                                        |                                                                                           |
| For screening deletion of <i>slp7</i> ORF                              |                                                                                           |
| orf_fw                                                                 | CTCAGGCTCTGCTTGGATTC (SEQ ID NO:398)                                                      |
| orf_rev                                                                | ATGCCAAAAAGACTGCTGCT (SEQ ID NO:399)                                                      |

**Table 29-2: Primers for generating *slp7* deletion plasmids.**

| <b>Deletion plasmid pTTv269 (<math>\Delta</math><i>slp7-pyr4-hph</i>), vector backbone pRS426</b> |                                                                                              |
|---------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| <b>Primer</b>                                                                                     | <b>Sequence</b>                                                                              |
| T1088_slp7_5flkfw_vector                                                                          | GTAACGCCAGGGTTTTCCAGTCACGACGGTTTAAAC<br>TCCCATATGCCTCTTGAAGG (SEQ ID NO:518)                 |
| T1089_slp7_5flkrev_pyr4Prom                                                                       | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATG<br>CTAGGCGGCCGCTTTCGAGCAAGATGTCGTTT (SEQ<br>ID NO: 391) |
| T1090_slp7_3flkfw_pyr4loop                                                                        | CAACCAGCCGCGAGCCTCAGCCTCTCTCAGCCTCATCA<br>GCCGCGGCCGCTGGGTGATAAGCTTGGGTTT (SEQ ID<br>NO:519) |
| T1091_slp7_3flkrev_vector                                                                         | GCGGATAACAATTTACACAGGAAACAGCGTTTAAACA<br>TCATGATGACCCATCGACA (SEQ ID NO:520)                 |

**[0309]** The deletion plasmid for the aspartic protease *slp8* (TrelD58698) is based on 1123 bp of 5' flanking regions and 1062 bp of 3' flanking regions. Fragments are produced by PCR using the primers listed in Table 30-1. This deletion plasmid for *slp8* results in deletion in the *slp8* locus and covers the complete coding sequence of SLP8. Alternatively, a deletion plasmid pTTv330 for *slp8* was constructed with a double marker *pyr4-hph* and using 975 bp of 5' flanking region and 1038 bp of 3' flanking regions as the basis. A 298 bp stretch from the end of *slp8* 5' flank was used as the direct repeat fragment. These fragments were amplified by PCR using the primers listed in Table 30-2. The *pyr4-hph* cassette was obtained from pTTv210 ( $\Delta$ *sep1-pyr4-hph*) with NotI digestion. NotI restriction sites were introduced on both sides of the double marker cassette and Ascl site was introduced between the *slp8* 5'direct repeat and 3' flank. The deletion plasmid for *slp8* (pTTv330, Table 30-2) results in a 1433 bp deletion in the *slp8* locus and cover the complete coding sequence of SLP8.

**Table 30-1: Primers for generating *slp8* deletion plasmids.**

| <b>Deletion plasmid for <i>slp8 lp8</i> (TrelD58698), vector backbone pRS426</b> |                                                                                          |
|----------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| <b>Primer</b>                                                                    | <b>Sequence</b>                                                                          |
| 5flankfw_pRS426                                                                  | GTAACGCCAGGGTTTTCCAGTCACGACGGTTTAAACGCCT<br>CCCTGGTATTCAGACA (SEQ ID NO:400)             |
| 5flankrev_pyr4                                                                   | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTAG<br>GCCGCCGCGACGCCAGAAAGAAATGCTC (SEQ ID NO:401) |
| 3flankfw_pyr4                                                                    | CAACCAGCCGCGAGCCTCAGCCTCTCTCAGCCTCATCAGCC                                                |
|                                                                                  | GCGGCCGCGACCTGGTCAGCTGCTCTTT (SEQ ID NO:402)                                             |
| 3flankrev_pRS426                                                                 | GCGGATAACAATTTACACAGGAAACAGCGTTTAACTGGA<br>ACCACATCGACTTCAC (SEQ ID NO:403)              |

| Deletion plasmid for <i>slp8 lp8</i> (TrelD58698), vector backbone pRS426 |                                      |
|---------------------------------------------------------------------------|--------------------------------------|
| Primer                                                                    | Sequence                             |
|                                                                           |                                      |
| For screening integration of <i>slp8</i> deletion cassette                |                                      |
| Primer                                                                    | Sequence                             |
| scrn_5forw                                                                | AACCACCTTGTACCGTCTC (SEQ ID NO:404)  |
| T026_Pyr4_orf_5rev2                                                       | CCATGAGCTTGAACAGGTAA (SEQ ID NO:405) |
| scrn_3rev                                                                 | GTCGTCGAGGCTGCTTTATC (SEQ ID NO:406) |
| T028_Pyr4_flank_rev                                                       | CATCCTCAAGGCCTCAGAC (SEQ ID NO:407)  |
|                                                                           |                                      |
| For screening deletion of <i>slp8</i> ORF                                 |                                      |
| orf_fw                                                                    | GATCTCGAATCCGAGGACAA (SEQ ID NO:408) |
| orf_rev                                                                   | CCGGTAGCGTTAGAGAGACG (SEQ ID NO:258) |

**Table 30-2: Primers for generating *slp8* deletion plasmid.**

| Deletion plasmid pTTv330 ( $\Delta$ <i>slp8-pyr4-hph</i> ), vector backbone pRS426 |                                                                                              |
|------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| Primer                                                                             | Sequence                                                                                     |
| T1203_slp8_5f_f                                                                    | GATTAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGAAG<br>GTTTAAACATCGTGCTTGGGCTATTCTG (SEQ ID NO:521)      |
| T1204_slp8_5f_r                                                                    | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCGATGCTA<br>G<br>GCGGCCGCGGAAAGACGCCAGAAAGAAA (SEQ ID NO:522) |
| T1205_slp8_5dr_f                                                                   | GTACACTTGTTTAGAGGTAATCCTTCTTTCTAGAAGGAGA<br>GCGGCCGCGCTCGATGTGGATGATACT (SEQ ID NO:523)      |
| T1206_slp8_5dr_r                                                                   | ATCTATACTGTCTGCACCAAAAGTACAACAACGCAAA<br>CCGGGCGCGCCGAAAGACGCCAGAAAGAAA (SEQ ID NO:524)      |
| T1207_slp8_3f_f                                                                    | CGGTTTGCGTTGTTGTACTT (SEQ ID NO:525)                                                         |
| T1208_slp8_3f_r                                                                    | TGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGC<br>GTTTAAACACAACCAACGTTCTCTCGT (SEQ ID NO:526)       |

[0310] The *pyr4* blaster cassette is obtained from pTTv71 with *NotI* digestion. Templates to be used in the PCR of the flanking regions is from the *T. reesei* wild type strain QM6a; the vector backbone is *EcoRI/XhoI* digested pRS426 and the plasmids are constructed using the yeast homologous recombination method described above.

#### EXAMPLE 6

**Generation of MAB01 antibody producing seven-fold protease deletion strain M507**

[0311] To generate an MAB01 antibody producing strain in the seven-fold protease deletion strain, M486 ( $\Delta pep1\Delta tsp1\Delta slp1\Delta gap1\Delta gap2\Delta pep4\Delta pep3$ ) as described in US provisional application 61/583,559 or PCT/EP2013/050126 and in example 4 was transformed with MAB01 light and heavy chain tandem construct (pTTv223) using acetamide selection for the transformation. Transformants were screened by PCR for correct integration into the *cbh1* locus and purified to single cell clones. One MAB01 antibody producing strain was designated with the number M507. To reuse *pyr4* as the selection marker, removal of the *pyr4* blaster cassette from the *pep3* deletion locus was carried out. Spores were spread onto minimal medium plates containing 20 g/l glucose, 2 g/l proteose peptone, 1 ml/l Triton X-100, 5 mM uridine and 1.5 g/l 5-FOA, pH 4.8. 5-FOA resistant colonies were picked after 5-7 days to 0.9% NaCl, suspended thoroughly by vortexing and filtrated through a cotton-filled pipette tip. To purify clones to single cell clones, filtrates were spread again onto plates described above. Purified clones were sporulated on plates containing 39 g/l potato dextrose agarose. These clones were tested for uridine auxotrophy by plating spores onto minimal medium plates (20 g/l glucose, 1 ml/l Triton X-100) where no growth was observed, indicating that the selected clones were *pyr4*<sup>-</sup>. Clones were further tested by PCR for the removal of the blaster cassette and were shown to be correct. One clone was designated with strain number M564.

**Generation of fucosylated G0 in seven-fold protease deletion strain**

[0312] The *PmeI* fragments of pTTv224 and pTTv225 or pTTv226 plasmids from example 2 are transformed into the seven-fold protease deletion *Trichoderma reesei* strain M564 ( $\Delta pep1\Delta tsp1\Delta slp1\Delta gap1\Delta gap2\Delta pep4\Delta pep3pyr4^-$ ) expressing codon optimized MAB01 antibody, essentially as described in Example 2. 5 µg of purified expression cassette DNA is co-transformed into protoplasts of the strain M564. Preparation of protoplasts and transformation are carried out essentially according to methods in Penttilä et al. (1987, Gene 61:155-164) and Gruber et al (1990, Curr. Genet. 18:71-76) for *pyr4* selection. The transformed protoplasts are plated onto *Trichoderma* minimal media (TrMM) plates containing sorbitol.

[0313] Transformants are streaked onto TrMM plates with 0.1% TritonX-100. Transformants growing fast as selective streaks are screened by PCR using the primers listed in Table 4. DNA from mycelia are purified and analyzed by PCR to look at the integration of the 5' and 3' flanks of cassette and the existence of the *pep4* ORF, as explained in example 2. Positively integrated transformants are purified to single cell clones.

[0314] The *pyr4* marker of pTTv110 (Example 1), containing chimeric GnTII/GnTI sequence, is changed to hygromycin resistance marker by *NotI* digestion and ligation. 5 µg of purified expression cassette DNA from this plasmid is transformed into protoplasts of a strain containing pTTv224 and pTTv225 or pTTv226. The transformed protoplasts are plated onto *Trichoderma* minimal media (TrMM) plates containing sorbitol and hygromycin (150 µg/ml). Transformants are streaked onto TrMM plates containing hygromycin (125 µg/ml) and 0.1% TritonX-100 and screened by PCR for correct integration into the *alg3* locus and loss of the *alg3* ORF. Positively integrated transformants are purified to single cell clones. Pure strains are cultivated in shake flask cultures as described in Example 2.

[0315] Samples are taken from shake flask cultures in days 3, 5, and 7 and MAB01 is purified with Protein G affinity chromatography. PNGase F reactions are performed for ~10 µg of denatured protein. The released N-glycans are first purified with Hypersep C-18 and then with Hypersep Hypercarb (both from Thermo Scientific). The purification steps are performed in 96-well format. Neutral N-glycans are analyzed by MALDITOF MS as described in Example 2.



**Generation of G0 producing strain M629**

[0316] Generation of G0 producing strain M629 is described in the International Patent Application PCT/EP2013/050126. Briefly, the vector pTTg173 (having *T. reesei* Kre2 signal peptide fused to human GnT1 and native human GnT2 targeted to *T. reesei* *alg3* locus) was transformed to *T. reesei*. Transformants were picked onto selective plates and on the basis of PCR screening clones with positive results were selected for single spore platings and re-screening for integration and *alg3* deletion. PCR-screened strains were subjected to shake flask cultivation and glycan analysis. Final G0 MAB01 producing strain was named as M629.

**Generation of MAB01 antibody producing double protease deletion strains M292 and M295**

[0317] To generate the MAB01 antibody producing strain, the *pep1* deletion strain M181 was transformed with MAB01 light and heavy chain constructs (pTTv98+pTTv67) using hygromycin and acetamide in selection. The removal of the *pyr4* blaster cassette from *pep1* locus was carried out essentially as described for M195 above. This *pyr4*- strain was designated with number M285.

[0318] To remove vector sequence, plasmids pTTv115 and pTTv116 were digested with *PmeI* and approximately 5 µg of either deletion cassette was used to transform M285 separately. Colonies growing on transformation plates were picked as selective streaks and clones growing fast were screened by PCR using the primers listed in Table 30-3 for the correct integration. Putative disruptants were purified to single cell clones. No pure clones were obtained even after repeated purification steps. However, clones having  $\Delta pep1\Delta slp2$  and  $\Delta pep1\Delta slp3$  were designated as M292 and M295, respectively.

**Table 30-3: Primers for screening *slp2* (pTTv115) and *slp3* (pTTv116) integration and strain purity.**

| For screening integration of pTTv115      |                                        |
|-------------------------------------------|----------------------------------------|
| Primer                                    | Sequence                               |
|                                           | GATGCACCGCTGCGGCC (SEQ ID NO: 327)     |
| T026_Pyr4_orf_5rev2                       | CCATGAGCTTGAACAGGTAA (SEQ ID NO: 328)  |
| T028_Pyr4_flank_rev                       | CATCCTCAAGGCCTCAGAC (SEQ ID NO: 329)   |
| T055_slp2_3screen_R                       | GGCGTTGCTCCCCATGCG (SEQ ID NO: 330)    |
|                                           |                                        |
| For screening deletion of <i>slp2</i> ORF |                                        |
| T111_slp2_ORF_F                           | ATGCGGTCCGTTGTCGCC (SEQ ID NO: 331)    |
| T112_slp2_ORF_R                           | TTACTCGGAGAGCTCAGAGA (SEQ ID NO: 332)  |
|                                           |                                        |
| For screening integration of pTTv116      |                                        |
| Primer                                    | Sequence                               |
| T056_slp3_5screen_F                       | GTGAATGGGTGGCAACATGA (SEQ ID NO: 333)  |
| T026_Pyr4_orf_5rev2                       | CCATGAGCTTGAACAGGTAA (SEQ ID NO: 334)  |
| T028_Pyr4_flank_rev                       | CATCCTCAAGGCCTCAGAC (SEQ ID NO: 335)   |
| T057_slp3_3screen_R                       | CATCAAGTTGACCACCATTGT (SEQ ID NO: 336) |
|                                           |                                        |
| For screening deletion of <i>slp3</i> ORF |                                        |
| T113_slp3_ORF_F                           | ATGCGGTTGTCCGTCCTCC (SEQ ID NO: 337)   |

**For screening deletion of *slp3* ORF**

T114\_slp3\_ORF\_R

TTAACCGGAAGGGTTGCCGT (SEQ ID NO: 338)

**Generation of MAB01 producing triple protease deletion strain M304**

[0319] To generate the MAB01 antibody producing strain the  $\Delta pep1\Delta tsp1$  double protease deletion strain M194 was transformed with MAB01 light and heavy chain constructs (pTTv99+pTTv67) using hygromycin and acetamide in selection. This MAB01 strain with  $\Delta pep1\Delta tsp1$  was designated with number M252. Removal of the *pyr4* blaster cassette from *pep1* locus was carried out essentially as described above. This *pyr4*<sup>-</sup> strain was designated with number M284.

[0320] The third protease deletion to M284 was obtained by using *slp1* deletion construct pTTv128. This construct contains a native KEX2 overexpression cassette targeted to the *slp1* locus. The resulting strain was designated M304. M304 comprises MAB01 light chain fused to *T. reesei* truncated CBH1 carrier with NVISKR Kex2 cleavage sequence and MAB01 heavy chain fused to *T. reesei* truncated CBH1 carrier with AXE1 [DGETVVKR] Kex2 cleavage sequence.

**Generation of MAB01 producing quadruple protease deletion strain M371**

[0321] Removal of the *pyr4* blaster cassette from *slp1* locus from strain M304 was carried out essentially as described above. This *pyr4*<sup>-</sup> strain was designated with number M317. *Gap1* was deleted from M317 using deletion construct pTTv117 above. A strain producing MAB01 and having quadruple protease deletion was designated as M371.

**Generation of MAB01 producing 8-fold protease deletion strain M646**

[0322] The M646 *slp2* deletion strain was made by transforming the pTTv115 deletion cassette into M564 (*pyr4*<sup>-</sup> version of M507). The M564 *pyr4*<sup>-</sup> strain was created essentially as described above. Approximately 5 µg of the deletion cassette was used to transform the MAB01 production strain M564. Growing streaks were screened by PCR (using the primers listed in Table 30-4) for correct integration and loss of *slp2* ORF. Clones giving the expected signals were purified to single cell clones and rescreened by PCR using the primers listed in Table 30-4. A correct clone was designated as strain M646.

**Table 30-4: Primers for screening pTTv115/ $\Delta$ *slp2*-*pyr4* cassette integration and strain purity.**

| <b>For screening integration of pTTv115 (<math>\Delta</math><i>slp2</i>-<i>pyr4</i>)</b> |                                       |
|------------------------------------------------------------------------------------------|---------------------------------------|
| Primer                                                                                   | Sequence                              |
| T054_slp2_5screen_F                                                                      | GATGCACCGCTGCGGCC (SEQ ID NO: 527)    |
| T1084_screen_5flk_pyr_rev                                                                | TCTTGAGCACGACAATCGAC (SEQ ID NO: 528) |
| T055_slp2_3screen_R                                                                      | GGCGTTGCTCCCCATGCG (SEQ ID NO: 529)   |
| T028_Pyr4_flank_rev                                                                      | CATCCTCAAGGCCTCAGAC (SEQ ID NO: 530)  |
| <b>For screening deletion of <i>slp2</i> (tr123244) ORF</b>                              |                                       |
| T111_slp2_ORF_F                                                                          | ATGCGGTCCGTTGTCGCC (SEQ ID NO: 531)   |
| T112_slp2_ORF_R                                                                          | TTACTCGGAGAGCTCAGAGA (SEQ ID NO: 532) |

**Generation of antibody fragments**

[0323] Generation of various antibody fragments is described in the International Patent Application PCT/EP2013/050126.

**Table 31-1. Overview of generation of the protease deletion strains having having MAB01 antibody.** pTTv99/67 plasmids include MAB01 light chain fused to *T. reesei* truncated CBH1 carrier with NVISKR Kex2 cleavage sequence and MAB01 heavy chain fused to *T. reesei* truncated CBH1 carrier with AXE1 [DGETVVKR] Kex2 cleavage sequence.

| Strain | Vector        | Clone    | Strain transformed | Proteases k/o                              | Markers in strain |
|--------|---------------|----------|--------------------|--------------------------------------------|-------------------|
| M181   | pTTv71        | 9-20A-1  | M127               | pep1                                       | pyr4              |
| M194   | pTTv42        | 13-172D  | M181               | pep1 tsp1                                  | pyr4 bar          |
| M252   | pTTv99/67     | 6.14A    | M194               | pep1 tsp1                                  | AmdS/HygR         |
| M284   | 5-FOA of M252 | 3A       | pyr4-of M252       | pep1 tsp1                                  | none              |
| M304   | pTTv128       | 12A      | M284               | pep1 tsp1 slp1                             | pyr4              |
| M317   | 5-FOA of M304 | 1A       | pyr4-of M304       | pep1 tsp1 slp1                             | None              |
| M371   | pTTv117       | 44A      | M317               | pep1 tsp1 slp1 gap1                        | pyr4              |
|        |               |          |                    |                                            |                   |
| M244   | pTTv98/67     | 1.3Apep1 | M181               | pep1                                       | AmdS/HygR         |
| M285   | 5-FOA of M244 | 2A       | pyr4-of M244       | pep1                                       | none              |
| M292   | pTTv115       | 11A      | M285               | pep1 slp2                                  | pyr4              |
| M295   | pTTv116       | 82A      | M285               | pep1 slp3                                  | pyr4              |
|        |               |          |                    |                                            |                   |
| M507   | pTTv223       | 19B      | M486               | pep1 tsp1 slp1 gap1<br>gap2 pep4 pep3      | AmdS              |
| M564   | 5-FOA of M507 |          | pyr4-of M507       | pep1 tsp1 slp1 gap1<br>gap2 pep4 pep3      | none              |
| M646   | pTTv115       |          | M564               | pep1 tsp1 slp1 gap1<br>gap2 pep4 pep3 slp2 | pyr4              |

**EXAMPLE 7****Fungal strain producing Man5**

[0324] A filamentous fungal cell of the invention can also be engineered to produce Man5 as the substrate for fucosylation and production of FG0 glycans (i.e traditional pathway). If a filamentous fungus does not produce endogenously sufficient levels of Man5, an  $\alpha$ 1-2-mannosidase expression can be introduced to the filamentous fungal cell.

[0325] In addition to introducing GnTI and GnTII or a recombinant GnTII/I fusion protein into a Man5-producing strain, a mannosidase II activity is further needed to remove two mannoses from the GlcNAcMan5 glycan structure so that GnTII can use GlcNAcMan3 as an acceptor molecule.

[0326] Mannosidase II activity is introduced to the e.g. MAB01 antibody expressing *Trichoderma reesei* strain by designing a mannosidase II-containing expression cassette with a promoter for driving the mannosidase II expression. Useful promoters are those from e.g. *gpdA* or *cbh1*. Mannosidase II activity can be transformed by random or targeted integration followed by screening of strains with most suitable expression level. The expression cassette is linked with a proprietary selection marker gene, or a selection marker is co-transformed as a separate expression cassette. Transformation is performed according to methods described above.

[0327] For ER/Golgi expression, the catalytic domain of the mannosidase II may be fused with an N-terminal targeting peptide. Exemplary targeting peptide is an N-terminal portion of *T. reesei* KRE2.

[0328] After transformation of *Trichoderma* with the mannosidase II construct described above, *Trichoderma* strains are selected, streaked on selective medium for two successive rounds, and tested by PCR for integration of the expression constructs into the genome. Selected transformants of *Trichoderma* strains producing Man5 and expressing the GnTI and GnTII, mannosidase II, and MAB01 antibody are then cultured in shake flasks or fermentor conditions and analyzed for glycan content as described above.

[0329] The resulting strains are then subjected to transformation of GMD, FX and FUT8.

#### EXAMPLE 8

##### ***Generation of a fungal strain producing galactosylated glycans on an antibody***

[0330] In order for a fungal strain to produce galactosylated and fucosylated glycans on an antibody, a  $\beta$ -1,4-galactosyltransferase gene is generated, cloned into a fungal expression plasmid followed by transformation into the fungal cell expressing an antibody of the above examples.

[0331] For example, a human  $\beta$ -1,4-galactosyltransferase I gene (GenBank accession P15291; gene NM\_001497) is artificially synthesized and cloned into a *Trichoderma* expression cassette with a promoter for driving the galactosyltransferase expression. Useful promoters are those from *gpdA* or *cbh1*. In order to enhance galactosyltransferase targeting to ER/Golgi, targeting peptide derived from *Trichoderma* (or host cell) Kre2/Mnt1 (described in Schwientek et al. (1996)) can be used to generate a fusion construct. Targeting peptide is ligated in-frame to an N-terminal amino acid deletion of the galactosyltransferase. The encoded fusion protein localizes in the ER/Golgi by means of the KRE2 targeting peptide sequence while retaining its galactosyltransferase catalytic domain activity and is capable of transferring galactose onto GlcNAc(1-2)Man3GlcNAc2. The KRE2 targeting peptide may comprise the amino acids from about 1 to about 106 or from about 1 to about 83, or shorter, e.g. from about 1 to about 51 amino acids.

Kre2 (Tre21576) aa 1-106

MASTNARYVRYLLIAFFTILVFYFVSNKYEGVDLNKGTFTAPDSTKTTKPPATG  
DAKDFPLALTPNDPGFNDLVGIAPGPRMNATFVTLARNSDVWDIARSIRQ (SEQ  
ID NO: 229)

Kre2 aa 1-83

MASTNARYVRYLLIAFFTILVFYFVSNKYEGVDLNKGTFTAPDSTKTTKPPATG  
DAKDFPLALTPNDPGFNDLVGIAPGPR (SEQ ID NO: 230)

Kre2 aa 1-51

MASTNARYVRYLLIAFFTILVFYFVSNSKYEGVDLNKGTFTAPDSTKTPK (SEQ ID NO: 231)

[0332] The galactosyltransferase expression cassette can be targeted to, for example, the *cbh2* or a protease locus of *T. reesei*, using methods essentially as described above. Alternatively, galactosyltransferase activity can be transformed by random integration.

[0333] After transformation of *Trichoderma* with the galactosyltransferase construct described above, *Trichoderma* strains are selected, streaked on selective medium for two successive rounds, and tested by PCR for integration of the expression constructs into the genome. Selected transformants of *Trichoderma* strains producing galactosylated (and fucosylated) antibody are then cultured in shake flasks or fermentor conditions and analyzed for glycan content as described above.

[0334] Optionally, the fungal strain in the examples can be made to express UDP-galactose (UDP-Gal) transporter. Human UDP-galactose (UDP-Gal) transporter has been cloned and shown to be active in *S. cerevisiae*. (e.g. GenBank accession NP\_005651; Kainuma, M., et al. 1999 Glycobiology 9(2): 133-141).

[0335] Further, to increase endogenous pool of UDP galactose expression of a UDP-galactose 4 epimerase (e.g. GenBank accession AAB86498) in a fungal cell may be introduced.

#### EXAMPLE 9

##### *Generation of fungal strain producing sialylated glycans*

[0336] The galactosylated glycans of previous example are substrates in the formation of sialylated glycoproteins produced in a filamentous fungus. The fungal cell is engineered to express enzymes needed in production and transport of sialylation pathway molecules. The following genes may be introduced, for example, into the fungal cell producing galactosylated and fucosylated glycans.

**Table 31-2.**

| Gene name                                                                  | Species                                                                                              | GenBank accession #          |
|----------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|------------------------------|
| Glucosamine UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase | <i>Homo sapiens</i> glucosamine UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (GNE) | Q6QNY5 (SEQ ID NO:232)       |
|                                                                            |                                                                                                      | NP_001121699 (SEQ ID NO:233) |
| N-acetylneuraminic acid synthase                                           | <i>Homo sapiens</i> N-acetylneuraminic acid synthase (NANS)                                          | NP_061819 (SEQ ID NO:234)    |
| N-acetylneuraminic acid phosphatase                                        | <i>Homo sapiens</i> N-acetylneuraminic acid phosphatase (NANP; optional)                             | NP_689880 (SEQ ID NO:235)    |
| Cytidine monophosphate N-acetylneuraminic acid synthetase                  | <i>Homo sapiens</i> cytidine monophosphate N-acetylneuraminic acid synthetase (CMAS)                 | NP_061156 (SEQ ID NO:236)    |
| CMP-sialic acid transporter                                                | <i>Mus musculus</i> solute carrier family 35 member 1 (SLC35A1)                                      | Q61420 (SEQ ID NO:237)       |
| Sialyltransferase                                                          | <i>Mus musculus</i> $\beta$ -galactoside $\alpha$ 2,6-sialyltransferase 1 (St6gal1)                  | NP_001239434 (SEQ ID NO:238) |

| Gene name | Species                                                                             | GenBank accession #       |
|-----------|-------------------------------------------------------------------------------------|---------------------------|
|           | <i>Homo sapiens</i> $\beta$ -galactoside $\alpha$ 2,3-sialyltransferase 4 (ST3GAL4) | NP_006269 (SEQ ID NO:239) |

[0337] Briefly, open reading frames for the above genes UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (human, GNE), N-acetylneuraminic acid synthase (human, NANS), N-acetylneuraminic acid phosphatase (human, NANP, this enzyme is optional), cytidine monophosphate N-acetylneuraminic acid synthetase (human, CMAS), solute carrier family 35 member 1 (*M. musculus*, SLC35A1),  $\beta$ -galactoside  $\alpha$ 2,6-sialyltransferase 1 (*M. musculus*, ST6GAL1) and  $\beta$ -galactoside  $\alpha$ 2,3-sialyltransferase 4 (human, ST3GAL4) are codon-optimized and synthesized for *Trichoderma* expression. The resultant synthetic DNAs for GNE, NANS, NANP, CMAS, SLC35A1, ST6GAL1 and ST3GAL4 are generated with appropriate restriction sites and cloned into expression vector(s). Tandem constructs/expression cassettes may be also generated with two or more genes to reduce number of transformations and loci to be transformed.

[0338] Sialyltransferase catalytic domain may be fused to a *Trichoderma* targeting peptide, for example, Kre2 described above.

[0339] The sialylation pathway gene expression cassettes can be targeted to, for example, the *cbh2*, *egl2*, or a protease locus of *T. reesei*, using methods essentially as described above. Alternatively, sialylation pathway genes can be transformed by random integration.

[0340] After transformation of *Trichoderma* with the sialylation pathway gene constructs described above, *Trichoderma* strains are selected, streaked on selective medium for two successive rounds, and tested by PCR for integration of the expression constructs into the genome. Selected transformants of *Trichoderma* strains producing sialylated (and fucosylated) antibody are then cultured in shake flasks or fermentor conditions and analyzed for glycan content as described above. Neutral N-glycans are detected in positive ion reflector mode as [M + Na]<sup>+</sup> ions, and acidic N-glycans are detected in negative ion linear mode as [M-H]<sup>-</sup> ions and as described above.

#### EXAMPLE 10

[0341] Proteases of the invention may also be silenced using RNAi technology. Examples of RNAi constructs to silence slp2 is described in the International Patent Application PCT/EP2013/050126.

#### EXAMPLE 11 - Generation of fucosylated antibody glycoform producing *T. reesei* strains

[0342] A pyr4<sup>-</sup> strain was created from M629 and the expression cassettes of pTTv224 and pTTv225 were transformed to the strain, however, N-glycan analysis showed that GNT2 activity was lost during the pyr4<sup>-</sup> loopout and therefore a clone (60-1; M905) was chosen to be retransformed with GnTs and GalT.

[0343] The coding sequence of human  $\beta$ -1,4-galactosyltransferase 1 (GalTI, SEQ ID NO:409) was optimized for *T. reesei* expression. A plasmid containing expression cassettes for human GalTI and chimeric GnTII/GnTI was generated (GnTII/GnTI fusion protein has been described in WO2012069593). The plasmid was cloned in two steps using yeast homologous recombination; first plasmid pTTv363 to which expression cassette for GNT2/1 was added generating plasmid pTTv434 (see Figure 13). For pTTv363 plasmid pTTv264 (described in International patent application PCT/EP2013/050126) was used as backbone vector. This plasmid contains

integration flanks for *egl2* locus, *gpdA* promoter, *cbh1* terminator and hygromycin marker. The plasmid was digested with *PacI*-*NotI* and all three fragments were utilised in cloning the plasmid pTTv363. For targeting of the GalTI to Golgi, the transmembrane region of a *T. reesei* native mannosyltransferase KRE2 (MNT1) was used. The first 85 amino acids of the KRE2 were fused to human *GalTI* without the transmembrane domain (amino acids 1-77). The *kre2* localisation fragment was generated by PCR from genomic DNA and the *GAITI* fragment was generated by PCR from the vector containing the optimised GalTI sequence (Table 32). *pyr4* marker, *egl2* direct repeat and *cbh2* terminator fragments were generated by PCR from genomic DNA, in order to generate a plasmid containing *pyr4*-hygromycin double marker. In addition to pTTv363, alternative GalTI plasmid pTTv362 was cloned. This plasmid has shorter truncation in the N-terminus of the human GalTI (amino acids 1-43). Otherwise the plasmid is like pTTv363 described above. The clonings of these plasmids were performed as described in Example 2. Clones were sequenced to verify the sequence and one correct clone for each plasmid were chosen to be the final vectors pTTv362 and pTTv363.

**Table 32. List of primers used for cloning vectors pTTv362 and pTTv363.**

| Fragment         | Primer                    | Primer sequence                                                                            |
|------------------|---------------------------|--------------------------------------------------------------------------------------------|
| KRE2             | T1372_kre2_recgpdA_for    | ACTAACAGCTACCCGCTTGAGCAGACATCA<br>TGGCGTCAACAAATGCGCG (SEQ ID NO:533)                      |
|                  | T337_21576_r              | GTTCATTCGAGGGCCGGGAG (SEQ ID NO:534)                                                       |
| GalTI Δ43        | T1373_galt_44_for         | GTCGGCATCGCTCCCGGCCCTCGAATGAACG<br>GCAGGGACCTCAGCCGCCT (SEQ ID NO:535)                     |
|                  | T1374_galt_rev            | ACCGGTGCGTCAGGCTTTCGCCACGGAGCTT<br>CAGCTGGGGGTGCCGATGT (SEQ ID NO:536)                     |
| GalTI Δ77        | T1375_galt_78_for         | GTCGGCATCGCTCCCGGCCCTCGAATGAACC<br>GAACCGGCGGCGCCCGCCC (SEQ ID NO:537)                     |
|                  | T1374_galt_rev            | ACCGGTGCGTCAGGCTTTCGCCACGGAGCTT<br>CAGCTGGGGGTGCCGATGT (SEQ ID NO:538)                     |
| <i>pyr4</i>      | T1369_pyr4_for            | CTAGCATCGACTACTGCTGC (SEQ ID NO:539)                                                       |
|                  | T1370_pyr4_rechphn ew rev | AAGGGGACCGGCCGCTAGTCTCACCGTTATC<br>ATGCAAAGATACACATCAA (SEQ ID NO:540)                     |
| <i>egl2</i> 3DR  | T1367_egl2_3dr_for        | TCCGTTGCGAGGCCAACTTGCAATTGCTGTCAAGA<br>CGATGAGGATCCCACTCTGAGCTGAATGCAGA<br>(SEQ ID NO:541) |
|                  | T1368_egl2_3dr_rev        | GCGCTGGCAACGAGAGCAGAGCAGCAGTAGTCG<br>ATGCTAGGCGGCCGCTGCGACAACTACGGATGC<br>(SEQ ID NO:542)  |
| <i>cbh2</i> term | T161_tcbh2_seq_f2         | CAGCTGCGGAGCATGAGCCT (SEQ ID NO:543)                                                       |
|                  | T1371_tcbh2_recelg 2_rev  | TGGCGAGGCTTCTGCATTGAGCTCAGAGTGG<br>CGGCCGCGTGTGCGGAATCATTATCA (SEQ ID<br>NO:544)           |

[0344] The pTTv363 vector was linearised with *FseI*, and the human chimeric GnTII/GnTI, together with the *cbh1* promoter and terminator, was inserted by yeast recombination with long overlapping primers as described in example 2 (Table 33). The GnTII/GnTI expression cassette was digested from plasmid pTTv110 with *SgfI* (pTTv110 is described in WO2012069593). The presence of the GnTII/GnTI gene was confirmed by digesting the prepared plasmids with *SacI* and one correct clone was chosen to be the final vector pTTv434.

**Table 33. List of primers used for cloning vector pTTv434.**

| Fragment                     | Primer | Primer sequence                                                                                               |
|------------------------------|--------|---------------------------------------------------------------------------------------------------------------|
| GNT2/1 overlapping oligos 5' | T1560  | ACACTCTCAGAATAAATTCATCGCCAATTTGACAGGCCGGCC<br>ATTCTCACGGTGAATGTAGGCCTTTGTAGGGTAGGAATT<br>(SEQ ID NO:545)      |
|                              | T1561  | AATTCCTACCTACAAAAGGCCTACATTCACCGTGAGAATGG<br>CCGGCCTGTCAAATTGGCGATGAATTTATTCTGAGAGTGT<br>(SEQ ID NO:546)      |
| GNT2/1 overlapping oligos 3' | T1562  | TTGCATTGCTGTCAAGACGATGACAACGTAGCCGAGGACCG<br>GCCGGCCCTTGTATCTCTACACAGGCTCAAATCAATAAG<br>AAG (SEQ ID NO:547)   |
|                              | T1563  | CTTCTTATTGATTGAGCCTGTGTGTAGAGATACAAGGGGGCC<br>GGCCGGTCTCGGCTACGTTGTCATCGTCTTGACAGCAATG<br>CAA (SEQ ID NO:548) |

[0345] The *PmeI* expression construct fragment of pTTv434 was prepared and transformed to the M905 strain essentially as described in Example 2. Transformation plates contained hygromycin (150 µg/ml). Transformants were streaked onto TrMM plates containing hygromycin (125 µg/ml) and 0.1% TritonX-100 and visible amounts of the 2<sup>nd</sup> streaks (total sum 51) were denaturised by boiling in 1% SDS followed by addition of 10% n-octyl-β-D-glucopyranoside to eliminate SDS. PNGase F (*Elizabethkingia meningosepticum*, Prozyme) was performed as an overnight reaction in 20 mM sodium phosphate buffer, pH 7.3. The released N-glycans were purified using Hypersep C18 and Hypersep Hypercarb 10 mg (Thermo Scientific) and analysed by MALDI-TOF MS. Clones positive for galactosylated N-glycans were selected for shake flask cultures. Streaks were also screened by PCR for correct integration into the *egl2* locus and loss of the *egl2* ORF (Table 34). Promising transformants were cultivated in a 50 ml volume for seven days at +28°C in a media containing TrMM, pH 5.5, supplemented with 40 g/l lactose, 20 g/l spent grain extract, 9 g/l casamino acids and 100 mM PIPPS.

**Table 34. List of primers used for PCR screening of *T. reesei* transformants**

|                             |                                      |
|-----------------------------|--------------------------------------|
| 5' flank screening primers: | 1932 bp product                      |
| T1410_egl2_5int_f3          | GCTCGAGACGTACGATTCAC (SEQ ID NO:549) |
| T176_pcbh1_seq_r4           | CTCCGGGTTTCGACGAGCTT (SEQ ID NO:550) |
| 3' flank screening primers: | 1168 bp product                      |
| T1158_egl2_3pr_intR2        | GGCGAAATAAGCTCACTCAG (SEQ ID NO:551) |
| T1411_cbh2t_end_f           | CCAATAGCCCGGTGATAGTC (SEQ ID NO:552) |
| egl1 ORF primers:           | 368 bp product                       |
| T1412_egl2_orf_f1           | AACAAGTCCGTGGCTCCATT (SEQ ID NO:553) |
| T1413_egl2_orf_r1           | CCAACTTTTCAGCCAGCAAC (SEQ ID NO:554) |



[0346] For N-glycan analysis the antibody was purified from day 5 culture supernatants using Protein G HP MultiTrap 96-well filter plate (GE Healthcare) basically according to manufacturer's instructions, but as an elution buffer 0.1 M citrate buffer, pH 2.6, was used. The antibody concentrations were determined via UV absorbance against MAB01 standard curve and the N-glycans were analysed from 15-20 µg of purified antibody as described in Example 2. N-glycans were analysed from total of eleven pTTv434 clones and four of them turned out to produce fucosylated or fucosylated and galactosylated glycoforms on antibody (Table 35).

**Table 35. Relative proportions of neutral N-glycans from purified antibody from pTTv434 clones #3, #41, #48 and #64.**

|                 |         |          | #3   | #41  | #48  | #64  |
|-----------------|---------|----------|------|------|------|------|
| Composition     | Short   | m/z      | %    | %    | %    | %    |
| Hex3HexNAc2     | Man3    | 933.310  | 31.7 | 0.0  | 4.7  | 20.2 |
| Hex4HexNAc2     | Man4    | 1095.370 | 3.9  | 0.0  | 3.0  | 5.5  |
| Hex3HexNAc3     | GnMan3  | 1136.400 | 3.3  | 0.0  | 0.0  | 17.9 |
| Hex5HexNAc2     | Man5    | 1257.420 | 4.4  | 12.4 | 22.9 | 5.3  |
| Hex3HexNAc3dHex | FGnMan3 | 1282.450 | 0.0  | 0.0  | 0.0  | 8.3  |
| Hex3HexNAc4     | G0      | 1339.480 | 18.6 | 0.0  | 0.0  | 0.0  |
| Hex6HexNAc2     | Hex6    | 1419.480 | 32.5 | 48.3 | 39.3 | 42.7 |
| Hex3HexNAc4dHex | FG0     | 1485.530 | 3.3  | 0.0  | 0.0  | 0.0  |
| Hex4HexNAc4     | G1      | 1501.530 | 0.9  | 0.0  | 0.0  | 0.0  |
| Hex7HexNAc2     | Hex7    | 1581.530 | 1.4  | 16.1 | 13.1 | 0.0  |
| Hex5HexNAc4     | G2      | 1663.580 | 0.0  | 7.9  | 1.9  | 0.0  |
| Hex8HexNAc2     | Hex8    | 1743.580 | 0.0  | 8.3  | 8.9  | 0.0  |
| Hex5HexNAc4dHex | FG2     | 1809.640 | 0.0  | 6.9  | 1.8  | 0.0  |
| Hex9HexNAc2     | Hex9    | 1905.630 | 0.0  | 0.0  | 3.5  | 0.0  |
| Hex10HexNAc2    | Hex10   | 2067.690 | 0.0  | 0.0  | 0.9  | 0.0  |

## EXAMPLE 12

### Protease activity measurement of protease deficient T reesei strains

[0347] The protein concentrations were determined from supernatant samples from day 2-7 of 1-7x protease deficient strains according to EnzChek protease assay kit (Molecular probes #E6638, green fluorescent casein substrate). Briefly, the supernatants were diluted in sodium citrate buffer to equal total protein concentration and equal amounts of the diluted supernatants were added into a black 96 well plate, using 3 replicate wells per sample. Casein FL diluted stock made in sodium citrate buffer was added to each supernatant containing well and the plates were incubated covered in plastic bag at 37°C. The fluorescence from the wells was measured after 2, 3, and 4 hours. The readings were done on the Varioskan fluorescent plate reader using 485 nm excitation and 530 nm emission. Some protease activity measurements were performed using succinylated casein (QuantiCleave protease assay kit, Pierce #23263) according to the manufacturer's protocol.

[0348] The *pep1* single deletion reduced the protease activity by 1.7-fold, the *pep1/tsp1* double deletion

reduced the protease activity by 2-fold, the *pep1/tsp1/slp1* triple deletion reduced the protease activity by 3.2-fold, the *pep1/tsp1/slp1/gap1* quadruple deletion reduced the protease activity by 7.8-fold compared to the wild type M124 strain, the *pep1/tsp1/slp1/gap1/gap2* 5-fold deletion reduced the protease activity by 10-fold, the *pep1/tsp1/slp1/gap1/gap2/pep4* 6-fold deletion reduced the protease activity by 15.9-fold, and the *pep1/tsp1/slp1/gap1/gap2/pep4/pep3* 7-fold deletion reduced the protease activity by 18.2-fold.

[0349] The Figure 14 graphically depicts normalized protease activity data from culture supernatants from each of the protease deletion supernatants (from 1-fold to 7-fold deletion mutant) and the parent strain M124. Protease activity was measured at pH 5.5 in first 5 strains and at pH 4.5 in the last three deletion strains. Protease activity is against green fluorescent casein. The six-fold protease deletion strain has only 6% of the wild type parent strain and the 7-fold protease deletion strain protease activity was about 40% less than the 6-fold protease deletion strain activity.

**EXAMPLE 13: Useful polynucleotide and amino acid sequences for practicing the invention**

[0350]

| SEQ |                                                                                                        |
|-----|--------------------------------------------------------------------------------------------------------|
| 1   | Human <i>FUT8</i> optimized coding sequence (as present in transformed strains)                        |
| 2   | <i>C. elegans</i> GMD optimized coding sequence (as present in transformed strains)                    |
| 3   | <i>C. elegans</i> FX optimized coding sequence (as present in transformed strains)                     |
| 4   | <i>C. elegans</i> GDP-fucose transporter optimized coding sequence (as present in transformed strains) |
| 5   | <i>T. reesei</i> KRE2/MNT1 coding sequence used for Golgi targeting                                    |
| 6   | Human <i>FUT8</i> protein sequence                                                                     |
| 7   | <i>C. elegans</i> GMD protein sequence                                                                 |
| 8   | <i>C. elegans</i> FX protein sequence                                                                  |
| 9   | <i>C. elegans</i> GDP-fucose transporter                                                               |
| 10  | <i>T. reesei</i> KRE2/MNT1 protein sequence used for Golgi targeting                                   |
| 11  | 5' flank nucleic acid sequence of <i>pep4</i> in the cassette used for integration                     |
| 12  | 3' flank nucleic acid sequence of <i>pep4</i> in the cassette used for integration                     |
| 13  | <i>H. pylori</i> FX protein sequence                                                                   |
| 14  | <i>H. pylori</i> optimized FX coding sequence                                                          |
| 15  | <i>H. pylori</i> GMD protein sequence                                                                  |
| 16  | <i>H. pylori</i> optimized GMD coding sequence                                                         |
| 17  | <i>T. reesei</i> amino acid <i>pep1</i>                                                                |
| 18  | <i>T. reesei</i> amino acid <i>pep2</i>                                                                |
| 19  | <i>T. reesei</i> amino acid <i>pep3</i>                                                                |
| 20  | <i>T. reesei</i> amino acid <i>pep4</i>                                                                |
| 21  | <i>T. reesei</i> amino acid <i>pep5</i>                                                                |
| 22  | T078_74156_3orf_pcr (primer)                                                                           |
| 23  | <i>T. reesei</i> amino acid <i>pep7</i>                                                                |
| 24  | <i>T. reesei</i> amino acid <i>tsp1</i>                                                                |
| 25  | <i>T. reesei</i> amino acid <i>slp1</i>                                                                |
| 26  | <i>T. reesei</i> amino acid <i>slp2</i>                                                                |

| SEQ |                                  |
|-----|----------------------------------|
| 27  | <i>T. reesei</i> amino acid slp3 |
| 28  | <i>T. reesei</i> amino acid slp5 |
| 29  | <i>T. reesei</i> amino acid slp6 |
| 30  | <i>T. reesei</i> amino acid slp7 |
| 31  | <i>T. reesei</i> amino acid slp8 |
| 32  | <i>T. reesei</i> amino acid gap1 |
| 33  | <i>T. reesei</i> amino acid gap2 |
| 34  | <i>T. reesei</i> amino acid tpp1 |
| 35  | T298_77579_5f(primer)            |
| 36  | T_758_pTTv224_1(primer)          |
| 37  | T729_pTTv227_3(primer)           |
| 38  | T730_pTTv227_4(primer)           |
| 39  | T731_pTTv227_5(primer)           |
| 40  | T732_pTTv227_6(primer)           |
| 41  | T733_pTTv227_7(primer)           |
| 42  | T734_pTTv227_8(primer)           |
| 43  | T735_pTTv227_9(primer)           |
| 44  | T759_pTTv224_2(primer)           |
| 45  | T760_pTTv224_3(primer)           |
| 46  | T761_pTTv224_4(primer)           |
| 47  | T762_pTTv225_1(primer)           |
| 48  | T763_pTTv225_2(primer)           |
| 49  | T764_pTTv225_3(primer)           |
| 50  | T759_pTTv224_2(primer)           |
| 51  | T765_pTTv225_4(primer)           |
| 52  | T738_pTTv228_2(primer)           |
| 53  | T739_pTTv228_3(primer)           |
| 54  | T740_pTTv228_4(primer)           |
| 55  | T741_pTTv228_5(primer)           |
| 56  | T742_pTTv228_6(primer)           |
| 57  | T743_pTTv228_7(primer)           |
| 58  | T744_pTTv228_8(primer)           |
| 59  | T766_pTTv225_5(primer)           |
| 60  | T301_77579_3r (primer)           |
| 61  | T780_pTTv226_1(primer)           |
| 62  | T781_pTTv226_2(primer)           |
| 63  | T782_pTTv226_3(primer)           |
| 64  | T783_pTTv226_4(primer)           |
| 65  | T023_pRS426_5.1sekv(primer)      |
| 66  | T228_pRS426_3.1sekv(primer)      |
| 67  | T094_pyr4_F(primer)              |

| SEQ |                                  |
|-----|----------------------------------|
| 68  | T770_alg3_3pr_int_pyr4_F(primer) |
| 69  | T785(primer)                     |
| 70  | T786(primer)                     |
| 71  | T787(primer)                     |
| 72  | T790(primer)                     |
| 73  | T791(primer)                     |
| 74  | T792(primer)                     |
| 75  | T793(primer)                     |
| 76  | T794(primer)                     |
| 77  | T795(primer)                     |
| 78  | T796(primer)                     |
| 79  | T797(primer)                     |
| 80  | T798(primer)                     |
| 81  | T799(primer)                     |
| 82  | T800(primer)                     |
| 83  | T801(primer)                     |
| 84  | T802(primer)                     |
| 85  | T803(primer)                     |
| 86  | T804(primer)                     |
| 87  | T805(primer)                     |
| 88  | T809(primer)                     |
| 89  | T810(primer)                     |
| 90  | T811(primer)                     |
| 91  | T812(primer)                     |
| 92  | T813(primer)                     |
| 93  | T814(primer)                     |
| 94  | T815(primer)                     |
| 95  | T816(primer)                     |
| 96  | T817(primer)                     |
| 97  | T818(primer)                     |
| 98  | T819(primer)                     |
| 99  | T820(primer)                     |
| 100 | T821(primer)                     |
| 101 | T824(primer)                     |
| 102 | T825(primer)                     |
| 103 | T302_77579_5int(primer)          |
| 104 | T018_pgpdA_5rev(primer)          |
| 105 | T816(primer)                     |
| 106 | T415_77579_3screen(primer)       |
| 107 | T821(primer)                     |

| SEQ |                                                                                                          |
|-----|----------------------------------------------------------------------------------------------------------|
| 108 | T416_77579_probeF(primer)                                                                                |
| 109 | T417_77579_probeR(primer)                                                                                |
| 110 | Hp GMD forw(primer)                                                                                      |
| 111 | Hp GMD rev(primer)                                                                                       |
| 112 | gi 324519268 gb ADY47333.1  GDP-L-fucose synthase [Ascaris suum]                                         |
| 113 | gi 170576679 ref XP_001893725.1  GDP-L-fucose synthetase [Brugia malayi]                                 |
| 114 | gi 363731098 ref XP_418405.3  PREDICTED: GDP-L-fucose synthase isoform 2 [Gallus gallus]                 |
| 115 | gi 116267961 ref NP_001070752.1  uncharacterized protein LOC768139 [Danio rerio]                         |
| 116 | gi 116063448 gb AAI23105.1  LOC398450 protein [Xenopus laevis]                                           |
| 117 | gi 114051291 ref NP_001039604.1  GDP-L-fucose synthase [Bos taurus]                                      |
| 118 | 149757572 ref XP_001505053.1  PREDICTED: GDP-L-fucose synthase [Equus caballus]                          |
| 119 | gi 73974844 ref XP_532346.2  PREDICTED: GDP-L-fucose synthase [Canis lupus familiaris]                   |
| 120 | gi 4507709 ref NP_003304.1  GDP-L-fucose synthase [Homo sapiens]                                         |
| 121 | gi 350538233 ref NP_001233708.1  GDP-L-fucose synthase [Cricetulus griseus]                              |
| 122 | gi 188536096 ref NP_001120927.1  GDP-L-fucose synthase [Rattus norvegicus]                               |
| 123 | gi 13654268 ref NP_112478.1  GDP-L-fucose synthase [Mus musculus]                                        |
| 124 | gi 312070424 ref XP_003138140.1  GDP-mannose 4,6-dehydratase [Loa loa]                                   |
| 125 | gi 170587907 ref XP_001898715.1  GDP-mannose 4,6-dehydratase [Brugia malayi]                             |
| 126 | gi 118778930 ref XP_308963.3  AGAP006783-PA [Anopheles gambiae str. PEST]                                |
| 127 | gi 57108166 ref XP_001650108.1  gdp mannose-4,6-dehydratase [Aedes aegypti]                              |
| 128 | gi 156523058 ref NP_001095945.1  GDP-mannose 4,6 dehydratase isoform 1 [Danio rerio]                     |
| 129 | gi 213515196 ref NP_001134845.1  GDP-mannose 4,6 dehydratase [Salmo salar]                               |
| 130 | gi 328790131 ref XP_395164.3  PREDICTED: GDP-mannose 4,6 dehydratase-like [Apis mellifera]               |
| 131 | gi 24158427 ref NP_608888.2  GDP-mannose 4,6-dehydratase [Drosophila melanogaster]                       |
| 132 | gi 147899928 ref NP_001080352.1  GDP-mannose 4,6-dehydratase [Xenopus laevis]                            |
| 133 | gi 122692409 ref NP_001073800.1  GDP-mannose 4,6 dehydratase [Bos taurus]                                |
| 134 | Article I. gi 350539705 ref NP_001233625.1  GDP-mannose 4,6 dehydratase [Cricetulus griseus]             |
| 135 | gi 22122523 ref NP_666153.1  GDP-mannose 4,6 dehydratase [Mus musculus]                                  |
| 136 | gi 88853855 ref NP_001034695.1  GDP-mannose 4,6 dehydratase [Rattus norvegicus]                          |
| 137 | gi 4504031 ref NP_001491.1  GDP-mannose 4,6 dehydratase isoform 1 [Homo sapiens]                         |
| 138 | gi 198423994 ref XP_002131034.1  PREDICTED: similar to GDP-mannose 4, 6-dehydratase [Ciona intestinalis] |
| 139 | gi 337754860 ref YP_004647371.1  GDP-mannose 4,6-dehydratase [Francisella sp. TX077308]                  |
| 140 | gi 380558719 gb EIA81894.1  GDP-D-mannose dehydratase [Campylobacter coli 59-2]                          |
| 141 | gi 325287517 ref YP-004263307.1  GDP-mannose 4,6-dehydratase [Cellulophaga]                              |

|         |                                                                                                                     |
|---------|---------------------------------------------------------------------------------------------------------------------|
| SEQ     |                                                                                                                     |
|         | lytica DSM 7489]                                                                                                    |
| 142     | gi 296482935 gb DAA25050.1  alpha-(1,6)-fucosyltransferase [Bos taurus]                                             |
| 143     | gi 52546726 ref NP_001005262.1  alpha-(1,6)-fucosyltransferase [Canis lupus familiaris]                             |
| 144     | gi 148704495 gb EDL36442.1  fucosyltransferase 8, isoform CRA_b [Mus musculus]                                      |
| 145     | gi 149051511 gb EDM03684.1  rCG62185, isoform CRA_a [Rattus norvegicus]                                             |
| 146     | gi 354479164 ref XP_003501783.1  PREDICTED: alpha-(1,6)-fucosyltransferase [Cricetulus griseus]                     |
| 147     | gi 52345460 ref NP_001004766.1  alpha-(1,6)-fucosyltransferase [Gallus gallus]                                      |
| 148     | gi 47575776 ref NP_001001232.1  alpha-(1,6)-fucosyltransferase [Xenopus (Silurana) tropicalis]                      |
| 149     | gi 51467976 ref NP_001003855.1  alpha-(1,6)-fucosyltransferase [Danio rerio]                                        |
| 150     | gi 324513182 gb ADY45426.1  GDP-fucose transporter [Ascaris suum]                                                   |
| 151     | gi 312066547 ref XP_003136322.1  GDP-fucose transporter [Loa loa]                                                   |
| 152     | gi 157820319 ref NP_001101218.1  GDP-fucose transporter 1 [Rattus norvegicus]                                       |
| 153     | gi 350538845 ref NP_001233737.1  GDP-fucose transporter [Cricetulus griseus]                                        |
| 154     | gi 74194961 dbj BAE26053.1  unnamed protein product [Mus musculus]                                                  |
| 155     | gi 22003876 ref NP_665831.1  GDP-fucose transporter 1 isoform 2 [Mus musculus]                                      |
| 156     | gi 313851048 ref NP_001186580.1  GDP-fucose transporter 1 [Gallus gallus]                                           |
| 157     | gi 301609135 ref XP_002934120.1  PREDICTED: GDP-fucose transporter 1-like isoform 1 [Xenopus (Silurana) tropicalis] |
| 158     | gi 56693251 ref NP_001008590.1  GDP-fucose transporter 1 [Danio rerio]                                              |
| 159     | gi 155372141 ref NP_001094680.1  GDP-fucose transporter 1 [Bos taurus]                                              |
| 160     | gi 308235937 ref NP_001184118.1  GDP-fucose transporter 1 [Canis lupus familiaris]                                  |
| 161     | gi 223671915 ref NP_060859.4  GDP-fucose transporter 1 isoform a [Homo sapiens]                                     |
| 162     | gi 223671917 ref NP_001138737.1  GDP-fucose transporter 1 isoform b [Homo sapiens]                                  |
| 163     | Nucleic acid sequence of <i>T. reesei</i> alg3 gene                                                                 |
| 164     | Amino acid sequence of <i>T. reesei</i> alg3 protein                                                                |
| 165     | Human GnTI amino acid sequence                                                                                      |
| 166     | Human GnTII amino acid sequence                                                                                     |
| 167     | Hp FX forw(primer)                                                                                                  |
| 168     | Hp FX rev(primer)                                                                                                   |
| 169     | Ann 79 hph(primer)                                                                                                  |
| 170     | Hph-gene 3.1 PCR(primer)                                                                                            |
| 171-173 | KRE2 targeting peptide<br>(cytoplasmic, transmembrane, luminal)                                                     |
| 174-176 | KRE2 alternative1<br>(cytoplasmic, transmembrane, luminal)                                                          |
| 177-179 | OCH1<br>(cytoplasmic, transmembrane, luminal)                                                                       |
| 180-182 | OCH1 alternative1                                                                                                   |

|         |                                                                                                                                                               |
|---------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SEQ     |                                                                                                                                                               |
|         | (cytoplasmic, transmembrane, luminal)                                                                                                                         |
| 183-185 | MNN9 targeting peptide                                                                                                                                        |
|         | (cytoplasmic, transmembrane, luminal)                                                                                                                         |
| 186-188 | MNN9 alternativel targeting peptide                                                                                                                           |
|         | (cytoplasmic, transmembrane, luminal)                                                                                                                         |
| 189-191 | MNN9 alternative2 targeting peptide (cytoplasmic, transmembrane, luminal)                                                                                     |
| 192-194 | MNN10 targeting peptide                                                                                                                                       |
|         | (cytoplasmic, transmembrane, luminal)                                                                                                                         |
| 195-197 | MNN10 alternativel targeting peptide                                                                                                                          |
|         | (cytoplasmic, transmembrane, luminal)                                                                                                                         |
| 198-200 | MNS1 targeting peptide                                                                                                                                        |
|         | (cytoplasmic, transmembrane, luminal)                                                                                                                         |
| 201-203 | MNS1 alternativel targeting peptide                                                                                                                           |
|         | (cytoplasmic, transmembrane, luminal)                                                                                                                         |
| 204-206 | MNS1 alternative2 targeting peptide                                                                                                                           |
|         | (cytoplasmic, transmembrane, luminal)                                                                                                                         |
| 207-209 | MNS1 alternative3 targeting peptide                                                                                                                           |
|         | (cytoplasmic, transmembrane, luminal)                                                                                                                         |
| 210-211 | MNS1 alternative4 targeting peptide                                                                                                                           |
|         | (transmembrane, luminal)                                                                                                                                      |
| 212-214 | VAN1 targeting peptide                                                                                                                                        |
|         | (cytoplasmic, transmembrane, luminal)                                                                                                                         |
| 215-217 | VAN1 alternativel targeting peptide                                                                                                                           |
|         | (cytoplasmic, transmembrane, luminal)                                                                                                                         |
| 218-220 | VAN1 alternative2 targeting peptide                                                                                                                           |
|         | (cytoplasmic, transmembrane, luminal)                                                                                                                         |
| 221-223 | Other01 targeting peptide                                                                                                                                     |
|         | (cytoplasmic, transmembrane, luminal)                                                                                                                         |
| 224-226 | Other02 targeting peptide                                                                                                                                     |
|         | (cytoplasmic, transmembrane, luminal)                                                                                                                         |
| 227     | >gi 74583855 sp Q12520.1 HUT1_YEAST RecName: Full=UDP-galactose transporter homolog 1; AltName: Full=Multicopy suppressor of leflunomide-sensitivty protein 6 |
| 228     | >gi 1119217 gb AAB86498.1  UDP-galactose-4-epimerase [Homo sapiens]                                                                                           |
| 229     | Kre2 (Tre21576) amino acids 1-106                                                                                                                             |
| 230     | Kre2 (Tre21576) amino acids 1-83                                                                                                                              |
| 231     | Kre2 (Tre21576) amino acids 1-51                                                                                                                              |
| 232     | Homo sapiens glucosamine UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (GNE) Q6QNY5                                                          |
| 233     | Homo sapiens glucosamine UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (GNE) NP_001121699                                                    |
| 234     | Homo sapiens N-acetylneuraminic acid synthase (NANS) NP_061819                                                                                                |
| 235     | Homo sapiens N-acetylneuraminic acid phosphatase (NANP) NP_689880                                                                                             |

| SEQ |                                                                                            |
|-----|--------------------------------------------------------------------------------------------|
| 236 | Homo sapiens cytidine monophosphate N-acetylneuraminic acid synthetase (CMAS) NP_ 061156   |
| 237 | Mus musculus solute carrier family 35 member 1 (SLC35A1) Q61420                            |
| 238 | Mus musculus $\beta$ -galactoside $\alpha$ 2,6-sialyltransferase 1 (St6gal1) NP_ 001239434 |
| 239 | Homo sapiens $\beta$ -galactoside $\alpha$ 2,3-sialyltransferase 4 (ST3GAL4) NP_ 006269    |
| 240 | Chimeric GnTII/GnTI amino acid sequence                                                    |
| 241 | 5flankfw                                                                                   |
| 242 | 5flankrev                                                                                  |
| 243 | 3flankfw                                                                                   |
| 244 | 3flankrev                                                                                  |
| 245 | PTfwd                                                                                      |
| 246 | PTrev                                                                                      |
| 247 | T315_pyr4_for                                                                              |
| 248 | T316_pyr4_rev                                                                              |
| 249 | T317_pyr4_loop_for                                                                         |
| 250 | T318_pyr4_loop_rev                                                                         |
| 251 | T075_74156_5int                                                                            |
| 252 | T032_Bar_end_for                                                                           |
| 253 | T076_74156_3int                                                                            |
| 254 | T031_Bar_begin_rev2                                                                        |
| 255 | T075_74156_5int                                                                            |
| 256 | T027_Pyr4_orf_start_rev                                                                    |
| 257 | T077_74156_5orf_pcr                                                                        |
| 258 | orf_rev                                                                                    |
| 259 | T303_71322_5f                                                                              |
| 260 | T304_71322_5r_pt                                                                           |
| 261 | T305_71322_3Cpt                                                                            |
| 262 | T306_71322_3r                                                                              |
| 263 | T083_74156_5a_seq                                                                          |
| 264 | T084_74156_3a_seq                                                                          |
| 265 | T307_71322_5int                                                                            |
| 266 | T027_Pyr4_orf_start_rev                                                                    |
| 267 | T308_71322_3int                                                                            |
| 268 | T028_Pyr4_flank_rev                                                                        |
| 269 | T309_71322_5orfpcr                                                                         |
| 270 | T310_71322_3orfpcr                                                                         |
| 271 | 5flankfw_vect                                                                              |
| 272 | slp1_5flankrev_pyr4Prom                                                                    |
| 273 | slp1_3flankfw_pyr4Term                                                                     |
| 274 | 3flankrev_vect                                                                             |
| 275 | T307_71322_5int                                                                            |



| SEQ |                           |
|-----|---------------------------|
| 276 | T026_Pyr4_orf_5rev2       |
| 277 | T308_71322_3int           |
| 278 | T028_Pyr4_flank_rev       |
| 279 | T079_slp1_scrn_5forw      |
| 280 | T026_Pyr4_orf_5rev2       |
| 281 | T080_slp1_scrn_3rev       |
| 282 | T028_Pyr4_flank_rev       |
| 283 | T081_slp1_orf_fw          |
| 284 | T082_slp1_orf_rev         |
| 285 | JJ-045 primer             |
| 286 | JJ-046 primer             |
| 287 | JJ-047 primer             |
| 288 | JJ-048 primer             |
| 289 | T079_slp1_scrn_5forw      |
| 290 | T026_Pyr4_orf_5rev2       |
| 291 | T080_slp1_scrn_3rev       |
| 292 | T028_Pyr4_flank_rev       |
| 293 | T052_gap1_5screen_F       |
| 294 | T026_Pyr4_orf_5rev2       |
| 295 | T053_gap1_3screen_R       |
| 296 | T028_Pyr4_flank_rev       |
| 297 | T109_gap1_ORF_F           |
| 298 | T110_gap1_ORF_R           |
| 299 | T101_gap2_5flank_F_pRS426 |
| 300 | T102_gap2_5flank_R_pyr4   |
| 301 | T103gap2-loop_F_pyr4      |
| 302 | T104gap2-loop_R           |
| 303 | T105gap2_3flank_F_loop    |
| 304 | T106_gap2_3flank_R_pRS426 |
| 305 | T052_gap1_5screen_F       |
| 306 | T026_Pyr4_orf_5rev2       |
| 307 | T053_gap1_3screen_R       |
| 308 | T028_Pyr4_flank_rev       |
| 309 | T048_gap2_5screen_F       |
| 310 | T026_Pyr4_orf_5rev2       |
| 311 | T049_gap2_3screen_R       |
| 312 | T028_Pyr4_flank_rev       |
| 313 | T107_gap2_ORF_F           |
| 314 | T108_gap2_ORF_R           |
| 315 | T209_pyr4_f_recpep4_5f    |

| SEQ |                               |
|-----|-------------------------------|
| 316 | T211_pep4_loop_f_recpyr4      |
| 317 | T212_pep4_loop_r_recpep4_3f   |
| 318 | T222_gap2_5f_f2               |
| 319 | T049_gap2_3screen_R           |
| 320 | T302_77579_5int               |
| 321 | T027_Pyr4_orf_start_rev       |
| 322 | T415_77579_3screen            |
| 323 | T061_pyr4_orf_screen_2F       |
| 324 | T416_77579_probeF             |
| 325 | T417_77579_probeR             |
| 326 | T346_pep3_5f_for              |
| 327 | T347_pep3_5f_rev              |
| 328 | T348_pep3_loop_for            |
| 329 | T349_pep3_loop_rev            |
| 330 | T350_pep3_3f_for              |
| 331 | T351_pep3_3f_rev              |
| 332 | T389_cDNApromoter_pep3flank   |
| 333 | T138_cDNA1_Rev                |
| 334 | T139_123561For_cDNA1          |
| 335 | 123561 Rev                    |
| 336 | trpCtermFor_123561            |
| 337 | T390_trpCtermR_AmdS           |
| 338 | T391_AmdS_endR                |
| 339 | T390_trpCtermR_AmdS           |
| 340 | T428_pep3_3flankDR_F-trpCterm |
| 341 | T429_pep3_3flankDR_R-pyr4     |
| 342 | T094_pyr4_F                   |
| 343 | T430_pyr4_R-pep3_3flank       |
| 344 | T302_77579_5int               |
| 345 | T214_pep4_3f_seq_r1           |
| 346 | T625_pep3_5int_new            |
| 347 | T140_cDNA1 promoter_seqR1     |
| 348 | T626_pep3_3int_new            |
| 349 | T061_pyr4_orf_screen_2F       |
| 350 | T352_pep3_orf_for             |
| 351 | T353_pep3_orf_rev             |
| 352 | T372_pep5_5f_for              |
| 353 | T373_pep5_5f_rev              |
| 354 | T376_pep5_5DR_for             |
| 355 | T377_pep5_5DR_rev             |
| 356 | T378_pep5_3f_for              |

| SEQ |                       |
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| 357 | T379_pep5_3f_rev      |
| 358 | T374_bar_recpyr4_for2 |
| 359 | T375_bar_rev          |
| 360 | 5flankfw_pRS426       |
| 361 | 5flankrev_pyr4        |
| 362 | 3flankfw_pyr4         |
| 363 | 3flankrev_pRS426      |
| 364 | scrn_5forw            |
| 365 | T026_Pyr4_orf_5rev2   |
| 366 | scrn_3rev             |
| 367 | T028_Pyr4_flank_rev   |
| 368 | orf_fw                |
| 369 | orf_rev               |
| 370 | 5flankfw_pRS426       |
| 371 | 5flankrev_pyr4        |
| 372 | 3flankfw_pyr4         |
| 373 | 3flankrev_pRS426      |
| 374 | scrn_5forw            |
| 375 | T026_Pyr4_orf_5rev2   |
| 376 | scrn_3rev             |
| 377 | T028_Pyr4_flank_rev   |
| 378 | orf_fw                |
| 379 | orf_rev               |
| 380 | 5flankfw_pRS426       |
| 381 | 5flankrev_pyr4        |
| 382 | 3flankfw_pyr4         |
| 383 | 3flankrev_pRS426      |
| 384 | scrn_5forw            |
| 385 | T026_Pyr4_orf_5rev2   |
| 386 | scrn_3rev             |
| 387 | T028_Pyr4_flank_rev   |
| 388 | orf_fw                |
| 389 | orf_rev               |
| 390 | 5flankfw_pRS426       |
| 391 | 5flankrev_pyr4        |
| 392 | 3flankfw_pyr4         |
| 393 | 3flankrev_pRS426      |
| 394 | scrn_5forw            |
| 395 | T026_Pyr4_orf_5rev2   |
| 396 | scrn_3rev             |

| SEQ |                                |
|-----|--------------------------------|
| 397 | T028_Pyr4_flank_rev            |
| 398 | orf_fw                         |
| 399 | orf_rev                        |
| 400 | 5flankfw_pRS426                |
| 401 | 5flankrev_pyr4                 |
| 402 | 3flankfw_pyr4                  |
| 403 | 3flankrev_pRS426               |
| 404 | scrn_5forw                     |
| 405 | T026_Pyr4_orf_5rev2            |
| 406 | scrn_3rev                      |
| 407 | T028_Pyr4_flank_rev            |
| 408 | orf_fw                         |
| 409 | Coding sequence of human GalT1 |
| 410 | Trichoderma reesei pep8        |
| 411 | Trichoderma reesei pep11       |
| 412 | Trichoderma reesei pep12       |
| 413 | T047_trpC_term_end_F           |
| 414 | T854_pep3_3f_r2                |
| 415 | T488_pyr4_5utr_rev             |
| 416 | T061_pyr4_orf_screen_2F        |
| 417 | T855_pep3_orf f3               |
| 418 | T754_pep3_orf_rev2             |
| 419 | T627_pep5_5int_new             |
| 420 | T488_pyr4_5utr_rev             |
| 421 | T061_pyr4_orf_screen_2F        |
| 422 | T628_pep5_3int_new             |
| 423 | T418_pep5_orf_for              |
| 424 | T419_pep5_orf_rev              |
| 425 | T859_pep5_orf_f2               |
| 426 | T860_pep5_orf_f3               |
| 427 | T861_pep5_orf_r2               |
| 428 | T477_pep12_5f_for              |
| 429 | T478_pep12_5f_rev              |
| 430 | T479_pep12_DR_for              |
| 431 | T480_pep12_DR_rev              |
| 432 | T481_pep12_3f_for              |
| 433 | T482_pep12_3f_rev              |
| 434 | T858_pep5_5f_f3                |
| 435 | T755_pep5_3f_rev3              |
| 436 | T627_pep5_5int_new             |
| 437 | T488_pyr4_5utr_rev             |

| SEQ |                              |
|-----|------------------------------|
| 438 | T860_pep5_orf_f3             |
| 439 | T861_pep5_orf_r2             |
| 440 | T517_pep12_5int              |
| 441 | T026_Pyr4_orf_5rev2          |
| 442 | T061_pyr4_orf_screen_2F      |
| 443 | T518_pep12_3int              |
| 444 | T486_pep12_orf_probef        |
| 445 | T487_pep12_orf_prober        |
| 446 | T1057_pep12_orf_probef2      |
| 447 | T1058_pep12_orf_prober2      |
| 448 | T431_pep2-5flankF-pRS426     |
| 449 | T629_pep2_5f_rev_pyr4        |
| 450 | T630_pep2_5DR_for_trpC       |
| 451 | T631_pep2_5DR_rev_cDNA1      |
| 452 | T632_pep2_3f_for_tcbh2       |
| 453 | T633_pep2_3f_rev             |
| 454 | T491_hph_recpyr4_for3        |
| 455 | T492_hph_rev2                |
| 456 | T495_cDNA1_for               |
| 457 | T138_cDNA1_Rev               |
| 458 | T139_123561 For_cDNA1        |
| 459 | T516_123561 Rev              |
| 460 | T496_tcbh2_for               |
| 461 | T497_tcbh2_rev               |
| 462 | T858_pep5_5f_f3              |
| 463 | T755_pep5_3f_rev3            |
| 464 | T627_pep5_5int_new           |
| 465 | T488_pyr4_5utr_rev           |
| 466 | T860_pep5_orf_f3             |
| 467 | T861_pep5_orf_r2             |
| 468 | T596_pep2 fwd 5'flank screen |
| 469 | T026_Pyr4_orf_5rev2          |
| 470 | T061_pyr4_orf_screen_2F      |
| 471 | T600_pep2 rev 3'flank screen |
| 472 | T601_pep2 fwd                |
| 473 | T623_pep2 rev                |
| 474 | T1077_pep2_orf_probef2       |
| 475 | T1078_pep2_orf_prober2       |
| 476 | T1009_pep11_5flkfw_vector    |
| 477 | T1010_pep11_5flkrev_pyr4Prom |

| SEQ |                              |
|-----|------------------------------|
| 478 | T1144_ pep11_5dr_for         |
| 479 | T1145_ pep11_5dr_rev         |
| 480 | T1146_ pep1_3f_for           |
| 481 | T1012_ pep11_3flkrev_vector  |
| 482 | T1162_ pep2_5f_f2            |
| 483 | T1163_ pep2_3f_r2            |
| 484 | T1162_ pep2_5f_f2            |
| 485 | T488_ pyr4_5utr_rev          |
| 486 | T601_ pep2 fwd               |
| 487 | T623_ pep2 rev               |
| 488 | T1013_ pep11_screen_5flk_fwd |
| 489 | T488_ pyr4_5utr_rev          |
| 490 | T061_ pyr4_orf_screen_2F     |
| 491 | T1016_ pep11_screen_3flk_rev |
| 492 | T1017_ pep11_orf_fwd         |
| 493 | T1018_ pep11_orf_rev         |
| 494 | T1019_ pep8_5flkfw_vector    |
| 495 | T1020_ pep8_5flkrev_pyr4Prom |
| 496 | T1167_ pep8_5DR_for          |
| 497 | T1168_ pep8_5DR_rev          |
| 498 | T1169_ pep8_3f_for2          |
| 499 | T1022_ pep8_3flkrev_vector   |
| 500 | T1019_ pep8_5flkfw_vector    |
| 501 | T1020_ pep8_5flkrev_pyr4Prom |
| 502 | T1021_ pep8_3flkfw_pvr4loop  |
| 503 | T1022_ pep8_3flkrev_vector   |
| 504 | T311_82623_5for              |
| 505 | T1190_ tpp1_5f_rev2          |
| 506 | T1191_ tpp1_5dr_for          |
| 507 | T1192_ tpp1_5dr_rev          |
| 508 | T1193_ tpp1_3f_for2          |
| 509 | T314_82623_3rev              |
| 510 | JJ-037 primer                |
| 511 | JJ-038 primer                |
| 512 | JJ-039 primer                |
| 513 | JJ-040 primer                |
| 514 | JJ-041 primer                |
| 515 | JJ-042 primer                |
| 516 | JJ-043 primer                |
| 517 | JJ-044 primer                |
| 518 | T1088_ slp7_5flkfw_vector    |

| SEQ |                            |
|-----|----------------------------|
| 519 | T1090_slp7_3flkfw_pvr4loop |
| 520 | T1091_slp7_3flkrev_vector  |
| 521 | T1203_slp8_5f_f            |
| 522 | T1204_slp8_5f_r            |
| 523 | T1205_slp8_5dr_f           |
| 524 | T1206_slp8_5dr_r           |
| 525 | T1207_slp8_3f_f            |
| 526 | T1208_slp8_3f_r            |
| 527 | T054_slp2_5screen_F        |
| 528 | T1084_screen_5flk_pyr_rev  |
| 529 | T055_slp2_3screen_R        |
| 530 | T028_Pyr4_flank_rev        |
| 531 | T111_slp2_ORF_F            |
| 532 | T112_slp2_ORF_R            |
| 533 | T1372_kre2_recgpa_for      |
| 534 | T337_21576_r               |
| 535 | T1373_qalt_44_for          |
| 536 | T1374_galt_rev             |
| 537 | T1375_qalt_78_for          |
| 538 | T1374_qalt_rev             |
| 539 | T1369_pvr4_for             |
| 540 | T1370_pvr4_rechphnew_rev   |
| 541 | T1367_egl2_3dr_for         |
| 542 | T1368_egl2_3dr_rev         |
| 543 | T161_tcbh2_seq_f2          |
| 544 | T1371_tcbh2_recelg2_rev    |
| 545 | T1560                      |
| 546 | T1561                      |
| 547 | T1562                      |
| 548 | T1563                      |
| 549 | T1410_egl2_5int_f3         |
| 550 | T176_pcbh1_seq_r4          |
| 551 | T1158_egl2 3pr intR2       |
| 552 | T1411_cbh2t_end_f          |
| 553 | T1412_egl2_orf_f1          |
| 554 | T1413_egl2_orf_r1          |

## SEQUENCE LISTING

[0351]

&lt;110&gt; Novartis

&lt;120&gt; PRODUCTION OF FUCOSYLATED GLYCOPROTEINS

&lt;130&gt; PAT055114

&lt;160&gt; 554

&lt;170&gt; PatentIn version 3.5

&lt;210&gt; 1

&lt;211&gt; 1728

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 1

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|------------|------------|-------------|-------------|------------|-------------|------|
| atgcgccctt | ggaccggcag | ctggcgggtg  | atcatgctca  | ttctgttcgc | ctgggggtacg | 60   |
| ctcctttttt | acatcggcgg | acacctggtc  | cgagacaacg  | accaccctga | tcattcctct  | 120  |
| cgcgagttgt | ctaagatcct | ggctaagctc  | gagaggtctc  | agcagcagaa | cgaggacctg  | 180  |
| cgcggtatgg | cgaagacctt | tgcgcatccc  | gaggggcccga | ttgaccaagg | cccagcgatc  | 240  |
| ggcggagtca | gagtgcctcg | ggaacagctg  | gttaaggcca  | aagagcagat | tgagaattac  | 300  |
| aagaagcaga | ctcgggaacg | cctcggtaag  | gatcacgaga  | tcttgcgccg | taggatcgag  | 360  |
| aacggcgcaa | aagaactctg | gttcttcctg  | cagtccgagc  | tgaagaagct | caagaacctt  | 420  |
| gagggaaacg | agctgcacac | ccacgcccgc  | gagtttctct  | tggaacctgg | ccaccatgaa  | 480  |
| cgatcaatta | tgaccgatct | ctactatctc  | tgcgagacag  | acggcgctgg | cgactggcgc  | 540  |
| gagaaagagg | ccaaggacct | gaccgagctt  | gtccagcgcc  | ggatcacgta | cctccagaac  | 600  |
| cccaaggatt | gcagcaaggc | gaagaagctg  | gtctgcaata  | ttaacaaggg | ctgcggttac  | 660  |
| ggatgtcaac | tccaccacgt | cgtgtattgc  | ttcatgatcg  | cctacggcac | ccagcgaacg  | 720  |
| ctgatcttgg | agtcccagaa | ctggcggtac  | gcaaccggcg  | ggtgggaaac | tgttttccgc  | 780  |
| cctgtcagtg | agacatgcac | ggacagggtc  | ggcattagca  | ccggccactg | gtcgggtgag  | 840  |
| gtcaaggaca | agaacgtcca | ggtggtgagg  | cttccgatcg  | ttgactccct | ccatcccaga  | 900  |
| cccccttacc | tccccctggc | cgtcccggaa  | gatctcgctg  | accgcctggg | ccgagtgcac  | 960  |
| ggcgacccag | ccgtctggtg | ggtcagccag  | tttggttaagt | atctcatccg | cccccaacct  | 1020 |
| tggttgagga | aagagattga | ggaagcgact  | aagaagctgg  | gattcaagca | ccccgtcatc  | 1080 |
| ggcgtccacg | tgccgcccgc | cgataagggt  | ggaaccgagg  | ccgctttcca | cccgatcgaa  | 1140 |
| gagtacatgg | tccacgtgga | ggaacatttt  | cagctgcttg  | ccgtaggat  | gcaggtcgac  | 1200 |
| aagaagcgcg | tctacctcgc | cacggacgac  | ccctcgctgc  | tcaaagaggg | aaagacgaag  | 1260 |
| taccctaact | atgagttcat | cagcgataat  | tccatttctt  | ggtcagccgg | cctccacaac  | 1320 |
| cgtacacacg | agaactccct | gcgcgggtgt  | atccttgaca  | ttcacttttt | gtcgcaggcg  | 1380 |
| gacttcctcg | tctgtacttt | cagctcccag  | gtctgccggg  | tggtttacga | gatcatgcaa  | 1440 |
| acactgcac  | cagatgccag | cgccaaactt  | cactctctcg  | acgacatcta | ctatttcggc  | 1500 |
| ggccagaacg | cccacaacca | gattgctatc  | tacgcgcacc  | agcccagaac | cgccgacgag  | 1560 |
| atccccatgg | aaccggggcg | tatcattggg  | gtcgcaggca  | atcattggga | cggatactcg  | 1620 |
| aaggggggtg | accgcaagct | cggccgtacg  | ggcctgtacc  | cttcctataa | ggtcgagag   | 1680 |
| aagatcgaga | ctgttaagta | cccgaacctac | cccgaggccg  | agaagtaa   |             | 1728 |



&lt;210&gt; 2

&lt;211&gt; 1200

&lt;212&gt; DNA

<213> *Caenorhabditis elegans*

&lt;400&gt; 2

|                                                                    |      |
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| tctaaagttg agggctctga agcgtgtatc ggaatgagcc acgaagtgtc cactactcca  | 120  |
| gctgctgagt tggctgcttt cagagcacgc aagggtgctc tgattactgg aatttctgga  | 180  |
| caagacggtt catatcttgc tgagcttctt ctttcaaaag gatacaaagt tcacggaatc  | 240  |
| atccgtcgtt catcttcatt caataccgca agaatcgaac atctgtacag taatccaatt  | 300  |
| actcatcacg gtgattcttc attctctctt cactatggag atatgactga ttcattctgt  | 360  |
| ctcatcaagc tcattttcaac tatcgagcca accgaagtgt atcatttggc tgctcaatct | 420  |
| cacgtcaaag tgtcttttga ttgtccagaa tacactgctg aagtagacgc tgttggaact  | 480  |
| cttcgccttc togatgcaat ccattgctgt cgtttgactg aaaaagtgc attctatcaa   | 540  |
| gcattcccat ccgaattgta tggaaaagtt caagagattc cacaagtgga gaagactcca  | 600  |
| ttctatccaa gatctccata tgctgttgcc aagatgtacg ggtactggat tgttggtaac  | 660  |
| tatcgtgaag cctacaatat gtttgcctgc aatggaattt tgttcaacca tgagagtcca  | 720  |
| agaagaggag agacattcgt gactcgtgaag atcactcgtt cagtcgctaa aatcagtttg | 780  |
| gggcaacagg aaagcattga gctcggaaat ctgagtgtc ttcgtgattg gggacacgcc   | 840  |
| agagagtatg ttgaagcaat gtggagaatt cttcaacacg actcaccaga tgattttgtg  | 900  |
| attgcaactg gaaaacaatt cagtgtcaga gagttttgta atcttgcaat tgctgaaatt  | 960  |
| ggagaagtat tgcaattgga aggagaaggt gttgaggaag ttggaaagaa taaagatgga  | 1020 |
| gttattcgag tttaaagttag tccaaagtac tacagaccaa ctgaagtgga gactttattg | 1080 |
| ggaaatgctg aaaaagcaaa gaagactctt ggatgggaag caaagggtac ggttccgga   | 1140 |
| ctcgtcaaag agatgggtgc aagcgacatt atccttatga agtccaatcc aatggcttaa  | 1200 |

&lt;210&gt; 3

&lt;211&gt; 948

&lt;212&gt; DNA

<213> *Caenorhabditis elegans*

&lt;400&gt; 3

|                                                                    |     |
|--------------------------------------------------------------------|-----|
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| gttgagacaa ccgagaagcg cgacgatgaa aagtgggtct ttatcggtc caaggactgc   | 120 |
| gacctcgaga accttgagga aacgcgagag ctgttcgagt cgggtcaagcc caccacgtg  | 180 |
| atccatttgg ccgtatggt cgggggcctc ttccacaacc tcgcgcacaa tctgcagttc   | 240 |
| tttcggaaga acatggccat caacgacaac gttctcgac tgtgccacga gttcgatgtc   | 300 |
| atcaagtgcg tgagctgcct ttctacctgt atcttcctg acaagacgtc ctacccgatt   | 360 |
| gacgagacta tgggtccacct cggccacccc catgactcaa actttggcta ctcgtatgcc | 420 |
| aagcgcatga tcgatgtcct gaacaaggtt tacgtctcaag agcacggacg taagtacacc | 480 |
| agcgtggttc cctgcaatgt ctttggccct cagcacaact acaacttgca gtccggccat  | 540 |
| gtcctcccg cctgatcca caaggcctat gtcgcgcaga gggacggcac gcccttcag     | 600 |
| gtgtacggca gtggtacacc cctgcgcag ttcatctact ctattgatct cgtcgcctg    | 660 |
| ttcattcgag tcgttagaga atacgaggac gtcgagccta tcatcctcag cgtgaacgag  | 720 |
| tccgacgaag tctcgattcg cgacgcctc agcgcctgg ttaaggcaat cgacttcacc    | 780 |
| ggggacgtcg agtatgatac ttccaaagcg gatggacagt tcaagaaaac cgcctcgaac  | 840 |

gagaagctcc tgaagttggt tccggacttc caattcacgc cctttgagca ggctatccaa 900  
gagtcagtc agtggttcgt tgacaactac gaaacccccc ggaagtaa 948

<210> 4

<211> 1092

<212> DNA

<213> Caenorhabditis elegans

<400> 4

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tcgattggcc tcgttttcct gaacaagtat cttttgtcta gcgtccagct cgacgcccct 180  
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gcttcggagg cgaagtctga caaggtcaag ctccctggga gagatggtaa gcgcccgag 1080  
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<210> 5

<211> 1431

<212> DNA

<213> Trichoderma reesei

<400> 5

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tcctcgtctt ctactttgtc tccaattcaa agtatgaggg cgtcgatctc aacaagggca 180  
ccttcacagc tcgggattcg accaagacga caccaaagcc gccagccact ggcgatgcca 240  
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gggtgttcct caacgacaag ccgtttgaca acaccttcaa gaaggtcacg acgtccctgg 480  
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```

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tgaagaacca tccggagcac attgcccccg acaactcgat gcgcttcctg agcgatgacg      900
gcgcgagagac gtacaacaac tgccacttcg taagcttccc tgcccgttga cttttctctc      960
tctctctttt gtctgccttc caccgggcaa catgatggct aacatgagga tagtgggtcca     1020
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&lt;210&gt; 6

&lt;211&gt; 575

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 6

```

Met Arg Pro Trp Thr Gly Ser Trp Arg Trp Ile Met Leu Ile Leu Phe
1           5           10          15

```

```

Ala Trp Gly Thr Leu Leu Phe Tyr Ile Gly Gly His Leu Val Arg Asp
          20           25           30

```

```

Asn Asp His Pro Asp His Ser Ser Arg Glu Leu Ser Lys Ile Leu Ala
          35           40           45

```

```

Lys Leu Glu Arg Leu Lys Gln Gln Asn Glu Asp Leu Arg Arg Met Ala
          50           55           60

```

```

Glu Ser Leu Arg Ile Pro Glu Gly Pro Ile Asp Gln Gly Pro Ala Ile
          65           70           75           80

```

```

Gly Arg Val Arg Val Leu Glu Glu Gln Leu Val Lys Ala Lys Glu Gln
          85           90           95

```

```

Ile Glu Asn Tyr Lys Lys Gln Thr Arg Asn Gly Leu Gly Lys Asp His
          100          105          110

```

```

Glu Ile Leu Arg Arg Arg Ile Glu Asn Gly Ala Lys Glu Leu Trp Phe
          115          120          125

```

```

Phe Leu Gln Ser Glu Leu Lys Lys Leu Lys Asn Leu Glu Gly Asn Glu
          130          135          140

```

```

Leu Gln Arg His Ala Asp Glu Phe Leu Leu Asp Leu Gly His His Glu
          145          150          155          160

```

```

Arg Ser Ile Met Thr Asp Leu Tyr Tyr Leu Ser Gln Thr Asp Gly Ala
          165          170          175

```

```

Gly Asp Trp Arg Glu Lys Glu Ala Lys Asp Leu Thr Glu Leu Val Gln

```

180

185

190

```

Arg Arg Ile Thr Tyr Leu Gln Asn Pro Lys Asp Cys Ser Lys Ala Lys

```

| 195                                                                                | 200 | 205 |
|------------------------------------------------------------------------------------|-----|-----|
| Lys Leu Val Cys Asn Ile Asn Lys Gly Cys Gly Tyr Gly Cys Gln Leu<br>210 215 220     |     |     |
| His His Val Val Tyr Cys Phe Met Ile Ala Tyr Gly Thr Gln Arg Thr<br>225 230 235 240 |     |     |
| Leu Ile Leu Glu Ser Gln Asn Trp Arg Tyr Ala Thr Gly Gly Trp Glu<br>245 250 255     |     |     |
| Thr Val Phe Arg Pro Val Ser Glu Thr Cys Thr Asp Arg Ser Gly Ile<br>260 265 270     |     |     |
| Ser Thr Gly His Trp Ser Gly Glu Val Lys Asp Lys Asn Val Gln Val<br>275 280 285     |     |     |
| Val Glu Leu Pro Ile Val Asp Ser Leu His Pro Arg Pro Pro Tyr Leu<br>290 295 300     |     |     |
| Pro Leu Ala Val Pro Glu Asp Leu Ala Asp Arg Leu Val Arg Val His<br>305 310 315 320 |     |     |
| Gly Asp Pro Ala Val Trp Trp Val Ser Gln Phe Val Lys Tyr Leu Ile<br>325 330 335     |     |     |
| Arg Pro Gln Pro Trp Leu Glu Lys Glu Ile Glu Glu Ala Thr Lys Lys<br>340 345 350     |     |     |
| Leu Gly Phe Lys His Pro Val Ile Gly Val His Val Arg Arg Thr Asp<br>355 360 365     |     |     |
| Lys Val Gly Thr Glu Ala Ala Phe His Pro Ile Glu Glu Tyr Met Val<br>370 375 380     |     |     |
| His Val Glu Glu His Phe Gln Leu Leu Ala Arg Arg Met Gln Val Asp<br>385 390 395 400 |     |     |
| Lys Lys Arg Val Tyr Leu Ala Thr Asp Asp Pro Ser Leu Leu Lys Glu<br>405 410 415     |     |     |
| Ala Lys Thr Lys Tyr Pro Asn Tyr Glu Phe Ile Ser Asp Asn Ser Ile<br>420 425 430     |     |     |
| Ser Trp Ser Ala Gly Leu His Asn Arg Tyr Thr Glu Asn Ser Leu Arg<br>435 440 445     |     |     |
| Gly Val Ile Leu Asp Ile His Phe Leu Ser Gln Ala Asp Phe Leu Val<br>450 455 460     |     |     |
| Cys Thr Phe Ser Ser Gln Val Cys Arg Val Ala Tyr Glu Ile Met Gln<br>465 470 475 480 |     |     |
| Thr Leu His Pro Asp Ala Ser Ala Asn Phe His Ser Leu Asp Asp Ile<br>485 490 495     |     |     |
| Tyr Tyr Phe Gly Gly Gln Asn Ala His Asn Gln Ile Ala Ile Tyr Ala<br>500 505 510     |     |     |
| His Gln Pro Arg Thr Ala Asp Glu Ile Pro Met Glu Pro Gly Asp Ile<br>515 520 525     |     |     |
| Ile Gly Val Ala Gly Asn His Trp Asp Gly Tyr Ser Lys Gly Val Asn<br>530 535 540     |     |     |
| Val Val Val Glu Val Phe Gln Val Thr Phe Ser Thr Val Val Glu<br>545 550 555         |     |     |

Arg Lys Leu Gly Arg Thr Gly Leu Tyr Pro Ser Tyr Lys Val Arg Glu  
545 550 555 560

Lys Ile Glu Thr Val Lys Tyr Pro Thr Tyr Pro Glu Ala Glu Lys  
565 570 575

<210> 7

<211> 399

<212> PRT

<213> Caenorhabditis elegans

<400> 7

Met Pro Thr Gly Lys Ser Glu Ser Ser Asp Ile Ser Glu Val Val Gly  
1 5 10 15

Asn Met Glu Ile Ser Lys Val Glu Gly Leu Glu Ala Cys Ile Gly Met  
20 25 30

Ser His Glu Val Ser Thr Thr Pro Ala Ala Glu Leu Ala Ala Phe Arg  
35 40 45

Ala Arg Lys Val Ala Leu Ile Thr Gly Ile Ser Gly Gln Asp Gly Ser  
50 55 60

Tyr Leu Ala Glu Leu Leu Ser Lys Gly Tyr Lys Val His Gly Ile  
65 70 75 80

Ile Arg Arg Ser Ser Ser Phe Asn Thr Ala Arg Ile Glu His Leu Tyr  
85 90 95

Ser Asn Pro Ile Thr His His Gly Asp Ser Ser Phe Ser Leu His Tyr  
100 105 110

Gly Asp Met Thr Asp Ser Ser Cys Leu Ile Lys Leu Ile Ser Thr Ile  
115 120 125

Glu Pro Thr Glu Val Tyr His Leu Ala Ala Gln Ser His Val Lys Val  
130 135 140

Ser Phe Asp Leu Pro Glu Tyr Thr Ala Glu Val Asp Ala Val Gly Thr  
145 150 155 160

Leu Arg Leu Leu Asp Ala Ile His Ala Cys Arg Leu Thr Glu Lys Val  
165 170 175

Arg Phe Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly Lys Val Gln Glu  
180 185 190

Ile Pro Gln Ser Glu Lys Thr Pro Phe Tyr Pro Arg Ser Pro Tyr Ala  
195 200 205

Val Ala Lys Met Tyr Gly Tyr Trp Ile Val Val Asn Tyr Arg Glu Ala  
210 215 220

Tyr Asn Met Phe Ala Cys Asn Gly Ile Leu Phe Asn His Glu Ser Pro  
225 230 235 240

Arg Arg Gly Glu Thr Phe Val Thr Arg Lys Ile Thr Arg Ser Val Ala  
245 250 255

Lys Ile Ser Leu Gly Gln Gln Glu Ser Ile Glu Leu Gly Asn Leu Ser  
260 265 270

Ala Leu Arg Asp Trp Gly His Ala Arg Glu Tyr Val Glu Ala Met Trp  
275 280 285

Arg Ile Leu Gln His Asp Ser Pro Asp Asp Phe Val Ile Ala Thr Gly  
290 295 300

Lys Gln Phe Ser Val Arg Glu Phe Cys Asn Leu Ala Phe Ala Glu Ile  
305 310 315 320

Gly Glu Val Leu Gln Trp Glu Gly Glu Gly Val Glu Glu Val Gly Lys  
325 330 335

Asn Lys Asp Gly Val Ile Arg Val Lys Val Ser Pro Lys Tyr Tyr Arg  
340 345 350

Pro Thr Glu Val Glu Thr Leu Leu Gly Asn Ala Glu Lys Ala Lys Lys  
355 360 365

Thr Leu Gly Trp Glu Ala Lys Val Thr Val Pro Glu Leu Val Lys Glu  
370 375 380

Met Val Ala Ser Asp Ile Ile Leu Met Lys Ser Asn Pro Met Ala  
385 390 395

<210> 8

<211> 315

<212> PRT

<213> Caenorhabditis elegans

<400> 8

Met Lys Thr Ile Leu Val Thr Gly Gly Thr Gly Leu Val Gly Ser Ala  
1 5 10 15

Ile Lys Lys Val Val Glu Thr Thr Glu Lys Arg Asp Asp Glu Lys Trp  
20 25 30

Val Phe Ile Gly Ser Lys Asp Cys Asp Leu Glu Asn Leu Glu Glu Thr  
35 40 45

Arg Glu Leu Phe Glu Ser Val Lys Pro Thr His Val Ile His Leu Ala  
50 55 60

Ala Met Val Gly Gly Leu Phe His Asn Leu Ala His Asn Leu Gln Phe  
65 70 75 80

Phe Arg Lys Asn Met Ala Ile Asn Asp Asn Val Leu Ala Leu Cys His  
85 90 95

Glu Phe Asp Val Ile Lys Cys Val Ser Cys Leu Ser Thr Cys Ile Phe  
100 105 110

Pro Asp Lys Thr Ser Tyr Pro Ile Asp Glu Thr Met Val His Leu Gly  
115 120 125

Pro Pro His Asp Ser Asn Phe Gly Tyr Ser Tyr Ala Lys Arg Met Ile  
130 135 140

Asp Val Leu Asn Lys Gly Tyr Ala Gln Glu His Gly Arg Lys Tyr Thr  
145 150 155 160

Ser Val Val Pro Cys Asn Val Phe Gly Pro His Asp Asn Tyr Asn Leu  
165 170 175

Gln Ser Gly His Val Leu Pro Ala Leu Ile His Lys Ala Tyr Val Ala  
180 185 190

Gln Arg Asp Gly Thr Pro Leu Gln Val Tyr Gly Ser Gly Thr Pro Leu  
195 200 205

Arg Gln Phe Ile Tyr Ser Ile Asp Leu Ala Arg Leu Phe Ile Arg Val  
210 215 220

Val Arg Glu Tyr Glu Asp Val Glu Pro Ile Ile Leu Ser Val Asn Glu  
225 230 235 240

Ser Asp Glu Val Ser Ile Arg Asp Ala Val Ser Ala Val Val Lys Ala  
245 250 255

Ile Asp Phe Thr Gly Asp Val Glu Tyr Asp Thr Ser Lys Ala Asp Gly  
260 265 270

Gln Phe Lys Lys Thr Ala Ser Asn Glu Lys Leu Leu Lys Leu Phe Pro  
275 280 285

Asp Phe Gln Phe Thr Pro Phe Glu Gln Ala Ile Gln Glu Ser Val Gln  
290 295 300

Trp Phe Val Asp Asn Tyr Glu Thr Ala Arg Lys  
305 310 315

<210> 9

<211> 363

<212> PRT

<213> caenorhabditis elegans

<400> 9

Met Lys Leu His Glu Glu Asn Asn Lys Ala Leu Phe Glu Ala Met Arg  
1 5 10 15

Asn Arg Glu Asn Pro Thr Lys Trp Glu Ser Tyr Lys Gln Val Ile Thr  
20 25 30

Ala Val Ser Ala Tyr Trp Val Phe Ser Ile Gly Leu Val Phe Leu Asn  
35 40 45

Lys Tyr Leu Leu Ser Ser Val Gln Leu Asp Ala Pro Leu Phe Ile Thr  
50 55 60

Trp Tyr Gln Cys Leu Val Thr Val Phe Leu Cys Leu Phe Leu Ser Lys  
65 70 75 80

Thr Ser Lys Ala Tyr Gly Leu Phe Lys Phe Pro Ser Met Pro Ile Asp  
85 90 95

Ala Lys Ile Ser Arg Glu Val Leu Pro Leu Ser Val Val Phe Val Ala  
100 105 110

Met Ile Ser Phe Asn Asn Leu Cys Leu Lys Tyr Val Gly Val Ser Phe  
115 120 125

Tyr Tyr Val Gly Arg Ser Leu Thr Thr Val Phe Asn Val Val Cys Thr  
130 135 140

Tyr Leu Ile Leu Gly Gln Lys Thr Ser Gly Gln Ala Ile Gly Cys Cys  
145 150 155 160

Ala Leu Ile Ile Phe Gly Phe Leu Leu Gly Val Asp Gln Glu Gly Val  
165 170 175

Thr Gly Thr Leu Ser Tyr Thr Gly Val Ile Phe Gly Val Leu Ala Ser  
180 185 190

Leu Ser Val Ala Leu Asn Ala Ile Tyr Thr Arg Lys Val Leu Ser Ser  
195 200 205

Val Gly Asp Cys Leu Trp Arg Leu Thr Met Tyr Asn Asn Leu Asn Ala  
210 215 220

Leu Val Leu Phe Leu Pro Leu Met Leu Phe Asn Gly Glu Phe Gly Ala  
225 230 235 240

Val Phe Tyr Phe Asp Lys Leu Phe Asp Thr Thr Phe Trp Ile Leu Met  
245 250 255

Thr Leu Gly Gly Val Phe Gly Phe Met Met Gly Tyr Val Thr Gly Trp  
260 265 270

Gln Ile Gln Ala Thr Ser Pro Leu Thr His Asn Ile Ser Gly Thr Ala  
275 280 285

Lys Ala Ala Ala Gln Thr Val Met Ala Val Val Trp Tyr Ser Glu Leu  
290 295 300

Lys Thr Leu Leu Trp Trp Thr Ser Asn Phe Val Val Leu Phe Gly Ser  
305 310 315 320

Gly Met Tyr Thr Tyr Val Gln Lys Arg Val Met Asp Lys Lys Asn Ser  
325 330 335

Gly Ala Ser Pro Ala Ser Glu Ala Lys Ser Asp Lys Val Lys Leu Leu  
340 345 350

Gly Arg Asp Gly Asn Ala Ala Glu Glu Ser Val  
355 360

<210> 10

<211> 406

<212> PRT

<213> Trichoderma reesei

<400> 10

Met Ala Ser Thr Asn Ala Arg Tyr Val Arg Tyr Leu Leu Ile Ala Phe  
1 5 10 15

Phe Thr Ile Leu Val Phe Tyr Phe Val Ser Asn Ser Lys Tyr Glu Gly  
20 25 30

Val Asp Leu Asn Lys Gly Thr Phe Thr Ala Pro Asp Ser Thr Lys Thr  
35 40 45

Thr Pro Lys Pro Pro Ala Thr Gly Asp Ala Lys Asp Phe Pro Leu Ala  
50 55 60

Leu Thr Pro Asn Asp Pro Gly Phe Asn Asp Leu Val Gly Ile Ala Pro  
65 70 75 80

Gly Pro Arg Met Asn Ala Thr Phe Val Thr Leu Ala Arg Asn Ser Asp  
85 90 95

Val Trp Asp Ile Ala Arg Ser Ile Arg Gln Val Glu Asp Arg Phe Asn  
100 105 110

Arg Arg Tyr Asn Tyr Asp Trp Val Phe Leu Asn Asp Lys Pro Phe Asp  
115 120 125

Asn Thr Phe Lys Lys Val Thr Thr Ser Leu Val Ser Gly Lys Thr His  
130 135 140

Met Gly Gly Ile Ala Pro Gly Val Met Ser Phe Pro Asn Thr Ile Asn



Tyr Gly Glu Ile Ala Pro Glu His Trp Ser Phe Pro Asp Trp Ile Asp  
145 150 155 160

Gln Asp Lys Ala Lys Lys Val Arg Glu Asp Met Ala Glu Arg Lys Ile  
165 170 175

Ile Tyr Gly Asp Ser Val Ser Tyr Arg His Met Cys Arg Phe Glu Ser  
180 185 190

Gly Phe Phe Phe Arg Gln Pro Leu Met Met Asn Tyr Glu Tyr Tyr Trp  
195 200 205

Arg Val Glu Pro Ser Ile Glu Leu Tyr Cys Asp Ile His Tyr Asp Pro  
210 215 220

Phe Arg Leu Met Val Glu Gln Gly Lys Lys Tyr Ser Phe Val Ile Ser  
225 230 235 240

Leu Tyr Glu Tyr Pro Ala Thr Ile Ala Thr Leu Trp Glu Ser Thr Lys  
245 250 255

Lys Phe Met Lys Asn His Pro Glu His Ile Ala Pro Asp Asn Ser Met  
260 265 270

Arg Phe Leu Ser Asp Asp Gly Gly Glu Thr Tyr Asn Asn Cys His Phe  
275 280 285

Trp Ser Asn Phe Glu Ile Gly Ser Leu Glu Trp Leu Arg Ser Lys Gln  
290 295 300

Tyr Ile Asp Phe Phe Glu Ser Leu Asp Lys Asp Gly Gly Phe Phe Tyr  
305 310 315 320

Glu Arg Trp Gly Asp Ala Pro Val His Ser Ile Ala Ala Gly Leu Met  
325 330 335

Leu Asn Arg Ser Glu Ile His Phe Phe Asn Asp Ile Ala Tyr Trp His  
340 345 350

Val Pro Phe Thr His Cys Pro Thr Gly Glu Lys Thr Arg Leu Asp Leu  
355 360 365

Lys Cys His Cys Asp Pro Lys Glu Asn Phe Asp Trp Lys Gly Tyr Ser  
370 375 380

Cys Thr Ser Arg Phe Phe Glu Met Asn Gly Met Asp Lys Pro Glu Gly  
385 390 395 400

Trp Glu Asn Gln Gln Asp  
405

<210> 11

<211> 959

<212> DNA

<213> Trichoderma reesei

<400> 11

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gtttgaagca tccattcgca gcgcgcgaga ccgtactgc ttcgtgacta tacagtacat 180

gtaataccaa caggagtcga ggatacaggt actaagtacc acctattaat ctgctgctcg 240

tgctctgtcc gcattgaaag cgaggtaag taggcagaac cgctgaccgc tcccccatca 300

ctctctctctc ccccccctctc ctctctctctc cccctctctc tccctctctc ctctctctctc 360

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yytgaatgata caacagggta gtaattgcygt gacattgaga caaagtaaac ctcttgcctt 300
ggacgcgcggc aacggtagcc caatccgagg tccaaatctg ctgcgcgggc ctgaggcaga 420
gacaccaccg tttgttgatc tcggttgccc catcagccag ccagcgcacc tcagtttaggc 480
tcggacctgg gcaaaagtgt gggttaacaaa ggccggcggc caaagcacc aagtacctct 540
gcccgccatt gcggttgtag aggtgacttc cgcgggccaat cacgggtggc ccccttgtag 600
ggagtgggtg gtcattcggt cgtcagcacg gccaatagac gcaaccggcg gccctgcttt 660
caatgttgac tgccccaggc caggtccagt gaccaccga tagcaccatc gcagccacc 720
caaatgcgac ctgcaccgcg ctggccaggg cccttcgggg cgcctgttag tgccctgcgt 780
tgctcggcgg gcgggctgct taactgtctt tgcgtgctga gctgcctgcc tgccgcgcgc 840
tcgccttacc taatgccgcg tacctctcct ccgtactccg tccctacaaa gaggtggtt 900
cctagcttgc tcactctcca agttcccttc ctctggcagc aatcgaacca tccattca 959

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&lt;210&gt; 12

&lt;211&gt; 992

&lt;212&gt; DNA

<213> *Trichoderma reesei*

&lt;400&gt; 12

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atgctgggta gttttgggta tttgggcagt ttgagagcgg aagacagtcc tactgctgcg 120
gaggagtctg gatcaagatt gcaacgtcgt ttatgtaata actataatgg agactggccg 180
tcgtctgctg ccgctatttg gttcgggtgc atgatctcgt gcctttgcga ggcgctcatc 240
tcgattgatt gattgattgg cctgtctcga catgtcgata ctaacctgc cgcggccgaa 300
cgattccatt tttgcttgct tgggtcaattg tactggtgtc gccgggacct ttgtcagagc 360
gagctgcccg tacctaccaa ctacctagta ggtgccatca aatgacgtgc tgcaagctat 420
cgcgaccagc caggtcagcc gcgtgtcaca tgtaaggta gagctaataa gatgcgacat 480
tctgtgcatt gctagcaccg ccaatactag cagcaaacgg ctttggcacc tcagtggcag 540
gccgaagtgc gcgtgggatg ggatccattt attaccctgc attatacggg gagagctaca 600
ggctctgagc gagtatcatc caacgggcag ttgtttataa ggatcaaagg acaggttggt 660
aataccgaat ttacattaag aagtagaatg caagatgagt tgtgaactgt aatctcctgt 720
gtttactgac caaggtagggt ggcttagcat gttagcagca atacattott acctgtagca 780
tctggcgcgg ctacctagta tcaatatgat ccaggcacta aggcgtgttc cgcctcgact 840
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acagacaagc cgaatcagtc cgcgtcagtt ca 992

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&lt;210&gt; 13

&lt;211&gt; 310

&lt;212&gt; PRT

<213> *Helicobacter pylori*

&lt;400&gt; 13

```

Met Asn Glu Ile Ile Leu Ile Thr Gly Ala Tyr Gly Met Val Gly Gln
1           5           10          15

Asn Thr Ala Leu Tyr Phe Lys Lys Asn Lys Pro Asp Val Thr Leu Leu
20          25          30

Thr Pro Lys Lys Ser Glu Leu Cys Leu Leu Asp Lys Asp Asn Val Gln
35          40          45

```

Ala Tyr Leu Lys Glu Tyr Lys Pro Thr Gly Ile Ile His Cys Ala Gly  
50 55 60

Arg Val Gly Gly Ile Val Ala Asn Met Asn Asp Leu Ser Thr Tyr Met  
65 70 75 80

Val Glu Asn Leu Leu Met Gly Leu Tyr Leu Phe Ser Ser Ala Leu Asp  
85 90 95

Ser Gly Val Lys Lys Ala Ile Asn Leu Ala Ser Ser Cys Ala Tyr Pro  
100 105 110

Lys Phe Ala Pro Asn Pro Leu Lys Glu Ser Asp Leu Leu Asn Gly Ser  
115 120 125

Leu Glu Pro Thr Asn Glu Gly Tyr Ala Leu Ala Lys Leu Ser Val Met  
130 135 140

Lys Tyr Cys Glu Tyr Val Ser Ala Glu Lys Gly Val Phe Tyr Lys Thr  
145 150 155 160

Leu Val Pro Cys Asn Leu Tyr Gly Glu Phe Asp Lys Phe Glu Glu Lys  
165 170 175

Ile Ala His Met Ile Pro Gly Leu Ile Ala Arg Met His Thr Ala Lys  
180 185 190

Leu Lys Asn Glu Lys Glu Phe Ala Met Trp Gly Asp Gly Thr Ala Arg  
195 200 205

Arg Glu Tyr Leu Asn Ala Lys Asp Leu Ala Arg Phe Ile Ser Leu Ala  
210 215 220

Tyr Glu Asn Ile Ala Ser Ile Pro Ser Val Met Asn Val Gly Ser Gly  
225 230 235 240

Val Asp Tyr Ser Ile Glu Glu Tyr Tyr Glu Lys Val Ala Gln Val Leu  
245 250 255

Asp Tyr Lys Gly Val Phe Val Lys Asp Leu Ser Lys Pro Val Gly Met  
260 265 270

Gln Gln Lys Leu Met Asp Ile Ser Lys Gln Arg Ala Leu Lys Trp Glu  
275 280 285

Leu Glu Ile Pro Leu Glu Gln Gly Ile Lys Glu Ala Tyr Glu Tyr Tyr  
290 295 300

Leu Lys Leu Leu Glu Val  
305 310

<210> 14

<211> 933

<212> DNA

<213> Helicobacter pylori

<400> 14

|                                                                    |     |
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| tacttcaaga agaacaagcc cgacgttacc ctcttactc ctaagaaaag cgagctgtgc   | 120 |
| ctcttggaaca aggataatgt ccaggcctac ctgaaagaat ataagccgac aggaatcatt | 180 |
| cactgcgcag gccgcgtcgg cgggatcgtg gcgaacatga acgacctctc cacgtacatg  | 240 |
| ctggagagag ttatgataag catatagata ttttggagag caatagata tcatatagag   | 300 |

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gtcagagaacc cctccatggy cctgtaccctg cctccagagcg cctccagacc cggctgtgaag      300
aaggccatca acttggcctc atcctgtgct tatcccaagt tcgcgcccaa tccactgaaa      360
gagtcgggac tgctcaacgg cagcctcgag cctaccaacg agggatacgc ccttgctaag      420
ctgtccgtca tgaagtactg cgaatacgtt agtgccgaga agggcgtggt ctataagacc      480
ctcgtcccggt gcaacttgta cggcgagttc gacaagtttg aagaaaagat cgcacacatg      540

atccccggcc tcattgcccg aatgcatacg gcgaagctga agaacgagaa agaatttgcc      600
atgtggggcg acggtactgc tcgccgggag tacctcaacg ccaaggatct ggcccgtttc      660
atttctcttg catacgagaa tatcgctagc attccctctg tcatgaacgt gggatcgggc      720
gttgactata gcatcgagga atactacgag aaggtcgcgc aagtgtctga ctacaagggg      780
gtgttcgtca aggacctgtc caagcctgtc ggtatgcagc aaaagctcat ggatatctcg      840
aagcagaggg ctctgaagtg ggagttggag attccgctcg agcagggcat caaagaggcc      900
tacgagtact atctgaagct gcttgaggtg taa      933

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&lt;210&gt; 15

&lt;211&gt; 381

&lt;212&gt; PRT

&lt;213&gt; Helicobacter pylori

&lt;400&gt; 15

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Met Lys Glu Lys Ile Ala Leu Ile Thr Gly Val Thr Gly Gln Asp Gly
1          5          10          15

Ser Tyr Leu Ala Glu Tyr Leu Leu Asn Leu Gly Tyr Glu Val His Gly
20        25        30

Leu Lys Arg Arg Ser Ser Ser Ile Asn Thr Ser Arg Ile Asp His Leu
35        40        45

Tyr Glu Asp Leu His Ser Glu His Lys Arg Arg Phe Phe Leu His Tyr
50        55        60

Gly Asp Met Thr Asp Ser Ser Asn Leu Ile His Leu Ile Ala Thr Thr
65        70        75        80

Lys Pro Thr Glu Ile Tyr Asn Leu Ala Ala Gln Ser His Val Lys Val
85          90          95

Ser Phe Glu Thr Pro Glu Tyr Thr Ala Asn Ala Asp Gly Ile Gly Thr
100       105       110

Leu Arg Ile Leu Glu Ala Met Arg Ile Leu Gly Leu Glu Lys Lys Thr
115       120       125

Arg Phe Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly Glu Val Leu Glu
130       135       140

Thr Pro Gln Asn Glu Asn Thr Pro Phe Asn Pro Arg Ser Pro Tyr Ala
145       150       155       160

Val Ala Lys Met Tyr Ala Phe Tyr Ile Thr Lys Asn Tyr Arg Glu Ala
165       170       175

Tyr Asn Leu Phe Ala Val Asn Gly Ile Leu Phe Asn His Glu Ser Arg
180       185       190

Val Arg Gly Glu Thr Phe Val Thr Arg Lys Ile Thr Arg Ala Ala Ser
195       200       205

Ala Ile Ala Tyr Asn Leu Thr Asp Cys Leu Tyr Leu Gly Asn Leu Asp

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210          215          220
Ala Lys Arg Asp Trp Gly His Ala Lys Asp Tyr Val Lys Met Met His
225          230          235          240
Leu Met Leu Gln Ala Pro Thr Pro Gln Asp Tyr Val Ile Ala Thr Gly
245          250          255
Lys Thr Thr Ser Val Arg Asp Phe Val Lys Met Ser Phe Glu Phe Ile
260          265          270
Gly Ile Asp Leu Glu Phe Gln Asn Thr Gly Ile Lys Glu Ile Gly Leu
275          280          285
Ile Lys Ser Val Asp Glu Lys Arg Ala Asn Ala Leu Gln Leu Asn Leu
290          295          300
Ser His Leu Lys Thr Gly Lys Ile Val Val Arg Ile Asp Glu His Tyr
305          310          315          320
Phe Arg Pro Thr Glu Val Asp Leu Leu Leu Gly Asp Pro Thr Gly Ala
325          330          335
Glu Lys Glu Leu Gly Trp Val Arg Glu Tyr Asp Leu Lys Glu Leu Val
340          345          350
Lys Asp Met Leu Glu Tyr Asp Leu Lys Glu Cys Gln Lys Asn Leu Tyr
355          360          365
Leu Gln Asp Gly Gly Tyr Thr Leu Arg Asn Phe Tyr Glu
370          375          380

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&lt;210&gt; 16

&lt;211&gt; 1146

&lt;212&gt; DNA

&lt;213&gt; Helicobacter pylori

&lt;400&gt; 16

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atgaagaga agatcgccct cattaccggc gtcacgggcc aggacggtag ctacctggct      60
gagtacctcc tgaaccttgg atatgaagtg cacggcttga agcgccgata ctcgagcata      120
aacactttctc ggatcgatca cctctacgag gacctccatt ccgagcacia gcgccgtttc      180
tttctgcact acggcgacat gaccgactcg tcaaattctga tccacctcat tgccaccaag      240
aagcccacag agatotacaa ccttgccgcg cagagccatg tcaaggttag tttcgaaacc      300
cctgagtata cggccaacgc agatggcatt gggactctca ggatcttgga ggctatgcgc      360
atcctgggcc tcgagaagaa aaccagattc taccaagcct ccacgtctga gctgtacggt      420
gaggtcctgg aaaccccgca gaacgagaac acacccttta acccccgcag ccataacgcc      480
gtcgcgaaga tgtacgcttt ctatattacg aagaattacc gagaggccta caacctcttt      540
gcagtgaacg gaatcctttt caaccacgag tcgcgcgtcc ggggcgaaac cttcgttacc      600
cgtaagatta ctagggccgc ctccgctatc gcgtacaacc tcacggactg cctgtatctg      660
ggcaatttgg acgccaagcg cgattggggc cacgccaagg actacgtcaa gatgatgcac      720
ctcatgctcc aggcacctac cccgcaggac tacgtgatcg ccaccggtaa gactacaagc      780
gtccgagact tcgtcaagat gtccttcgag ttatctcgca ttgatcttga gtttcagaac      840
acgggaatca aagagattgg cctgatcaag tcggttgacg agaagcgcgc gaacgcgctc      900
caactcaacc tgtctcatct caagaccggg aagatcgtcg tgcggattga cgaacactat      960
ttccgccccca ctgaggtcga cctgttgctg ggcgatccca cgggtgcga gaaagagctt     1020

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ggctgggtcc gagagtacga cctcaaagaa ctggtgaagg acatggtcga gtacgatctg 1080  
 aaagagtgcc agaagaacct ctacttcgag gacggcggat atacccttcg gaatttctac 1140  
 gagtaa 1146

<210> 17

<211> 407

<212> PRT

<213> Trichoderma reesei

<400> 17

Met Gln Thr Phe Gly Ala Phe Leu Val Ser Phe Leu Ala Ala Ser Gly  
 1 5 10 15

Leu Ala Ala Ala Leu Pro Thr Glu Gly Gln Lys Thr Ala Ser Val Glu  
 20 25 30

Val Gln Tyr Asn Lys Asn Tyr Val Pro His Gly Pro Thr Ala Leu Phe  
 35 40 45

Lys Ala Lys Arg Lys Tyr Gly Ala Pro Ile Ser Asp Asn Leu Lys Ser  
 50 55 60

Leu Val Ala Ala Arg Gln Ala Lys Gln Ala Leu Ala Lys Arg Gln Thr  
 65 70 75 80

Gly Ser Ala Pro Asn His Pro Ser Asp Ser Ala Asp Ser Glu Tyr Ile  
 85 90 95

Thr Ser Val Ser Ile Gly Thr Pro Ala Gln Val Leu Pro Leu Asp Phe  
 100 105 110

Asp Thr Gly Ser Ser Asp Leu Trp Val Phe Ser Ser Glu Thr Pro Lys  
 115 120 125

Ser Ser Ala Thr Gly His Ala Ile Tyr Thr Pro Ser Lys Ser Ser Thr  
 130 135 140

Ser Lys Lys Val Ser Gly Ala Ser Trp Ser Ile Ser Tyr Gly Asp Gly  
 145 150 155 160

Ser Ser Ser Ser Gly Asp Val Tyr Thr Asp Lys Val Thr Ile Gly Gly  
 165 170 175

Phe Ser Val Asn Thr Gln Gly Val Glu Ser Ala Thr Arg Val Ser Thr  
 180 185 190

Glu Phe Val Gln Asp Thr Val Ile Ser Gly Leu Val Gly Leu Ala Phe  
 195 200 205

Asp Ser Gly Asn Gln Val Arg Pro His Pro Gln Lys Thr Trp Phe Ser  
 210 215 220

Asn Ala Ala Ser Ser Leu Ala Glu Pro Leu Phe Thr Ala Asp Leu Arg  
 225 230 235 240

His Gly Gln Asn Gly Ser Tyr Asn Phe Gly Tyr Ile Asp Thr Ser Val  
 245 250 255

Ala Lys Gly Pro Val Ala Tyr Thr Pro Val Asp Asn Ser Gln Gly Phe  
 260 265 270

Trp Glu Phe Thr Ala Ser Gly Tyr Ser Val Gly Gly Gly Lys Leu Asn  
 275 280 285

Arg Asn Ser Ile Asp Gly Ile Ala Asp Thr Gly Thr Thr Leu Leu Leu  
290 295 300

Leu Asp Asp Asn Val Val Asp Ala Tyr Tyr Ala Asn Val Gln Ser Ala  
305 310 315 320

Gln Tyr Asp Asn Gln Gln Glu Gly Val Val Phe Asp Cys Asp Glu Asp  
325 330 335

Leu Pro Ser Phe Ser Phe Gly Val Gly Ser Ser Thr Ile Thr Ile Pro  
340 345 350

Gly Asp Leu Leu Asn Leu Thr Pro Leu Glu Glu Gly Ser Ser Thr Cys  
355 360 365

Phe Gly Gly Leu Gln Ser Ser Ser Gly Ile Gly Ile Asn Ile Phe Gly  
370 375 380

Asp Val Ala Leu Lys Ala Ala Leu Val Val Phe Asp Leu Gly Asn Glu  
385 390 395 400

Arg Leu Gly Trp Ala Gln Lys  
405

<210> 18

<211> 399

<212> PRT

<213> Trichoderma reesei

<400> 18

Met Gln Pro Ser Phe Gly Ser Phe Leu Val Thr Val Leu Ser Ala Ser  
1 5 10 15

Met Ala Ala Gly Ser Val Ile Pro Ser Thr Asn Ala Asn Pro Gly Ser  
20 25 30

Phe Glu Ile Lys Arg Ser Ala Asn Lys Ala Phe Thr Gly Arg Asn Gly  
35 40 45

Pro Leu Ala Leu Ala Arg Thr Tyr Ala Lys Tyr Gly Val Glu Val Pro  
50 55 60

Lys Thr Leu Val Asp Ala Ile Gln Leu Val Lys Ser Ile Gln Leu Ala  
65 70 75 80

Lys Arg Asp Ser Ala Thr Val Thr Ala Thr Pro Asp His Asp Asp Ile  
85 90 95

Glu Tyr Leu Val Pro Val Lys Ile Gly Thr Pro Pro Gln Thr Leu Asn  
100 105 110

Leu Asp Phe Asp Thr Gly Ser Ser Asp Leu Trp Val Phe Ser Ser Asp  
115 120 125

Val Asp Pro Thr Ser Ser Gln Gly His Asp Ile Tyr Thr Pro Ser Lys  
130 135 140

Ser Thr Ser Ser Lys Lys Leu Glu Gly Ala Ser Trp Asn Ile Thr Tyr  
145 150 155 160

Gly Asp Arg Ser Ser Ser Ser Gly Asp Val Tyr His Asp Ile Val Ser  
165 170 175

Val Gly Asn Leu Thr Val Lys Ser Gln Ala Val Glu Ser Ala Arg Asn  
180 185 190

Val Ser Ala Gln Phe Thr Gln Gly Asn Asn Asp Gly Leu Val Gly Leu  
195 200 205

Ala Phe Ser Ser Ile Asn Thr Val Lys Pro Thr Pro Gln Lys Thr Trp  
210 215 220

Tyr Asp Asn Ile Val Gly Ser Leu Asp Ser Pro Val Phe Val Ala Asp  
225 230 235 240

Leu Arg His Asp Thr Pro Gly Ser Tyr His Phe Gly Ser Ile Pro Ser  
245 250 255

Glu Ala Ser Lys Ala Phe Tyr Ala Pro Ile Asp Asn Ser Lys Gly Phe  
260 265 270

Trp Gln Phe Ser Thr Ser Ser Asn Ile Ser Gly Gln Phe Asn Ala Val  
275 280 285

Ala Asp Thr Gly Thr Thr Leu Leu Leu Ala Ser Asp Asp Leu Val Lys  
290 295 300

Ala Tyr Tyr Ala Lys Val Gln Gly Ala Arg Val Asn Val Phe Leu Gly  
305 310 315 320

Gly Tyr Val Phe Asn Cys Thr Thr Gln Leu Pro Asp Phe Thr Phe Thr  
325 330 335

Val Gly Glu Gly Asn Ile Thr Val Pro Gly Thr Leu Ile Asn Tyr Ser  
340 345 350

Glu Ala Gly Asn Gly Gln Cys Phe Gly Gly Ile Gln Pro Ser Gly Gly  
355 360 365

Leu Pro Phe Ala Ile Phe Gly Asp Ile Ala Leu Lys Ala Ala Tyr Val  
370 375 380

Ile Phe Asp Ser Gly Asn Lys Gln Val Gly Trp Ala Gln Lys Lys  
385 390 395

<210> 19

<211> 452

<212> PRT

<213> Trichoderma reesei

<400> 19

Met Glu Ala Ile Leu Gln Ala Gln Ala Lys Phe Arg Leu Asp Arg Gly  
1 5 10 15

Leu Gln Lys Ile Thr Ala Val Arg Asn Lys Asn Tyr Lys Arg His Gly  
20 25 30

Pro Lys Ser Tyr Val Tyr Leu Leu Asn Arg Phe Gly Phe Glu Pro Thr  
35 40 45

Lys Pro Gly Pro Tyr Phe Gln Gln His Arg Ile His Gln Arg Gly Leu  
50 55 60

Ala His Pro Asp Phe Lys Ala Ala Val Gly Gly Arg Val Thr Arg Gln  
65 70 75 80

Lys Val Leu Ala Lys Lys Val Lys Glu Asp Gly Thr Val Asp Ala Gly  
85 90 95

Gly Ser Lys Thr Gly Glu Val Asp Ala Glu Asp Gln Gln Asn Asp Ser  
100 105 110



Glu Tyr Leu Cys Glu Val Thr Ile Gly Thr Pro Gly Gln Lys Leu Met  
 115 120 125  
 Leu Asp Phe Asp Thr Gly Ser Ser Asp Leu Trp Val Phe Ser Thr Glu  
 130 135 140  
 Leu Ser Lys His Leu Gln Glu Asn His Ala Ile Phe Asp Pro Lys Lys  
 145 150 155 160  
 Ser Ser Thr Phe Lys Pro Leu Lys Asp Gln Thr Trp Gln Ile Ser Tyr  
 165 170 175  
 Gly Asp Gly Ser Ser Ala Ser Gly Thr Cys Gly Ser Asp Thr Val Thr  
 180 185 190  
 Leu Gly Gly Leu Ser Ile Lys Asn Gln Thr Ile Glu Leu Ala Ser Lys  
 195 200 205  
 Leu Ala Pro Gln Phe Ala Gln Gly Thr Gly Asp Gly Leu Leu Gly Leu  
 210 215 220  
 Ala Trp Pro Gln Ile Asn Thr Val Gln Thr Asp Gly Arg Pro Thr Pro  
 225 230 235 240  
 Ala Asn Thr Pro Val Ala Asn Met Ile Gln Gln Asp Asp Ile Pro Ser  
 245 250 255  
 Asp Ala Gln Leu Phe Thr Ala Ala Phe Tyr Ser Glu Arg Asp Glu Asn  
 260 265 270  
 Ala Glu Ser Phe Tyr Thr Phe Gly Tyr Ile Asp Gln Asp Leu Val Ser  
 275 280 285  
 Ala Ser Gly Gln Glu Ile Ala Trp Thr Asp Val Asp Asn Ser Gln Gly  
 290 295 300  
 Phe Trp Met Phe Pro Ser Thr Lys Thr Thr Ile Asn Gly Lys Asp Ile  
 305 310 315 320  
 Ser Gln Glu Gly Asn Thr Ala Ile Ala Asp Thr Gly Thr Thr Leu Ala  
 325 330 335  
 Leu Val Ser Asp Glu Val Cys Glu Ala Leu Tyr Lys Ala Ile Pro Gly  
 340 345 350  
 Ala Lys Tyr Asp Asp Asn Gln Gln Gly Tyr Val Phe Pro Ile Asn Thr  
 355 360 365  
 Asp Ala Ser Ser Leu Pro Glu Leu Lys Val Ser Val Gly Asn Thr Gln  
 370 375 380  
 Phe Val Ile Gln Pro Glu Asp Leu Ala Phe Ala Pro Ala Asp Asp Ser  
 385 390 395 400  
 Asn Trp Tyr Gly Gly Val Gln Ser Arg Gly Ser Asn Pro Phe Asp Ile  
 405 410 415  
 Leu Gly Asp Val Phe Leu Lys Ser Val Tyr Ala Ile Phe Asp Gln Gly  
 420 425 430  
 Asn Gln Arg Phe Gly Ala Val Pro Lys Ile Gln Ala Lys Gln Asn Leu  
 435 440 445  
 Gln Pro Pro Gln

450

&lt;210&gt; 20

&lt;211&gt; 395

&lt;212&gt; PRT

&lt;213&gt; Trichoderma reesei

&lt;400&gt; 20

Met Lys Ser Ala Leu Leu Ala Ala Ala Ala Leu Val Gly Ser Ala Gln  
 1 5 10 15

Ala Gly Ile His Lys Met Lys Leu Gln Lys Val Ser Leu Glu Gln Gln  
 20 25 30

Leu Glu Gly Ser Ser Ile Glu Ala His Val Gln Gln Leu Gly Gln Lys  
 35 40 45

Tyr Met Gly Val Arg Pro Thr Ser Arg Ala Glu Val Met Phe Asn Asp  
 50 55 60

Lys Pro Pro Lys Val Gln Gly Gly His Pro Val Pro Val Thr Asn Phe  
 65 70 75 80

Met Asn Ala Gln Tyr Phe Ser Glu Ile Thr Ile Gly Thr Pro Pro Gln  
 85 90 95

Ser Phe Lys Val Val Leu Asp Thr Gly Ser Ser Asn Leu Trp Val Pro  
 100 105 110

Ser Gln Ser Cys Asn Ser Ile Ala Cys Phe Leu His Ser Thr Tyr Asp  
 115 120 125

Ser Ser Ser Ser Ser Thr Tyr Lys Pro Asn Gly Ser Asp Phe Glu Ile  
 130 135 140

His Tyr Gly Ser Gly Ser Leu Thr Gly Phe Ile Ser Asn Asp Val Val  
 145 150 155 160

Thr Ile Gly Asp Leu Lys Ile Lys Gly Gln Asp Phe Ala Glu Ala Thr  
 165 170 175

Ser Glu Pro Gly Leu Ala Phe Ala Phe Gly Arg Phe Asp Gly Ile Leu  
 180 185 190

Gly Leu Gly Tyr Asp Thr Ile Ser Val Asn Gly Ile Val Pro Pro Phe  
 195 200 205

Tyr Gln Met Val Asn Gln Lys Leu Ile Asp Glu Pro Val Phe Ala Phe  
 210 215 220

Tyr Leu Gly Ser Ser Asp Glu Gly Ser Glu Ala Val Phe Gly Gly Val  
 225 230 235 240

Asp Asp Ala His Tyr Glu Gly Lys Ile Glu Tyr Ile Pro Leu Arg Arg  
 245 250 255

Lys Ala Tyr Trp Glu Val Asp Leu Asp Ser Ile Ala Phe Gly Asp Glu  
 260 265 270

Val Ala Glu Leu Glu Asn Thr Gly Ala Ile Leu Asp Thr Gly Thr Ser  
 275 280 285

Leu Asn Val Leu Pro Ser Gly Leu Ala Glu Leu Leu Asn Ala Glu Ile  
290 295 300

Gly Ala Lys Lys Gly Phe Gly Gly Gln Tyr Thr Val Asp Cys Ser Lys  
305 310 315 320

Arg Asp Ser Leu Pro Asp Ile Thr Phe Ser Leu Ala Gly Ser Lys Tyr  
325 330 335

Ser Leu Pro Ala Ser Asp Tyr Ile Ile Glu Met Ser Gly Asn Cys Ile  
340 345 350

Ser Ser Phe Gln Gly Met Asp Phe Pro Glu Pro Val Gly Pro Leu Val  
355 360 365

Ile Leu Gly Asp Ala Phe Leu Arg Arg Tyr Tyr Ser Val Tyr Asp Leu  
370 375 380

Gly Arg Asp Ala Val Gly Leu Ala Lys Ala Lys  
385 390 395

<210> 21

<211> 426

<212> PRT

<213> Trichoderma reesei

<400> 21

Met Lys Phe His Ala Ala Ala Leu Thr Leu Ala Cys Leu Ala Ser Ser  
1 5 10 15

Ala Ser Ala Gly Val Ala Gln Pro Arg Ala Asp Glu Val Glu Ser Ala  
20 25 30

Glu Gln Gly Lys Thr Phe Ser Leu Glu Gln Ile Pro Asn Glu Arg Tyr  
35 40 45

Lys Gly Asn Ile Pro Ala Ala Tyr Ile Ser Ala Leu Ala Lys Tyr Ser  
50 55 60

Pro Thr Ile Pro Asp Lys Ile Lys His Ala Ile Glu Ile Asn Pro Asp  
65 70 75 80

Leu His Arg Lys Phe Ser Lys Leu Ile Asn Ala Gly Asn Met Thr Gly  
85 90 95

Thr Ala Val Ala Ser Pro Pro Pro Gly Ala Asp Ala Glu Tyr Val Leu  
100 105 110

Pro Val Lys Ile Gly Thr Pro Pro Gln Thr Leu Pro Leu Asn Leu Asp  
115 120 125

Thr Gly Ser Ser Asp Leu Trp Val Ile Ser Thr Asp Thr Tyr Pro Pro  
130 135 140

Gln Val Gln Gly Gln Thr Arg Tyr Asn Val Ser Ala Ser Thr Thr Ala  
145 150 155 160

Gln Arg Leu Ile Gly Glu Ser Trp Val Ile Arg Tyr Gly Asp Gly Ser  
165 170 175

Ser Ala Asn Gly Ile Val Tyr Lys Asp Arg Val Gln Ile Gly Asn Thr  
180 185 190

Phe Phe Asn Gln Gln Ala Val Glu Ser Ala Val Asn Ile Ser Asn Glu  
195 200 205

Ile Ser Asp Asp Ser Phe Ser Ser Gly Leu Leu Gly Ala Ala Ser Ser  
210 215 220

Ala Ala Asn Thr Val Arg Pro Asp Arg Gln Thr Thr Tyr Leu Glu Asn  
225 230 235 240

Ile Lys Ser Gln Leu Ala Arg Pro Val Phe Thr Ala Asn Leu Lys Lys  
245 250 255

Gly Lys Pro Gly Asn Tyr Asn Phe Gly Tyr Ile Asn Gly Ser Glu Tyr  
260 265 270

Ile Gly Pro Ile Gln Tyr Ala Ala Ile Asn Pro Ser Ser Pro Leu Trp  
275 280 285

Glu Val Ser Val Ser Gly Tyr Arg Val Gly Ser Asn Asp Thr Lys Tyr  
290 295 300

Val Pro Arg Val Trp Asn Ala Ile Ala Asp Thr Gly Thr Thr Leu Leu  
305 310 315 320

Leu Val Pro Asn Asp Ile Val Ser Ala Tyr Tyr Ala Gln Val Lys Gly  
325 330 335

Ser Thr Phe Ser Asn Asp Val Gly Met Met Leu Val Pro Cys Ala Ala  
340 345 350

Thr Leu Pro Asp Phe Ala Phe Gly Leu Gly Asn Tyr Arg Gly Val Ile  
355 360 365

Pro Gly Ser Tyr Ile Asn Tyr Gly Arg Met Asn Lys Thr Tyr Cys Tyr  
370 375 380

Gly Gly Ile Gln Ser Ser Glu Asp Ala Pro Phe Ala Val Leu Gly Asp  
385 390 395 400

Ile Ala Leu Lys Ala Gln Phe Val Val Phe Asp Met Gly Asn Lys Val  
405 410 415

Val Gly Phe Ala Asn Lys Asn Thr Asn Val  
420 425

<210> 22

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 22

acccaaagcg tccttcatta 20

<210> 23

<211> 446

<212> PRT

<213> Trichoderma reesei

<400> 23

Met Thr Leu Pro Val Pro Leu Arg Glu His Asp Leu Pro Phe Leu Lys  
1 5 10 15

Glu Lys Arg Lys Leu Pro Ala Asp Asp Ile Pro Ser Gly Thr Tyr Thr  
 20 25 30  
 Leu Pro Ile Ile His Ala Arg Arg Pro Lys Leu Ala Ser Arg Ala Ile  
 35 40 45  
 Glu Val Gln Val Glu Asn Arg Ser Asp Val Ser Tyr Tyr Ala Gln Leu  
 50 55 60  
 Asn Ile Gly Thr Pro Pro Gln Thr Val Tyr Ala Gln Ile Asp Thr Gly  
 65 70 75 80  
 Ser Phe Glu Leu Trp Val Asn Pro Asn Cys Ser Asn Val Gln Ser Ala  
 85 90 95  
 Asp Gln Arg Phe Cys Arg Ala Ile Gly Phe Tyr Asp Pro Ser Ser Ser  
 100 105 110  
 Ser Thr Ala Asp Val Thr Ser Gln Ser Ala Arg Leu Arg Tyr Gly Ile  
 115 120 125  
 Gly Ser Ala Asp Val Thr Tyr Val His Asp Thr Ile Ser Leu Pro Gly  
 130 135 140  
 Ser Gly Ser Gly Ser Lys Ala Met Lys Ala Val Gln Phe Gly Val Ala  
 145 150 155 160  
 Asp Thr Ser Val Asp Glu Phe Ser Gly Ile Leu Gly Leu Gly Ala Gly  
 165 170 175  
 Asn Gly Ile Asn Thr Glu Tyr Pro Asn Phe Val Asp Glu Leu Ala Ala  
 180 185 190  
 Gln Gly Val Thr Ala Thr Lys Ala Phe Ser Leu Ala Leu Gly Ser Lys  
 195 200 205  
 Ala Glu Glu Glu Gly Val Ile Ile Phe Gly Gly Val Asp Thr Ala Lys  
 210 215 220  
 Phe His Gly Glu Leu Ala His Leu Pro Ile Val Pro Ala Asp Asp Ser  
 225 230 235 240  
 Pro Asp Gly Val Ala Arg Tyr Trp Val Lys Met Lys Ser Ile Ser Leu  
 245 250 255  
 Thr Pro Pro Pro Pro Ser Ser Ser Gly Ser Thr Asp Asp Asn Asn Asn  
 260 265 270  
 Lys Pro Val Ala Phe Pro Gln Thr Ser Met Thr Val Phe Leu Asp Ser  
 275 280 285  
 Gly Ser Thr Leu Thr Leu Leu Pro Pro Ala Leu Val Arg Gln Ile Ala  
 290 295 300  
 Ser Ala Leu Gly Ser Thr Gln Thr Asp Glu Ser Gly Phe Phe Val Val  
 305 310 315 320  
 Asp Cys Ala Leu Ala Ser Gln Asp Gly Thr Ile Asp Phe Glu Phe Asp  
 325 330 335  
 Gly Val Thr Ile Arg Val Pro Tyr Ala Glu Met Ile Arg Gln Val Ser  
 340 345 350  
 Thr Leu Pro Pro His Cys Tyr Leu Gly Met Met Gly Ser Thr Gln Phe  
 355 360 365

Ala Leu Leu Gly Asp Thr Phe Leu Arg Ser Ala Tyr Ala Val Phe Asp  
370 375 380

Leu Thr Ser Asn Val Val His Leu Ala Pro Tyr Ala Asn Cys Gly Thr  
385 390 395 400

Asn Val Lys Ser Ile Thr Ser Thr Ser Ser Leu Ser Asn Leu Val Gly  
405 410 415

Thr Cys Asn Asp Pro Ser Lys Pro Ser Ser Ser Pro Ser Pro Ser Gln  
420 425 430

Thr Pro Ser Ala Ser Pro Ser Ser Thr Ala Thr Gln Lys Ala  
435 440 445

<210> 24

<211> 259

<212> PRT

<213> Trichoderma reesei

<400> 24

Met Ala Pro Ala Ser Gln Val Val Ser Ala Leu Met Leu Pro Ala Leu  
1 5 10 15

Ala Leu Gly Ala Ala Ile Gln Pro Arg Gly Ala Asp Ile Val Gly Gly  
20 25 30

Thr Ala Ala Ser Leu Gly Glu Phe Pro Tyr Ile Val Ser Leu Gln Asn  
35 40 45

Pro Asn Gln Gly Gly His Phe Cys Gly Gly Val Leu Val Asn Ala Asn  
50 55 60

Thr Val Val Thr Ala Ala His Cys Ser Val Val Tyr Pro Ala Ser Gln  
65 70 75 80

Ile Arg Val Arg Ala Gly Thr Leu Thr Trp Asn Ser Gly Gly Thr Leu  
85 90 95

Val Gly Val Ser Gln Ile Ile Val Asn Pro Ser Tyr Asn Asp Arg Thr  
100 105 110

Thr Asp Phe Asp Val Ala Val Trp His Leu Ser Ser Pro Ile Arg Glu  
115 120 125

Ser Ser Thr Ile Gly Tyr Ala Thr Leu Pro Ala Gln Gly Ser Asp Pro  
130 135 140

Val Ala Gly Ser Thr Val Thr Thr Ala Gly Trp Gly Thr Thr Ser Glu  
145 150 155 160

Asn Ser Asn Ser Ile Pro Ser Arg Leu Asn Lys Val Ser Val Pro Val  
165 170 175

Val Ala Arg Ser Thr Cys Gln Ala Asp Tyr Arg Ser Gln Gly Leu Ser  
180 185 190

Val Thr Asn Asn Met Phe Cys Ala Gly Leu Thr Gln Gly Gly Lys Asp  
195 200 205

Ser Cys Ser Gly Asp Ser Gly Gly Pro Ile Val Asp Ala Asn Gly Val  
210 215 220

Leu Gln Gly Val Val Ser Trp Gly Ile Gly Cys Ala Glu Ala Gly Phe

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225          230          235          240
Pro Gly Val Tyr Thr Arg Ile Gly Asn Phe Val Asn Tyr Ile Asn Gln
      245          250          255

Asn Leu Ala

<210> 25
<211> 882
<212> PRT
<213> Trichoderma reesei

<400> 25
Met Val Arg Ser Ala Leu Phe Val Ser Leu Leu Ala Thr Phe Ser Gly
1          5          10          15

Val Ile Ala Arg Val Ser Gly His Gly Ser Lys Ile Val Pro Gly Ala
      20          25          30

Tyr Ile Phe Glu Phe Glu Asp Ser Gln Asp Thr Ala Asp Phe Tyr Lys
      35          40          45

Lys Leu Asn Gly Glu Gly Ser Thr Arg Leu Lys Phe Asp Tyr Lys Leu
      50          55          60

Phe Lys Gly Val Ser Val Gln Leu Lys Asp Leu Asp Asn His Glu Ala
      65          70          75          80

Lys Ala Gln Gln Met Ala Gln Leu Pro Ala Val Lys Asn Val Trp Pro
      85          90          95

Val Thr Leu Ile Asp Ala Pro Asn Pro Lys Val Glu Trp Val Ala Gly
      100          105          110

Ser Thr Ala Pro Thr Leu Glu Ser Arg Ala Ile Lys Lys Pro Pro Ile
      115          120          125

Pro Asn Asp Ser Ser Asp Phe Pro Thr His Gln Met Thr Gln Ile Asp
      130          135          140

Lys Leu Arg Ala Lys Gly Tyr Thr Gly Lys Gly Val Arg Val Ala Val
      145          150          155          160

Ile Asp Thr Gly Ile Asp Tyr Thr His Pro Ala Leu Gly Gly Cys Phe
      165          170          175

Gly Arg Gly Cys Leu Val Ser Phe Gly Thr Asp Leu Val Gly Asp Asp
      180          185          190

Tyr Thr Gly Phe Asn Thr Pro Val Pro Asp Asp Asp Pro Val Asp Cys
      195          200          205

Ala Gly His Gly Ser His Val Ala Gly Ile Ile Ala Ala Gln Glu Asn
      210          215          220

Pro Tyr Gly Phe Thr Gly Gly Ala Pro Asp Val Thr Leu Gly Ala Tyr
      225          230          235          240

Arg Val Phe Gly Cys Asp Gly Gln Ala Gly Asn Asp Val Leu Ile Ser
      245          250          255

Ala Tyr Asn Gln Ala Phe Glu Asp Gly Ala Gln Ile Ile Thr Ala Ser
      260          265          270

Ile Gly Gly Pro Ser Gly Trp Ala Glu Glu Pro Trp Ala Val Ala Val
      275          280          285

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Thr Arg Ile Val Glu Ala Gly Val Pro Cys Thr Val Ser Ala Gly Asn  
290 295 300

Glu Gly Asp Ser Gly Leu Phe Phe Ala Ser Thr Ala Ala Asn Gly Lys  
305 310 315 320

Lys Val Ile Ala Val Ala Ser Val Asp Asn Glu Asn Ile Pro Ser Val  
325 330 335

Leu Ser Val Ala Ser Tyr Lys Ile Asp Ser Gly Ala Ala Gln Asp Phe  
340 345 350

Gly Tyr Val Ser Ser Ser Lys Ala Trp Asp Gly Val Ser Lys Pro Leu  
355 360 365

Tyr Ala Val Ser Phe Asp Thr Thr Ile Pro Asp Asp Gly Cys Ser Pro  
370 375 380

Leu Pro Asp Ser Thr Pro Asp Leu Ser Asp Tyr Ile Val Leu Val Arg  
385 390 395 400

Arg Gly Thr Cys Thr Phe Val Gln Lys Ala Gln Asn Val Ala Ala Lys  
405 410 415

Gly Ala Lys Tyr Leu Leu Tyr Tyr Asn Asn Ile Pro Gly Ala Leu Ala  
420 425 430

Val Asp Val Ser Ala Val Pro Glu Ile Glu Ala Val Gly Met Val Asp  
435 440 445

Asp Lys Thr Gly Ala Thr Trp Ile Ala Ala Leu Lys Asp Gly Lys Thr  
450 455 460

Val Thr Leu Thr Leu Thr Asp Pro Ile Glu Ser Glu Lys Gln Ile Gln  
465 470 475 480

Phe Ser Asp Asn Pro Thr Thr Gly Gly Ala Leu Ser Gly Tyr Thr Thr  
485 490 495

Trp Gly Pro Thr Trp Glu Leu Asp Val Lys Pro Gln Ile Ser Ser Pro  
500 505 510

Gly Gly Asn Ile Leu Ser Thr Tyr Pro Val Ala Leu Gly Gly Tyr Ala  
515 520 525

Thr Leu Ser Gly Thr Ser Met Ala Cys Pro Leu Thr Ala Ala Ala Val  
530 535 540

Ala Leu Ile Gly Gln Ala Arg Gly Thr Phe Asp Pro Ala Leu Ile Asp  
545 550 555 560

Asn Leu Leu Ala Thr Thr Ala Asn Pro Gln Leu Phe Asn Asp Gly Glu  
565 570 575

Lys Phe Tyr Asp Phe Leu Ala Pro Val Pro Gln Gln Gly Gly Gly Leu  
580 585 590

Ile Gln Ala Tyr Asp Ala Ala Phe Ala Thr Thr Leu Leu Ser Pro Ser  
595 600 605

Ser Leu Ser Phe Asn Asp Thr Asp His Phe Ile Lys Lys Lys Gln Ile  
610 615 620



Thr Leu Lys Asn Thr Ser Lys Gln Arg Val Thr Tyr Lys Leu Asn His  
625 630 635 640

Val Pro Thr Asn Thr Phe Tyr Thr Leu Ala Pro Gly Asn Gly Tyr Pro  
645 650 655

Ala Pro Phe Pro Asn Asp Ala Val Ala Ala His Ala Asn Leu Lys Phe  
660 665 670

Asn Leu Gln Gln Val Thr Leu Pro Ala Gly Arg Ser Ile Thr Val Asp  
675 680 685

Val Phe Pro Thr Pro Pro Arg Asp Val Asp Ala Lys Arg Leu Ala Leu  
690 695 700

Trp Ser Gly Tyr Ile Thr Val Asn Gly Thr Asp Gly Thr Ser Leu Ser  
705 710 715 720

Val Pro Tyr Gln Gly Leu Thr Gly Ser Leu His Lys Gln Lys Val Leu  
725 730 735

Tyr Pro Glu Asp Ser Trp Ile Ala Asp Ser Thr Asp Glu Ser Leu Ala  
740 745 750

Pro Val Glu Asn Gly Thr Val Phe Thr Ile Pro Ala Pro Gly Asn Ala  
755 760 765

Gly Pro Asp Asp Lys Leu Pro Ser Leu Val Val Ser Pro Ala Leu Gly  
770 775 780

Ser Arg Tyr Val Arg Val Asp Leu Val Leu Leu Ser Ala Pro Pro His  
785 790 795 800

Gly Thr Lys Leu Lys Thr Val Lys Phe Leu Asp Thr Thr Ser Ile Gly  
805 810 815

Gln Pro Ala Gly Ser Pro Leu Leu Trp Ile Ser Arg Gly Ala Asn Pro  
820 825 830

Ile Ala Trp Thr Gly Glu Leu Ser Asp Asn Lys Phe Ala Pro Pro Gly  
835 840 845

Thr Tyr Lys Ala Val Phe His Ala Leu Arg Ile Phe Gly Asn Glu Lys  
850 855 860

Lys Lys Glu Asp Trp Asp Val Ser Glu Ser Pro Ala Phe Thr Ile Lys  
865 870 875 880

Tyr Ala

<210> 26

<211> 541

<212> PRT

<213> Trichoderma reesei

<400> 26

Met Arg Ser Val Val Ala Leu Ser Met Ala Ala Val Ala Gln Ala Ser  
1 5 10 15

Thr Phe Gln Ile Gly Thr Ile His Glu Lys Ser Ala Pro Val Leu Ser  
20 25 30

Asn Val Glu Ala Asn Ala Ile Pro Asp Ala Tyr Ile Ile Lys Phe Lys  
35 40 45

Asp His Val Gly Glu Asp Asp Ala Ser Lys His His Asp Trp Ile Gln  
 50 55 60

Ser Ile His Thr Asn Val Glu Gln Glu Arg Leu Glu Leu Arg Lys Arg  
 65 70 75 80

Ser Asn Val Phe Gly Ala Asp Asp Val Phe Asp Gly Leu Lys His Thr  
 85 90 95

Phe Lys Ile Gly Asp Gly Phe Lys Gly Tyr Ala Gly His Phe His Glu  
 100 105 110

Ser Val Ile Glu Gln Val Arg Asn His Pro Asp Val Glu Tyr Ile Glu  
 115 120 125

Arg Asp Ser Ile Val His Thr Met Leu Pro Leu Glu Ser Lys Asp Ser  
 130 135 140

Ile Ile Val Glu Asp Ser Cys Asn Gly Glu Thr Glu Lys Gln Ala Pro  
 145 150 155 160

Trp Gly Leu Ala Arg Ile Ser His Arg Glu Thr Leu Asn Phe Gly Ser  
 165 170 175

Phe Asn Lys Tyr Leu Tyr Thr Ala Asp Gly Gly Glu Gly Val Asp Ala  
 180 185 190

Tyr Val Ile Asp Thr Gly Thr Asn Ile Glu His Val Asp Phe Glu Gly  
 195 200 205

Arg Ala Lys Trp Gly Lys Thr Ile Pro Ala Gly Asp Glu Asp Glu Asp  
 210 215 220

Gly Asn Gly His Gly Thr His Cys Ser Gly Thr Val Ala Gly Lys Lys  
 225 230 235 240

Tyr Gly Val Ala Lys Lys Ala His Val Tyr Ala Val Lys Val Leu Arg  
 245 250 255

Ser Asn Gly Ser Gly Thr Met Ser Asp Val Val Lys Gly Val Glu Tyr  
 260 265 270

Ala Ala Leu Ser His Ile Glu Gln Val Lys Lys Ala Lys Lys Gly Lys  
 275 280 285

Arg Lys Gly Phe Lys Gly Ser Val Ala Asn Met Ser Leu Gly Gly Gly  
 290 295 300

Lys Thr Gln Ala Leu Asp Ala Ala Val Asn Ala Ala Val Arg Ala Gly  
 305 310 315 320

Val His Phe Ala Val Ala Ala Gly Asn Asp Asn Ala Asp Ala Cys Asn  
 325 330 335

Tyr Ser Pro Ala Ala Ala Thr Glu Pro Leu Thr Val Gly Ala Ser Ala  
 340 345 350

Leu Asp Asp Ser Arg Ala Tyr Phe Ser Asn Tyr Gly Lys Cys Thr Asp  
 355 360 365

Ile Phe Ala Pro Gly Leu Ser Ile Gln Ser Thr Trp Ile Gly Ser Lys  
 370 375 380

Tyr Ala Val Asn Thr Ile Ser Gly Thr Ser Met Ala Ser Pro His Ile  
 385 390 395 400

Cys Gly Leu Leu Ala Tyr Tyr Leu Ser Leu Gln Pro Ala Gly Asp Ser  
405 410 415

Glu Phe Ala Val Ala Pro Ile Thr Pro Lys Lys Leu Lys Glu Ser Val  
420 425 430

Ile Ser Val Ala Thr Lys Asn Ala Leu Ser Asp Leu Pro Asp Ser Asp  
435 440 445

Thr Pro Asn Leu Leu Ala Trp Asn Gly Gly Gly Cys Ser Asn Phe Ser  
450 455 460

Gln Ile Val Glu Ala Gly Ser Tyr Thr Val Lys Pro Lys Gln Asn Lys  
465 470 475 480

Gln Ala Lys Leu Pro Ser Thr Ile Glu Glu Leu Glu Glu Ala Ile Glu  
485 490 495

Gly Asp Phe Glu Val Val Ser Gly Glu Ile Val Lys Gly Ala Lys Ser  
500 505 510

Phe Gly Ser Lys Ala Glu Lys Phe Ala Lys Lys Ile His Asp Leu Val  
515 520 525

Glu Glu Glu Ile Glu Glu Phe Ile Ser Glu Leu Ser Glu  
530 535 540

<210> 27

<211> 391

<212> PRT

<213> Trichoderma reesei

<400> 27

Met Arg Leu Ser Val Leu Leu Ser Val Leu Pro Leu Val Leu Ala Ala  
1 5 10 15

Pro Ala Ile Glu Lys Arg Ala Glu Pro Ala Pro Leu Leu Val Pro Thr  
20 25 30

Thr Lys His Gly Leu Val Ala Asp Lys Tyr Ile Val Lys Phe Lys Asp  
35 40 45

Gly Ser Ser Leu Gln Ala Val Asp Glu Ala Ile Ser Gly Leu Val Ser  
50 55 60

Asn Ala Asp His Val Tyr Gln His Val Phe Arg Gly Phe Ala Ala Thr  
65 70 75 80

Leu Asp Lys Glu Thr Leu Glu Ala Leu Arg Asn His Pro Glu Val Asp  
85 90 95

Tyr Ile Glu Gln Asp Ala Val Val Lys Ile Asn Ala Tyr Val Ser Gln  
100 105 110

Thr Gly Ala Pro Trp Gly Leu Gly Arg Ile Ser His Lys Ala Arg Gly  
115 120 125

Ser Thr Thr Tyr Val Tyr Asp Asp Ser Ala Gly Ala Gly Thr Cys Ser  
130 135 140

Tyr Val Ile Asp Thr Gly Val Asp Ala Thr His Pro Asp Phe Glu Gly  
145 150 155 160

Arg Ala Thr Leu Leu Arg Ser Phe Val Ser Gly Gln Asn Thr Asp Gly  
165 170 175

Asn Gly His Gly Thr His Val Ser Gly Thr Ile Gly Ser Arg Thr Tyr  
180 185 190

Gly Val Ala Lys Lys Thr Gln Ile Tyr Gly Val Lys Val Leu Asp Asn  
195 200 205

Ser Gly Ser Gly Ser Phe Ser Thr Val Ile Ala Gly Met Asp Tyr Val  
210 215 220

Ala Ser Asp Ser Gln Thr Arg Asn Cys Pro Asn Gly Ser Val Ala Asn  
225 230 235 240

Met Ser Leu Gly Gly Gly Tyr Thr Ala Ser Val Asn Gln Ala Ala Ala  
245 250 255

Arg Leu Ile Gln Ala Gly Val Phe Leu Ala Val Ala Ala Gly Asn Asp  
260 265 270

Gly Val Asp Ala Arg Asn Thr Ser Pro Ala Ser Glu Pro Thr Val Cys  
275 280 285

Thr Val Gly Ala Ser Thr Ser Ser Asp Ala Arg Ala Ser Phe Ser Asn  
290 295 300

Tyr Gly Ser Val Val Asp Ile Phe Ala Pro Gly Gln Asp Ile Leu Ser  
305 310 315 320

Thr Trp Pro Asn Arg Gln Thr Asn Thr Ile Ser Gly Thr Ser Met Ala  
325 330 335

Thr Pro His Ile Val Gly Leu Gly Ala Tyr Leu Ala Gly Leu Glu Gly  
340 345 350

Phe Ser Asp Pro Gln Ala Leu Cys Ala Arg Ile Gln Ser Leu Ala Asn  
355 360 365

Arg Asn Leu Leu Ser Gly Ile Pro Ser Gly Thr Ile Asn Ala Ile Ala  
370 375 380

Phe Asn Gly Asn Pro Ser Gly  
385 390

<210> 28

<211> 387

<212> PRT

<213> Trichoderma reesei

<400> 28

Met Gly Leu Val Thr Asn Pro Phe Ala Lys Asn Ile Ile Pro Asn Arg  
1 5 10 15

Tyr Ile Val Val Tyr Asn Asn Ser Phe Gly Glu Glu Ala Ile Ser Ala  
20 25 30

Lys Gln Ala Gln Phe Ala Ala Lys Ile Ala Lys Arg Asn Leu Gly Lys  
35 40 45

Arg Gly Leu Phe Gly Asn Glu Leu Ser Thr Ala Ile His Ser Phe Ser  
50 55 60

Met His Thr Trp Arg Ala Met Ala Leu Asp Ala Asp Asp Ile Met Ile  
65 70 75 80

Lys Asp Ile Phe Asp Ala Glu Glu Val Ala Tyr Ile Glu Ala Asp Thr  
85 90 95

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Lys Val Gln His Ala Ala Leu Val Ala Gln Thr Asn Ala Ala Pro Gly
100                      105                      110

Leu Ile Arg Leu Ser Asn Lys Ala Val Gly Gly Gln Asn Tyr Ile Phe
115                      120                      125

Asp Asn Ser Ala Gly Ser Asn Ile Thr Ala Tyr Val Val Asp Thr Gly
130                      135                      140

Ile Arg Ile Thr His Ser Glu Phe Glu Gly Arg Ala Thr Phe Gly Ala
145                      150                      155                      160

Asn Phe Val Asn Asp Asp Thr Asp Glu Asn Gly His Gly Ser His Val
165                      170                      175

Ala Gly Thr Ile Gly Gly Ala Thr Phe Gly Val Ala Lys Asn Val Glu
180                      185                      190

Leu Val Ala Val Lys Val Leu Asp Ala Asp Gly Ser Gly Ser Asn Ser
195                      200                      205

Gly Val Leu Asn Gly Met Gln Phe Val Val Asn Asp Val Gln Ala Lys
210                      215                      220

Lys Arg Ser Gly Lys Ala Val Met Asn Met Ser Leu Gly Gly Ser Phe
225                      230                      235                      240

Ser Thr Ala Val Asn Asn Ala Ile Thr Ala Leu Thr Asn Ala Gly Ile
245                      250                      255

Val Pro Val Val Ala Ala Gly Asn Glu Asn Gln Asp Thr Ala Asn Thr
260                      265                      270

Ser Pro Gly Ser Ala Pro Gln Ala Ile Thr Val Gly Ala Ile Asp Ala
275                      280                      285

Thr Thr Asp Ile Arg Ala Gly Phe Ser Asn Phe Gly Thr Gly Val Asp
290                      295                      300

Ile Tyr Ala Pro Gly Val Asp Val Leu Ser Val Gly Ile Lys Ser Asp
305                      310                      315                      320

Ile Asp Thr Ala Val Leu Ser Gly Thr Ser Met Ala Ser Pro His Val
325                      330                      335

Ala Gly Leu Ala Ala Tyr Leu Met Ala Leu Glu Gly Val Ser Asn Val
340                      345                      350

Asp Asp Val Ser Asn Leu Ile Lys Asn Leu Ala Ala Lys Thr Gly Ala
355                      360                      365

Ala Val Lys Gln Asn Ile Ala Gly Thr Thr Ser Leu Ile Ala Asn Asn
370                      375                      380

Gly Asn Phe
385

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&lt;210&gt; 29

&lt;211&gt; 409

&lt;212&gt; PRT

&lt;213&gt; Trichoderma reesei

&lt;400&gt; 29

Met Ala Ser Leu Arg Arg Leu Ala Leu Tyr Leu Gly Ala Leu Leu Pro  
 1 5 10 15

Ala Val Leu Ala Ala Pro Ala Val Asn Tyr Lys Leu Pro Glu Ala Val  
 20 25 30

Pro Asn Lys Phe Ile Val Thr Leu Lys Asp Gly Ala Ser Val Asp Thr  
 35 40 45

Asp Ser His Leu Thr Trp Val Lys Asp Leu His Arg Arg Ser Leu Gly  
 50 55 60

Lys Arg Ser Thr Ala Gly Val Glu Lys Thr Tyr Asn Ile Asp Ser Trp  
 65 70 75 80

Asn Ala Tyr Ala Gly Glu Phe Asp Glu Glu Thr Val Lys Gln Ile Lys  
 85 90 95

Ala Asn Pro Asp Val Ala Ser Val Glu Pro Asp Tyr Ile Met Trp Leu  
 100 105 110

Ser Asp Ile Val Glu Asp Lys Arg Ala Leu Thr Thr Gln Thr Gly Ala  
 115 120 125

Pro Trp Gly Leu Gly Thr Val Ser His Arg Thr Pro Gly Ser Thr Ser  
 130 135 140

Tyr Ile Tyr Asp Thr Ser Ala Gly Ser Gly Thr Phe Ala Tyr Val Val  
 145 150 155 160

Asp Ser Gly Ile Asn Ile Ala His Gln Gln Phe Gly Gly Arg Ala Ser  
 165 170 175

Leu Gly Tyr Asn Ala Ala Gly Gly Asp His Val Asp Thr Leu Gly His  
 180 185 190

Gly Thr His Val Ser Gly Thr Ile Gly Gly Ser Thr Tyr Gly Val Ala  
 195 200 205

Lys Gln Ala Ser Leu Ile Ser Val Lys Val Phe Gln Gly Asn Ser Ala  
 210 215 220

Ser Thr Ser Val Ile Leu Asp Gly Tyr Asn Trp Ala Val Asn Asp Ile  
 225 230 235 240

Val Ser Arg Asn Arg Ala Ser Lys Ser Ala Ile Asn Met Ser Leu Gly  
 245 250 255

Gly Pro Ala Ser Ser Thr Trp Ala Thr Ala Ile Asn Ala Ala Phe Asn  
 260 265 270

Lys Gly Val Leu Thr Ile Val Ala Ala Gly Asn Gly Asp Ala Leu Gly  
 275 280 285

Asn Pro Gln Pro Val Ser Ser Thr Ser Pro Ala Asn Val Pro Asn Ala  
 290 295 300

Ile Thr Val Ala Ala Leu Asp Ile Asn Trp Arg Thr Ala Ser Phe Thr  
 305 310 315 320

Asn Tyr Gly Ala Gly Val Asp Val Phe Ala Pro Gly Val Asn Ile Leu  
 325 330 335

Ser Ser Trp Ile Gly Ser Asn Thr Ala Thr Asn Thr Ile Ser Gly Thr  
340 345 350

Ser Met Ala Thr Pro His Val Val Gly Leu Ala Leu Tyr Leu Gln Ala  
355 360 365

Leu Glu Gly Leu Ser Thr Pro Thr Ala Val Thr Asn Arg Ile Lys Ala  
370 375 380

Leu Ala Thr Thr Gly Arg Val Thr Gly Ser Leu Asn Gly Ser Pro Asn  
385 390 395 400

Thr Leu Ile Phe Asn Gly Asn Ser Ala  
405

<210> 30

<211> 555

<212> PRT

<213> Trichoderma reesei

<400> 30

Met Arg Ala Cys Leu Phe Leu Gly Ile Thr Ala Leu Ala Thr Ala  
1 5 10 15

Ile Pro Ala Leu Lys Pro Pro His Gly Ser Pro Asp Arg Ala His Thr  
20 25 30

Thr Gln Leu Ala Lys Val Ser Ile Ala Leu Gln Pro Glu Cys Arg Glu  
35 40 45

Leu Leu Glu Gln Ala Leu His His Leu Ser Asp Pro Ser Ser Pro Arg  
50 55 60

Tyr Gly Arg Tyr Leu Gly Arg Glu Glu Ala Lys Ala Leu Leu Arg Pro  
65 70 75 80

Arg Arg Glu Ala Thr Ala Ala Val Lys Arg Trp Leu Ala Arg Ala Gly  
85 90 95

Val Pro Ala His Asp Val Leu Thr Asp Gly Gln Phe Ile His Val Arg  
100 105 110

Thr Leu Ala Glu Lys Ala Gln Ala Leu Leu Gly Phe Glu Tyr Asn Ser  
115 120 125

Thr Leu Gly Ser Gln Thr Ile Ala Ile Ser Thr Leu Pro Gly Lys Ile  
130 135 140

Arg Lys His Val Met Thr Val Gln Tyr Val Pro Leu Trp Thr Glu Ala  
145 150 155 160

Asp Trp Glu Glu Cys Lys Thr Ile Ile Thr Pro Ser Cys Leu Lys Arg  
165 170 175

Leu Tyr His Val Asp Ser Tyr Arg Ala Lys Tyr Glu Ser Ser Ser Leu  
180 185 190

Phe Gly Ile Val Gly Phe Ser Gly Gln Ala Ala Gln His Asp Glu Leu  
195 200 205

Asp Lys Phe Leu His Asp Phe Ala Pro Tyr Ser Thr Asn Ala Asn Phe  
210 215 220

225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300

Ser Ile Glu Ser Val Asn Gly Gly Gln Ser Pro Gln Gly Met Asn Glu  
225 230 235 240

Pro Ala Ser Glu Ala Asn Gly Asp Val Gln Tyr Ala Val Ala Met Gly  
245 250 255

Tyr His Val Pro Val Arg Tyr Tyr Ala Val Gly Gly Glu Asn His Asp  
260 265 270

Ile Ile Pro Asp Leu Asp Leu Val Asp Thr Thr Glu Glu Tyr Leu Glu  
275 280 285

Pro Phe Leu Glu Phe Ala Ser His Leu Leu Asp Leu Asp Asp Asp Glu  
290 295 300

Leu Pro Arg Val Val Ser Ile Ser Tyr Gly Ala Asn Glu Gln Leu Phe  
305 310 315 320

Pro Arg Ser Tyr Ala His Gln Val Cys Asp Met Phe Gly Gln Leu Gly  
325 330 335

Ala Arg Gly Val Ser Ile Val Val Ala Ala Gly Asp Leu Gly Pro Gly  
340 345 350

Val Ser Cys Gln Ser Asn Asp Gly Ser Ala Arg Pro Lys Phe Ile Pro  
355 360 365

Ser Phe Pro Ala Thr Cys Pro Tyr Val Thr Ser Val Gly Ser Thr Arg  
370 375 380

Gly Ile Met Pro Glu Val Ala Ala Ser Phe Ser Ser Gly Gly Phe Ser  
385 390 395 400

Asp Tyr Phe Ala Arg Pro Ala Trp Gln Asp Arg Ala Val Gly Ala Tyr  
405 410 415

Leu Gly Ala His Gly Glu Glu Trp Glu Gly Phe Tyr Asn Pro Ala Gly  
420 425 430

Arg Gly Phe Pro Asp Val Ala Ala Gln Gly Val Asn Phe Arg Phe Arg  
435 440 445

Ala His Gly Asn Glu Ser Leu Ser Ser Gly Thr Ser Leu Ser Ser Pro  
450 455 460

Val Phe Ala Ala Leu Ile Ala Leu Leu Asn Asp His Arg Ser Lys Ser  
465 470 475 480

Gly Met Pro Pro Met Gly Phe Leu Asn Pro Trp Ile Tyr Thr Val Gly  
485 490 495

Ser His Ala Phe Thr Asp Ile Ile Glu Ala Arg Ser Glu Gly Cys Pro  
500 505 510

Gly Gln Ser Val Glu Tyr Leu Ala Ser Pro Tyr Ile Pro Asn Ala Gly  
515 520 525

Trp Ser Ala Val Pro Gly Trp Asp Pro Val Thr Gly Trp Gly Thr Pro  
530 535 540

Leu Phe Asp Arg Met Leu Asn Leu Ser Leu Val  
545 550 555

<210> 31

<211> 388

<212> PRT



&lt;213&gt; Trichoderma reesei

&lt;400&gt; 31

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Met Ala Trp Leu Lys Lys Leu Ala Leu Val Leu Leu Ala Ile Val Pro
1      5      10      15

Tyr Ala Thr Ala Ser Pro Ala Leu Ser Pro Arg Ser Arg Glu Ile Leu
20      25      30

Ser Leu Glu Asp Leu Glu Ser Glu Asp Lys Tyr Val Ile Gly Leu Lys
35      40      45

Gln Gly Leu Ser Pro Thr Asp Leu Lys Lys His Leu Leu Arg Val Ser
50      55      60

Ala Val Gln Tyr Arg Asn Lys Asn Ser Thr Phe Glu Gly Gly Thr Gly
65      70      75      80

Val Lys Arg Thr Tyr Ala Ile Gly Asp Tyr Arg Ala Tyr Thr Ala Val
85      90      95

Leu Asp Arg Asp Thr Val Arg Glu Ile Trp Asn Asp Thr Leu Glu Lys
100     105     110

Pro Pro Trp Gly Leu Ala Thr Leu Ser Asn Lys Lys Pro His Gly Phe
115     120     125

Leu Tyr Arg Tyr Asp Lys Ser Ala Gly Glu Gly Thr Phe Ala Tyr Val
130     135     140

Leu Asp Thr Gly Ile Asn Ser Lys His Val Asp Phe Glu Gly Arg Ala
145     150     155     160

Tyr Met Gly Phe Ser Pro Pro Lys Thr Glu Pro Thr Asp Ile Asn Gly
165     170     175

His Gly Thr His Val Ala Gly Ile Ile Gly Gly Lys Thr Phe Gly Val
180     185     190

Ala Lys Lys Thr Gln Leu Ile Gly Val Lys Val Phe Leu Asp Asp Glu
195     200     205

Ala Thr Thr Ser Thr Leu Met Glu Gly Leu Glu Trp Ala Val Asn Asp
210     215     220

Ile Thr Thr Lys Gly Arg Gln Gly Arg Ser Val Ile Asn Met Ser Leu
225     230     235     240

Gly Gly Pro Tyr Ser Gln Ala Leu Asn Asp Ala Ile Asp His Ile Ala
245     250     255

Asp Met Gly Ile Leu Pro Val Ala Ala Ala Gly Asn Lys Gly Ile Pro
260     265     270

Ala Thr Phe Ile Ser Pro Ala Ser Ala Asp Lys Ala Met Thr Val Gly
275     280     285

Ala Ile Asn Ser Asp Trp Gln Glu Thr Asn Phe Ser Asn Phe Gly Pro
290     295     300

Gln Val Asn Ile Leu Ala Pro Gly Glu Asp Val Leu Ser Ala Tyr Val
305     310     315     320

Ser Thr Asn Thr Ala Thr Arg Val Leu Ser Gly Thr Ser Met Ala Ala
325     330     335

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Pro His Val Ala Gly Leu Ala Leu Tyr Leu Met Ala Leu Glu Glu Phe  
340 345 350

Asp Ser Thr Gln Lys Leu Thr Asp Arg Ile Leu Gln Leu Gly Met Lys  
355 360 365

Asn Lys Val Val Asn Leu Met Thr Asp Ser Pro Asn Leu Ile Ile His  
370 375 380

Asn Asn Val Lys  
385

<210> 32

<211> 256

<212> PRT

<213> Trichoderma reesei

<400> 32

Met Phe Ile Ala Gly Val Ala Leu Ser Ala Leu Leu Cys Ala Asp Thr  
1 5 10 15

Val Leu Ala Gly Val Ala Gln Asp Arg Gly Leu Ala Ala Arg Leu Ala  
20 25 30

Arg Arg Ala Gly Arg Arg Ser Ala Pro Phe Arg Asn Asp Thr Ser His  
35 40 45

Ala Thr Val Gln Ser Asn Trp Gly Gly Ala Ile Leu Glu Gly Ser Gly  
50 55 60

Phe Thr Ala Ala Ser Ala Thr Val Asn Val Pro Arg Gly Gly Gly Gly  
65 70 75 80

Ser Asn Ala Ala Gly Ser Ala Trp Val Gly Ile Asp Gly Ala Ser Cys  
85 90 95

Gln Thr Ala Ile Leu Gln Thr Gly Phe Asp Trp Tyr Gly Asp Gly Thr  
100 105 110

Tyr Asp Ala Trp Tyr Glu Trp Tyr Pro Glu Phe Ala Ala Asp Phe Ser  
115 120 125

Gly Ile Asp Ile Arg Gln Gly Asp Gln Ile Ala Met Ser Val Val Ala  
130 135 140

Thr Ser Leu Thr Gly Gly Ser Ala Thr Leu Glu Asn Leu Ser Thr Gly  
145 150 155 160

Gln Lys Val Thr Gln Asn Phe Asn Arg Val Thr Ala Gly Ser Leu Cys  
165 170 175

Glu Thr Ser Ala Glu Phe Ile Ile Glu Asp Phe Glu Glu Cys Asn Ser  
180 185 190

Asn Gly Ser Asn Cys Gln Pro Val Pro Phe Ala Ser Phe Ser Pro Ala  
195 200 205

Ile Thr Phe Ser Ser Ala Thr Ala Thr Arg Ser Gly Arg Ser Val Ser  
210 215 220

Leu Ser Gly Ala Glu Ile Thr Glu Val Ile Val Asn Asn Gln Asp Leu  
225 230 235 240

Thr Arg Cys Ser Val Ser Gly Ser Ser Thr Leu Thr Cys Ser Tyr Val  
245 250 255

<210> 33

<211> 236

<212> PRT

<213> Trichoderma reesei

<400> 33

Met Asp Ala Ile Arg Ala Arg Ser Ala Ala Arg Arg Ser Asn Arg Phe  
1 5 10 15

Gln Ala Gly Ser Ser Lys Asn Val Asn Gly Thr Ala Asp Val Glu Ser  
20 25 30

Thr Asn Trp Ala Gly Ala Ala Ile Thr Thr Ser Gly Val Thr Glu Val  
35 40 45

Ser Gly Thr Phe Thr Val Pro Arg Pro Ser Val Pro Ala Gly Gly Ser  
50 55 60

Ser Arg Glu Glu Tyr Cys Gly Ala Ala Trp Val Gly Ile Asp Gly Tyr  
65 70 75 80

Ser Asp Ala Asp Leu Ile Gln Thr Gly Val Leu Trp Cys Val Glu Asp  
85 90 95

Gly Glu Tyr Leu Tyr Glu Ala Trp Tyr Glu Tyr Leu Pro Ala Ala Leu  
100 105 110

Val Glu Tyr Ser Gly Ile Ser Val Thr Ala Gly Ser Val Val Thr Val  
115 120 125

Thr Ala Thr Lys Thr Gly Thr Asn Ser Gly Val Thr Thr Leu Thr Ser  
130 135 140

Gly Gly Lys Thr Val Ser His Thr Phe Ser Arg Gln Asn Ser Pro Leu  
145 150 155 160

Pro Gly Thr Ser Ala Glu Trp Ile Val Glu Asp Phe Thr Ser Gly Ser  
165 170 175

Ser Leu Val Pro Phe Ala Asp Phe Gly Ser Val Thr Phe Thr Gly Ala  
180 185 190

Thr Ala Val Val Asn Gly Ala Thr Val Thr Ala Gly Gly Asp Ser Pro  
195 200 205

Val Ile Ile Asp Leu Glu Asp Ser Arg Gly Asp Ile Leu Thr Ser Thr  
210 215 220

Thr Val Ser Gly Ser Thr Val Thr Val Glu Tyr Glu  
225 230 235

<210> 34

<211> 612

<212> PRT

<213> Trichoderma reesei

<400> 34

Met Ala Lys Leu Ser Thr Leu Arg Leu Ala Ser Leu Leu Ser Leu Val  
1 5 10 15

Ser Val Gln Val Ser Ala Ser Val His Leu Leu Glu Ser Leu Glu Lys  
20 25 30

20                      25                      30  
 Leu Pro His Gly Trp Lys Ala Ala Glu Thr Pro Ser Pro Ser Ser Gln  
                          35                      40                      45  
 Ile Val Leu Gln Val Ala Leu Thr Gln Gln Asn Ile Asp Gln Leu Glu  
                          50                      55                      60  
 Ser Arg Leu Ala Ala Val Ser Thr Pro Thr Ser Ser Thr Tyr Gly Lys  
                          65                      70                      75                      80  
 Tyr Leu Asp Val Asp Glu Ile Asn Ser Ile Phe Ala Pro Ser Asp Ala  
                          85                      90                      95  
 Ser Ser Ser Ala Val Glu Ser Trp Leu Gln Ser His Gly Val Thr Ser  
                          100                      105                      110  
 Tyr Thr Lys Gln Gly Ser Ser Ile Trp Phe Gln Thr Asn Ile Ser Thr  
                          115                      120                      125  
 Ala Asn Ala Met Leu Ser Thr Asn Phe His Thr Tyr Ser Asp Leu Thr  
                          130                      135                      140  
 Gly Ala Lys Lys Val Arg Thr Leu Lys Tyr Ser Ile Pro Glu Ser Leu  
                          145                      150                      155                      160  
 Ile Gly His Val Asp Leu Ile Ser Pro Thr Thr Tyr Phe Gly Thr Thr  
                          165                      170                      175  
 Lys Ala Met Arg Lys Leu Lys Ser Ser Gly Val Ser Pro Ala Ala Asp  
                          180                      185                      190  
 Ala Leu Ala Ala Arg Gln Glu Pro Ser Ser Cys Lys Gly Thr Leu Val  
                          195                      200                      205  
 Phe Glu Gly Glu Thr Phe Asn Val Phe Gln Pro Asp Cys Leu Arg Thr  
                          210                      215                      220  
 Glu Tyr Ser Val Asp Gly Tyr Thr Pro Ser Val Lys Ser Gly Ser Arg  
                          225                      230                      235                      240  
 Ile Gly Phe Gly Ser Phe Leu Asn Glu Ser Ala Ser Phe Ala Asp Gln  
                          245                      250                      255  
 Ala Leu Phe Glu Lys His Phe Asn Ile Pro Ser Gln Asn Phe Ser Val  
                          260                      265                      270  
 Val Leu Ile Asn Gly Gly Thr Asp Leu Pro Gln Pro Pro Ser Asp Ala  
                          275                      280                      285  
 Asn Asp Gly Glu Ala Asn Leu Asp Ala Gln Thr Ile Leu Thr Ile Ala  
                          290                      295                      300  
 His Pro Leu Pro Ile Thr Glu Phe Ile Thr Ala Gly Ser Pro Pro Tyr  
                          305                      310                      315                      320  
 Phe Pro Asp Pro Val Glu Pro Ala Gly Thr Pro Asn Glu Asn Glu Pro  
                          325                      330                      335  
 Tyr Leu Gln Tyr Tyr Glu Phe Leu Leu Ser Lys Ser Asn Ala Glu Ile  
                          340                      345                      350  
 Pro Gln Val Ile Thr Asn Ser Tyr Gly Asp Glu Glu Gln Thr Val Pro  
                          355                      360                      365  
 Arg Ser Tyr Ala Val Arg Val Cys Asn Leu Ile Glu Leu Leu Gly Leu

Arg Ser Tyr Ala Val Arg Val Cys Asn Ser Ile Gly Ser Ser Gly Ser  
 370 375 380  
 Arg Gly Ile Ser Val Leu His Ser Ser Gly Asp Glu Gly Val Gly Ala  
 385 390 395 400  
 Ser Cys Val Ala Thr Asn Ser Thr Thr Pro Gln Phe Asn Pro Ile Phe  
 405 410 415  
 Pro Ala Thr Cys Pro Tyr Val Thr Ser Val Gly Gly Thr Val Ser Phe  
 420 425 430  
 Asn Pro Glu Val Ala Trp Ala Gly Ser Ser Gly Gly Phe Ser Tyr Tyr  
 435 440 445  
 Phe Ser Arg Pro Trp Tyr Gln Gln Glu Ala Val Gly Thr Tyr Leu Glu  
 450 455 460  
 Lys Tyr Val Ser Ala Glu Thr Lys Lys Tyr Tyr Gly Pro Tyr Val Asp  
 465 470 475 480  
 Phe Ser Gly Arg Gly Phe Pro Asp Val Ala Ala His Ser Val Ser Pro  
 485 490 495  
 Asp Tyr Pro Val Phe Gln Gly Gly Glu Leu Thr Pro Ser Gly Gly Thr  
 500 505 510  
 Ser Ala Ala Ser Pro Val Val Ala Ala Ile Val Ala Leu Leu Asn Asp  
 515 520 525  
 Ala Arg Leu Arg Glu Gly Lys Pro Thr Leu Gly Phe Leu Asn Pro Leu  
 530 535 540  
 Ile Tyr Leu His Ala Ser Lys Gly Phe Thr Asp Ile Thr Ser Gly Gln  
 545 550 555 560  
 Ser Glu Gly Cys Asn Gly Asn Asn Thr Gln Thr Gly Ser Pro Leu Pro  
 565 570 575  
 Gly Ala Gly Phe Ile Ala Gly Ala His Trp Asn Ala Thr Lys Gly Trp  
 580 585 590  
 Asp Pro Thr Thr Gly Phe Gly Val Pro Asn Leu Lys Lys Leu Leu Ala  
 595 600 605  
 Leu Val Arg Phe  
 610

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&lt;213&gt; Artificial Sequence

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&lt;223&gt; Primer

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gccgagtttg 70

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&lt;211&gt; 65

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

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agcgt 65

&lt;210&gt; 43

&lt;211&gt; 63

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

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agc 63

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&lt;211&gt; 27

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 44

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&lt;210&gt; 45

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&lt;223&gt; Primer

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&lt;211&gt; 320

&lt;212&gt; PRT

&lt;213&gt; Ascaris suum

&lt;400&gt; 112

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20 25 30

Pro Asn Glu Arg Trp Ile Phe Ile Gly Ser Lys Asp Cys Asp Leu Ser  
35 40 45

Asp Leu Glu Ala Thr Arg Lys Leu Phe Ala Lys His Lys Pro Thr His  
50 55 60

Val Ile His Leu Ala Ala Met Val Gly Gly Leu Phe His Asn Leu Gln  
65 70 75 80

His Asn Leu Gln Phe Phe Arg Lys Asn Met Gln Ile Asn Asp Asn Val  
85 90 95

Leu Ala Val Cys Asp Glu Asn Asp Ile Glu Lys Cys Val Ser Cys Leu  
100 105 110

Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr Pro Ile Asp Glu Thr  
115 120 125

Met Val His Asn Gly Pro Pro His Asp Ser Asn Phe Gly Tyr Ser Tyr  
130 135 140

Ala Lys Arg Met Ile Asp Val Leu Asn Arg Gly Tyr Ala Gln Glu Arg  
145 150 155 160

Gly Arg Lys Tyr Thr Ser Val Val Pro Cys Asn Val Phe Gly Pro Tyr  
165 170 175

Asp Asn Tyr Asn Leu Glu Tyr Gly His Val Ile Pro Ala Leu Ile His  
180 185 190

Lys Thr Phe Ile Ala Lys Arg Asp Gly Lys Pro Leu Glu Val Phe Gly  
195 200 205

Ser Gly Ala Pro Leu Arg Gln Phe Ile Tyr Ser Leu Asp Leu Ala Arg  
210 215 220

Leu Phe Val Trp Val Leu Arg Asn Tyr Glu Glu Ile Glu Pro Ile Ile  
225 230 235 240

Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile Met Asp Ala Val Asn  
245 250 255

Ala Ile Val Lys Ala Phe Asp Phe Lys Gly Gly Ile Val Gln Asp Lys  
260 265 270

Ser Lys Ala Asp Gly Gln Tyr Lys Lys Thr Ala Ser Asn Ala Lys Leu  
275 280 285

Arg Lys Tyr Leu Pro Asp Phe Lys Phe Thr Pro Phe Asp Val Ala Ile  
290 295 300

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20 25 30

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35 40 45

Lys Gly Ala Phe Lys Val Ala Ile Asp Phe Pro Val Glu Tyr Pro Phe  
50 55 60

Lys Pro Pro Lys Ile Thr Phe Leu Thr Lys Ile Tyr His Pro Asn Val  
65 70 75 80

Asp Glu Lys Gly Gln Val Cys Leu Pro Ile Ile Ser Pro Asp Asn Trp  
85 90 95

Lys Pro Ala Thr Lys Thr Glu Gln Val Met Asn Ala Leu Leu Gly Leu  
100 105 110

Ile Thr Glu Pro Glu Pro Asp His Pro Leu Arg Ala Asp Leu Ala Glu  
115 120 125

Glu Phe Thr Lys Asp Arg Lys Lys Phe Asn Lys Thr Ala Glu Asp Tyr  
130 135 140

Thr Lys Lys Tyr Ala Val Lys Arg Pro Asp Gly Trp Phe Glu Thr Arg  
145 150 155 160

His Lys Ile Met Asp Arg Glu Gln Ser Met Thr Val Leu Val Thr Gly  
165 170 175

Gly Thr Gly Leu Val Gly Arg Ser Ile Glu Lys Ile Ile Thr Thr Glu  
180 185 190

Glu Ala Arg Pro Asn Glu Thr Trp Ile Phe Val Gly Arg Asn Asp Cys  
195 200 205

Asp Leu Thr Asp Ile Glu Ala Thr Arg Lys Leu Phe Met Lys Cys Arg  
210 215 220

Pro Ser His Val Ile His Leu Ala Ala Met Val Gly Gly Leu Phe His  
225 230 235 240

Asn Leu His Cys Asn Leu Gln Phe Phe Arg Lys Asn Met Gln Ile Asn  
245 250 255

Asp Asn Val Leu Met Ala Cys Asn Glu Phe Asp Val Val Lys Cys Ile  
260 265 270

Ser Cys Leu Ser Thr Cys Val Phe Pro Asp Lys Thr Thr Tyr Pro Ile  
275 280 285 290

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 Tyr Ser Tyr Ala Lys Arg Met Ile Asp Val Leu Asn Arg Gly Tyr Ala  
 305                      310                      315                      320  
 Gln Glu Phe Gly Arg Lys Tyr Thr Ser Val Ile Pro Cys Asn Val Phe  
 325                      330                      335  
 Gly Pro His Asp Asn Tyr Asn Leu Lys Asp Gly His Val Ile Pro Ala  
 340                      345                      350  
 Leu Ile His Lys Thr Tyr Ile Ala Lys His Glu Gly Thr Pro Leu Glu  
 355                      360                      365  
 Val Phe Gly Ser Gly Thr Pro Leu Arg Gln Phe Ile Tyr Ser Leu Asp  
 370                      375                      380  
 Leu Ala Arg Leu Phe Ile Trp Val Ala Arg Ser Tyr Glu Glu Ile Asp  
 385                      390                      395                      400  
 Pro Ile Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile Met Asp  
 405                      410                      415  
 Ala Val His Ala Val Val Arg Ala Phe Asp Phe Lys Gly Glu Ile Val  
 420                      425                      430  
 His Asp Lys Thr Lys Ala Asp Gly Gln Tyr Lys Lys Thr Ala Ser Asn  
 435                      440                      445  
 Ala Lys Leu Arg Lys Tyr Leu Pro Asn Phe Lys Phe Thr Pro Phe Glu  
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 Glu Gly Arg Pro Asp Glu Glu Trp Val Phe Val Ser Ser Arg Asp Ala  
 35                      40                      45  
 Asp Leu Thr Ser Ala Val Glu Thr Lys Ala Leu Phe Glu Lys His Lys  
 50                      55                      60  
 Pro Thr His Val Ile His Leu Ala Ala Met Val Gly Gly Leu Phe Lys  
 65                      70                      75                      80  
 Asn Ile Arg Cys Asn Leu Asp Phe Trp Arg Arg Asn Ile His Ile Asn  
 85                      90                      95  
 Asn Asn Val Leu His Ser Ala Tyr Glu Cys Glu Val Gln Lys Val Val

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100      105      110
Ser Cys Leu Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr Pro Ile
115      120      125

Asp Glu Thr Met Ile His Asn Gly Pro Pro His Ser Ser Asn Phe Gly
130      135      140

Tyr Ser Tyr Ala Lys Arg Met Ile Asp Val Gln Asn Arg Gly Tyr Phe
145      150      155      160

Glu Gln His Gly Cys Arg Phe Thr Ala Val Ile Pro Thr Asn Val Phe
165      170      175

Gly Pro His Asp Asn Phe Asn Ile Glu Asp Gly His Val Leu Pro Gly
180      185      190

Leu Ile His Lys Val Tyr Leu Ala Lys Gln Asn Gly Ser Ala Leu Thr
195      200      205

Val Trp Gly Thr Gly Lys Pro Arg Arg Gln Phe Ile Tyr Ser Leu Asp
210      215      220

Leu Ala Arg Leu Phe Val Trp Val Leu Arg Glu Tyr Glu Glu Val Glu
225      230      235      240

Pro Ile Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile Arg Glu
245      250      255

Ala Ala Glu Ala Ile Val Glu Ala Met Asp Phe Arg Gly Glu Leu Ile
260      265      270

Phe Asp Thr Thr Lys Ala Asp Gly Gln Phe Lys Lys Thr Ala Ser Asn
275      280      285

Ala Lys Leu Arg His Tyr Leu Pro Asn Phe Gln Phe Thr Pro Phe Arg
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Glu Gly Glu Glu Trp Thr Phe Leu Ser Ser Lys Asp Ala Asn Leu Leu
35      40      45

Ser Ala Glu Glu Thr Arg Ala Ile Phe Gln Lys Tyr Arg Pro Thr His
50      55      60

Val Ile His Leu Ala Ala Met Val Gly Gly Leu Phe Arg Asn Met Arg
65      70      75      80

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Gln Asn Leu Asp Phe Trp Arg Asn Asn Val Phe Ile Asn Asp Asn Val  
85 90 95

Leu Gln Thr Ala Asn Glu Phe Gly Val Val Lys Val Val Ser Cys Leu  
100 105 110

Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr Pro Ile Asp Glu Thr  
115 120 125

Met Ile His Asn Gly Pro Pro His Asp Ser Asn Phe Gly Tyr Ala Phe  
130 135 140

Ala Lys Arg Met Ile Asp Val Gln Asn Arg Thr Cys Phe Lys Gln Tyr  
145 150 155 160

Gly Arg Arg Tyr Thr Ser Val Ile Pro Thr Asn Val Phe Gly Ala His  
165 170 175

Asp Asn Phe Asn Ile Asp Asp Gly His Val Leu Pro Gly Leu Ile His  
180 185 190

Lys Thr Tyr Leu Ala Lys Lys Glu Gly Lys Pro Leu Gln Val Trp Gly  
195 200 205

Ser Gly Lys Pro Leu Arg Gln Phe Ile Tyr Ser Leu Asp Leu Ala Arg  
210 215 220

Leu Phe Leu Trp Val Leu Arg Glu Tyr Asp Glu Val Asp Pro Ile Ile  
225 230 235 240

Leu Ser Val Gly Glu Glu Asp Glu Leu Ser Ile Lys Asp Cys Ala Asp  
245 250 255

Ala Val Val Asp Ala Leu Gly Phe Lys Gly Asp Val Ile Tyr Asp Thr  
260 265 270

Ser Lys Ala Asp Gly Gln Phe Lys Lys Thr Ala Ser Asn Ala Lys Leu  
275 280 285

Arg Gln Tyr Leu Pro Asp Phe Gln Phe Thr Pro Phe Arg Glu Ala Ile  
290 295 300

Lys Glu Thr Cys Asp Trp Phe Val Ala Asn Tyr Asp Ile Ala Arg Lys  
305 310 315 320

<210> 116

<211> 317

<212> PRT

<213> Xenopus laevis

<400> 116

Met Glu Gly Lys Arg Ile Leu Val Thr Gly Gly Ser Gly Leu Val Gly  
1 5 10 15

Lys Ala Ile Glu Lys Val Val Ala Asp Gly Glu Gly Arg Pro Asp Glu  
20 25 30

Gln Trp Ile Phe Ile Ser Ser Lys Asp Ala Asp Leu Thr Asn Ala Ala  
35 40 45

Asp Thr Lys Cys Leu Phe Glu Lys His Lys Pro Thr His Val Ile His  
50 55 60

Val Ala Ala Met Val Gln Gln Tyr Phe Asp Ser Met Tyr Thr Lys Tyr

Leu Ala Ala Met Val Gly Gly Leu Phe Arg Asn Met Lys Tyr Asn Leu  
65 70 75 80

Asp Phe Leu Arg Asn Asn Leu His Ile Asn Asp Asn Val Leu His Ser  
85 90 95

Ala Tyr Glu Met Gly Val Gln Lys Val Val Ser Cys Leu Ser Thr Cys  
100 105 110

Ile Phe Pro Asp Lys Thr Thr Tyr Pro Ile Asp Glu Thr Met Ile His  
115 120 125

Asn Gly Pro Pro His Thr Ser Asn Phe Gly Tyr Ser Tyr Ala Lys Arg  
130 135 140

Met Ile Asp Val Gln Asn Arg Ala Tyr Tyr Glu Gln His Gly Cys Lys

145 150 155 160

Phe Thr Ser Val Ile Pro Thr Asn Val Phe Gly Pro His Asp Asn Phe  
165 170 175

Asn Ile Asp Asp Gly His Val Leu Pro Gly Leu Ile His Lys Val Tyr  
180 185 190

Ser Ala Lys Gln Asn Gly Thr Ala Leu Ser Ile Trp Gly Thr Gly Gln  
195 200 205

Pro Arg Arg Gln Phe Ile Tyr Ser Leu Asp Leu Ala Arg Leu Phe Ile  
210 215 220

Trp Val Leu Arg Glu Tyr Asn Glu Val Asp Pro Ile Ile Leu Ser Val  
225 230 235 240

Gly Glu Glu Asp Glu Val Ser Ile Lys Glu Ala Ala Glu Ser Ile Val  
245 250 255

Ala Ala Met Glu Phe Lys Gly Glu Leu Ile Phe Asp Ser Thr Lys Ser  
260 265 270

Asp Gly Gln Phe Lys Lys Thr Ala Ser Asn His Lys Leu Arg Lys Tyr  
275 280 285

Leu Pro Asp Phe Gln Phe Thr Pro Phe Asn Lys Ala Val Gln Glu Thr  
290 295 300

Cys Asn Trp Phe Asn Ser Asn Tyr Ala Gln Ala Arg Lys  
305 310 315

<210> 117

<211> 321

<212> PRT

<213> Bos taurus

<400> 117

Met Gly Asp Pro Arg Gly Thr Arg Arg Ile Leu Val Thr Gly Gly Ser  
1 5 10 15

Gly Leu Val Gly Arg Ala Ile Gln Lys Val Val Glu Asp Gly Ala Arg  
20 25 30

Leu Pro Gly Glu Asp Trp Val Phe Val Ser Ser Lys Asp Ala Asp Leu  
35 40 45

Thr Asp Ala Ala Gln Thr Arg Ala Leu Phe Gln Gln Val Gln Pro Thr

50

55

60

His Val Ile His Leu Ala Ala Met Val Gly Gly Leu Phe Arg Asn Ile  
65 70 75 80

Lys Tyr Asn Leu Asp Phe Trp Arg Lys Asn Ile His Ile Asn Asp Asn  
85 90 95

Val Leu His Ser Ala Phe Glu Val Gly Val Arg Lys Val Val Ser Cys  
100 105 110

Leu Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr Pro Ile Asp Glu  
115 120 125

Thr Met Ile His Asn Gly Pro Pro His Ser Ser Asn Phe Gly Tyr Ser  
130 135 140

Tyr Ala Lys Arg Met Ile Asp Val Gln Asn Arg Ala Tyr Phe Gln Gln  
145 150 155 160

His Gly Cys Thr Phe Thr Ala Val Ile Pro Thr Asn Val Phe Gly Pro  
165 170 175

His Asp Asn Phe Ser Ile Glu Asp Gly His Val Leu Pro Gly Leu Ile  
180 185 190

His Lys Val His Leu Ala Lys Ser Ser Gly Ser Ala Leu Thr Val Trp  
195 200 205

Gly Thr Gly Arg Pro Arg Arg Gln Phe Ile Tyr Ser Leu Asp Leu Ala  
210 215 220

Arg Leu Phe Ile Trp Ala Leu Arg Glu Tyr Asp Glu Val Glu Pro Ile  
225 230 235 240

Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Val Gln Glu Ala Ala  
245 250 255

Glu Ala Val Val Glu Ala Met Asp Phe His Gly Glu Val Thr Phe Asp  
260 265 270

Thr Thr Lys Ser Asp Gly Gln Phe Lys Lys Thr Ala Ser Asn Ala Lys  
275 280 285

Leu Arg Ala Tyr Leu Pro Asp Phe Arg Phe Thr Pro Phe Lys Gln Ala  
290 295 300

Val Lys Glu Thr Cys Ala Trp Phe Thr Asp Asn Tyr Glu Gln Ala Arg  
305 310 315 320

Lys

<210> 118

<211> 321

<212> PRT

<213> Equus caballus

<400> 118

Met Gly Glu Pro Gln Gly Ser Met Arg Ile Leu Val Thr Gly Ser Ser  
1 5 10 15

Gly Leu Val Gly Arg Ala Ile Gln Lys Val Val Ala Asp Gly Ala Arg  
20 25 30

Leu Pro Gly Glu Asp Trp Val Phe Val Ser Ser Lys Asp Ala Asp Leu  
35 40 45

Thr Asp Ala Ala Gln Thr Arg Ala Leu Phe Glu Lys Val Arg Pro Thr  
50 55 60

His Val Ile His Leu Ala Ala Met Val Gly Gly Leu Phe Arg Asn Ile  
65 70 75 80

Lys Tyr Asn Leu Asp Phe Trp Arg Lys Asn Val His Ile Asn Asp Asn  
85 90 95

Val Leu His Ser Ala Phe Glu Val Gly Ala Arg Lys Val Val Ser Cys  
100 105 110

Leu Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr Pro Ile Asp Glu  
115 120 125

Thr Met Ile His Asn Gly Pro Pro His Ser Ser Asn Phe Gly Tyr Ser  
130 135 140

Tyr Ala Lys Arg Met Ile Asp Val Gln Asn Arg Ala Tyr Phe Gln Gln  
145 150 155 160

His Gly Cys Thr Phe Thr Ala Val Ile Pro Thr Asn Val Phe Gly Pro  
165 170 175

His Asp Asn Phe Asn Ile Glu Asp Gly His Val Leu Pro Gly Leu Ile  
180 185 190

His Lys Val His Leu Ala Lys Ser Ser Gly Ser Ala Leu Thr Val Trp  
195 200 205

Gly Thr Gly Lys Pro Arg Arg Gln Phe Ile Tyr Ser Leu Asp Leu Ala  
210 215 220

Arg Leu Phe Ile Trp Val Leu Arg Glu Tyr Asn Glu Val Glu Pro Ile  
225 230 235 240

Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile Gln Glu Ala Ala  
245 250 255

Glu Ala Val Val Glu Ala Met Asp Phe His Gly Asp Val Thr Phe Asp  
260 265 270

Thr Thr Lys Ser Asp Gly Gln Phe Lys Lys Thr Ala Ser Asn Gly Lys  
275 280 285

Leu Arg Ala Tyr Leu Pro Asp Phe Arg Phe Thr Pro Phe Lys Gln Ala  
290 295 300

Val Lys Glu Thr Cys Ala Trp Phe Thr Asp Asn Tyr Glu Gln Ala Arg  
305 310 315 320

Lys

<210> 119

<211> 321

<212> PRT

<213> Canis lupus familiaris

<400> 119

Met Ser Glu Pro Gly Gly Ser Val Arg Ile Leu Val Thr Gly Gly Ser

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1             5             10             15

Gly Leu Val Gly Arg Ala Ile Gln Lys Val Val Ala Asp Gly Ala Gly
  20             25             30

Leu Pro Gly Glu Asp Trp Val Phe Val Ser Ser Lys Asp Ala Asp Leu
  35             40             45

Thr Asp Ala Ala Gln Thr Arg Ala Leu Phe Glu Lys Val Arg Pro Thr
  50             55             60

His Val Ile His Leu Ala Ala Met Val Gly Gly Leu Phe Arg Asn Ile
  65             70             75             80

Lys Tyr Asn Leu Asp Phe Trp Arg Lys Asn Val His Ile Asn Asp Asn
  85             90             95

Val Leu His Ser Ala Phe Glu Val Gly Val Arg Lys Val Val Ser Cys
  100            105            110

Leu Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr Pro Ile Asp Glu
  115            120            125

Thr Met Ile His Asn Gly Pro Pro His Asn Ser Asn Phe Gly Tyr Ser
  130            135            140

Tyr Ala Lys Arg Met Ile Asp Val Gln Asn Arg Ala Tyr Phe Gln Gln
  145            150            155            160

His Gly Cys Thr Phe Thr Ala Val Ile Pro Thr Asn Val Phe Gly Pro
  165            170            175

His Asp Asn Phe Asn Ile Glu Asp Gly His Val Leu Pro Gly Leu Ile
  180            185            190

His Lys Val His Leu Ala Lys Ser Ser Gly Ser Ala Leu Thr Val Trp
  195            200            205

Gly Thr Gly Lys Pro Arg Arg Gln Phe Ile Tyr Ser Leu Asp Leu Ala
  210            215            220

Arg Leu Phe Ile Trp Val Leu Arg Glu Tyr Asn Glu Val Glu Pro Ile
  225            230            235            240

Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile Gln Glu Ala Ala
  245            250            255

Glu Ala Val Val Glu Ala Met Asp Phe His Gly Glu Val Thr Phe Asp
  260            265            270

Thr Thr Lys Ser Asp Gly Gln Phe Lys Lys Thr Ala Ser Asn Gly Lys
  275            280            285

Leu Arg Thr Tyr Leu Pro Asp Phe Arg Phe Thr Pro Phe Lys Gln Ala
  290            295            300

Val Lys Glu Thr Cys Ala Trp Phe Thr His Asn Tyr Glu Gln Ala Arg
  305            310            315            320

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Lys

<210> 120

<211> 321

<212> PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 120

Met Gly Glu Pro Gln Gly Ser Met Arg Ile Leu Val Thr Gly Gly Ser  
 1 5 10 15

Gly Leu Val Gly Lys Ala Ile Gln Lys Val Val Ala Asp Gly Ala Gly  
 20 25 30

Leu Pro Gly Glu Asp Trp Val Phe Val Ser Ser Lys Asp Ala Asp Leu  
 35 40 45

Thr Asp Thr Ala Gln Thr Arg Ala Leu Phe Glu Lys Val Gln Pro Thr  
 50 55 60

His Val Ile His Leu Ala Ala Met Val Gly Gly Leu Phe Arg Asn Ile  
 65 70 75 80

Lys Tyr Asn Leu Asp Phe Trp Arg Lys Asn Val His Met Asn Asp Asn  
 85 90 95

Val Leu His Ser Ala Phe Glu Val Gly Ala Arg Lys Val Val Ser Cys  
 100 105 110

Leu Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr Pro Ile Asp Glu  
 115 120 125

Thr Met Ile His Asn Gly Pro Pro His Asn Ser Asn Phe Gly Tyr Ser  
 130 135 140

Tyr Ala Lys Arg Met Ile Asp Val Gln Asn Arg Ala Tyr Phe Gln Gln  
 145 150 155 160

Tyr Gly Cys Thr Phe Thr Ala Val Ile Pro Thr Asn Val Phe Gly Pro  
 165 170 175

His Asp Asn Phe Asn Ile Glu Asp Gly His Val Leu Pro Gly Leu Ile  
 180 185 190

His Lys Val His Leu Ala Lys Ser Ser Gly Ser Ala Leu Thr Val Trp  
 195 200 205

Gly Thr Gly Asn Pro Arg Arg Gln Phe Ile Tyr Ser Leu Asp Leu Ala  
 210 215 220

Gln Leu Phe Ile Trp Val Leu Arg Glu Tyr Asn Glu Val Glu Pro Ile  
 225 230 235 240

Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile Lys Glu Ala Ala  
 245 250 255

Glu Ala Val Val Glu Ala Met Asp Phe His Gly Glu Val Thr Phe Asp  
 260 265 270

Thr Thr Lys Ser Asp Gly Gln Phe Lys Lys Thr Ala Ser Asn Ser Lys  
 275 280 285

Leu Arg Thr Tyr Leu Pro Asp Phe Arg Phe Thr Pro Phe Lys Gln Ala  
 290 295 300

Val Lys Glu Thr Cys Ala Trp Phe Thr Asp Asn Tyr Glu Gln Ala Arg  
 305 310 315 320

Lys

&lt;210&gt; 121

&lt;211&gt; 321

&lt;212&gt; PRT

&lt;213&gt; Cricetulus griseus

&lt;400&gt; 121

Met Gly Glu Pro Gln Gly Ser Arg Arg Ile Leu Val Thr Gly Gly Ser  
 1 5 10 15

Gly Leu Val Gly Arg Ala Ile Gln Lys Val Val Ala Asp Gly Ala Gly  
 20 25 30

Leu Pro Gly Glu Glu Trp Val Phe Val Ser Ser Lys Asp Ala Asp Leu  
 35 40 45

Thr Asp Ala Ala Gln Thr Gln Ala Leu Phe Gln Lys Val Gln Pro Thr  
 50 55 60

His Val Ile His Leu Ala Ala Met Val Gly Gly Leu Phe Arg Asn Ile  
 65 70 75 80

Lys Tyr Asn Leu Asp Phe Trp Arg Lys Asn Val His Ile Asn Asp Asn  
 85 90 95

Val Leu His Ser Ala Phe Glu Val Gly Thr Arg Lys Val Val Ser Cys  
 100 105 110

Leu Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr Pro Ile Asp Glu  
 115 120 125

Thr Met Ile His Asn Gly Pro Pro His Ser Ser Asn Phe Gly Tyr Ser  
 130 135 140

Tyr Ala Lys Arg Met Ile Asp Val Gln Asn Arg Ala Tyr Phe Gln Gln  
 145 150 155 160

His Gly Cys Thr Phe Thr Ala Val Ile Pro Thr Asn Val Phe Gly Pro  
 165 170 175

His Asp Asn Phe Asn Ile Glu Asp Gly His Val Leu Pro Gly Leu Ile  
 180 185 190

His Lys Val His Leu Ala Lys Ser Asn Gly Ser Ala Leu Thr Val Trp  
 195 200 205

Gly Thr Gly Lys Pro Arg Arg Gln Phe Ile Tyr Ser Leu Asp Leu Ala  
 210 215 220

Arg Leu Phe Ile Trp Val Leu Arg Glu Tyr Asn Glu Val Glu Pro Ile  
 225 230 235 240

Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile Lys Glu Ala Ala  
 245 250 255

Glu Ala Val Val Glu Ala Met Asp Phe Cys Gly Glu Val Thr Phe Asp  
 260 265 270

Ser Thr Lys Ser Asp Gly Gln Tyr Lys Lys Thr Ala Ser Asn Gly Lys  
 275 280 285

Leu Arg Ala Tyr Leu Pro Asp Phe Arg Phe Thr Pro Phe Lys Gln Ala  
 290 295 300

Val Lys Glu Thr Cys Ala Trp Phe Thr Asp Asn Tyr Glu Gln Ala Arg

305 - - 310 - - 315 - - 320

Lys

<210> 122

<211> 321

<212> PRT

<213> Rattus norvegicus

<400> 122

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Met Gly Glu Pro His Gly Ser Met Arg Ile Leu Val Thr Gly Gly Ser
 1          5          10          15

Gly Leu Val Gly Arg Ala Ile Gln Lys Val Val Ala Asp Gly Ala Gly
 20          25          30

Leu Pro Gly Glu Glu Trp Val Phe Val Ser Ser Lys Asp Ala Asp Leu
 35          40          45

Thr Asp Ala Ala Gln Thr Gln Ala Leu Phe Gln Lys Val Gln Pro Thr
 50          55          60

His Val Ile His Leu Ala Ala Met Val Gly Gly Leu Phe Arg Asn Ile
 65          70          75          80

Lys Tyr Asn Leu Asp Phe Trp Arg Lys Asn Val His Ile Asn Asp Asn
 85          90          95

Val Leu His Ser Ala Phe Glu Val Gly Thr Arg Lys Val Val Ser Cys
100          105          110

Leu Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr Pro Ile Asp Glu
115          120          125

Thr Met Ile His Asn Gly Pro Pro His Ser Ser Asn Phe Gly Tyr Ser
130          135          140

Tyr Ala Lys Arg Met Ile Asp Val Gln Asn Arg Ala Tyr Phe Gln Gln
145          150          155          160

His Gly Cys Thr Phe Thr Ser Val Ile Pro Thr Asn Val Phe Gly Pro
165          170          175

Tyr Asp Asn Phe Asn Ile Glu Asp Gly His Val Leu Pro Gly Leu Ile
180          185          190

His Lys Val His Leu Ala Lys Ser Ser Gly Ser Ala Leu Thr Val Trp
195          200          205

Gly Thr Gly Lys Pro Arg Arg Gln Phe Ile Tyr Ser Leu Asp Leu Ala
210          215          220

Arg Leu Phe Ile Trp Val Leu Arg Glu Tyr Asn Glu Val Glu Pro Ile
225          230          235          240

Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile Lys Glu Ala Ala
245          250          255

Glu Ala Val Val Glu Ala Met Asp Phe Ser Gly Glu Val Thr Phe Asp
260          265          270

Ser Thr Lys Ser Asp Gly Gln Tyr Lys Lys Thr Ala Ser Asn Gly Lys
275          280          285

Leu Arg Ser Tyr Leu Pro Asp Phe Cys Phe Thr Pro Phe Lys Gln Ala
290          295          300

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Val Lys Glu Thr Cys Ala Trp Phe Thr Glu Asn Tyr Glu Gln Ala Arg  
305 310 315 320

Lys

<210> 123

<211> 321

<212> PRT

<213> Mus musculus

<400> 123

Met Gly Glu Pro His Gly Ser Met Arg Ile Leu Val Thr Gly Gly Ser  
1 5 10 15

Gly Leu Val Gly Arg Ala Ile Gln Lys Val Val Ala Asp Gly Ala Gly  
20 25 30

Leu Pro Gly Glu Glu Trp Val Phe Val Ser Ser Lys Asp Ala Asp Leu  
35 40 45

Thr Asp Ala Ala Gln Thr Gln Ala Leu Phe Gln Lys Val Gln Pro Thr  
50 55 60

His Val Ile His Leu Ala Ala Met Val Gly Gly Leu Phe Arg Asn Ile  
65 70 75 80

Lys Tyr Asn Leu Asp Phe Trp Arg Lys Asn Val His Ile Asn Asp Asn  
85 90 95

Val Leu His Ser Ala Phe Glu Val Gly Ala Arg Lys Val Val Ser Cys  
100 105 110

Leu Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr Pro Ile Asp Glu  
115 120 125

Thr Met Ile His Asn Gly Pro Pro His Ser Ser Asn Phe Gly Tyr Ser  
130 135 140

Tyr Ala Lys Arg Met Ile Asp Val Gln Asn Arg Ala Tyr Phe Gln Gln  
145 150 155 160

His Gly Cys Thr Phe Thr Ala Val Ile Pro Thr Asn Val Phe Gly Pro  
165 170 175

Tyr Asp Asn Phe Asn Ile Glu Asp Gly His Val Leu Pro Gly Leu Ile  
180 185 190

His Lys Val His Leu Ala Lys Ser Ser Asp Ser Ala Leu Thr Val Trp  
195 200 205

Gly Thr Gly Lys Pro Arg Arg Gln Phe Ile Tyr Ser Leu Asp Leu Ala  
210 215 220

Arg Leu Phe Ile Trp Val Leu Arg Glu Tyr Ser Glu Val Glu Pro Ile  
225 230 235 240

Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile Lys Glu Ala Ala  
245 250 255

Glu Ala Val Val Glu Ala Met Asp Phe Asn Gly Glu Val Thr Phe Asp  
260 265 270

Ser Phe Tyr Ser Asn Glu Glu Met Tyr Tyr Tyr Phe Ala Ser Asn Glu Tyr

Ser Thr Lys Ser Asp Gly Gln Tyr Lys Lys Thr Ala Ser Asn Gly Lys  
 275 280 285

Leu Arg Ser Tyr Leu Pro Asp Phe Arg Phe Thr Pro Phe Lys Gln Ala  
290 295 300

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Lys | Glu | Thr | Cys | Thr | Trp | Phe | Thr | Asp | Asn | Tyr | Glu | Gln | Ala | Arg |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |

Lys

<210> 124

<211> 380

&lt;212&gt; PRT

<213> Loa loa

<400> 124

Met Asn Thr Glu Ala Leu Glu Asp Cys Leu Gly Asn Arg His Ser Met  
1 5 10 15

Lys Pro Ile Ser Glu Val Glu Lys Cys Lys Phe Arg Thr Pro Lys Ile  
20 25 30

Ala Leu Ile Thr Gly Ile Ser Gly Gln Asp Gly Ser Tyr Leu Ala Glu  
35 40 45

Leu Leu Leu Ser Lys Gly Tyr Glu Val His Gly Ile Ile Arg Arg Ser  
50 55 60

Ser Ser Phe Asn Thr Ser Arg Ile Glu His Leu Tyr Ser Asn Pro Val  
65 70 75 80

Thr His His Ala Asp Ser Ser Phe Thr Leu His Tyr Gly Asp Met Thr  
85 90 95

Asp Ser Ser Cys Leu Ile Lys Leu Val Ser Gln Ile Gln Pro Thr Glu  
100 105 110

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Tyr | His | Leu | Ala | Ala | Gln | Ser | His | Val | Lys | Val | Ser | Phe | Asp | Leu |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |

Pro Glu Tyr Thr Ala Glu Val Asp Ala Val Gly Thr Leu Arg Leu Leu  
130 135 140

Asp Ser Ile His Ala Cys Gly Leu Thr Asn Lys Val Arg Phe Tyr Gln  
145 150 155 160

Ala Ser Thr Ser Glu Leu Tyr Gly Lys Val Gln Glu Val Pro Gln Lys  
165 170 175

Glu Thr Thr Pro Phe Tyr Pro Arg Ser Pro Tyr Ala Ala Ala Lys Leu  
180 185 190

Tyr Ser Tyr Trp Ile Val Val Asn Tyr Arg Glu Ala Tyr Asn Met Phe  
195 200 205

Ala Cys Asn Gly Ile Leu Phe Asn His Glu Ser Pro Arg Arg Gly Glu  
210 215 220

Thr Phe Val Thr Arg Lys Ile Thr Arg Ala Val Ala Lys Ile Ala Leu  
225 230 235 240

Gly Gln Gln Glu Ile Leu Glu Leu Gly Asn Leu Ser Ser Ser Arg Asp  
245 250 255

[illegible]

Trp Gly His Ala Lys Glu Tyr Val Glu Ala Met Trp Lys Ile Leu Gln  
260 265 270

Tyr Asp Gln Pro Asp Asp Phe Val Ile Ala Thr Gly Lys Ser Tyr Thr  
275 280 285

Val Arg Arg Phe Ala Glu Leu Ala Phe Glu Glu Ile Gly Lys Thr Ile  
290 295 300

Ile Trp Glu Gly Glu Gly Val His Glu Val Gly Lys Glu Lys Asp Thr  
305 310 315 320

Gly Ile Ile Arg Val Arg Val Ser Pro Lys Tyr Tyr Arg Pro Thr Glu  
325 330 335

Val Asp Leu Leu Ile Gly Asp Pro Thr Lys Ala Lys Gln Lys Leu Asn  
340 345 350

Trp Glu Ala Lys Ile Thr Leu Glu Glu Leu Val Lys Glu Met Val Ala  
355 360 365

Ser Asp Leu Gln Leu Met Lys Ser Asn Pro Met Ala  
370 375 380

<210> 125

<211> 387

<212> PRT

<213> Brugia malayi

<400> 125

Met Thr Ile Asn Ala Thr Lys Met Asn Thr Glu Thr Gln Glu Asn Cys  
1 5 10 15

Leu Gly Asn Arg Ser Ser Met Lys Thr Ile Ser Glu Val Glu Arg Tyr  
20 25 30

Lys Cys Arg Ala Arg Lys Ile Ala Leu Ile Thr Gly Ile Ser Gly Gln  
35 40 45

Asp Gly Ser Tyr Leu Ala Glu Leu Leu Leu Ser Lys Gly Tyr Glu Val  
50 55 60

His Gly Ile Ile Arg Arg Ser Ser Ser Phe Asn Thr Ala Arg Ile Glu  
65 70 75 80

His Leu Tyr Ser Asn Pro Ile Thr His His Ala Asp Ser Ser Phe Thr  
85 90 95

Leu His Tyr Gly Asp Met Thr Asp Ser Ser Cys Leu Ile Lys Ile Val  
100 105 110

Ser Gln Ile Gln Pro Thr Glu Val Tyr His Leu Ala Ala Gln Ser His  
115 120 125

Val Lys Val Ser Phe Asp Leu Pro Glu Tyr Thr Ala Glu Val Asp Ala  
130 135 140

Val Gly Thr Leu Arg Leu Leu Asp Ser Ile His Ala Cys Gly Leu Thr  
145 150 155 160

Asn Lys Val Lys Phe Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly Lys  
165 170 175

Val Gln Glu Val Pro Gln Lys Glu Thr Thr Pro Phe Tyr Pro Arg Ser  
180 185 190

Pro Tyr Ala Ala Ala Lys Leu Tyr Ser Tyr Trp Ile Val Val Asn Tyr  
195 200 205

Arg Glu Ala Tyr Ser Met Phe Ala Cys Asn Gly Ile Leu Phe Asn His  
210 215 220

Glu Ser Pro Arg Arg Gly Glu Thr Phe Val Thr Arg Lys Ile Thr Arg  
225 230 235 240

Ala Val Ala Lys Ile Ala Leu Gly Gln Gln Glu Val Leu Glu Leu Gly  
245 250 255

Asn Leu Ser Ser Ser Arg Asp Trp Gly His Ala Lys Glu Tyr Val Glu  
260 265 270

Ala Met Trp Arg Ile Leu Gln Tyr Asp Lys Pro Asp Asp Phe Val Ile  
275 280 285

Ala Thr Gly Lys Ser Tyr Thr Val Arg Arg Phe Ala Glu Leu Ala Phe  
290 295 300

Glu Glu Ile Gly Lys Lys Ile Ile Trp Glu Gly Glu Gly Val His Glu  
305 310 315 320

Val Gly Lys Glu Glu Asp Thr Gly Ile Val Arg Val Arg Val Ser Ser  
325 330 335

Lys Tyr Tyr Arg Pro Thr Glu Val Asp Leu Leu Ile Gly Asp Ser Thr  
340 345 350

Lys Ala Lys Gln Lys Leu Asn Trp Glu Ala Lys Thr Thr Leu Glu Glu  
355 360 365

Leu Val Lys Glu Met Val Ala Ser Asp Leu Gln Leu Met Lys Ser Asn  
370 375 380

Pro Met Ala  
385

<210> 126

<211> 362

<212> PRT

<213> Anopheles gambiae str. PEST

<400> 126

Met Glu Gly Glu Ala Ala Val Thr Asn Pro Val Asp Arg Lys Val Ala  
1 5 10 15

Leu Ile Thr Gly Ile Thr Gly Gln Asp Gly Ser Tyr Leu Ala Glu Phe  
20 25 30

Leu Leu Asp Lys Gly Tyr Glu Val His Gly Ile Ile Arg Arg Ala Ser  
35 40 45

Thr Phe Asn Thr Ser Arg Ile Glu His Leu Tyr Ala Asp Pro Arg Thr  
50 55 60

His Arg Glu Gly Lys Met Lys Leu His Tyr Gly Asp Met Thr Asp Ser  
65 70 75 80

Ser Ala Leu Val Lys Ile Ile Ala Gln Val Arg Pro Ser Glu Ile Tyr  
85 90 95

05                      30                      35  
 Asn Leu Ala Ala Gln Ser His Val Lys Ile Ser Phe Asp Leu Ser Glu  
                          100                                      105                                      110  
 Tyr Thr Ala Glu Val Asp Ala Val Gly Thr Leu Arg Leu Leu Asp Ala  
                          115                                      120                                      125  
 Ile Arg Thr Val Gly Gln Glu Arg Thr Val Arg Phe Tyr Gln Ala Ser  
                          130                                      135                                      140  
 Thr Ser Glu Leu Tyr Gly Lys Val Ala Glu Thr Pro Gln Asn Glu Lys  
                          145                                      150                                      155                                      160  
 Thr Pro Phe Tyr Pro Arg Ser Pro Tyr Ala Cys Ala Lys Met Tyr Gly  
                          165                                      170                                      175  
 Tyr Trp Ile Val Ile Asn Tyr Arg Glu Ala Tyr Asp Met Phe Ala Cys  
                          180                                      185                                      190  
 Asn Gly Ile Leu Phe Asn His Glu Ser Pro Arg Arg Gly Glu Asn Phe  
                          195                                      200                                      205  
 Val Thr Arg Lys Ile Thr Arg Ser Val Ala Lys Ile Ser Leu Gly Gln  
                          210                                      215                                      220  
 Gln Glu Tyr Leu Glu Leu Gly Asn Leu Asp Ser Lys Arg Asp Trp Gly  
                          225                                      230                                      235                                      240  
 His Ala Lys Asp Tyr Val Glu Ala Met Trp Leu Met Met Gln Gln Glu  
                          245                                      250                                      255  
 Arg Pro Glu Asp Phe Val Ile Ala Thr Gly Glu Thr His Ser Val Arg  
                          260                                      265                                      270  
 Glu Phe Val Glu Gln Ala Phe Arg Tyr Ile Gly Arg Glu Ile Glu Trp  
                          275                                      280                                      285  
 Arg Gly Thr Gly Val Asp Glu Val Gly Val Glu Lys Gly Thr Asp Thr  
                          290                                      295                                      300  
 Val Arg Val Arg Ile Asn Pro Lys Phe Phe Arg Pro Thr Glu Val Asp  
                          305                                      310                                      315                                      320  
 Leu Leu Leu Gly Asp Ala Ser Lys Ala Lys Ala Gln Leu Gly Trp Ser  
                          325                                      330                                      335  
 Pro Lys Val Thr Phe Leu Glu Leu Ile Ala Asp Met Met Ala Ala Asp  
                          340                                      345                                      350  
 Ile Gln Leu Met Lys Asn Asn Pro Ser Ala  
                          355                                      360  
 <210> 127  
 <211> 382  
 <212> PRT  
 <213> Aedes aegypti  
 <400> 127  
 Met Ser Leu Glu Asn Gly Ser Asn Lys Lys Arg Arg Leu Ser Leu Glu  
                          1                                      5                                      10                                      15  
 Asn His Leu Ala Thr Glu Ser Glu Gly Ser Ser Ser Ser Asp Pro Asn  
                          20                                      25                                      30

Arg Arg Val Ala Leu Ile Thr Gly Ile Thr Gly Gln Asp Gly Ser Tyr  
 35 40 45  
 Leu Ala Glu Phe Leu Leu Lys Lys Asp Tyr Glu Val His Gly Ile Ile  
 50 55 60  
 Arg Arg Ala Ser Thr Phe Asn Thr Ser Arg Ile Glu His Leu Tyr Ala  
 65 70 75 80  
 Asp Pro His Ser His Lys Gln Gly Lys Met Lys Leu His Tyr Gly Asp  
 85 90 95  
 Met Thr Asp Ser Ser Cys Leu Val Lys Ile Ile Ser Ser Val Arg Pro  
 100 105 110  
 Ser Glu Ile Tyr Asn Leu Ala Ala Gln Ser His Val Lys Val Ser Phe  
 115 120 125  
 Asp Leu Ser Glu Tyr Thr Ala Glu Val Asp Ala Val Gly Thr Leu Arg  
 130 135 140  
 Leu Leu Asp Ala Ile Arg Thr Cys Gly Leu Glu Lys Ser Val Arg Phe  
 145 150 155 160  
 Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly Lys Val Val Glu Thr Pro  
 165 170 175  
 Gln Asn Glu Lys Thr Pro Phe Tyr Pro Arg Ser Pro Tyr Ala Cys Ala  
 180 185 190  
 Lys Met Tyr Gly Tyr Trp Ile Val Ile Asn Tyr Arg Glu Ala Tyr Asn  
 195 200 205  
 Met Phe Ala Cys Asn Gly Ile Leu Phe Asn His Glu Ser Pro Arg Arg  
 210 215 220  
 Gly Glu Asn Phe Val Thr Arg Lys Ile Thr Arg Ser Val Ala Lys Ile  
 225 230 235 240  
 Ser Leu Asn Gln Met Asp Cys Leu Glu Leu Gly Asn Leu Asp Ser Lys  
 245 250 255  
 Arg Asp Trp Gly His Ala Gln Asp Tyr Val Glu Ala Met Trp Met Met  
 260 265 270  
 Leu Gln Gln Pro Gln Pro Gln Asp Tyr Val Ile Ala Thr Gly Glu Cys  
 275 280 285  
 His Ser Val Arg Glu Phe Val Glu His Ser Phe Arg His Ile Gly Arg  
 290 295 300  
 Glu Ile Glu Trp Arg Gly Glu Gly Leu Asn Glu Val Gly Val Glu Lys  
 305 310 315 320  
 Gly Thr Asp Thr Val Arg Val Arg Ile Asn Pro Lys Phe Phe Arg Pro  
 325 330 335  
 Thr Glu Val Asp Leu Leu Leu Gly Asp Ala Ser Lys Ala Lys Arg Glu  
 340 345 350  
 Leu Gly Trp Thr Pro Lys Val Ser Phe Leu Gln Leu Val Ser Asp Met  
 355 360 365  
 Met Val Ala Asp Ile Glu Leu Met Lys Lys Asn Pro Asn Ala  
 370 375 380

&lt;210&gt; 128

&lt;211&gt; 370

&lt;212&gt; PRT

&lt;213&gt; Danio rerio

&lt;400&gt; 128

Met Ala Gln Cys Thr Ala Thr Thr Thr Gly Gly Gly Met Asn Gly Asp  
 1 5 10 15

Ser Lys Arg Lys Arg Lys Val Ala Ile Ile Thr Gly Ile Thr Gly Gln  
 20 25 30

Asp Gly Ser Tyr Leu Ala Glu Leu Leu Leu Ala Lys Gly Tyr Glu Val  
 35 40 45

His Gly Ile Leu Arg Arg Ser Ser Ser Phe Asn Thr Gly Arg Ile Glu  
 50 55 60

His Leu Tyr His Asn Pro Gln Thr His Thr Glu Gly Asn Met Lys Leu  
 65 70 75 80

His Tyr Gly Asp Leu Thr Asp Ser Thr Cys Leu Val Lys Ile Ile Asn  
 85 90 95

Glu Val Lys Pro Thr Glu Ile Tyr Asn Leu Gly Ala Gln Ser His Val  
 100 105 110

Lys Ile Ser Phe Asp Leu Ala Glu Tyr Thr Ala Asp Val Asp Gly Val  
 115 120 125

Gly Thr Leu Arg Leu Leu Asp Ala Val Lys Thr Cys Gly Leu Thr Asp  
 130 135 140

Thr Val Arg Phe Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly Lys Val  
 145 150 155 160

Gln Glu Ile Pro Gln Lys Glu Thr Thr Pro Phe Tyr Pro Arg Ser Pro  
 165 170 175

Tyr Gly Ala Ala Lys Leu Tyr Ala Tyr Trp Ile Val Ile Asn Phe Arg  
 180 185 190

Glu Ala Tyr Asn Leu Phe Ala Val Asn Gly Ile Leu Phe Asn His Glu  
 195 200 205

Ser Pro Arg Arg Gly Ser Asn Phe Val Thr Arg Lys Ile Ser Arg Ser  
 210 215 220

Val Ala Lys Ile His Leu Gly Gln Leu Glu Cys Phe Ser Leu Gly Asn  
 225 230 235 240

Leu Asp Ser Met Arg Asp Trp Gly His Ala Lys Asp Tyr Val Glu Ala  
 245 250 255

Met Trp Leu Met Leu Gln Gln Glu Glu Pro Val Asp Phe Val Ile Ala  
 260 265 270

Thr Gly Glu Val His Ser Val Arg Glu Phe Val Glu Arg Ala Phe Lys  
 275 280 285

His Val Gly Lys Thr Ile Val Trp Glu Gly Lys Asp Glu Lys Glu Val  
 290 295 300

Gly Arg Cys Gln Glu Thr Gly Val Ile His Val Arg Val Asp Pro Lys

305                      310                      315                      320  
 Tyr Tyr Arg Pro Thr Glu Val Asp Tyr Leu Gln Gly Asp Ser Ser Lys  
                                  325                      330                      335  
 Ala Phe Lys Val Leu Gly Trp Lys Pro Arg Val Thr Phe Glu Glu Leu  
                                  340                      345                      350  
 Val Lys Glu Met Val Asp Ala Asp Ile Lys Leu Met Gln Asn Asn Pro  
                                  355                      360                      365  
 Asn Ala  
                  370  
 <210> 129  
 <211> 370  
 <212> PRT  
 <213> Salmo salar  
 <400> 129  
 Met Ala Gln Cys Thr Glu Pro Ser Thr Ser Thr Gly Ala Asn Gly Glu  
 1                                   5                                   10                                   15  
 Leu Lys Lys Pro Arg Lys Val Ala Val Ile Thr Gly Ile Thr Gly Gln  
                                  20                                   25                                   30  
 Asp Gly Ser Tyr Leu Ala Glu Phe Leu Leu Glu Lys Gly Tyr Glu Val  
                                  35                                   40                                   45  
 His Gly Ile Leu Arg Arg Ser Ser Ser Phe Asn Thr Gly Arg Ile Glu  
                                  50                                   55                                   60  
 His Leu Tyr Gln Asn Pro Gln Thr His Thr Glu Gly Asn Met Lys Leu  
 65                                   70                                   75                                   80  
 His Tyr Gly Asp Leu Thr Asp Ser Thr Cys Leu Val Lys Ile Ile Asn  
                                  85                                   90                                   95  
 Gln Val Lys Pro Thr Glu Ile Tyr Asn Leu Gly Ala Gln Ser His Val  
                                  100                                   105                                   110  
 Lys Ile Ser Phe Glu Leu Ala Glu Tyr Thr Ala Asn Val Asp Gly Val  
                                  115                                   120                                   125  
 Gly Thr Leu Arg Leu Leu Asp Ala Ile Lys Thr Cys Gly Leu Thr Asn  
                                  130                                   135                                   140  
 Ser Val Lys Phe Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly Lys Val  
 145                                   150                                   155                                   160  
 Gln Glu Ile Pro Gln Lys Glu Thr Thr Pro Phe Tyr Pro Arg Ser Pro  
                                  165                                   170                                   175  
 Tyr Gly Ala Ala Lys Leu Tyr Ala Tyr Trp Ile Val Val Asn Phe Arg  
                                  180                                   185                                   190  
 Glu Ala Tyr Asn Leu Phe Ala Val Asn Gly Ile Leu Phe Asn His Glu  
                                  195                                   200                                   205  
 Ser Pro Arg Arg Gly Ala Asn Phe Val Thr Arg Lys Ile Ser Arg Ser  
                                  210                                   215                                   220  
 Val Ala Lys Ile His Leu Gly Gln Leu Glu Ser Phe Ser Leu Gly Asn  
 225                                   230                                   235                                   240



Leu Asp Ser Lys Arg Asp Trp Gly His Ala Lys Asp Tyr Val Glu Ala  
245 250 255

Met Trp Leu Met Leu Gln Gln Glu Glu Pro Glu Asp Leu Val Ile Ala  
260 265 270

Thr Gly Glu Val His Ser Val Arg Glu Phe Val Glu Lys Ser Phe Lys  
275 280 285

His Val Gly Lys Thr Ile Val Trp Glu Gly Lys Asp Glu Asn Glu Val  
290 295 300

Gly Arg Cys Gln Glu Thr Gly Val Ile His Val Lys Val Asp Ser Lys  
305 310 315 320

Tyr Tyr Arg Pro Thr Glu Val Glu Tyr Leu Gln Gly Asp Ser Thr Lys  
325 330 335

Ala Leu Thr Lys Leu Gly Trp Lys Ala Lys Ile Thr Phe Glu Glu Leu  
340 345 350

Val Lys Glu Met Val Asp Ala Asp Ile His Leu Met Lys Asn Asn Pro  
355 360 365

Asn Ala  
370

<210> 130

<211> 358

<212> PRT

<213> Apis mellifera

<400> 130

Met Lys Met Ala Thr Asp Ser Arg Arg Arg Val Ala Leu Ile Thr Gly  
1 5 10 15

Ile Thr Gly Gln Asp Gly Ser Tyr Leu Ala Glu Phe Leu Leu Glu Lys  
20 25 30

Gly Tyr Asp Val His Gly Ile Ile Arg Arg Ala Ser Ser Phe Asn Thr  
35 40 45

Ala Arg Ile Gln His Leu Tyr Glu Asp Pro Lys Cys His Arg Gln Gly  
50 55 60

Lys Met Lys Leu His Tyr Gly Asp Met Thr Asp Ser Ser Ser Leu Ile  
65 70 75 80

Lys Val Ile Ser Ser Val Gln Pro Thr Glu Ile Tyr Asn Leu Ala Ala  
85 90 95

Gln Ser His Val Met Val Ser Phe Glu Val Ser Glu Tyr Thr Ala Glu  
100 105 110

Val Asp Ala Val Gly Thr Val Arg Leu Leu Asp Ala Ile Arg Thr Cys  
115 120 125

Gly Leu Glu Lys Ser Val Lys Phe Tyr His Ala Ser Thr Ser Glu Leu  
130 135 140

Tyr Gly Arg Val Thr Gln Val Pro Gln Asn Glu Lys Thr Pro Phe Tyr  
145 150 155 160

Pro Arg Ser Pro Tyr Ala Cys Ala Lys Leu Tyr Ser Phe Trp Ile Val  
165 170 175 180 185 190

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165              170              175

Ile Asn Tyr Arg Glu Ala Tyr Asn Met Phe Ala Cys Asn Gly Ile Leu
180              185              190

Phe Asn His Glu Ser Pro Arg Arg Gly Glu Asn Phe Val Thr Arg Lys
195              200              205

Val Thr Arg Ser Ile Ala Lys Ile His Leu Gly Leu Gln Asp Ile Leu
210              215              220

Glu Leu Gly Asn Leu Asp Ala Lys Arg Asp Trp Gly His Ala Lys Asp
225              230              235              240

Tyr Val Glu Ala Met Trp Leu Met Leu Gln Gln Pro Thr Ala Asp Asp
245              250              255

Tyr Val Ile Ala Thr Gly Glu Thr His Ser Val Arg Glu Phe Val Glu
260              265              270

Ala Ala Phe Gln Tyr Val Gly Arg Thr Ile Lys Trp Glu Gly Glu Gly
275              280              285

Ile Asn Glu Ile Gly Gln Asp Val Gln Thr Gly Gln Val Leu Val Lys
290              295              300

Val Asn Pro Lys Tyr Phe Arg Pro Thr Glu Val Asp Val Leu Leu Gly
305              310              315              320

Asp Ala Thr Lys Ala Lys Glu Lys Ile Gly Trp Lys Pro Thr Ile Thr
325              330              335

Phe Glu Asn Leu Val Lys Asp Met Met Asp Ser Asp Leu Glu Leu Met
340              345              350

Ser Lys Asn Pro Asn Ala
355

<210> 131
<211> 395
<212> PRT
<213> Drosophila melanogaster

<400> 131
Met Leu Asn Thr Arg Leu Ile Ala Met Ser Thr Ser Asp Gly Ala Pro
1      5      10      15

Glu Thr Lys Lys Gln Arg Pro Glu Ser Ser Ser Asn Gly Ser Lys Asp
20     25     30

Gln Asn Gly Thr Glu Ala Gly Ala Glu Gly Asp Ser Arg Asp Lys Val
35     40     45

Ala Leu Ile Thr Gly Ile Thr Gly Gln Asp Gly Ser Tyr Leu Ala Glu
50     55     60

Phe Leu Leu Lys Lys Asp Tyr Glu Val His Gly Ile Ile Arg Arg Ala
65     70     75     80

Ser Thr Phe Asn Thr Thr Arg Ile Glu His Leu Tyr Ala Asp Pro Lys
85     90     95

Ala His Lys Gly Gly Arg Met Lys Leu His Tyr Gly Asp Met Thr Asp
100    105    110

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 Ser Ser Ser Leu Val Lys Ile Ile Asn Met Val Lys Pro Thr Glu Ile  
 115 120 125

Tyr Asn Leu Ala Ala Gln Ser His Val Lys Val Ser Phe Asp Leu Ser  
 130 135 140

Glu Tyr Thr Ala Glu Val Asp Ala Val Gly Thr Leu Arg Ile Leu Asp  
 145 150 155 160

Ala Ile Arg Thr Cys Gly Met Glu Lys Asn Val Arg Phe Tyr Gln Ala  
 165 170 175

Ser Thr Ser Glu Leu Tyr Gly Lys Val Val Glu Thr Pro Gln Asn Glu  
 180 185 190

Gln Thr Pro Phe Tyr Pro Arg Ser Pro Tyr Ala Cys Ala Lys Met Tyr  
 195 200 205

Gly Phe Trp Ile Val Ile Asn Tyr Arg Glu Ala Tyr Asn Met Tyr Ala  
 210 215 220

Cys Asn Gly Ile Leu Phe Asn His Glu Ser Pro Arg Arg Gly Glu Asn  
 225 230 235 240

Phe Val Thr Arg Lys Ile Thr Arg Ser Val Ala Lys Ile Tyr His Lys  
 245 250 255

Gln Met Glu Tyr Phe Glu Leu Gly Asn Leu Asp Ser Lys Arg Asp Trp  
 260 265 270

Gly His Ala Ser Asp Tyr Val Glu Ala Met Trp Met Met Leu Gln Arg  
 275 280 285

Glu Ser Pro Ser Asp Tyr Val Ile Ala Thr Gly Glu Thr His Ser Val  
 290 295 300

Arg Glu Phe Val Glu Ala Ala Phe Lys His Ile Asp Arg Glu Ile Thr  
 305 310 315 320

Trp Lys Gly Lys Gly Val Asp Glu Val Gly Val Glu Asn Gly Thr Gly  
 325 330 335

Ile Val Arg Val Arg Ile Asn Pro Lys Tyr Phe Arg Pro Thr Glu Val  
 340 345 350

Asp Leu Leu Gln Gly Asp Ala Ser Lys Ala Asn Arg Glu Leu Asn Trp  
 355 360 365

Thr Pro Lys Val Thr Phe Val Glu Leu Val Ser Asp Met Met Lys Ala  
 370 375 380

Asp Ile Glu Leu Met Arg Lys Asn Pro Ile Ala  
 385 390 395

<210> 132

<211> 369

<212> PRT

<213> Xenopus laevis

<400> 132

Met Ala Gln Asn Gln Gly Asn Cys Ser Cys Ser Pro Ser Asn Ser Ser

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1           5           10           15

Gly Lys Pro Arg Lys Val Ala Leu Ile Thr Gly Ile Thr Gly Gln Asp
    20           25           30

Gly Ser Tyr Leu Ala Glu Phe Leu Leu Glu Lys Gly Tyr Glu Val His
    35           40           45

Gly Ile Val Arg Arg Ser Ser Ser Phe Asn Thr Gly Arg Ile Glu His
    50           55           60

Leu Tyr Lys Asn Pro His Ala His Thr Glu Gly Asn Met Lys Leu His
    65           70           75           80

Tyr Gly Asp Leu Thr Asp Ser Thr Cys Leu Val Lys Ile Ile Asn Glu
    85           90           95

Val Lys Pro Thr Glu Ile Tyr Asn Leu Gly Ala Gln Ser His Val Lys
    100          105          110

Ile Ser Phe Asp Leu Ala Glu Tyr Thr Ala Asp Val Asp Gly Leu Gly
    115          120          125

Thr Leu Arg Leu Leu Asp Ala Thr Lys Thr Cys Gly Leu Ile Asn Thr
    130          135          140

Val Lys Phe Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly Lys Val Gln
    145          150          155          160

Glu Ile Pro Gln Lys Glu Thr Thr Pro Phe Tyr Pro Arg Ser Pro Tyr
    165          170          175

Gly Ala Ala Lys Leu Tyr Ala Tyr Trp Ile Val Val Asn Phe Arg Glu
    180          185          190

Ala Tyr Asn Leu Phe Ala Val Asn Gly Ile Leu Phe Asn His Glu Ser
    195          200          205

Pro Arg Arg Gly Ala Asn Phe Val Thr Arg Lys Ile Ser Arg Ser Val
    210          215          220

Ala Lys Ile His Leu Gly Gln Met Glu Ser Phe Ser Leu Gly Asn Leu
    225          230          235          240

Asp Ala Lys Arg Asp Trp Gly His Ala Lys Asp Tyr Val Glu Ala Met
    245          250          255

Trp Leu Met Leu Gln Thr Asp Glu Pro Glu Asp Phe Val Ile Ser Thr
    260          265          270

Gly Glu Val His Ser Val Arg Glu Phe Val Glu Lys Ala Phe Lys His
    275          280          285

Ile Gly Lys Thr Ile Val Trp Glu Gly Lys Asn Glu Asn Glu Val Gly
    290          295          300

Arg Cys Ser Glu Thr Gly Lys Ile His Val Lys Val Asp Leu Lys Tyr
    305          310          315          320

Tyr Arg Pro Thr Glu Val Asp Phe Leu Gln Gly Asp Cys Ser Gln Ala
    325          330          335

Lys Asn Lys Leu Gly Trp Thr Pro Lys Val Ser Phe Asp Glu Leu Val
    340          345          350

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Lys Glu Met Val Glu Ser Asp Val Lys Leu Met Ala Thr Asn Pro Asn  
 355 360 365

Ala

<210> 133

<211> 372

<212> PRT

<213> Bos taurus

<400> 133

Met Ala Gln Ala Ala Ala His Tyr Pro Gly Ala Cys Gly Ala Gly Asp  
 1 5 10 15

Ala Glu Thr Gly Lys Pro Arg Lys Val Ala Leu Ile Thr Gly Ile Thr  
 20 25 30

Gly Gln Asp Gly Ser Tyr Leu Ala Glu Phe Leu Leu Glu Lys Gly Tyr  
 35 40 45

Glu Val His Gly Ile Val Arg Arg Ser Ser Ser Phe Asn Thr Gly Arg  
 50 55 60

Ile Glu His Leu Tyr Lys Asn Pro Gln Ala His Ile Glu Gly Asn Met  
 65 70 75 80

Lys Leu His Tyr Gly Asp Leu Thr Asp Ser Thr Cys Leu Val Lys Ile  
 85 90 95

Ile Asn Glu Val Lys Pro Thr Glu Ile Tyr Asn Leu Gly Ala Gln Ser  
 100 105 110

His Val Lys Ile Ser Phe Asp Leu Ala Glu Tyr Thr Ala Asp Val Asp  
 115 120 125

Gly Val Gly Thr Leu Arg Leu Leu Asp Ala Val Lys Thr Cys Gly Leu  
 130 135 140

Ile Ser Ser Val Arg Phe Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly  
 145 150 155 160

Lys Val Gln Glu Ile Pro Gln Lys Glu Thr Thr Pro Phe Tyr Pro Arg  
 165 170 175

Ser Pro Tyr Gly Ala Ala Lys Leu Tyr Ala Tyr Trp Ile Val Val Asn  
 180 185 190

Phe Arg Glu Ala Tyr Asn Leu Phe Ala Val Asn Gly Ile Leu Phe Asn  
 195 200 205

His Glu Ser Pro Arg Arg Gly Ala Asn Phe Val Thr Arg Lys Ile Ser  
 210 215 220

Arg Ser Val Ala Lys Ile His Leu Gly Gln Leu Glu Cys Phe Ser Leu  
 225 230 235 240

Gly Asn Leu Asp Ala Lys Arg Asp Trp Gly His Ala Lys Asp Tyr Val  
 245 250 255

Glu Ala Met Trp Leu Met Leu Gln Lys Asp Glu Pro Glu Asp Phe Val  
 260 265 270

Ile Ala Thr Gly Glu Val His Ser Val Arg Glu Phe Val Glu Lys Ser  
 275 280 285

Phe Leu His Ile Gly Lys Thr Ile Val Trp Glu Gly Lys Asn Glu Asn  
290 295 300

Glu Val Gly Arg Cys Lys Glu Thr Gly Lys Ile His Val Thr Val Asp  
305 310 315 320

Leu Lys Tyr Tyr Arg Pro Thr Glu Val Asp Phe Leu Gln Gly Asp Cys  
325 330 335

Ser Lys Ala Arg Gln Lys Leu Ser Trp Lys Pro Arg Val Ala Phe Asp  
340 345 350

Glu Leu Val Arg Glu Met Val Glu Ala Asp Val Glu Leu Met Arg Thr  
355 360 365

Asn Pro His Ala  
370

<210> 134

<211> 372

<212> PRT

<213> Cricetulus griseus

<400> 134

Met Ala His Ala Pro Ala Ser Cys Pro Ser Ser Arg Asn Ser Gly Asp  
1 5 10 15

Gly Asp Lys Gly Lys Pro Arg Lys Val Ala Leu Ile Thr Gly Ile Thr  
20 25 30

Gly Gln Asp Gly Ser Tyr Leu Ala Glu Phe Leu Leu Glu Lys Gly Tyr  
35 40 45

Glu Val His Gly Ile Val Arg Arg Ser Ser Ser Phe Asn Thr Gly Arg  
50 55 60

Ile Glu His Leu Tyr Lys Asn Pro Gln Ala His Ile Glu Gly Asn Met  
65 70 75 80

Lys Leu His Tyr Gly Asp Leu Thr Asp Ser Thr Cys Leu Val Lys Ile  
85 90 95

Ile Asn Glu Val Lys Pro Thr Glu Ile Tyr Asn Leu Gly Ala Gln Ser  
100 105 110

His Val Lys Ile Ser Phe Asp Leu Ala Glu Tyr Thr Ala Asp Val Asp  
115 120 125

Gly Val Gly Thr Leu Arg Leu Leu Asp Ala Ile Lys Thr Cys Gly Leu  
130 135 140

Ile Asn Ser Val Lys Phe Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly  
145 150 155 160

Lys Val Gln Glu Ile Pro Gln Lys Glu Thr Thr Pro Phe Tyr Pro Arg  
165 170 175

Ser Pro Tyr Gly Ala Ala Lys Leu Tyr Ala Tyr Trp Ile Val Val Asn  
180 185 190

Phe Arg Glu Ala Tyr Asn Leu Phe Ala Val Asn Gly Ile Leu Phe Asn  
195 200 205

His Glu Ser Pro Arg Arg Gly Ala Asn Phe Val Thr Arg Lys Ile Ser  
210 215 220

Arg Ser Val Ala Lys Ile Tyr Leu Gly Gln Leu Glu Cys Phe Ser Leu  
225 230 235 240

Gly Asn Leu Asp Ala Lys Arg Asp Trp Gly His Ala Lys Asp Tyr Val  
245 250 255

Glu Ala Met Trp Leu Met Leu Gln Asn Asp Glu Pro Glu Asp Phe Val  
260 265 270

Ile Ala Thr Gly Glu Val His Ser Val Arg Glu Phe Val Glu Lys Ser  
275 280 285

Phe Met His Ile Gly Lys Thr Ile Val Trp Glu Gly Lys Asn Glu Asn  
290 295 300

Glu Val Gly Arg Cys Lys Glu Thr Gly Lys Ile His Val Thr Val Asp  
305 310 315 320

Leu Lys Tyr Tyr Arg Pro Thr Glu Val Asp Phe Leu Gln Gly Asp Cys  
325 330 335

Ser Lys Ala Gln Gln Lys Leu Asn Trp Lys Pro Arg Val Ala Phe Asp  
340 345 350

Glu Leu Val Arg Glu Met Val Gln Ala Asp Val Glu Leu Met Arg Thr  
355 360 365

Asn Pro Asn Ala  
370

<210> 135

<211> 372

<212> PRT

<213> Mus musculus

<400> 135

Met Ala Gln Ala Pro Ala Lys Cys Pro Ser Tyr Pro Gly Ser Gly Asp  
1 5 10 15

Gly Glu Met Gly Lys Leu Arg Lys Val Ala Leu Ile Thr Gly Ile Thr  
20 25 30

Gly Gln Asp Gly Ser Tyr Leu Ala Glu Phe Leu Leu Glu Lys Gly Tyr  
35 40 45

Glu Val His Gly Ile Val Arg Arg Ser Ser Ser Phe Asn Thr Gly Arg  
50 55 60

Ile Glu His Leu Tyr Lys Asn Pro Gln Ala His Ile Glu Gly Asn Met  
65 70 75 80

Lys Leu His Tyr Gly Asp Leu Thr Asp Ser Thr Cys Leu Val Lys Ile  
85 90 95

Ile Asn Glu Val Lys Pro Thr Glu Ile Tyr Asn Leu Gly Ala Gln Ser  
100 105 110

His Val Lys Ile Ser Phe Asp Leu Ala Glu Tyr Thr Ala Asp Val Asp  
115 120 125

Gly Val Gly Thr Leu Arg Leu Leu Asp Ala Ile Lys Thr Cys Gly Leu  
130 135 140

Ile Asn Ser Val Lys Phe Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly  
145 150 155 160

140                      150                      155                      160  
 Lys Val Gln Glu Ile Pro Gln Lys Glu Thr Thr Pro Phe Tyr Pro Arg  
                                  165                                   170                                   175  
 Ser Pro Tyr Gly Ala Ala Lys Leu Tyr Ala Tyr Trp Ile Val Val Asn  
                                  180                                   185                                   190  
 Phe Arg Glu Ala Tyr Asn Leu Phe Ala Val Asn Gly Ile Leu Phe Asn  
                                  195                                   200                                   205  
 His Glu Ser Pro Arg Arg Gly Ala Asn Phe Val Thr Arg Lys Ile Ser  
                                  210                                   215                                   220  
 Arg Ser Val Ala Lys Ile Tyr Leu Gly Gln Leu Glu Cys Phe Ser Leu  
                                  225                                   230                                   235                                   240  
 Gly Asn Leu Asp Ala Lys Arg Asp Trp Gly His Ala Lys Asp Tyr Val  
                                  245                                   250                                   255  
 Glu Ala Met Trp Leu Met Leu Gln Asn Asp Glu Pro Glu Asp Phe Val  
                                  260                                   265                                   270  
 Ile Ala Thr Gly Glu Val His Ser Val Arg Glu Phe Val Glu Lys Ser  
                                  275                                   280                                   285  
 Phe Met His Ile Gly Lys Thr Ile Val Trp Glu Gly Lys Asn Glu Asn  
                                  290                                   295                                   300  
 Glu Val Gly Arg Cys Lys Glu Thr Gly Lys Val His Val Thr Val Asp  
                                  305                                   310                                   315                                   320  
 Leu Lys Tyr Tyr Arg Pro Thr Glu Val Asp Phe Leu Gln Gly Asp Cys  
                                  325                                   330                                   335  
 Ser Lys Ala Gln Gln Lys Leu Asn Trp Lys Pro Arg Val Ala Phe Asp  
                                  340                                   345                                   350  
 Glu Leu Val Arg Glu Met Val Gln Ala Asp Val Glu Leu Met Arg Thr  
                                  355                                   360                                   365  
 Asn Pro Asn Ala  
                                  370  
 <210> 136  
 <211> 372  
 <212> PRT  
 <213> Rattus norvegicus  
 <400> 136  
 Met Ala His Ala Pro Ala Ser Cys Arg Arg Tyr Pro Gly Ser Gly Asp  
                                  1                                   5                                   10                                   15  
 Gly Glu Met Gly Lys Leu Arg Lys Val Ala Leu Ile Thr Gly Ile Thr  
                                  20                                   25                                   30  
 Gly Gln Asp Gly Ser Tyr Leu Ala Glu Phe Leu Leu Glu Lys Gly Tyr  
                                  35                                   40                                   45  
 Glu Val His Gly Ile Val Arg Arg Ser Ser Ser Phe Asn Thr Gly Arg  
                                  50                                   55                                   60  
 Ile Glu His Leu Tyr Lys Asn Pro Gln Ala His Ile Glu Gly Asn Met  
                                  65                                   70                                   75                                   80  
 Tyr Leu His Thr Gln Arg Leu Phe Arg Ser Phe Gln Tyr Val Tyr Ile



Lys Leu His Tyr Gly Asp Leu Thr Asp Ser Thr Cys Leu Val Lys Ile  
 85 90 95  
 Ile Asn Glu Val Lys Pro Thr Glu Ile Tyr Asn Leu Gly Ala Gln Ser  
 100 105 110  
 His Val Lys Ile Ser Phe Asp Leu Ala Glu Tyr Thr Ala Asp Val Asp  
 115 120 125  
 Gly Val Gly Thr Leu Arg Leu Leu Asp Ala Ile Lys Thr Cys Gly Leu  
 130 135 140  
 Ile Asn Ser Val Lys Phe Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly  
 145 150 155 160  
 Lys Val Gln Glu Ile Pro Gln Lys Glu Thr Thr Pro Phe Tyr Pro Arg  
 165 170 175  
 Ser Pro Tyr Gly Ala Ala Lys Leu Tyr Ala Tyr Trp Ile Val Val Asn  
 180 185 190  
 Phe Arg Glu Ala Tyr Asn Leu Phe Ala Val Asn Gly Ile Leu Phe Asn  
 195 200 205  
 His Glu Ser Pro Arg Arg Gly Ala Asn Phe Val Thr Arg Lys Ile Ser  
 210 215 220  
 Arg Ser Val Ala Lys Ile Tyr Leu Gly Gln Leu Glu Cys Phe Ser Leu  
 225 230 235 240  
 Gly Asn Leu Asp Ala Lys Arg Asp Trp Gly His Ala Lys Asp Tyr Val  
 245 250 255  
 Glu Ala Met Trp Leu Met Leu Gln Asn Asp Glu Pro Glu Asp Phe Val  
 260 265 270  
 Ile Ala Thr Gly Glu Val His Ser Val Arg Glu Phe Val Glu Lys Ser  
 275 280 285  
 Phe Met His Ile Gly Lys Thr Ile Val Trp Glu Gly Lys Asn Glu Asn  
 290 295 300  
 Glu Val Gly Arg Cys Lys Glu Thr Gly Lys Ile His Val Thr Val Asp  
 305 310 315 320  
 Leu Lys Tyr Tyr Arg Pro Thr Glu Val Asp Phe Leu Gln Gly Asp Cys  
 325 330 335  
 Ser Lys Ala Gln Gln Lys Leu Asn Trp Lys Pro Arg Val Ala Phe Asp  
 340 345 350  
 Glu Leu Val Arg Glu Met Val Gln Ala Asp Val Glu Leu Met Arg Thr  
 355 360 365  
 Asn Pro Asn Ala  
 370  
 <210> 137  
 <211> 372  
 <212> PRT  
 <213> Homo sapiens  
 <400> 137  
 Met Ala His Ala Pro Ala Arg Cys Pro Ser Ala Arg Gly Ser Gly Asp  
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```

1           5           10           15

Gly Glu Met Gly Lys Pro Arg Asn Val Ala Leu Ile Thr Gly Ile Thr
      20           25           30

Gly Gln Asp Gly Ser Tyr Leu Ala Glu Phe Leu Leu Glu Lys Gly Tyr
      35           40           45

Glu Val His Gly Ile Val Arg Arg Ser Ser Ser Phe Asn Thr Gly Arg
      50           55           60

Ile Glu His Leu Tyr Lys Asn Pro Gln Ala His Ile Glu Gly Asn Met
      65           70           75           80

Lys Leu His Tyr Gly Asp Leu Thr Asp Ser Thr Cys Leu Val Lys Ile
      85           90           95

Ile Asn Glu Val Lys Pro Thr Glu Ile Tyr Asn Leu Gly Ala Gln Ser
      100          105          110

His Val Lys Ile Ser Phe Asp Leu Ala Glu Tyr Thr Ala Asp Val Asp
      115          120          125

Gly Val Gly Thr Leu Arg Leu Leu Asp Ala Val Lys Thr Cys Gly Leu
      130          135          140

Ile Asn Ser Val Lys Phe Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly
      145          150          155          160

Lys Val Gln Glu Ile Pro Gln Lys Glu Thr Thr Pro Phe Tyr Pro Arg
      165          170          175

Ser Pro Tyr Gly Ala Ala Lys Leu Tyr Ala Tyr Trp Ile Val Val Asn
      180          185          190

Phe Arg Glu Ala Tyr Asn Leu Phe Ala Val Asn Gly Ile Leu Phe Asn
      195          200          205

His Glu Ser Pro Arg Arg Gly Ala Asn Phe Val Thr Arg Lys Ile Ser
      210          215          220

Arg Ser Val Ala Lys Ile Tyr Leu Gly Gln Leu Glu Cys Phe Ser Leu
      225          230          235          240

Gly Asn Leu Asp Ala Lys Arg Asp Trp Gly His Ala Lys Asp Tyr Val
      245          250          255

Glu Ala Met Trp Leu Met Leu Gln Asn Asp Glu Pro Glu Asp Phe Val
      260          265          270

Ile Ala Thr Gly Glu Val His Ser Val Arg Glu Phe Val Glu Lys Ser
      275          280          285

Phe Leu His Ile Gly Lys Thr Ile Val Trp Glu Gly Lys Asn Glu Asn
      290          295          300

Glu Val Gly Arg Cys Lys Glu Thr Gly Lys Val His Val Thr Val Asp
      305          310          315          320

Leu Lys Tyr Tyr Arg Pro Thr Glu Val Asp Phe Leu Gln Gly Asp Cys
      325          330          335

Thr Lys Ala Lys Gln Lys Leu Asn Trp Lys Pro Arg Val Ala Phe Asp
      340          345          350

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340 345 350

Glu Leu Val Arg Glu Met Val His Ala Asp Val Glu Leu Met Arg Thr  
355 360 365

Asn Pro Asn Ala  
370

<210> 138  
<211> 373  
<212> PRT  
<213> Ciona intestinalis

<400> 138

Met Ala Asp Ser Ser Cys Asn Gly Lys Arg Lys Asn Gly Asp Ser Cys  
1 5 10 15

Asp Thr Glu Thr Lys Lys Gln Lys Lys Thr Ala Leu Val Thr Gly Ile  
20 25 30

Thr Gly Gln Asp Gly Ser Tyr Leu Ala Glu Leu Leu Ile Glu Lys Gly  
35 40 45

Tyr Glu Val His Gly Ile Ile Arg Arg Ala Ser Gln Pro Asn Thr Ala  
50 55 60

Arg Ile Glu His Leu Tyr Ala Asp Arg Ala Thr His Lys Glu Gly Ser  
65 70 75 80

Met Lys Leu His Tyr Gly Asp Leu Thr Asp Ser Ser Cys Leu Val Lys  
85 90 95

Ile Ile Asn Gln Val Lys Pro Asn Glu Ile Tyr Asn Leu Gly Ala Met  
100 105 110

Ser His Val Lys Val Ser Phe Asp Leu Ala Glu Tyr Thr Ala Asp Val  
115 120 125

Asp Gly Val Gly Thr Leu Arg Leu Leu Asp Ala Ile Arg Thr Cys Gly  
130 135 140

Met Ser Asp Ser Val Arg Phe Tyr Gln Ala Ser Thr Ser Glu Met Phe  
145 150 155 160

Gly Lys Val Gln Glu Ile Pro Gln Lys Glu Thr Thr Pro Phe Tyr Pro  
165 170 175

Arg Ser Pro Tyr Ala Ala Ala Lys Val Tyr Ala Tyr Trp Ile Val Val  
180 185 190

Asn Tyr Arg Glu Ala Tyr Gly Met His Ala Ser Asn Gly Ile Leu Phe  
195 200 205

Asn His Glu Ser Pro Arg Arg Gly Phe Asn Phe Val Thr Arg Lys Ile  
210 215 220

Thr Arg Ser Val Ala Lys Ile His Leu Gly Leu Gln Glu Leu Ile Thr  
225 230 235 240

Leu Gly Asn Leu Asp Ser Lys Arg Asp Trp Gly His Ala Arg Asp Tyr  
245 250 255

Val Lys Gly Met Trp Met Met Met Gln His Asp Glu Pro Gly Asp Tyr  
260 265 270

Val Leu Ser Thr Asn Glu Thr His Ser Val Arg Glu Phe Val Glu Lys  
275 280 285

Ser Phe Lys His Ile Gly Val Glu Leu Glu Trp Gln Gly Glu Gly Val  
290 295 300

Asn Glu Ile Gly Val Asp Lys Ser Thr Gly Val Lys Arg Val Gln Ile  
305 310 315 320

Ser Glu Arg Tyr Tyr Arg Pro Thr Glu Val Glu Phe Leu Leu Gly Asp  
325 330 335

Tyr Ser Lys Ala Lys Lys Val Leu Gly Trp Ser Pro Asp Ile Lys Phe  
340 345 350

Asn Glu Leu Val Lys Glu Met Val Glu Ala Asp Ile Asn Leu Met Lys  
355 360 365

Lys Thr Pro Asn Ala  
370

<210> 139

<211> 379

<212> PRT

<213> Francisella sp. TX077308

<400> 139

Met Lys Lys Val Ala Leu Ile Thr Gly Ile Thr Gly Gln Asp Gly Ser  
1 5 10 15

Tyr Leu Ala Glu Phe Leu Leu Lys Lys Gly Tyr Ile Val His Gly Ile  
20 25 30

Lys Arg Arg Ser Ser Leu Phe Asn Thr Asp Arg Ile Asp His Leu Tyr  
35 40 45

Gln Asp Pro His Val Glu Asn Arg Asn Leu Ile Leu His Tyr Gly Asp  
50 55 60

Met Thr Asp Ser Met Asn Leu Thr Arg Val Ile Ala Asp Thr Gln Pro  
65 70 75 80

Asp Glu Ile Tyr Asn Leu Ala Ala Met Ser His Val His Val Ser Phe  
85 90 95

Glu Thr Pro Glu Tyr Val Ala Asn Ala Asp Gly Thr Gly Thr Leu Arg  
100 105 110

Ile Leu Glu Ala Val Arg Leu Leu Gly Leu Glu Lys Lys Thr Lys Ile  
115 120 125

Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly Lys Val Gln Ala Ile Pro  
130 135 140

Gln Ser Glu Thr Thr Pro Phe Tyr Pro Arg Ser Pro Tyr Ala Val Ala  
145 150 155 160

Lys Met Tyr Ala Phe Trp Ile Thr Val Asn Tyr Arg Glu Ala Tyr Asp  
165 170 175

Met Phe Ala Cys Asn Gly Ile Leu Phe Asn His Glu Ser Pro Val Arg  
180 185 190

Gln Glu Phe Phe Val Phe Ser Thr Ile Phe Ser Ile Ile Ser Thr Ile

Gly Glu Thr Phe Val Thr Arg Lys Ile Thr Arg Ala Ala Ser Lys Ile  
195 200 205

Ala Leu Asn Leu Gln Asp Lys Leu Tyr Leu Gly Asn Leu Asp Ala Lys  
210 215 220

Arg Asp Trp Gly His Ala Lys Asp Tyr Val Arg Met Met Trp Met Ile  
225 230 235 240

Leu Gln Ala Asp Gln Pro Glu Asp Trp Val Ile Ala Thr Gly Gln Thr  
245 250 255

Thr Thr Val Arg Asp Phe Val Lys Leu Ser Phe Ala Tyr Ala Gly Ile  
260 265 270

Asn Leu Arg Phe Glu Gly Glu Gly Val Asp Glu Ile Gly Val Val Asp  
275 280 285

Ser Val Asp Ser Ala Arg Ala Glu Arg Ala Gly Val Asp Leu Ser His  
290 295 300

Ile Ser Glu Gly Gln Val Val Val Cys Val Asp Pro Arg Tyr Phe Arg  
305 310 315 320

Pro Thr Glu Val Asp Leu Leu Leu Gly Asp Pro Thr Lys Ala Glu Gln  
325 330 335

Lys Leu Gly Trp Ser Arg Glu Tyr Asp Leu Glu His Leu Val Asn Asp  
340 345 350

Met Met Glu Ser Asp Leu Lys Leu Met Thr Lys Asp Val Tyr Leu Lys  
355 360 365

Asp Gly Gly Tyr Lys Ile Met Ser Tyr Phe Glu  
370 375

<210> 140

<211> 379

<212> PRT

<213> Campylobacter coli 59-2

<400> 140

Met Lys Lys Val Ala Leu Ile Thr Gly Val Thr Gly Gln Asp Gly Ala  
1 5 10 15

Tyr Leu Ser Glu Phe Leu Leu Asn Lys Gly Tyr Glu Val His Gly Ile  
20 25 30

Lys Arg Arg Ser Ser Leu Phe Asn Thr Asp Arg Ile Asp His Leu Phe  
35 40 45

Glu Gly His His Asn Lys Lys Asn Asp Phe Tyr Leu His Tyr Gly Asp  
50 55 60

Met Thr Asp Ser Met Asn Leu Thr Arg Ile Ile Ala Glu Ile Lys Pro  
65 70 75 80

Asp Glu Ile Tyr Asn Leu Ala Ala Met Ser His Val His Val Ser Phe  
85 90 95

Glu Thr Pro Glu Tyr Thr Ala Asn Ala Asp Gly Ile Gly Thr Leu Arg  
100 105 110

Ile Leu Asp Ala Val Arg Phe Leu Asn Leu Thr Gln Lys Thr Lys Ile  
115 120 125

Tyr Gln Ala Ser Thr Ser Glu Leu Phe Gly Lys Val Gln Glu Ile Pro  
130 135 140

Gln Asn Glu Lys Thr Pro Phe Tyr Pro Arg Ser Pro Tyr Ala Val Ala  
145 150 155 160

Lys Met Tyr Ala Tyr Trp Ile Thr Val Asn Tyr Arg Glu Ala Tyr Asp  
165 170 175

Met Phe Ala Cys Asn Gly Ile Leu Phe Asn His Glu Ser Pro Val Arg  
180 185 190

Gly Glu Thr Phe Val Thr Arg Lys Ile Thr Arg Ala Thr Ala Lys Ile  
195 200 205

Ala Leu Asn Leu Asp Asp Met Leu Tyr Leu Gly Asn Leu Asp Ala Lys  
210 215 220

Arg Asp Trp Gly His Ala Lys Asp Tyr Val Arg Met Met Trp Met Ile  
225 230 235 240

Leu Gln His Glu Lys Ala Asp Asp Trp Val Ile Ala Thr Gly Lys Thr  
245 250 255

Thr Thr Val Arg Asp Phe Val Lys Leu Ala Phe Glu Tyr Cys Gly Ile  
260 265 270

Lys Leu Asn Phe Lys Gly Val Gly Val Asp Glu Glu Gly Phe Ile Glu  
275 280 285

Asp Leu Asp Gln Gln Arg Ala Lys Glu Leu Ser Leu Ser Phe Asn His  
290 295 300

Leu Gln Lys Gly Gln Val Val Val Lys Val Asn Pro Arg Tyr Phe Arg  
305 310 315 320

Pro Thr Glu Val Asp Leu Leu Leu Gly Asp Pro Ser Lys Ala Glu Lys  
325 330 335

Glu Leu Gly Trp Asn Arg Glu Tyr Asp Leu Lys Asn Leu Val Asp Asp  
340 345 350

Met Met Glu Asn Asp Leu Lys Leu Met Thr Lys Asp Val Tyr Leu Lys  
355 360 365

Gln Gly Gly Tyr Lys Ile Met Arg Tyr Tyr Glu  
370 375

<210> 141

<211> 373

<212> PRT

<213> Cellulophaga lytica DSM 7489

<400> 141

Met Ser Asp Lys Lys Val Ala Leu Ile Thr Gly Val Thr Gly Gln Asp  
1 5 10 15

Gly Ala Tyr Leu Ser Glu Phe Leu Leu Lys Lys Gly Tyr Glu Val His  
20 25 30

Gly Leu Lys Arg Arg Ser Ser Leu Phe Asn Thr Asp Arg Ile Asp His  
35 40 45

Leu Tyr Gln Asp Pro His Ile Glu Asn Arg Asn Phe Ile Leu His Tyr  
50 55 60

Gly Asp Met Thr Asp Ser Thr Asn Leu Ile Arg Leu Ile Gln Glu Ile  
65 70 75 80

Gln Pro Asp Glu Ile Tyr Asn Leu Ala Ala Met Ser His Val His Val  
85 90 95

Ser Phe Glu Val Pro Glu Tyr Thr Ala Asn Ala Asp Gly Ile Gly Thr  
100 105 110

Leu Arg Ile Leu Asp Ala Val Arg Met Leu Gly Leu Ser Asn Lys Thr  
115 120 125

Arg Ile Tyr Gln Ala Ser Thr Ser Glu Leu Tyr Gly Lys Val Gln Glu  
130 135 140

Val Pro Gln Ser Glu Thr Thr Pro Phe Tyr Pro Arg Ser Pro Tyr Ala  
145 150 155 160

Val Ala Lys Met Tyr Ala Tyr Trp Ile Thr Val Asn Tyr Arg Glu Ala  
165 170 175

Tyr Asn Met Tyr Ala Cys Asn Gly Ile Leu Phe Asn His Glu Ser Pro  
180 185 190

Ile Arg Gly Glu Thr Phe Val Thr Arg Lys Ile Thr Arg Ala Thr Ala  
195 200 205

Lys Ile Ala Leu Gly Leu Gln Asp Lys Phe Tyr Leu Gly Asn Leu Asp  
210 215 220

Ala Glu Arg Asp Trp Gly His Ala Lys Asp Tyr Val Arg Met Met Trp  
225 230 235 240

Met Ile Leu Gln Ala Asp Glu Pro Glu Asp Trp Val Ile Ala Thr Gly  
245 250 255

Lys Thr Thr Lys Val Arg Glu Phe Val Lys Met Ser Phe Ala Glu Ala  
260 265 270

Gly Ile Glu Leu Glu Phe Lys Gly Ser Gly Val Glu Glu Lys Ala Tyr  
275 280 285

Val Val Lys Cys Ser Asn Pro Asp Tyr Gln Leu Glu Ile Gly Lys Glu  
290 295 300

Val Leu Ser Val Asp Pro Lys Tyr Phe Arg Pro Thr Glu Val Glu Leu  
305 310 315 320

Leu Ile Gly Asp Pro Thr Lys Ala Lys Thr Lys Leu Gly Trp Thr Val  
325 330 335

Glu Tyr Glu Leu Ala Asp Leu Val Lys Asp Met Val Gln Ser Asp Leu  
340 345 350

Lys Leu Met Gln Lys Glu Gln Tyr Leu Lys Asp Gly Gly Tyr Arg Ile  
355 360 365

Leu Asn Tyr Phe Glu  
370

<210> 142

<211> 575

<212> PRT

&lt;213&gt; Bos taurus

&lt;400&gt; 142

Met Arg Pro Trp Thr Gly Ser Trp Arg Trp Ile Met Leu Ile Leu Phe  
 1 5 10 15

Ala Trp Gly Thr Leu Leu Phe Tyr Ile Gly Gly His Leu Val Arg Asp  
 20 25 30

Asn Asp His Pro Asp His Ser Ser Arg Glu Leu Ser Lys Ile Leu Ala  
 35 40 45

Lys Leu Glu Arg Leu Lys Gln Gln Asn Glu Asp Leu Arg Arg Met Ala  
 50 55 60

Glu Ser Leu Arg Ile Pro Glu Gly Pro Ile Asp Gln Gly Pro Ala Ser  
 65 70 75 80

Gly Arg Ile Arg Ala Leu Glu Glu Gln Leu Val Lys Ala Lys Glu Gln  
 85 90 95

Ile Glu Asn Tyr Lys Lys Gln Thr Arg Asn Gly Leu Gly Lys Asp His  
 100 105 110

Glu Ile Leu Arg Arg Arg Ile Glu Asn Gly Ala Lys Glu Leu Trp Phe  
 115 120 125

Phe Leu Gln Ser Glu Leu Lys Lys Leu Lys Asn Leu Glu Gly Asn Glu  
 130 135 140

Leu Gln Arg His Ala Asp Glu Phe Leu Ser Asp Leu Gly His His Glu  
 145 150 155 160

Arg Ser Ile Met Thr Asp Leu Tyr Tyr Leu Ser Gln Thr Asp Gly Ala  
 165 170 175

Gly Asp Trp Arg Glu Lys Glu Ala Lys Asp Leu Thr Glu Leu Val Gln  
 180 185 190

Arg Arg Ile Thr Tyr Leu Gln Asn Pro Lys Asp Cys Ser Lys Ala Lys  
 195 200 205

Lys Leu Val Cys Asn Ile Asn Lys Gly Cys Gly Tyr Gly Cys Gln Leu  
 210 215 220

His His Val Val Tyr Cys Phe Met Ile Ala Tyr Gly Thr Gln Arg Thr  
 225 230 235 240

Leu Ile Leu Glu Ser His Asn Trp Arg Tyr Ala Thr Gly Gly Trp Glu  
 245 250 255

Thr Val Phe Arg Pro Val Ser Glu Thr Cys Thr Asp Arg Ser Gly Val  
 260 265 270

Ser Thr Gly His Trp Ser Gly Glu Ile Lys Asp Lys Asn Val Gln Val  
 275 280 285

Val Glu Leu Pro Ile Val Asp Ser Leu His Pro Arg Pro Pro Tyr Leu  
 290 295 300

Pro Leu Ala Val Pro Glu Asp Leu Ala Asp Arg Leu Val Arg Val His  
 305 310 315 320

Gly Asp Pro Ala Val Trp Trp Val Ser Gln Phe Val Lys Tyr Leu Ile  
 325 330 335



Arg Pro Gln Pro Trp Leu Glu Lys Glu Ile Glu Glu Ala Thr Lys Lys  
340 345 350

Leu Gly Phe Lys His Pro Val Ile Gly Val His Val Arg Arg Thr Asp  
355 360 365

Lys Val Gly Thr Glu Ala Ala Phe His Pro Ile Glu Glu Tyr Met Val  
370 375 380

His Val Glu Glu His Phe Gln Leu Leu Ala Arg Arg Met Gln Val Asp  
385 390 395 400

Lys Lys Arg Val Tyr Leu Ala Thr Asp Asp Pro Ser Leu Leu Lys Glu  
405 410 415

Ala Lys Thr Lys Tyr Pro His Tyr Glu Phe Ile Ser Asp Asn Ser Ile  
420 425 430

Ser Trp Ser Ala Gly Leu His Asn Arg Tyr Thr Glu Asn Ser Leu Arg  
435 440 445

Gly Val Ile Leu Asp Ile His Phe Leu Ser Gln Ala Asp Phe Leu Val  
450 455 460

Cys Thr Phe Ser Ser Gln Val Cys Arg Val Ala Tyr Glu Ile Met Gln  
465 470 475 480

Thr Leu His Pro Asp Ala Ser Ala Asn Phe His Ser Leu Asp Asp Ile  
485 490 495

Tyr Tyr Phe Gly Gly Gln Asn Ala His Asn Gln Ile Ala Ile Tyr Pro  
500 505 510

His Glu Pro Arg Thr Ala Asp Glu Ile Pro Met Glu Pro Gly Asp Ile  
515 520 525

Ile Gly Val Ala Gly Asn His Trp Asp Gly Tyr Ser Lys Gly Val Asn  
530 535 540

Arg Lys Leu Gly Arg Thr Gly Leu Tyr Pro Ser Tyr Lys Val Arg Glu  
545 550 555 560

Lys Ile Glu Thr Val Lys Tyr Pro Thr Tyr Pro Glu Ala Glu Lys  
565 570 575

<210> 143

<211> 575

<212> PRT

<213> Canis lupus familiaris

<400> 143

Met Arg Pro Trp Thr Gly Ser Trp Arg Trp Ile Met Leu Ile Leu Phe  
1 5 10 15

Ala Trp Gly Thr Leu Leu Phe Tyr Ile Gly Gly His Leu Val Arg Asp  
20 25 30

Asn Asp His Pro Asp His Ser Ser Arg Glu Leu Ser Lys Ile Leu Ala  
35 40 45

Lys Leu Glu Arg Leu Lys Gln Gln Asn Glu Asp Leu Arg Arg Met Ala  
50 55 60

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Ser | Leu | Arg | Ile | Pro | Glu | Gly | Pro | Ile | Asp | Gln | Ala | Pro | Ala | Ser |
| 65  |     |     |     |     | 70  |     |     |     | 75  |     |     |     | 80  |     |     |
| Gly | Arg | Val | Arg | Ala | Leu | Glu | Glu | Gln | Leu | Leu | Lys | Ala | Lys | Glu | Gln |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |
| Ile | Glu | Asn | Tyr | Lys | Lys | Gln | Thr | Arg | Asn | Gly | Leu | Gly | Lys | Asp | His |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Glu | Ile | Leu | Arg | Arg | Arg | Ile | Glu | Asn | Gly | Ala | Lys | Glu | Leu | Trp | Phe |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| Phe | Leu | Gln | Ser | Glu | Leu | Lys | Lys | Leu | Lys | Asn | Leu | Glu | Gly | Asn | Val |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Leu | Gln | Arg | His | Ala | Asp | Glu | Phe | Leu | Ser | Asp | Leu | Gly | His | His | Glu |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |
| Arg | Ser | Ile | Met | Thr | Asp | Leu | Tyr | Tyr | Leu | Ser | Gln | Thr | Asp | Gly | Ala |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |
| Gly | Asp | Trp | Arg | Glu | Lys | Glu | Ala | Lys | Asp | Leu | Thr | Glu | Leu | Val | Gln |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| Arg | Arg | Ile | Thr | Tyr | Leu | Gln | Asn | Pro | Lys | Asp | Cys | Ser | Lys | Ala | Lys |
|     |     |     | 195 |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Lys | Leu | Val | Cys | Asn | Ile | Asn | Lys | Gly | Cys | Gly | Tyr | Gly | Cys | Gln | Leu |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| His | His | Val | Val | Tyr | Cys | Phe | Met | Ile | Ala | Tyr | Gly | Thr | Gln | Arg | Thr |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Leu | Ile | Leu | Glu | Ser | Gln | Asn | Trp | Arg | Tyr | Ala | Thr | Gly | Gly | Trp | Glu |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Thr | Val | Phe | Arg | Pro | Val | Asn | Glu | Thr | Cys | Thr | Asp | Arg | Ser | Gly | Thr |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
| Ser | Thr | Gly | His | Trp | Ser | Gly | Glu | Val | Lys | Asp | Lys | Asn | Val | Gln | Val |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| Val | Glu | Leu | Pro | Ile | Val | Asp | Ser | Leu | His | Pro | Arg | Pro | Pro | Tyr | Leu |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
| Pro | Leu | Ala | Val | Pro | Glu | Asp | Leu | Ala | Asp | Arg | Leu | Val | Arg | Val | His |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
| Gly | Asp | Pro | Ala | Val | Trp | Trp | Val | Ser | Gln | Phe | Val | Lys | Tyr | Leu | Ile |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |
| Arg | Pro | Gln | Pro | Trp | Leu | Glu | Lys | Glu | Ile | Glu | Glu | Ala | Thr | Lys | Lys |
|     |     |     |     | 340 |     |     |     | 345 |     |     |     |     | 350 |     |     |
| Leu | Gly | Phe | Asn | Ile | Pro | Val | Ile | Gly | Val | His | Val | Arg | Arg | Thr | Asp |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |
| Lys | Val | Gly | Thr | Glu | Ala | Ala | Phe | His | Pro | Ile | Glu | Glu | Tyr | Met | Val |
|     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |
| His | Val | Glu | Glu | His | Phe | Gln | Leu | Leu | Ala | Arg | Arg | Met | Gln | Val | Asp |
| 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |
| Lys | Arg | Arg | Val | Tyr | Leu | Al  |     |     |     |     |     |     |     |     |     |

400 410 420  
 Ala Lys Thr Lys Tyr Pro Thr Tyr Glu Phe Ile Ser Asp Asn Ser Ile  
 420 425 430  
 Ser Trp Ser Ala Gly Leu His Asn Arg Tyr Thr Glu Asn Ser Leu Arg  
 435 440 445  
 Gly Val Ile Leu Asp Ile His Phe Leu Ser Gln Ala Asp Phe Leu Val  
 450 455 460  
 Cys Thr Phe Ser Ser Gln Val Cys Arg Val Ala Tyr Glu Ile Met Gln  
 465 470 475 480  
 Thr Leu His Pro Asp Ala Ser Ala Asn Phe His Ser Leu Asp Asp Ile  
 485 490 495  
 Tyr Tyr Phe Gly Gly Gln Asn Ala His Asn Gln Ile Ala Ile Tyr Pro  
 500 505 510  
 His Gln Pro Arg Thr Ala Asp Glu Ile Pro Met Glu Pro Gly Asp Ile  
 515 520 525  
 Ile Gly Val Ala Gly Asn His Trp Asp Gly Tyr Ser Lys Gly Val Asn  
 530 535 540  
 Arg Lys Leu Gly Arg Thr Gly Leu Tyr Pro Ser Tyr Lys Val Arg Glu  
 545 550 555 560  
 Lys Ile Glu Thr Val Lys Tyr Pro Thr Tyr Pro Glu Ala Glu Lys  
 565 570 575  
 <210> 144  
 <211> 599  
 <212> PRT  
 <213> Mus musculus  
 <400> 144  
 Leu Glu Pro Glu Tyr Asn Val Ser Ser Ser Leu Ser Ser Arg Lys Ile  
 1 5 10 15  
 Glu Asp Arg Val Glu Thr Leu Lys Met Arg Ala Trp Thr Gly Ser Trp  
 20 25 30  
 Arg Trp Ile Met Leu Ile Leu Phe Ala Trp Gly Thr Leu Leu Phe Tyr  
 35 40 45  
 Ile Gly Gly His Leu Val Arg Asp Asn Asp His Pro Asp His Ser Ser  
 50 55 60  
 Arg Glu Leu Ser Lys Ile Leu Ala Lys Leu Glu Arg Leu Lys Gln Gln  
 65 70 75 80  
 Asn Glu Asp Leu Arg Arg Met Ala Glu Ser Leu Arg Ile Pro Glu Gly  
 85 90 95  
 Pro Ile Asp Gln Gly Thr Ala Thr Gly Arg Val Arg Val Leu Glu Glu  
 100 105 110  
 Gln Leu Val Lys Ala Lys Glu Gln Ile Glu Asn Tyr Lys Lys Gln Ala  
 115 120 125  
 Arg Asn Gly Leu Gly Lys Asp His Glu Ile Leu Arg Arg Arg Ile Glu  
 130 135 140  
 Ser Glu Ile Tyr Glu Tyr Thr Phe Phe Tyr Glu Ser Glu Tyr Tyr Tyr

Asn Gly Ala Lys Glu Leu Trp Phe Phe Leu Gln Ser Glu Leu Lys Lys  
 145 150 155 160  
 Leu Lys His Leu Glu Gly Asn Glu Leu Gln Arg His Ala Asp Glu Ile  
 165 170 175  
 Leu Leu Asp Leu Gly His His Glu Arg Ser Ile Met Thr Asp Leu Tyr  
 180 185 190  
 Tyr Leu Ser Gln Thr Asp Gly Ala Gly Asp Trp Arg Glu Lys Glu Ala  
 195 200 205  
 Lys Asp Leu Thr Glu Leu Val Gln Arg Arg Ile Thr Tyr Leu Gln Asn  
 210 215 220  
 Pro Lys Asp Cys Ser Lys Ala Arg Lys Leu Val Cys Asn Ile Asn Lys  
 225 230 235 240  
 Gly Cys Gly Tyr Gly Cys Gln Leu His His Val Val Tyr Cys Phe Met  
 245 250 255  
 Ile Ala Tyr Gly Thr Gln Arg Thr Leu Ile Leu Glu Ser Gln Asn Trp  
 260 265 270  
 Arg Tyr Ala Thr Gly Gly Trp Glu Thr Val Phe Arg Pro Val Ser Glu  
 275 280 285  
 Thr Cys Thr Asp Arg Ser Gly Leu Ser Thr Gly His Trp Ser Gly Glu  
 290 295 300  
 Val Asn Asp Lys Asn Ile Gln Val Val Glu Leu Pro Ile Val Asp Ser  
 305 310 315 320  
 Leu His Pro Arg Pro Pro Tyr Leu Pro Leu Ala Val Pro Glu Asp Leu  
 325 330 335  
 Ala Asp Arg Leu Leu Arg Val His Gly Asp Pro Ala Val Trp Trp Val  
 340 345 350  
 Ser Gln Phe Val Lys Tyr Leu Ile Arg Pro Gln Pro Trp Leu Glu Lys  
 355 360 365  
 Glu Ile Glu Glu Ala Thr Lys Lys Leu Gly Phe Lys His Pro Val Ile  
 370 375 380  
 Gly Val His Val Arg Arg Thr Asp Lys Val Gly Thr Glu Ala Ala Phe  
 385 390 395 400  
 His Pro Ile Glu Glu Tyr Met Val His Val Glu Glu His Phe Gln Leu  
 405 410 415  
 Leu Ala Arg Arg Met Gln Val Asp Lys Lys Arg Val Tyr Leu Ala Thr  
 420 425 430  
 Asp Asp Pro Thr Leu Leu Lys Glu Ala Lys Thr Lys Tyr Ser Asn Tyr  
 435 440 445  
 Glu Phe Ile Ser Asp Asn Ser Ile Ser Trp Ser Ala Gly Leu His Asn  
 450 455 460  
 Arg Tyr Thr Glu Asn Ser Leu Arg Gly Val Ile Leu Asp Ile His Phe  
 465 470 475 480  
 Leu Ser Gln Ala Asp Phe Leu Val Cys Thr Phe Ser Ser Gln Val Cys  
 485 490 495  
 Arg Val Ala Tyr Glu Ile Met Gln Thr Leu His Pro Asp Ala Ser Ala  
 500 505 510

300 305 310  
 Asn Phe His Ser Leu Asp Asp Ile Tyr Tyr Phe Gly Gly Gln Asn Ala  
 515 520 525  
 His Asn Gln Ile Ala Val Tyr Pro His Lys Pro Arg Thr Glu Glu Glu  
 530 535 540  
 Ile Pro Met Glu Pro Gly Asp Ile Ile Gly Val Ala Gly Asn His Trp  
 545 550 555 560  
 Asp Gly Tyr Ser Lys Gly Ile Asn Arg Lys Leu Gly Lys Thr Gly Leu  
 565 570 575  
 Tyr Pro Ser Tyr Lys Val Arg Glu Lys Ile Glu Thr Val Lys Tyr Pro  
 580 585 590  
 Thr Tyr Pro Glu Ala Glu Lys  
 595  
 <210> 145  
 <211> 575  
 <212> PRT  
 <213> Rattus norvegicus  
 <400> 145  
 Met Arg Ala Trp Thr Gly Ser Trp Arg Trp Ile Met Leu Ile Leu Phe  
 1 5 10 15  
 Ala Trp Gly Thr Leu Leu Phe Tyr Ile Gly Gly His Leu Val Arg Asp  
 20 25 30  
 Asn Asp His Pro Asp His Ser Ser Arg Glu Leu Ser Lys Ile Leu Ala  
 35 40 45  
 Lys Leu Glu Arg Leu Lys Gln Gln Asn Glu Asp Leu Arg Arg Met Ala  
 50 55 60  
 Glu Ser Leu Arg Ile Pro Glu Gly Pro Ile Asp Gln Gly Thr Ala Thr  
 65 70 75 80  
 Gly Arg Val Arg Val Leu Glu Glu Gln Leu Val Lys Ala Lys Glu Gln  
 85 90 95  
 Ile Glu Asn Tyr Lys Lys Gln Ala Arg Asn Gly Leu Gly Lys Asp His  
 100 105 110  
 Glu Ile Leu Arg Arg Arg Ile Glu Asn Gly Ala Lys Glu Leu Trp Phe  
 115 120 125  
 Phe Leu Gln Ser Glu Leu Lys Lys Leu Lys His Leu Glu Gly Asn Glu  
 130 135 140  
 Leu Gln Arg His Ala Asp Glu Ile Leu Leu Asp Leu Gly His His Glu  
 145 150 155 160  
 Arg Ser Ile Met Thr Asp Leu Tyr Tyr Leu Ser Gln Thr Asp Gly Ala  
 165 170 175  
 Gly Asp Trp Arg Glu Lys Glu Ala Lys Asp Leu Thr Glu Leu Val Gln  
 180 185 190  
 Arg Arg Ile Thr Tyr Leu Gln Asn Pro Lys Asp Cys Ser Lys Ala Arg  
 195 200 205

Lys Leu Val Cys Asn Ile Asn Lys Gly Cys Gly Tyr Gly Cys Gln Leu  
 210 215 220  
 His His Val Val Tyr Cys Phe Met Ile Ala Tyr Gly Thr Gln Arg Thr  
 225 230 235 240  
 Leu Ile Leu Glu Ser Gln Asn Trp Arg Tyr Ala Thr Gly Gly Trp Glu  
 245 250 255  
 Thr Val Phe Arg Pro Val Ser Glu Thr Cys Thr Asp Arg Ser Gly Leu  
 260 265 270  
 Ser Thr Gly His Trp Ser Gly Glu Val Asn Asp Lys Asn Ile Gln Val  
 275 280 285  
 Val Glu Leu Pro Ile Val Asp Ser Leu His Pro Arg Pro Pro Tyr Leu  
 290 295 300  
 Pro Leu Ala Val Pro Glu Asp Leu Ala Asp Arg Leu Val Arg Val His  
 305 310 315 320  
 Gly Asp Pro Ala Val Trp Trp Val Ser Gln Phe Val Lys Tyr Leu Ile  
 325 330 335  
 Arg Pro Gln Pro Trp Leu Glu Lys Glu Ile Glu Glu Ala Thr Lys Lys  
 340 345 350  
 Leu Gly Phe Lys His Pro Val Ile Gly Val His Val Arg Arg Thr Asp  
 355 360 365  
 Lys Val Gly Thr Glu Ala Ala Phe His Pro Ile Glu Glu Tyr Met Val  
 370 375 380  
 His Val Glu Glu His Phe Gln Leu Leu Ala Arg Arg Met Gln Val Asp  
 385 390 395 400  
 Lys Lys Arg Val Tyr Leu Ala Thr Asp Asp Pro Ala Leu Leu Lys Glu  
 405 410 415  
 Ala Lys Thr Lys Tyr Ser Asn Tyr Glu Phe Ile Ser Asp Asn Ser Ile  
 420 425 430  
 Ser Trp Ser Ala Gly Leu His Asn Arg Tyr Thr Glu Asn Ser Leu Arg  
 435 440 445  
 Gly Val Ile Leu Asp Ile His Phe Leu Ser Gln Ala Asp Phe Leu Val  
 450 455 460  
 Cys Thr Phe Ser Ser Gln Val Cys Arg Val Ala Tyr Glu Ile Met Gln  
 465 470 475 480  
 Thr Leu His Pro Asp Ala Ser Ala Asn Phe His Ser Leu Asp Asp Ile  
 485 490 495  
 Tyr Tyr Phe Gly Gly Gln Asn Ala His Asn Gln Ile Ala Val Tyr Pro  
 500 505 510  
 His Lys Pro Arg Thr Asp Glu Glu Ile Pro Met Glu Pro Gly Asp Ile  
 515 520 525  
 Ile Gly Val Ala Gly Asn His Trp Asp Gly Tyr Ser Lys Gly Val Asn  
 530 535 540  
 Arg Lys Leu Gly Lys Thr Gly Leu Tyr Pro Ser Tyr Lys Val Arg Glu

545 550 555 560

Lys Ile Glu Thr Val Lys Tyr Pro Thr Tyr Pro Glu Ala Glu Lys  
565 570 575

<210> 146

<211> 575

<212> PRT

<213> Cricetulus griseus

<400> 146

Met Arg Ala Trp Thr Gly Ser Trp Arg Trp Ile Met Leu Ile Leu Phe  
1 5 10 15

Ala Trp Gly Thr Leu Leu Phe Tyr Ile Gly Gly His Leu Val Arg Asp  
20 25 30

Asn Asp His Pro Asp His Ser Ser Arg Glu Leu Ser Lys Ile Leu Ala  
35 40 45

Lys Leu Glu Arg Leu Lys Gln Gln Asn Glu Asp Leu Arg Arg Met Ala  
50 55 60

Glu Ser Leu Arg Ile Pro Glu Gly Pro Ile Asp Gln Gly Thr Ala Thr  
65 70 75 80

Gly Arg Val Arg Val Leu Glu Glu Gln Leu Val Lys Ala Lys Glu Gln  
85 90 95

Ile Glu Asn Tyr Lys Lys Gln Ala Arg Asn Asp Leu Gly Lys Asp His  
100 105 110

Glu Ile Leu Arg Arg Arg Ile Glu Asn Gly Ala Lys Glu Leu Trp Phe  
115 120 125

Phe Leu Gln Ser Glu Leu Lys Lys Leu Lys Lys Leu Glu Gly Asn Glu  
130 135 140

Leu Gln Arg His Ala Asp Glu Ile Leu Leu Asp Leu Gly His His Glu  
145 150 155 160

Arg Ser Ile Met Thr Asp Leu Tyr Tyr Leu Ser Gln Thr Asp Gly Ala  
165 170 175

Gly Glu Trp Arg Glu Lys Glu Ala Lys Asp Leu Thr Glu Leu Val Gln  
180 185 190

Arg Arg Ile Thr Tyr Leu Gln Asn Pro Lys Asp Cys Ser Lys Ala Arg  
195 200 205

Lys Leu Val Cys Asn Ile Asn Lys Gly Cys Gly Tyr Gly Cys Gln Leu  
210 215 220

His His Val Val Tyr Cys Phe Met Ile Ala Tyr Gly Thr Gln Arg Thr  
225 230 235 240

Leu Ile Leu Glu Ser Gln Asn Trp Arg Tyr Ala Thr Gly Gly Trp Glu  
245 250 255

Thr Val Phe Arg Pro Val Ser Glu Thr Cys Thr Asp Arg Ser Gly Leu  
260 265 270

Ser Thr Gly His Trp Ser Gly Glu Val Lys Asp Lys Asn Val Gln Val  
275 280 285

Val Glu Leu Pro Ile Val Asp Ser Leu His Pro Arg Pro Pro Tyr Leu  
 290 295 300  
 Pro Leu Ala Val Pro Glu Asp Leu Ala Asp Arg Leu Leu Arg Val His  
 305 310 315 320  
 Gly Asp Pro Ala Val Trp Trp Val Ser Gln Phe Val Lys Tyr Leu Ile  
 325 330 335  
 Arg Pro Gln Pro Trp Leu Glu Arg Glu Ile Glu Glu Thr Thr Lys Lys  
 340 345 350  
 Leu Gly Phe Lys His Pro Val Ile Gly Val His Val Arg Arg Thr Asp  
 355 360 365  
 Lys Val Gly Thr Glu Ala Ala Phe His Pro Ile Glu Glu Tyr Met Val  
 370 375 380  
 His Val Glu Glu His Phe Gln Leu Leu Glu Arg Arg Met Lys Val Asp  
 385 390 395 400  
 Lys Lys Arg Val Tyr Leu Ala Thr Asp Asp Pro Ser Leu Leu Lys Glu  
 405 410 415  
 Ala Lys Thr Lys Tyr Ser Asn Tyr Glu Phe Ile Ser Asp Asn Ser Ile  
 420 425 430  
 Ser Trp Ser Ala Gly Leu His Asn Arg Tyr Thr Glu Asn Ser Leu Arg  
 435 440 445  
 Gly Val Ile Leu Asp Ile His Phe Leu Ser Gln Ala Asp Phe Leu Val  
 450 455 460  
 Cys Thr Phe Ser Ser Gln Val Cys Arg Val Ala Tyr Glu Ile Met Gln  
 465 470 475 480  
 Thr Leu His Pro Asp Ala Ser Ala Asn Phe His Ser Leu Asp Asp Ile  
 485 490 495  
 Tyr Tyr Phe Gly Gly Gln Asn Ala His Asn Gln Ile Ala Val Tyr Pro  
 500 505 510  
 His Gln Pro Arg Thr Lys Glu Glu Ile Pro Met Glu Pro Gly Asp Ile  
 515 520 525  
 Ile Gly Val Ala Gly Asn His Trp Asn Gly Tyr Ser Lys Gly Val Asn  
 530 535 540  
 Arg Lys Leu Gly Lys Thr Gly Leu Tyr Pro Ser Tyr Lys Val Arg Glu  
 545 550 555 560  
 Lys Ile Glu Thr Val Lys Tyr Pro Thr Tyr Pro Glu Ala Glu Lys  
 565 570 575  
 <210> 147  
 <211> 575  
 <212> PRT  
 <213> Gallus gallus  
 <400> 147  
 Met Arg Pro Trp Thr Gly Ser Trp Arg Trp Ile Met Leu Ile Leu Phe  
 1 5 10 15  
 Ala Trp Gly Thr Leu Leu Phe Tyr Ile Gly Gly His Leu Val Arg Asp  
 20 25 30



Ser Glu His Pro Asp His Ser Ser Arg Glu Leu Ser Lys Ile Leu Ala  
 35 40 45  
 Lys Leu Glu Arg Leu Lys Gln Gln Asn Glu Asp Leu Arg Arg Met Ala  
 50 55 60  
 Glu Ser Leu Arg Ile Pro Asp Gly Pro Ile Asp Gln Gly Pro Ala Ala  
 65 70 75 80  
 Gly Lys Val His Ala Leu Glu Glu Gln Leu Leu Lys Ala Lys Glu Gln  
 85 90 95  
 Ile Glu Asn Tyr Lys Lys Gln Thr Gly Asp Gly Leu Gly Lys Asp His  
 100 105 110  
 Glu Ile Leu Arg Arg Arg Ile Glu Asn Gly Ala Lys Glu Leu Trp Phe  
 115 120 125  
 Phe Leu Gln Ser Glu Leu Lys Lys Leu Lys Asn Leu Glu Gly Ser Glu  
 130 135 140  
 Leu Gln Arg Arg Ile Asp Glu Phe Leu Ser Asp Leu Gly His Gln Glu  
 145 150 155 160  
 Arg Ser Ile Met Thr Asp Leu Tyr Tyr Leu Ser Gln Thr Asp Gly Ala  
 165 170 175  
 Gly Asp Trp Arg Glu Lys Glu Ala Lys Asp Leu Thr Asp Leu Val Gln  
 180 185 190  
 Arg Arg Ile Thr Tyr Leu Gln Asn Pro Lys Asp Cys Ser Lys Ala Lys  
 195 200 205  
 Lys Leu Val Cys Asn Ile Asn Lys Gly Cys Gly Tyr Gly Cys Gln Leu  
 210 215 220  
 His His Val Val Tyr Cys Phe Met Ile Ala Tyr Gly Thr Gln Arg Thr  
 225 230 235 240  
 Leu Ile Leu Glu Ser Gln Asn Trp Arg Tyr Ala Thr Gly Gly Trp Glu  
 245 250 255  
 Thr Val Phe Arg Pro Val Ser Glu Thr Cys Thr Asp Arg Ser Gly Thr  
 260 265 270  
 Thr Thr Gly His Trp Ser Gly Glu Thr Asn Asp Lys Asp Val Gln Val  
 275 280 285  
 Val Glu Leu Pro Ile Val Asp Ser Leu His Pro Arg Pro Pro Tyr Leu  
 290 295 300  
 Pro Leu Ala Val Pro Glu Asp Leu Ala Asp Arg Leu Ile Arg Val His  
 305 310 315 320  
 Gly Asp Pro Ala Val Trp Trp Val Ser Gln Phe Val Lys Tyr Leu Ile  
 325 330 335  
 Arg Pro Gln Pro Trp Leu Glu Lys Glu Ile Glu Glu Ala Thr Arg Lys  
 340 345 350  
 Leu Gly Phe Lys His Pro Val Ile Gly Val His Val Arg Arg Thr Asp  
 355 360 365  
 Lys Val Gly Thr Glu Ala Ala Phe His Pro Ile Glu Glu Tyr Met Val  
 370 375 380

His Val Glu Glu Arg Phe Glu Leu Leu Ala Arg Arg Met His Val Asp  
385 390 395 400

Lys Lys Arg Val Tyr Leu Ala Thr Asp Asp Pro Ser Leu Leu Gln Glu  
405 410 415

Ala Lys Ser Lys Tyr Pro Asn Tyr Glu Phe Ile Ser Asp Asn Ser Ile  
420 425 430

Ser Trp Ser Ala Gly Leu His Asn Arg Tyr Thr Glu Asn Ser Leu Arg  
435 440 445

Gly Val Ile Leu Asp Ile His Phe Leu Ser Gln Ala Asp Phe Leu Val  
450 455 460

Cys Thr Phe Ser Ser Gln Val Cys Arg Val Pro Tyr Glu Ile Met Gln  
465 470 475 480

Thr Leu His Pro Asp Ala Ser Ala Tyr Phe His Ser Leu Asp Asp Ile  
485 490 495

Tyr Tyr Phe Gly Gly Gln Asn Ala His Asn Gln Ile Ala Val Tyr Ala  
500 505 510

His His Pro Arg Thr Ala Asp Glu Ile Pro Met Glu Pro Gly Asp Ile  
515 520 525

Ile Gly Val Ala Gly Asn His Trp Asp Gly Tyr Ser Lys Gly Ile Asn  
530 535 540

Arg Lys Leu Gly Lys Thr Gly Leu Tyr Pro Ser Tyr Lys Val Lys Glu  
545 550 555 560

Lys Ile Glu Thr Val Lys Tyr Pro Thr Tyr Pro Glu Ala Glu Lys  
565 570 575

<210> 148

<211> 578

<212> PRT

<213> *Xenopus (Silurana) tropicalis*

<400> 148

Met Arg Pro Trp Thr Gly Ser Trp Arg Trp Ile Met Leu Ile Leu Phe  
1 5 10 15

Ala Trp Gly Thr Leu Leu Phe Tyr Ile Gly Gly His Leu Val Arg Asp  
20 25 30

Asn Glu Asn Pro Asp His Ser Ser Arg Glu Leu Ser Lys Ile Leu Ala  
35 40 45

Lys Leu Glu Arg Leu Lys Gln Gln Asn Glu Asp Leu Arg Arg Met Ala  
50 55 60

Glu Ser Leu Arg Ile Pro Glu Gly Pro Ile Glu Gln Gly Ala Ala Ala  
65 70 75 80

Gly Arg Ile Arg Ala Leu Glu Glu Gln Leu Leu Lys Ala Lys Glu Gln  
85 90 95

Ile Glu Met Tyr Lys Gln Gln Ser Ser Asn Ala Val Ser Gly Leu Gly  
100 105 110

Lys Asp His Glu Ile Leu Arg Arg Ala Ile Glu Asn Gly Ala Lys Glu  
115 120 125

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110          120          130
Phe Trp Tyr Phe Val Gln Ser Glu Val Lys Lys Leu Lys His Leu Asp
130          135          140

Arg Asn Glu Leu Gln Arg His Val Asp Glu Ile Ile Ile Asp Met Gly
145          150          155          160

His Gln Gln Arg Ser Val Met Thr Asp Leu Tyr Tyr Leu Ser Gln Thr
165          170          175

Asp Gly Ala Gly Asp Trp Arg Glu Arg Glu Ala Lys Asp Leu Thr Asp
180          185          190

Leu Val Gln Arg Arg Ile Thr Tyr Leu Gln Asn Pro Lys Asp Cys Ser
195          200          205

Lys Ala Lys Lys Leu Val Cys Asn Ile Asn Lys Gly Cys Gly Tyr Gly
210          215          220

Cys Gln Leu His His Val Val Tyr Cys Phe Met Ile Ala Tyr Gly Thr
225          230          235          240

Gln Arg Thr Leu Ile Leu Glu Ser Gln Ser Trp Arg Tyr Ala Thr Gly
245          250          255

Gly Trp Glu Thr Val Phe Lys Pro Val Ser Glu Thr Cys Thr Asp Arg
260          265          270

Ser Gly Ser Ser Thr Gly His Trp Ser Gly Glu Ala Asn Asp Lys Asn
275          280          285

Val Gln Val Val Glu Leu Pro Ile Val Asp Ser Leu His Pro Arg Pro
290          295          300

Pro Tyr Leu Pro Leu Gly Val Pro Glu Asp Leu Ala Asp Arg Leu Ile
305          310          315          320

Arg Leu His Gly Asp Pro Ala Val Trp Trp Val Ser Gln Phe Val Lys
325          330          335

Tyr Leu Ile Arg Pro Gln Pro Trp Leu Glu Lys Glu Ile Glu Glu Ser
340          345          350

Thr Lys Lys Leu Gly Phe Lys His Pro Val Ile Gly Val His Val Arg
355          360          365

Arg Thr Asp Lys Val Gly Thr Glu Ala Ala Phe His Pro Ile Glu Glu
370          375          380

Tyr Met Val His Val Glu Glu His Phe Gln Leu Leu Ala Arg Arg Met
385          390          395          400

Gln Ile Asp Lys Lys Arg Val Tyr Leu Ala Thr Asp Asp Pro Thr Leu
405          410          415

Leu Gln Glu Ala Lys Ala Lys Tyr Pro Gln Tyr Glu Phe Ile Ser Asp
420          425          430

Asn Ser Ile Ser Trp Ser Ala Gly Leu His Asn Arg Tyr Thr Glu Asn
435          440          445

Ser Leu Arg Gly Val Ile Leu Asp Ile His Phe Leu Ser Gln Ala Asp
450          455          460

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Phe Leu Val Cys Thr Phe Ser Ser Gln Val Cys Arg Val Ala Tyr Glu  
465 470 475 480

Ile Met Gln Thr Leu His Pro Asp Ala Ser Ala His Phe His Ser Leu  
485 490 495

Asp Asp Ile Tyr Tyr Phe Gly Gly Gln Asn Ala His Asn Gln Leu Ala  
500 505 510

Ile Tyr Pro His Gln Pro Arg Asn Ala Glu Glu Ile Pro Leu Glu Pro  
515 520 525

Gly Asp Ile Ile Gly Val Ala Gly Asn His Trp Asp Gly Tyr Ser Lys  
530 535 540

Gly Ile Asn Arg Lys Leu Gly Arg Thr Gly Leu Tyr Pro Ser Tyr Lys  
545 550 555 560

Val Lys Glu Lys Ile Glu Thr Val Lys Tyr Pro Thr Tyr Gln Glu Ala  
565 570 575

Glu Lys

<210> 149

<211> 580

<212> PRT

<213> Danio rerio

<400> 149

Met Arg Pro Trp Thr Gly Ser Trp Arg Trp Ile Ala Leu Val Leu Leu  
1 5 10 15

Ala Trp Gly Thr Leu Leu Phe Tyr Ile Gly Gly His Leu Val Lys Asp  
20 25 30

Ser Glu His Ala Pro Arg Ser Ser Arg Glu Leu Ala Lys Ile Leu Thr  
35 40 45

Lys Leu Glu Arg Leu Lys Gln Gln Asn Glu Asp Leu Arg Arg Met Ala  
50 55 60

Gln Ser Leu Arg Ile Pro Glu Gly Gln Ser Asp Gly Pro Ile Ser Ser  
65 70 75 80

Gly Arg Leu Arg Ser Leu Glu Glu Gln Leu Ser Arg Ala Lys Gln Lys  
85 90 95

Ile Gln Ser Phe Gln Arg Leu Ser Gly Glu Gly Pro Gly Lys Asp His  
100 105 110

Glu Glu Leu Arg Arg Lys Val Glu Asn Gly Val Arg Glu Leu Trp Tyr  
115 120 125

Phe Val Arg Ser Glu Val Lys Lys Leu Pro Leu Met Glu Thr Gly Ala  
130 135 140

Met His Lys His Val Asp Thr Leu Met Gln Asp Leu Gly His Gln Gln  
145 150 155 160

Arg Ser Val Met Thr Asp Leu Tyr His Leu Ser Gln Ala Asp Gly Ala  
165 170 175

Gly Asp Trp Arg Glu Lys Glu Ala Asn Glu Leu Ser Asp Leu Val Gln  
180 185 190

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|
| Asn | Arg | Ile | Met | Tyr | Leu | Gln | Asn | Pro | Gln | Asp | Cys | Ser | Lys | Ala | Arg |  |  |
|     |     |     | 195 |     |     |     |     | 200 |     |     |     |     |     | 205 |     |  |  |
| Lys | Leu | Val | Cys | Asn | Ile | Asn | Lys | Gly | Cys | Gly | Tyr | Gly | Cys | Gln | Leu |  |  |
|     |     | 210 |     |     |     | 215 |     |     |     |     |     | 220 |     |     |     |  |  |
| His | His | Val | Val | Tyr | Cys | Phe | Met | Ile | Ala | Tyr | Gly | Thr | Gln | Arg | Thr |  |  |
|     |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |  |
| Leu | Ile | Leu | Glu | Ser | Gln | Asn | Trp | Arg | Tyr | Ala | Thr | Gly | Gly | Trp | Glu |  |  |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |  |  |
| Thr | Val | Phe | Lys | Pro | Val | Ser | Asp | Thr | Cys | Thr | Asp | Arg | Thr | Gly | Ala |  |  |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |  |  |
| Ser | Thr | Gly | His | Trp | Ser | Gly | Glu | Ala | His | Asp | Arg | Asp | Val | Gln | Val |  |  |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |  |  |
| Val | Glu | Leu | Pro | Ile | Val | Asp | Ser | Leu | His | Pro | Arg | Pro | Pro | Tyr | Leu |  |  |
|     |     | 290 |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |  |  |
| Pro | Leu | Ala | Val | Pro | Glu | Asp | Leu | Ala | Pro | Arg | Leu | Gln | Arg | Leu | His |  |  |
|     |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |  |  |
| Gly | Asp | Pro | Ser | Val | Trp | Trp | Val | Ser | Gln | Phe | Val | Lys | Phe | Leu | Val |  |  |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |  |  |
| Arg | Pro | Gln | Ala | Trp | Leu | Glu | Lys | Glu | Ile | Gln | Glu | Thr | Cys | Leu | Lys |  |  |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |  |  |
| Leu | Gly | Phe | Lys | His | Pro | Ile | Ile | Gly | Val | His | Val | Arg | Arg | Thr | Asp |  |  |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |  |  |
| Lys | Val | Gly | Thr | Glu | Ala | Ala | Phe | His | Pro | Ile | Glu | Glu | Tyr | Met | Val |  |  |
|     |     | 370 |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |  |  |
| His | Val | Glu | Asp | His | Tyr | Gln | Ser | Leu | Ala | Gln | Arg | Met | His | Val | Asp |  |  |
|     |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |  |  |
| Lys | Lys | Arg | Val | Tyr | Leu | Ala | Thr | Asp | Asp | Pro | Ser | Leu | Leu | Gln | Glu |  |  |
|     |     |     |     | 405 |     |     |     |     | 410 |     |     |     |     | 415 |     |  |  |
| Ala | Lys | Thr | Lys | Tyr | Pro | Asp | Tyr | Glu | Phe | Ile | Ser | Asp | Asn | Ser | Ile |  |  |
|     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |  |  |
| Ser | Trp | Ser | Ala | Gly | Leu | His | Asn | Arg | Tyr | Thr | Glu | Asn | Ser | Leu | Arg |  |  |
|     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |  |  |
| Gly | Val | Ile | Leu | Asp | Ile | His | Phe | Leu | Ser | Arg | Thr | Asn | Tyr | Leu | Val |  |  |
|     |     | 450 |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |  |  |
| Cys | Thr | Phe | Ser | Ser | Gln | Val | Cys | Arg | Val | Ala | Tyr | Glu | Ile | Met | Gln |  |  |
|     |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     | 480 |  |  |
| Thr | Leu | His | Pro | Asp | Ala | Ser | Ser | Tyr | Phe | Tyr | Ser | Leu | Asp | Asp | Ile |  |  |
|     |     |     |     | 485 |     |     |     |     | 490 |     |     |     |     | 495 |     |  |  |
| Tyr | Tyr | Phe | Gly | Gly | Gln | Asn | Ala | His | Asn | Gln | Ile | Ala | Ile | Tyr | Pro |  |  |
|     |     |     | 500 |     |     |     |     | 505 |     |     |     |     | 510 |     |     |  |  |
| His | Gln | Pro | Arg | Asn | Ser | Asp | Asp | Ile | Pro | Leu | Glu | Pro | Gly |     |     |  |  |

Arg Lys Thr Gly Arg Thr Gly Leu Tyr Pro Ser Tyr Lys Val Lys Glu  
545 550 555 560

Lys Ile Glu Thr Val Lys Tyr Pro Thr Tyr Pro Glu Ala Asp Lys Leu  
565 570 575

Leu Lys Lys Pro  
580

<210> 150

<211> 385

<212> PRT

<213> Ascaris suum

<400> 150

Met His His Asp Ser Lys Ile Thr Pro Val Ala Thr Ile His Leu Leu  
1 5 10 15

Pro Lys Gly Leu Gly Arg Gly Leu Leu Leu Gln Gln Phe Ala Met Lys  
20 25 30

Asn Gly Arg Gln His Glu Thr Leu Ala Gln Lys Ser Ile Lys Ile Val  
35 40 45

Leu Ala Val Ser Ala Tyr Trp Ile Cys Ser Ile Gly Leu Val Phe Leu  
50 55 60

Asn Lys Tyr Leu Leu Ser Ser Glu Asn Leu Lys Leu Asn Ala Pro Leu  
65 70 75 80

Phe Ile Thr Trp Tyr Gln Cys Leu Val Thr Val Val Leu Cys Tyr Thr  
85 90 95

Cys Ser Tyr Leu Ser Arg Ile Phe Pro Ser Arg Phe Ser Phe Pro Ser  
100 105 110

Ile Ala Phe Asp His Arg Ile Ser Arg Glu Val Leu Pro Leu Ser Phe  
115 120 125

Val Phe Val Ala Met Ile Thr Thr Asn Asn Leu Cys Leu Lys Tyr Val  
130 135 140

Gly Val Ser Phe Tyr Tyr Val Gly Arg Ser Leu Thr Thr Val Phe Asn  
145 150 155 160

Val Val Cys Ser Tyr Leu Ile Leu Gly Gln Gly Thr Ser Trp Arg Ala  
165 170 175

Leu Leu Cys Cys Ala Val Ile Ile Gly Gly Phe Phe Leu Gly Val Asp  
180 185 190

Gln Glu Asp Ala Ala Gly Ser Leu Ser Val Leu Gly Val Val Tyr Gly  
195 200 205

Val Ala Ala Ser Leu Cys Val Ala Leu Asn Ala Ile Tyr Thr Gln Arg  
210 215 220

Thr Leu Pro Ala Val Gly Asp Ser Val Ala Arg Leu Thr Met Tyr Asn  
225 230 235 240

Asn Thr Asn Ala Val Val Leu Phe Ile Pro Leu Met Leu Phe Ser Gly  
245 250 255

Glu Phe Gly Glu Ile Ile Tyr Phe Pro Tyr Leu Leu Ser Thr His Phe  
260 265 270

Trp Ala Leu Met Thr Ile Ser Gly Val Phe Gly Phe Leu Met Gly Tyr  
275 280 285

Val Thr Gly Trp Gln Ile Gln Val Thr Ser Pro Leu Thr His Asn Ile  
290 295 300

Ser Gly Thr Ala Lys Ala Ala Ala Gln Thr Val Ile Ala Val Ala Trp  
305 310 315 320

Trp Gln Glu Val Lys Ser Val Leu Trp Trp Val Ser Asn Val Val Val  
325 330 335

Leu Gly Gly Ser Ala Ala Tyr Thr Ala Val Lys Arg Lys Glu Met Ile  
340 345 350

Ala Asn His Glu Ser Asn Ala Lys Pro Arg Gly Ser Val Ser Pro Asp  
355 360 365

Arg Glu Pro Ile Leu Ser Ser Lys Ser Ser Gly Asp Thr Ser Glu Asp  
370 375 380

Val  
385

<210> 151

<211> 331

<212> PRT

<213> Loa loa

<400> 151

Met Ser Lys Tyr Ser Leu Ser Ser Ser Tyr Thr Gly Lys Leu Leu Asn  
1 5 10 15

Ala Pro Leu Phe Val Thr Trp Tyr Gln Cys Phe Val Thr Val Leu Leu  
20 25 30

Cys Cys Val Phe Cys Trp Val Ser Lys Gln Tyr Pro Ser Leu Val Thr  
35 40 45

Phe Pro Phe Val Gly Phe Asp His Arg Ile Ser Arg Glu Val Leu Pro  
50 55 60

Leu Ser Phe Val Phe Val Ala Met Ile Ala Thr Asn Asn Leu Cys Leu  
65 70 75 80

Lys Tyr Val Gly Val Ser Phe Tyr Tyr Ile Gly Arg Ser Leu Thr Thr  
85 90 95

Val Phe Asn Val Ile Cys Ser Tyr Ile Ile Leu Gly Gln Leu Thr Ser  
100 105 110

Leu Lys Thr Ile Leu Cys Cys Ala Leu Ile Ile Gly Gly Phe Val Leu  
115 120 125

Gly Val Asp Gln Glu Asp Ala Thr Ala Gln Phe Phe Leu Pro Arg Thr  
130 135 140

Phe Leu Gly Thr Leu Ser Val Thr Gly Val Ile Phe Gly Val Ala Ala  
145 150 155 160

Ser Met Phe Val Ala Leu Asn Ala Ile Tyr Thr Gln Arg Thr Leu Pro  
165 170 175

Ser Val Gly Asp Ser Ile Thr Gln Leu Thr Leu Tyr Asn Asn Ile Asn  
180 185 190

180 185 190  
 Ala Leu Val Leu Phe Ile Pro Val Met Leu Phe Ser Gly Asp Ile Ser  
 195 200 205  
 Glu Val Phe Tyr Phe Arg Tyr Ser Ser Ser Leu Arg Phe Trp Thr Leu  
 210 215 220  
 Met Thr Leu Ser Gly Ile Phe Gly Phe Leu Met Ser Tyr Val Thr Gly  
 225 230 235 240  
 Trp Gln Ile Gln Val Thr Ser Ser Leu Thr His Asn Ile Ser Gly Thr  
 245 250 255  
 Ala Lys Ala Ala Ala Gln Thr Val Ile Ala Val Val Trp Trp Gln Glu  
 260 265 270  
 Met Lys Ser Leu Leu Trp Trp Ile Ser Asn Val Ile Val Leu Gly Gly  
 275 280 285  
 Ser Ala Ile Tyr Thr Met Ile Lys Arg Lys Glu Met Val Asp Lys Tyr  
 290 295 300  
 Asp Thr Asn Lys Ser Lys Leu Asn Glu Asn Ile Glu Arg Gln Ala Val  
 305 310 315 320  
 Leu Phe Gly Leu Ser Asp Glu Asp Glu Thr Val  
 325 330  
 <210> 152  
 <211> 363  
 <212> PRT  
 <213> Rattus norvegicus  
 <400> 152  
 Met Asn Arg Val Pro Leu Lys Arg Ser Arg Ile Leu Arg Met Ala Leu  
 1 5 10 15  
 Thr Gly Ala Ser Ala Val Ser Glu Glu Ala Asp Ser Glu Asn Lys Pro  
 20 25 30  
 Phe Leu Leu Arg Ala Leu Gln Ile Ala Leu Val Val Ser Leu Tyr Trp  
 35 40 45  
 Val Thr Ser Ile Ser Met Val Phe Leu Asn Lys Tyr Leu Leu Asp Ser  
 50 55 60  
 Pro Ser Leu Gln Leu Asp Thr Pro Ile Phe Val Thr Phe Tyr Gln Cys  
 65 70 75 80  
 Leu Val Thr Ser Leu Leu Cys Lys Gly Leu Ser Thr Leu Ala Thr Cys  
 85 90 95  
 Cys Pro Gly Met Val Asp Phe Pro Thr Leu Asn Leu Asp Leu Lys Val  
 100 105 110  
 Ala Arg Ser Val Leu Pro Leu Ser Val Val Phe Ile Gly Met Ile Thr  
 115 120 125  
 Phe Asn Asn Leu Cys Leu Lys Tyr Val Gly Val Ala Phe Tyr Asn Val  
 130 135 140  
 Gly Arg Ser Leu Thr Thr Val Phe Asn Val Leu Leu Ser Tyr Leu Leu  
 145 150 155 160



Leu Lys Gln Thr Thr Ser Phe Tyr Ala Leu Leu Thr Cys Ala Ile Ile  
165 170 175

Ile Gly Gly Phe Trp Leu Gly Ile Asp Gln Glu Gly Ala Glu Gly Thr  
180 185 190

Leu Ser Leu Thr Gly Thr Ile Phe Gly Val Leu Ala Ser Leu Cys Val  
195 200 205

Ser Leu Asn Ala Ile Tyr Thr Lys Lys Val Leu Pro Ala Val Asp His  
210 215 220

Ser Ile Trp Arg Leu Thr Phe Tyr Asn Asn Val Asn Ala Cys Val Leu  
225 230 235 240

Phe Leu Pro Leu Met Val Val Leu Gly Glu Leu His Ala Leu Leu Ala  
245 250 255

Phe Ala His Leu Asn Ser Ala His Phe Trp Val Met Met Thr Leu Gly  
260 265 270

Gly Leu Phe Gly Phe Ala Ile Gly Tyr Val Thr Gly Leu Gln Ile Lys  
275 280 285

Phe Thr Ser Pro Leu Thr His Asn Val Ser Gly Thr Ala Lys Ala Cys  
290 295 300

Ala Gln Thr Val Leu Ala Val Leu Tyr Tyr Glu Glu Ile Lys Ser Phe  
305 310 315 320

Leu Trp Trp Thr Ser Asn Leu Met Val Leu Gly Gly Ser Ser Ala Tyr  
325 330 335

Thr Trp Val Arg Gly Trp Glu Met Gln Lys Thr Gln Glu Asp Pro Ser  
340 345 350

Ser Lys Glu Gly Glu Lys Ser Ala Ile Gly Val  
355 360

<210> 153

<211> 365

<212> PRT

<213> Cricetulus griseus

<400> 153

Met Asn Arg Ala Pro Leu Lys Arg Ser Arg Ile Leu Arg Met Ala Leu  
1 5 10 15

Thr Gly Gly Ser Thr Ala Ser Glu Glu Ala Asp Glu Asp Ser Arg Asn  
20 25 30

Lys Pro Phe Leu Leu Arg Ala Leu Gln Ile Ala Leu Val Val Ser Leu  
35 40 45

Tyr Trp Val Thr Ser Ile Ser Met Val Phe Leu Asn Lys Tyr Leu Leu  
50 55 60

Asp Ser Pro Ser Leu Gln Leu Asp Thr Pro Ile Phe Val Thr Phe Tyr  
65 70 75 80

Gln Cys Leu Val Thr Ser Leu Leu Cys Lys Gly Leu Ser Thr Leu Ala  
85 90 95

-- -- -- -- -- -- -- -- -- -- -- -- -- -- -- --

Thr Cys Cys Pro Gly Thr Val Asp Phe Pro Thr Leu Asn Leu Asp Leu  
100 105 110

Lys Val Ala Arg Ser Val Leu Pro Leu Ser Val Val Phe Ile Gly Met  
115 120 125

Ile Ser Phe Asn Asn Leu Cys Leu Lys Tyr Val Gly Val Ala Phe Tyr  
130 135 140

Asn Val Gly Arg Ser Leu Thr Thr Val Phe Asn Val Leu Leu Ser Tyr  
145 150 155 160

Leu Leu Leu Lys Gln Thr Thr Ser Phe Tyr Ala Leu Leu Thr Cys Gly  
165 170 175

Ile Ile Ile Gly Gly Phe Trp Leu Gly Ile Asp Gln Glu Gly Ala Glu  
180 185 190

Gly Thr Leu Ser Leu Ile Gly Thr Ile Phe Gly Val Leu Ala Ser Leu  
195 200 205

Cys Val Ser Leu Asn Ala Ile Tyr Thr Lys Lys Val Leu Pro Ala Val  
210 215 220

Asp Asn Ser Ile Trp Arg Leu Thr Phe Tyr Asn Asn Val Asn Ala Cys  
225 230 235 240

Val Leu Phe Leu Pro Leu Met Val Leu Leu Gly Glu Leu Arg Ala Leu  
245 250 255

Leu Asp Phe Ala His Leu Tyr Ser Ala His Phe Trp Leu Met Met Thr  
260 265 270

Leu Gly Gly Leu Phe Gly Phe Ala Ile Gly Tyr Val Thr Gly Leu Gln  
275 280 285

Ile Lys Phe Thr Ser Pro Leu Thr His Asn Val Ser Gly Thr Ala Lys  
290 295 300

Ala Cys Ala Gln Thr Val Leu Ala Val Leu Tyr Tyr Glu Glu Thr Lys  
305 310 315 320

Ser Phe Leu Trp Trp Thr Ser Asn Leu Met Val Leu Gly Gly Ser Ser  
325 330 335

Ala Tyr Thr Trp Val Arg Gly Trp Glu Met Gln Lys Thr Gln Glu Asp  
340 345 350

Pro Ser Ser Lys Glu Gly Glu Lys Ser Ala Ile Arg Val  
355 360 365

<210> 154

<211> 363

<212> PRT

<213> Mus musculus

<400> 154

Met Asn Arg Ala Pro Leu Lys Arg Ser Arg Ile Leu Arg Met Ala Leu  
1 5 10 15

Thr Gly Val Ser Ala Val Ser Glu Glu Ser Glu Ser Gly Asn Lys Pro  
20 25 30

Phe Leu Leu Arg Ala Leu Gln Ile Ala Leu Val Val Ser Leu Tyr Trp  
 35 40 45  
 Val Thr Ser Ile Ser Met Val Phe Leu Asn Lys Tyr Leu Leu Asp Ser  
 50 55 60  
 Pro Ser Leu Gln Leu Asp Thr Pro Ile Phe Val Thr Phe Tyr Gln Cys  
 65 70 75 80  
 Leu Val Thr Ser Leu Leu Cys Lys Gly Leu Ser Thr Leu Ala Thr Cys  
 85 90 95  
 Cys Pro Gly Met Val Asp Phe Pro Thr Leu Asn Leu Asp Val Lys Val  
 100 105 110  
 Ala Arg Ser Val Leu Pro Leu Ser Val Val Phe Ile Gly Met Ile Thr  
 115 120 125  
 Phe Asn Asn Leu Cys Leu Lys Tyr Val Gly Val Pro Phe Tyr Asn Val  
 130 135 140  
 Gly Arg Ser Leu Thr Thr Val Phe Asn Val Leu Leu Ser Tyr Leu Leu  
 145 150 155 160  
 Leu Lys Gln Thr Thr Ser Phe Tyr Ala Leu Leu Thr Cys Gly Val Ile  
 165 170 175  
 Ile Gly Gly Phe Trp Leu Gly Ile Asp Gln Glu Gly Ala Glu Gly Thr  
 180 185 190  
 Leu Ser Leu Thr Gly Thr Ile Phe Gly Val Leu Ala Ser Leu Cys Val  
 195 200 205  
 Ser Leu Asn Ala Ile Tyr Thr Lys Lys Val Leu Pro Ala Val Asp His  
 210 215 220  
 Ser Ile Trp Arg Leu Thr Phe Tyr Asn Asn Val Asn Ala Cys Val Leu  
 225 230 235 240  
 Phe Leu Pro Leu Met Ile Val Leu Gly Glu Leu Arg Ala Leu Leu Ala  
 245 250 255  
 Phe Thr His Leu Ser Ser Ala His Phe Trp Leu Met Met Thr Leu Gly  
 260 265 270  
 Gly Leu Phe Gly Phe Ala Ile Gly Tyr Val Thr Gly Leu Gln Ile Lys  
 275 280 285  
 Phe Thr Ser Pro Leu Thr His Asn Val Ser Gly Thr Ala Lys Ala Cys  
 290 295 300  
 Ala Gln Thr Val Leu Ala Val Leu Tyr Tyr Glu Glu Ile Lys Ser Phe  
 305 310 315 320  
 Leu Trp Trp Thr Ser Asn Leu Met Val Leu Gly Gly Ser Ser Ala Tyr  
 325 330 335  
 Thr Trp Val Arg Gly Trp Glu Met Gln Lys Thr Gln Glu Asp Pro Ser  
 340 345 350  
 Ser Lys Asp Gly Glu Lys Ser Ala Ile Arg Val  
 355 360

&lt;210&gt; 155

&lt;211&gt; 350

&lt;212&gt; PRT

&lt;213&gt; Mus musculus

&lt;400&gt; 155

Met Ala Leu Thr Gly Val Ser Ala Val Ser Glu Glu Ser Glu Ser Gly  
 1 5 10 15

Asn Lys Pro Phe Leu Leu Arg Ala Leu Gln Ile Ala Leu Val Val Ser  
 20 25 30

Leu Tyr Trp Val Thr Ser Ile Ser Met Val Phe Leu Asn Lys Tyr Leu  
 35 40 45

Leu Asp Ser Pro Ser Leu Gln Leu Asp Thr Pro Ile Phe Val Thr Phe  
 50 55 60

Tyr Gln Cys Leu Val Thr Ser Leu Leu Cys Lys Gly Leu Ser Thr Leu  
 65 70 75 80

Ala Thr Cys Cys Pro Gly Met Val Asp Phe Pro Thr Leu Asn Leu Asp  
 85 90 95

Leu Lys Val Ala Arg Ser Val Leu Pro Leu Ser Val Val Phe Ile Gly  
 100 105 110

Met Ile Thr Phe Asn Asn Leu Cys Leu Lys Tyr Val Gly Val Pro Phe  
 115 120 125

Tyr Asn Val Gly Arg Ser Leu Thr Thr Val Phe Asn Val Leu Leu Ser  
 130 135 140

Tyr Leu Leu Leu Lys Gln Thr Thr Ser Phe Tyr Ala Leu Leu Thr Cys  
 145 150 155 160

Gly Val Ile Ile Gly Gly Phe Trp Leu Gly Ile Asp Gln Glu Gly Ala  
 165 170 175

Glu Gly Thr Leu Ser Leu Thr Gly Thr Ile Phe Gly Val Leu Ala Ser  
 180 185 190

Leu Cys Val Ser Leu Asn Ala Ile Tyr Thr Lys Lys Val Leu Pro Ala  
 195 200 205

Val Asp His Ser Ile Trp Arg Leu Thr Phe Tyr Asn Asn Val Asn Ala  
 210 215 220

Cys Val Leu Phe Leu Pro Leu Met Ile Val Leu Gly Glu Leu Arg Ala  
 225 230 235 240

Leu Leu Ala Phe Thr His Leu Ser Ser Ala His Phe Trp Leu Met Met  
 245 250 255

Thr Leu Gly Gly Leu Phe Gly Phe Ala Ile Gly Tyr Val Thr Gly Leu  
 260 265 270

Gln Ile Lys Phe Thr Ser Pro Leu Thr His Asn Val Ser Gly Thr Ala  
 275 280 285

Lys Ala Cys Ala Gln Thr Val Leu Ala Val Leu Tyr Tyr Glu Glu Ile  
 290 295 300

Lys Ser Phe Leu Trp Trp Thr Ser Asn Leu Met Val Leu Gly Gly Ser  
 305 310 315 320

Ser Ala Tyr Thr Trp Val Arg Gly Trp Glu Met Gln Lys Thr Gln Glu  
 325 330 335

Asp Pro Ser Ser Lys Asp Gly Glu Lys Ser Ala Ile Arg Val  
 340 345 350

<210> 156

<211> 365

<212> PRT

<213> Gallus gallus

<400> 156

Met Ser Arg Ser Gln Leu Thr Arg Thr Gly Ile Leu Arg Met Ala Leu  
 1 5 10 15

Gly Gly Ala Ala Asp Pro Leu Leu Pro Ala Glu Gly Ala Gly Gly Arg  
 20 25 30

Arg Thr Pro Phe Val Leu Arg Ala Leu Arg Ile Ala Leu Val Val Ser  
 35 40 45

Leu Tyr Trp Phe Val Ser Ile Thr Met Val Phe Leu Asn Lys Tyr Leu  
 50 55 60

Leu Asp Ser Pro Ser Leu Arg Leu Asp Ala Pro Leu Phe Val Thr Phe  
 65 70 75 80

Phe Gln Cys Ala Val Thr Ala Ala Leu Cys Leu Gly Leu Ser Leu Gly  
 85 90 95

Ala Ala Cys Gly Pro Cys Ala Ala Leu Pro Ala Leu Arg Leu Asp Leu  
 100 105 110

Lys Val Ser Arg Ser Val Leu Pro Leu Ser Val Val Phe Ile Gly Met  
 115 120 125

Val Thr Ser Asn Asn Leu Cys Leu Lys His Val Gly Val Ala Phe Tyr  
 130 135 140

Asn Val Gly Arg Ser Leu Thr Thr Val Phe Asn Val Leu Leu Ser Tyr  
 145 150 155 160

Leu Leu Leu Lys Gln Thr Thr Ser Leu Tyr Ala Leu Leu Ala Cys Gly  
 165 170 175

Ile Ile Ile Gly Gly Phe Trp Leu Gly Val Asp Gln Glu Gly Ala Glu  
 180 185 190

Gly Thr Leu Ser Trp Thr Gly Ile Ile Phe Gly Ile Leu Ala Ser Leu  
 195 200 205

Cys Val Ser Leu Asn Ala Ile Tyr Thr Lys Lys Val Leu Pro Val Val  
 210 215 220

Asp Gly Ser Ile Trp His Leu Thr Phe Tyr Asn Asn Met Asn Ala Cys  
 225 230 235 240

Val Leu Phe Leu Pro Leu Met Met Ile Thr Gly Glu Phe His Thr Leu  
 245 250 255

Tyr His Phe Asp Lys Leu Gly Ser Pro Ser Phe Trp Gly Met Met Thr  
 260 265 270

Leu Gly Gly Val Phe Gly Phe Ala Ile Gly Tyr Val Thr Gly Leu Gln  
 275 280 285

Ile Lys Phe Thr Ser Pro Leu Thr His Asn Val Ser Gly Thr Ala Lys

290 295 300  
 Ala Cys Ala Gln Thr Val Leu Ala Val Val Tyr Phe Glu Glu Thr Lys  
 305 310 315 320  
 Ser Leu Leu Trp Trp Thr Ser Asn Leu Met Val Leu Gly Gly Ser Phe  
 325 330 335  
 Ala Tyr Thr Trp Val Lys Gly Leu Glu Met Arg Lys Ala Gln Glu Asp  
 340 345 350  
 Pro Asn Leu Lys Ser Ser Glu Lys Asn Glu Thr Gly Val  
 355 360 365  
 <210> 157  
 <211> 362  
 <212> PRT  
 <213> Xenopus (Silurana) tropicalis  
 <400> 157  
 Met Asn Phe Lys Arg Ser Ser Ile Leu Arg Met Ala Leu Met Gly Ala  
 1 5 10 15  
 Gly Glu Gly Asp Gln Glu Lys Val Ser Arg Glu Ser Phe Leu Val Arg  
 20 25 30  
 Ala Val Lys Ile Ala Leu Val Val Thr Leu Tyr Trp Phe Ile Ser Ile  
 35 40 45  
 Thr Met Val Phe Leu Asn Lys Tyr Leu Leu Asp Ser Pro Ser Leu Lys  
 50 55 60  
 Leu Asp Ala Pro Leu Phe Val Thr Phe Tyr Gln Cys Val Val Thr Val  
 65 70 75 80  
 Val Leu Cys Lys Gly Leu Ser Leu Leu Thr His Val Val Pro Ser His  
 85 90 95  
 Ile Leu Glu Phe Pro Ser Leu Arg Phe Asp Leu Lys Val Leu Arg Thr  
 100 105 110  
 Val Leu Pro Leu Ser Ile Val Phe Ile Gly Met Ile Thr Phe Asn Asn  
 115 120 125  
 Leu Cys Leu Lys Tyr Leu Gly Val Ala Phe Tyr Thr Val Gly Arg Cys  
 130 135 140  
 Leu Ser Thr Val Phe Asn Val Leu Leu Ser Tyr Ile Met Leu Lys Gln  
 145 150 155 160  
 Thr Thr Ser Met Tyr Ala Leu Met Ser Cys Gly Val Ile Leu Gly Gly  
 165 170 175  
 Phe Trp Leu Gly Ile Asp Gln Glu Gly Ala Glu Gly Thr Leu Ser Trp  
 180 185 190  
 Ala Gly Ile Phe Phe Gly Val Leu Ala Ser Leu Cys Val Ser Leu Asn  
 195 200 205  
 Ala Ile Tyr Thr Lys Lys Val Leu Pro Ala Val Asp Gly Ser Ile Trp  
 210 215 220  
 Arg Leu Thr Phe Tyr Asn Asn Val Asn Ala Cys Phe Leu Phe Thr Pro  
 225 230 235 240

Leu Leu Phe Ile Phe Gly Glu Val Gly Thr Leu Phe Thr Phe Asp Lys  
245 250 255

Leu Phe Ala Phe Ser Phe Trp Gly Met Met Thr Leu Gly Gly Ile Phe  
260 265 270

Gly Phe Ala Ile Gly Tyr Val Thr Gly Leu Gln Ile Gln Phe Thr Ser  
275 280 285

Pro Leu Thr His Asn Ile Ser Gly Thr Ala Lys Ala Cys Ala Gln Thr  
290 295 300

Val Leu Ala Val Met Tyr Tyr His Gln Ile Lys Ser Phe Leu Trp Trp  
305 310 315 320

Thr Ser Asn Leu Met Val Leu Gly Gly Ser Phe Ser Tyr Thr Trp Val  
325 330 335

Lys Gly Leu Glu Met Lys Lys Ser Gln Gly Glu Thr Asn Gln Ser Gln  
340 345 350

Ser Asn Gly Glu Lys Asn Ser Val Gly Val  
355 360

<210> 158

<211> 348

<212> PRT

<213> Danio rerio

<400> 158

Met Ala Phe Thr Asp Ser Thr Arg Pro Gly Asp Lys Glu Glu Pro Phe  
1 5 10 15

Phe Met Arg Ala Thr Lys Ile Ala Leu Val Val Thr Leu Tyr Trp Phe  
20 25 30

Ile Ser Ile Ser Met Val Phe Leu Asn Asn Tyr Leu Leu Asp Ser Lys  
35 40 45

Glu Leu Asp Ala Pro Val Phe Ile Thr Phe Phe Gln Cys Val Val Ser  
50 55 60

Val Gly Leu Cys Leu Leu Met Ser Phe Leu Ser Ser Leu Cys Pro Gly  
65 70 75 80

Ser Val Asp Phe Pro Ser Leu Lys Phe Asp Leu Arg Val Ser Arg Glu  
85 90 95

Ile Leu Pro Leu Thr Ile Val Phe Ile Ser Met Ile Thr Phe Asn Asn  
100 105 110

Leu Cys Leu Lys Tyr Val Gly Val Ala Phe Tyr Thr Val Gly Arg Ser  
115 120 125

Leu Ser Thr Val Phe Asn Val Ile Leu Ser Tyr Val Val Leu Lys Gln  
130 135 140

Thr Thr Ser Leu Tyr Ala Val Leu Cys Cys Gly Val Ile Leu Gly Gly  
145 150 155 160

Phe Trp Leu Gly Val Asp Gln Glu Ala Val Ala Gly Ser Leu Ser Trp  
165 170 175

Ala Gly Val Val Phe Gly Val Ile Ala Ser Leu Cys Val Ser Leu Asn  
180 185 190

Ala Ile Phe Thr Lys Lys Val Leu Pro Val Val Asp Gly Asn Ile Trp  
195 200 205

Lys Leu Ser Tyr Tyr Asn Asn Leu Asn Ala Ile Val Leu Phe Leu Pro  
210 215 220

Leu Leu Ile Ile Leu Gly Glu Val Lys Ser Val Phe Glu Phe Ser Arg  
225 230 235 240

Leu Thr Asp Leu His Phe Trp Gly Met Met Thr Leu Gly Gly Val Phe  
245 250 255

Gly Phe Ala Ile Gly Tyr Val Thr Gly Leu Gln Ile Lys Phe Thr Ser  
260 265 270

Pro Leu Thr His Asn Val Ser Gly Thr Ala Lys Ser Cys Ala Gln Thr  
275 280 285

Val Leu Ala Val Val Tyr Trp Ala Ser Glu Lys Ser Thr Leu Trp Trp  
290 295 300

Thr Ser Asn Leu Met Val Leu Gly Gly Ser Phe Ala Tyr Thr Trp Val  
305 310 315 320

Lys Gly Met Glu Met Lys Lys Ala Pro Val Pro Thr Glu Thr Gln Ser  
325 330 335

Leu Asn Pro Gln Lys Asn Lys Glu Asp Leu Gly Val  
340 345

<210> 159

<211> 364

<212> PRT

<213> Bos taurus

<400> 159

Met Asn Arg Ala Ser Leu Lys Arg Ser Lys Ile Leu His Met Ala Leu  
1 5 10 15

Met Gly Thr Ser Asp Pro Ser Gly Glu Ala Glu Ala Ser Gln Glu Lys  
20 25 30

Pro Phe Val Leu Arg Ala Leu Gln Ile Ala Leu Val Val Ser Leu Tyr  
35 40 45

Trp Val Thr Ser Ile Ser Met Val Phe Leu Asn Lys Tyr Leu Leu Asp  
50 55 60

Ser Pro Ser Leu Arg Leu Asp Thr Pro Ile Phe Val Thr Phe Tyr Gln  
65 70 75 80

Cys Leu Val Thr Val Leu Leu Cys Lys Gly Leu Ser Ser Leu Ala Thr  
85 90 95

Cys Cys Pro Gly Thr Val Asp Phe Pro Ala Leu His Leu Asp Leu Lys  
100 105 110

Val Ala Arg Ser Val Leu Pro Leu Ser Val Val Phe Ile Gly Met Ile  
115 120 125

Thr Phe Asn Asn Leu Cys Leu Lys Tyr Val Gly Val Ala Phe Tyr Asn  
130 135 140

Val Gly Arg Ser Leu Thr Thr Val Phe Asn Val Leu Leu Ser Tyr Leu  
145 150 155



145                      150                      155                      160  
 Leu Leu Lys Gln Thr Thr Ser Phe Tyr Ala Leu Leu Thr Cys Ser Val  
                                  165                                   170                                   175  
 Ile Ile Gly Gly Phe Trp Leu Gly Val Asp Gln Glu Gly Ala Glu Gly  
                                  180                                   185                                   190  
 Thr Leu Ser Trp Thr Gly Thr Leu Phe Gly Val Leu Ala Ser Leu Cys  
                                  195                                   200                                   205  
 Val Ser Leu Asn Ala Ile Tyr Thr Lys Lys Val Leu Pro Ala Val Asp  
                                  210                                   215                                   220  
 Gly Ser Ile Trp Arg Leu Thr Phe Tyr Asn Asn Ala Asn Ala Cys Val  
                                  225                                   230                                   235                                   240  
 Leu Phe Leu Pro Leu Leu Leu Ala Leu Gly Glu Leu Arg Ala Leu Leu  
                                  245                                   250                                   255  
 Ala Phe Pro Gln Leu Gly Ser Ala His Phe Trp Ala Met Met Thr Leu  
                                  260                                   265                                   270  
 Gly Gly Leu Phe Gly Phe Ala Ile Gly Tyr Val Thr Gly Leu Gln Ile  
                                  275                                   280                                   285  
 Lys Phe Thr Ser Pro Leu Thr His Asn Val Ser Gly Thr Ala Lys Ala  
                                  290                                   295                                   300  
 Cys Ala Gln Thr Val Leu Ala Val Leu Tyr Tyr Glu Glu Ala Lys Ser  
 305                                   310                                   315                                   320  
 Phe Leu Trp Trp Thr Ser Asn Met Met Val Leu Gly Gly Ser Ser Ala  
                                  325                                   330                                   335  
 Tyr Thr Trp Val Arg Gly Arg Glu Met Lys Lys Thr Gln Glu Glu Pro  
                                  340                                   345                                   350  
 His Pro Arg Glu Asn Glu Lys Ser Asn Met Glu Val  
                                  355                                   360  
 <210> 160  
 <211> 364  
 <212> PRT  
 <213> Canis lupus familiaris  
 <400> 160  
 Met Asn Arg Ala Pro Leu Lys Arg Ser Arg Ile Leu His Met Ala Leu  
 1                                   5                                   10                                   15  
 Met Gly Ala Ser Asp Pro Leu Gly Glu Ala Glu Ala Ile Lys Glu Lys  
                                  20                                   25                                   30  
 Pro Phe Leu Leu Arg Ala Leu Gln Ile Thr Leu Val Val Ser Leu Tyr  
                                  35                                   40                                   45  
 Trp Val Thr Ser Ile Ser Met Val Phe Leu Asn Lys Tyr Leu Leu Asp  
                                  50                                   55                                   60  
 Ser Pro Ser Leu Gln Leu Asp Thr Pro Ile Phe Val Thr Phe Tyr Gln  
 65                                   70                                   75                                   80  
 Cys Leu Val Thr Thr Leu Leu Cys Lys Ser Leu Ser Thr Leu Ala Ala  
                                  85                                   90                                   95

Phe Cys Pro Gly Ala Met Asp Phe Pro Thr Leu Arg Leu Asp Leu Arg  
100 105 110

Val Ala Arg Ser Val Leu Pro Leu Ser Val Val Phe Ile Gly Met Ile  
115 120 125

Thr Phe Asn Asn Leu Cys Leu Lys Tyr Val Gly Val Ala Phe Tyr Asn  
130 135 140

Val Gly Arg Ser Leu Thr Thr Val Phe Asn Val Leu Leu Ser Tyr Leu  
145 150 155 160

Leu Leu Lys Gln Thr Thr Ser Phe Tyr Ala Leu Leu Thr Cys Gly Ile  
165 170 175

Ile Ile Gly Gly Phe Trp Leu Gly Val Asp Gln Glu Gly Ala Glu Gly  
180 185 190

Thr Leu Ser Trp Thr Gly Thr Leu Phe Gly Val Leu Ala Ser Leu Cys  
195 200 205

Val Ser Leu Asn Ala Ile Tyr Thr Lys Lys Val Leu Pro Ala Val Asp  
210 215 220

Gly Ser Ile Trp Arg Leu Thr Phe Tyr Asn Asn Val Asn Ala Cys Val  
225 230 235 240

Leu Phe Leu Pro Leu Leu Leu Leu Leu Gly Glu Leu Gln Thr Leu Leu  
245 250 255

Asn Phe Ser Gln Leu Gly Ser Ala His Phe Trp Gly Met Met Thr Leu  
260 265 270

Gly Gly Leu Phe Gly Phe Ala Ile Gly Tyr Val Thr Gly Leu Gln Ile  
275 280 285

Lys Phe Thr Ser Pro Leu Thr His Asn Val Ser Gly Thr Ala Lys Ala  
290 295 300

Cys Ala Gln Thr Val Leu Ala Val Leu Tyr Tyr Glu Glu Thr Lys Ser  
305 310 315 320

Phe Leu Trp Trp Thr Ser Asn Met Met Val Leu Gly Gly Ser Ser Ala  
325 330 335

Tyr Thr Trp Val Arg Gly Trp Glu Met Lys Lys Ile Gln Glu Asp Pro  
340 345 350

Ser Pro Lys Glu Asp Glu Lys Ser Ser Met Gly Val  
355 360

<210> 161

<211> 364

<212> PRT

<213> Homo sapiens

<400> 161

Met Asn Arg Ala Pro Leu Lys Arg Ser Arg Ile Leu His Met Ala Leu  
1 5 10 15

Thr Gly Ala Ser Asp Pro Ser Ala Glu Ala Glu Ala Asn Gly Glu Lys

| 20                                                              | 25  | 30  |
|-----------------------------------------------------------------|-----|-----|
| Pro Phe Leu Leu Arg Ala Leu Gln Ile Ala Leu Val Val Ser Leu Tyr |     |     |
| 35                                                              | 40  | 45  |
| Trp Val Thr Ser Ile Ser Met Val Phe Leu Asn Lys Tyr Leu Leu Asp |     |     |
| 50                                                              | 55  | 60  |
| Ser Pro Ser Leu Arg Leu Asp Thr Pro Ile Phe Val Thr Phe Tyr Gln |     |     |
| 65                                                              | 70  | 80  |
| Cys Leu Val Thr Thr Leu Leu Cys Lys Gly Leu Ser Ala Leu Ala Ala |     |     |
|                                                                 | 85  | 90  |
| Cys Cys Pro Gly Ala Val Asp Phe Pro Ser Leu Arg Leu Asp Leu Arg |     |     |
|                                                                 | 100 | 105 |
| Val Ala Arg Ser Val Leu Pro Leu Ser Val Val Phe Ile Gly Met Ile |     |     |
|                                                                 | 115 | 120 |
| Thr Phe Asn Asn Leu Cys Leu Lys Tyr Val Gly Val Ala Phe Tyr Asn |     |     |
|                                                                 | 130 | 135 |
| Val Gly Arg Ser Leu Thr Thr Val Phe Asn Val Leu Leu Ser Tyr Leu |     |     |
| 145                                                             | 150 | 155 |
| Leu Leu Lys Gln Thr Thr Ser Phe Tyr Ala Leu Leu Thr Cys Gly Ile |     |     |
|                                                                 | 165 | 170 |
| Ile Ile Gly Gly Phe Trp Leu Gly Val Asp Gln Glu Gly Ala Glu Gly |     |     |
|                                                                 | 180 | 185 |
| Thr Leu Ser Trp Leu Gly Thr Val Phe Gly Val Leu Ala Ser Leu Cys |     |     |
|                                                                 | 195 | 200 |
| Val Ser Leu Asn Ala Ile Tyr Thr Thr Lys Val Leu Pro Ala Val Asp |     |     |
|                                                                 | 210 | 215 |
| Gly Ser Ile Trp Arg Leu Thr Phe Tyr Asn Asn Val Asn Ala Cys Ile |     |     |
| 225                                                             | 230 | 235 |
| Leu Phe Leu Pro Leu Leu Leu Leu Leu Gly Glu Leu Gln Ala Leu Arg |     |     |
|                                                                 | 245 | 250 |
| Asp Phe Ala Gln Leu Gly Ser Ala His Phe Trp Gly Met Met Thr Leu |     |     |
|                                                                 | 260 | 265 |
| Gly Gly Leu Phe Gly Phe Ala Ile Gly Tyr Val Thr Gly Leu Gln Ile |     |     |
|                                                                 | 275 | 280 |
| Lys Phe Thr Ser Pro Leu Thr His Asn Val Ser Gly Thr Ala Lys Ala |     |     |
|                                                                 | 290 | 295 |
| Cys Ala Gln Thr Val Leu Ala Val Leu Tyr Tyr Glu Glu Thr Lys Ser |     |     |
| 305                                                             | 310 | 315 |
| Phe Leu Trp Trp Thr Ser Asn Met Met Val Leu Gly Gly Ser Ser Ala |     |     |
|                                                                 | 325 | 330 |
| Tyr Thr Trp Val Arg Gly Trp Glu Met Lys Lys Thr Pro Glu Glu Pro |     |     |
|                                                                 | 340 | 345 |
| Ser Pro Lys Asp Ser Glu Lys Ser Ala Met Gly Val                 |     |     |
|                                                                 | 355 | 360 |

&lt;210&gt; 162

&lt;211&gt; 351

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 162

Met Ala Leu Thr Gly Ala Ser Asp Pro Ser Ala Glu Ala Glu Ala Asn  
 1 5 10 15

Gly Glu Lys Pro Phe Leu Leu Arg Ala Leu Gln Ile Ala Leu Val Val  
 20 25 30

Ser Leu Tyr Trp Val Thr Ser Ile Ser Met Val Phe Leu Asn Lys Tyr  
 35 40 45

Leu Leu Asp Ser Pro Ser Leu Arg Leu Asp Thr Pro Ile Phe Val Thr  
 50 55 60

Phe Tyr Gln Cys Leu Val Thr Thr Leu Leu Cys Lys Gly Leu Ser Ala  
 65 70 75 80

Leu Ala Ala Cys Cys Pro Gly Ala Val Asp Phe Pro Ser Leu Arg Leu  
 85 90 95

Asp Leu Arg Val Ala Arg Ser Val Leu Pro Leu Ser Val Val Phe Ile  
 100 105 110

Gly Met Ile Thr Phe Asn Asn Leu Cys Leu Lys Tyr Val Gly Val Ala  
 115 120 125

Phe Tyr Asn Val Gly Arg Ser Leu Thr Thr Val Phe Asn Val Leu Leu  
 130 135 140

Ser Tyr Leu Leu Leu Lys Gln Thr Thr Ser Phe Tyr Ala Leu Leu Thr  
 145 150 155 160

Cys Gly Ile Ile Ile Gly Gly Phe Trp Leu Gly Val Asp Gln Glu Gly  
 165 170 175

Ala Glu Gly Thr Leu Ser Trp Leu Gly Thr Val Phe Gly Val Leu Ala  
 180 185 190

Ser Leu Cys Val Ser Leu Asn Ala Ile Tyr Thr Thr Lys Val Leu Pro  
 195 200 205

Ala Val Asp Gly Ser Ile Trp Arg Leu Thr Phe Tyr Asn Asn Val Asn  
 210 215 220

Ala Cys Ile Leu Phe Leu Pro Leu Leu Leu Leu Gly Glu Leu Gln  
 225 230 235 240

Ala Leu Arg Asp Phe Ala Gln Leu Gly Ser Ala His Phe Trp Gly Met  
 245 250 255

Met Thr Leu Gly Gly Leu Phe Gly Phe Ala Ile Gly Tyr Val Thr Gly  
 260 265 270

Leu Gln Ile Lys Phe Thr Ser Pro Leu Thr His Asn Val Ser Gly Thr  
 275 280 285

Ala Lys Ala Cys Ala Gln Thr Val Leu Ala Val Leu Tyr Tyr Glu Glu  
 290 295 300

Thr Lys Ser Phe Leu Trp Trp Thr Ser Asn Met Met Val Leu Gly Gly

|                                                                    |     |     |     |      |
|--------------------------------------------------------------------|-----|-----|-----|------|
| 305                                                                | 310 | 315 | 320 |      |
| Ser Ser Ala Tyr Thr Trp Val Arg Gly Trp Glu Met Lys Lys Thr Pro    |     |     |     |      |
|                                                                    | 325 | 330 | 335 |      |
|                                                                    |     |     |     |      |
| Glu Glu Pro Ser Pro Lys Asp Ser Glu Lys Ser Ala Met Gly Val        |     |     |     |      |
|                                                                    | 340 | 345 | 350 |      |
|                                                                    |     |     |     |      |
| <210> 163                                                          |     |     |     |      |
| <211> 1263                                                         |     |     |     |      |
| <212> DNA                                                          |     |     |     |      |
| <213> Trichoderma reesei                                           |     |     |     |      |
|                                                                    |     |     |     |      |
| <400> 163                                                          |     |     |     |      |
| atggcgctcac tcatcaaaac tgccgtggac attgccaacg gccgccatgc gctgtccaga |     |     |     | 60   |
| tatgtcatct ttgggctctg gcttgccgat gcggtgctgt gcgggtgat tatctggaaa   |     |     |     | 120  |
| gtgccttata cggaatcga ctgggtcgcc tacatggagc aagtcaccca gtctgtccac   |     |     |     | 180  |
| ggagagcgag actacccaa gatggagggc ggcacagggc ccctggtgta tcccgcggcc   |     |     |     | 240  |
| catgtgtaca tctacacagc gctctactac ctgacgaaca agggcaccga catcctgctg  |     |     |     | 300  |
| gcgcagcagc tctttgccgt gctctacatg gctactctgg cggtcgtcat gacatgctac  |     |     |     | 360  |
| tcaaaggcca aggtcccgcc gtacatcttc ccgcttctca tctctccaa aagacttcac   |     |     |     | 420  |
| agcgtctctg tctgagatg ctccaacgac tgcttcgccg ccttcttcct ctggctctgc   |     |     |     | 480  |
| atcttcttct tccagaggcg agagtggacc atcggagctc tcgcatacag catcggcctg  |     |     |     | 540  |
| ggcgctcaaaa tgtcgtgtct actggttctc ccgcgctggc tcatcgtcct ctacctcggc |     |     |     | 600  |
| cgcggtctca agggcgccct gcggctgctc tggctcatgg tgcagggtcca gtcctcctc  |     |     |     | 660  |
| gccataccct tcatcacgac aaattggcgc ggctacctcg gccgtgcatt cgagctctcg  |     |     |     | 720  |
| aggcagttca agtttgaatg gacagtcaat tggcgcatgc tgggcgagga tctgttcctc  |     |     |     | 780  |
| agccggggct tctctatcac gctactggca ttccacgcca tcttcctcct cgcctttatc  |     |     |     | 840  |
| ctcgcccggt ggctgaagat tagggaacgc accgtactcg ggatgatccc ctatgtcatc  |     |     |     | 900  |
| cgattcagat cgccctttac cgagcaggaa gacgcgcgca tctccaaccg cgtcgtcacg  |     |     |     | 960  |
| cccggctatg tcatgtccac catcttgtcg gccaacgtgg tgggactgct gtttgcccg   |     |     |     | 1020 |
| tctctgcact accagttcta tgcatatctg gcgtgggcga cccctatct cctgtggaag   |     |     |     | 1080 |
| gcctgcccca atcttttggg ggtggcccc ctctgggcgg cgcaagaatg ggcctggaac   |     |     |     | 1140 |
| gtcttcccca gcacgcctct tagctcgagc gtcgtggtga gcgtgctggc cgtgacggtg  |     |     |     | 1200 |
| gccatggcgt ttgcaggttc aaatccgcag ccacgtgaaa catcgaagcc gaagcagcac  |     |     |     | 1260 |
| taa                                                                |     |     |     | 1263 |

<210> 164  
<211> 420  
<212> PRT  
<213> Trichoderma reesei

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<400> 164
Met Ala Ser Leu Ile Lys Thr Ala Val Asp Ile Ala Asn Gly Arg His
 1             5             10             15

Ala Leu Ser Arg Tyr Val Ile Phe Gly Leu Trp Leu Ala Asp Ala Val
      20             25             30

Leu Cys Gly Leu Ile Ile Trp Lys Val Pro Tyr Thr Glu Ile Asp Trp
      35             40             45

Val Ala Tyr Met Glu Gln Val Thr Gln Phe Val His Gly Glu Arg Asp

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50      55      60
Tyr Pro Lys Met Glu Gly Gly Thr Gly Pro Leu Val Tyr Pro Ala Ala
65      70      75      80
His Val Tyr Ile Tyr Thr Gly Leu Tyr Tyr Leu Thr Asn Lys Gly Thr
85      90      95
Asp Ile Leu Leu Ala Gln Gln Leu Phe Ala Val Leu Tyr Met Ala Thr
100     105     110
Leu Ala Val Val Met Thr Cys Tyr Ser Lys Ala Lys Val Pro Pro Tyr
115     120     125
Ile Phe Pro Leu Leu Ile Leu Ser Lys Arg Leu His Ser Val Phe Val
130     135     140
Leu Arg Cys Phe Asn Asp Cys Phe Ala Ala Phe Phe Leu Trp Leu Cys
145     150     155     160
Ile Phe Phe Phe Gln Arg Arg Glu Trp Thr Ile Gly Ala Leu Ala Tyr
165     170     175
Ser Ile Gly Leu Gly Val Lys Met Ser Leu Leu Leu Val Leu Pro Ala
180     185     190
Val Val Ile Val Leu Tyr Leu Gly Arg Gly Phe Lys Gly Ala Leu Arg
195     200     205
Leu Leu Trp Leu Met Val Gln Val Gln Leu Leu Leu Ala Ile Pro Phe
210     215     220
Ile Thr Thr Asn Trp Arg Gly Tyr Leu Gly Arg Ala Phe Glu Leu Ser
225     230     235     240
Arg Gln Phe Lys Phe Glu Trp Thr Val Asn Trp Arg Met Leu Gly Glu
245     250     255
Asp Leu Phe Leu Ser Arg Gly Phe Ser Ile Thr Leu Leu Ala Phe His
260     265     270
Ala Ile Phe Leu Leu Ala Phe Ile Leu Gly Arg Trp Leu Lys Ile Arg
275     280     285
Glu Arg Thr Val Leu Gly Met Ile Pro Tyr Val Ile Arg Phe Arg Ser
290     295     300
Pro Phe Thr Glu Gln Glu Glu Arg Ala Ile Ser Asn Arg Val Val Thr
305     310     315     320
Pro Gly Tyr Val Met Ser Thr Ile Leu Ser Ala Asn Val Val Gly Leu
325     330     335
Leu Phe Ala Arg Ser Leu His Tyr Gln Phe Tyr Ala Tyr Leu Ala Trp
340     345     350
Ala Thr Pro Tyr Leu Leu Trp Thr Ala Cys Pro Asn Leu Leu Val Val
355     360     365
Ala Pro Leu Trp Ala Ala Gln Glu Trp Ala Trp Asn Val Phe Pro Ser
370     375     380
Thr Pro Leu Ser Ser Ser Val Val Val Ser Val Leu Ala Val Thr Val
385     390     395     400
Ala Met Ala Phe Ala Gly Ser Asn Pro Gln Pro Arg Glu Thr Ser Lys

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405

410

415

Pro Lys Gln His  
420

&lt;210&gt; 165

&lt;211&gt; 445

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 165

Met Leu Lys Lys Gln Ser Ala Gly Leu Val Leu Trp Gly Ala Ile Leu  
1 5 10 15

Phe Val Ala Trp Asn Ala Leu Leu Leu Phe Phe Trp Thr Arg Pro  
20 25 30

Ala Pro Gly Arg Pro Pro Ser Val Ser Ala Leu Asp Gly Asp Pro Ala  
35 40 45

Ser Leu Thr Arg Glu Val Ile Arg Leu Ala Gln Asp Ala Glu Val Glu  
50 55 60

Leu Glu Arg Gln Arg Gly Leu Leu Gln Gln Ile Gly Asp Ala Leu Ser  
65 70 75 80

Ser Gln Arg Gly Arg Val Pro Thr Ala Ala Pro Pro Ala Gln Pro Arg  
85 90 95

Val Pro Val Thr Pro Ala Pro Ala Val Ile Pro Ile Leu Val Ile Ala  
100 105 110

Cys Asp Arg Ser Thr Val Arg Arg Cys Leu Asp Lys Leu Leu His Tyr  
115 120 125

Arg Pro Ser Ala Glu Leu Phe Pro Ile Ile Val Ser Gln Asp Cys Gly  
130 135 140

His Glu Glu Thr Ala Gln Ala Ile Ala Ser Tyr Gly Ser Ala Val Thr  
145 150 155 160

His Ile Arg Gln Pro Asp Leu Ser Ser Ile Ala Val Pro Pro Asp His  
165 170 175

Arg Lys Phe Gln Gly Tyr Tyr Lys Ile Ala Arg His Tyr Arg Trp Ala  
180 185 190

Leu Gly Gln Val Phe Arg Gln Phe Arg Phe Pro Ala Ala Val Val Val  
195 200 205

Glu Asp Asp Leu Glu Val Ala Pro Asp Phe Phe Glu Tyr Phe Arg Ala  
210 215 220

Thr Tyr Pro Leu Leu Lys Ala Asp Pro Ser Leu Trp Cys Val Ser Ala  
225 230 235 240

Trp Asn Asp Asn Gly Lys Glu Gln Met Val Asp Ala Ser Arg Pro Glu  
245 250 255

Leu Leu Tyr Arg Thr Asp Phe Phe Pro Gly Leu Gly Trp Leu Leu Leu  
260 265 270

Ala Glu Leu Trp Ala Glu Leu Glu Pro Lys Trp Pro Lys Ala Phe Trp  
275 280 285

Asp Asp Trp Met Arg Arg Pro Glu Gln Arg Gln Glv Arg Ala Cys Ile

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290                295                300
Arg Pro Glu Ile Ser Arg Thr Met Thr Phe Gly Arg Lys Gly Val Ser
305                310                315                320

His Gly Gln Phe Phe Asp Gln His Leu Lys Phe Ile Lys Leu Asn Gln
325                330                335

Gln Phe Val His Phe Thr Gln Leu Asp Leu Ser Tyr Leu Gln Arg Glu
340                345                350

Ala Tyr Asp Arg Asp Phe Leu Ala Arg Val Tyr Gly Ala Pro Gln Leu
355                360                365

Gln Val Glu Lys Val Arg Thr Asn Asp Arg Lys Glu Leu Gly Glu Val
370                375                380

Arg Val Gln Tyr Thr Gly Arg Asp Ser Phe Lys Ala Phe Ala Lys Ala
385                390                395                400

Leu Gly Val Met Asp Asp Leu Lys Ser Gly Val Pro Arg Ala Gly Tyr
405                410                415

Arg Gly Ile Val Thr Phe Gln Phe Arg Gly Arg Arg Val His Leu Ala
420                425                430

Pro Pro Leu Thr Trp Glu Gly Tyr Asp Pro Ser Trp Asn
435                440                445

<210> 166
<211> 447
<212> PRT
<213> Homo sapiens

<400> 166
Met Arg Phe Arg Ile Tyr Lys Arg Lys Val Leu Ile Leu Thr Leu Val
1          5          10          15

Val Ala Ala Cys Gly Phe Val Leu Trp Ser Ser Asn Gly Arg Gln Arg
20          25          30

Lys Asn Glu Ala Leu Ala Pro Pro Leu Leu Asp Ala Glu Pro Ala Arg
35          40          45

Gly Ala Gly Gly Arg Gly Gly Asp His Pro Ser Val Ala Val Gly Ile
50          55          60

Arg Arg Val Ser Asn Val Ser Ala Ala Ser Leu Val Pro Ala Val Pro
65          70          75          80

Gln Pro Glu Ala Asp Asn Leu Thr Leu Arg Tyr Arg Ser Leu Val Tyr
85          90          95

Gln Leu Asn Phe Asp Gln Thr Leu Arg Asn Val Asp Lys Ala Gly Thr
100         105         110

Trp Ala Pro Arg Glu Leu Val Leu Val Val Gln Val His Asn Arg Pro
115         120         125

Glu Tyr Leu Arg Leu Leu Leu Asp Ser Leu Arg Lys Ala Gln Gly Ile
130         135         140

Asp Asn Val Leu Val Ile Phe Ser His Asp Phe Trp Ser Thr Glu Ile
145         150         155         160

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Asn Gln Leu Ile Ala Gly Val Asn Phe Cys Pro Val Leu Gln Val Phe
    165                                170                                175

Phe Pro Phe Ser Ile Gln Leu Tyr Pro Asn Glu Phe Pro Gly Ser Asp
    180                                185                                190

Pro Arg Asp Cys Pro Arg Asp Leu Pro Lys Asn Ala Ala Leu Lys Leu
    195                                200                                205

Gly Cys Ile Asn Ala Glu Tyr Pro Asp Ser Phe Gly His Tyr Arg Glu
    210                                215                                220

Ala Lys Phe Ser Gln Thr Lys His His Trp Trp Trp Lys Leu His Phe
    225                                230                                235                                240

Val Trp Glu Arg Val Lys Ile Leu Arg Asp Tyr Ala Gly Leu Ile Leu
    245                                250                                255

Phe Leu Glu Glu Asp His Tyr Leu Ala Pro Asp Phe Tyr His Val Phe
    260                                265                                270

Lys Lys Met Trp Lys Leu Lys Gln Gln Glu Cys Pro Glu Cys Asp Val
    275                                280                                285

Leu Ser Leu Gly Thr Tyr Ser Ala Ser Arg Ser Phe Tyr Gly Met Ala
    290                                295                                300

Asp Lys Val Asp Val Lys Thr Trp Lys Ser Thr Glu His Asn Met Gly
    305                                310                                315                                320

Leu Ala Leu Thr Arg Asn Ala Tyr Gln Lys Leu Ile Glu Cys Thr Asp
    325                                330                                335

Thr Phe Cys Thr Tyr Asp Asp Tyr Asn Trp Asp Trp Thr Leu Gln Tyr
    340                                345                                350

Leu Thr Val Ser Cys Leu Pro Lys Phe Trp Lys Val Leu Val Pro Gln
    355                                360                                365

Ile Pro Arg Ile Phe His Ala Gly Asp Cys Gly Met His His Lys Lys
    370                                375                                380

Thr Cys Arg Pro Ser Thr Gln Ser Ala Gln Ile Glu Ser Leu Leu Asn
    385                                390                                395                                400

Asn Asn Lys Gln Tyr Met Phe Pro Glu Thr Leu Thr Ile Ser Glu Lys
    405                                410                                415

Phe Thr Val Val Ala Ile Ser Pro Pro Arg Lys Asn Gly Gly Trp Gly
    420                                425                                430

Asp Ile Arg Asp His Glu Leu Cys Lys Ser Tyr Arg Arg Leu Gln
    435                                440                                445

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&lt;210&gt; 167

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 167

agcgagctgt gcctcttgga 20

<210> 168

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 168

agtcgagcac ttgcgcgacc t 21

<210> 169

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 169

ttgttgcca tatttcctg c 21

<210> 170

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 170

tgccagtga tacacatggg 20

<210> 171

<211> 10

<212> PRT

<213> Trichoderma reesei

<400> 171

|     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ala | Ser | Thr | Asn | Ala | Arg | Tyr | Val | Arg |
| 1   |     |     |     | 5   |     |     |     | 10  |     |

<210> 172

<211> 17

<212> PRT

<213> Trichoderma reesei

<400> 172

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Leu | Leu | Ile | Ala | Phe | Phe | Thr | Ile | Leu | Val | Phe | Tyr | Phe | Val | Ser |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

Asn

<210> 173

<211> 379

<212> PRT

&lt;213&gt; Trichoderma reesei

&lt;400&gt; 173

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Ser Lys Tyr Glu Gly Val Asp Leu Asn Lys Gly Thr Phe Thr Ala Pro
1           5           10           15

Asp Ser Thr Lys Thr Thr Pro Lys Pro Pro Ala Thr Gly Asp Ala Lys
20           25           30

Asp Phe Pro Leu Ala Leu Thr Pro Asn Asp Pro Gly Phe Asn Asp Leu
35           40           45

Val Gly Ile Ala Pro Gly Pro Arg Met Asn Ala Thr Phe Val Thr Leu
50           55           60

Ala Arg Asn Ser Asp Val Trp Asp Ile Ala Arg Ser Ile Arg Gln Val
65           70           75           80

Glu Asp Arg Phe Asn Arg Arg Tyr Asn Tyr Asp Trp Val Phe Leu Asn
85           90           95

Asp Lys Pro Phe Asp Asn Thr Phe Lys Lys Val Thr Thr Ser Leu Val
100          105          110

Ser Gly Lys Thr His Tyr Gly Glu Ile Ala Pro Glu His Trp Ser Phe
115          120          125

Pro Asp Trp Ile Asp Gln Asp Lys Ala Lys Lys Val Arg Glu Asp Met
130          135          140

Ala Glu Arg Lys Ile Ile Tyr Gly Asp Ser Val Ser Tyr Arg His Met
145          150          155          160

Cys Arg Phe Glu Ser Gly Phe Phe Phe Arg Gln Pro Leu Met Met Asn
165          170          175

Tyr Glu Tyr Tyr Trp Arg Val Glu Pro Ser Ile Glu Leu Tyr Cys Asp
180          185          190

Ile His Tyr Asp Pro Phe Arg Leu Met Val Glu Gln Gly Lys Lys Tyr
195          200          205

Ser Phe Val Ile Ser Leu Tyr Glu Tyr Pro Ala Thr Ile Ala Thr Leu
210          215          220

Trp Glu Ser Thr Lys Lys Phe Met Lys Asn His Pro Glu His Ile Ala
225          230          235          240

Pro Asp Asn Ser Met Arg Phe Leu Ser Asp Asp Gly Gly Glu Thr Tyr
245          250          255

Asn Asn Cys His Phe Trp Ser Asn Phe Glu Ile Gly Ser Leu Glu Trp
260          265          270

Leu Arg Ser Lys Gln Tyr Ile Asp Phe Phe Glu Ser Leu Asp Lys Asp
275          280          285

Gly Gly Phe Phe Tyr Glu Arg Trp Gly Asp Ala Pro Val His Ser Ile
290          295          300

Ala Ala Gly Leu Met Leu Asn Arg Ser Glu Ile His Phe Phe Asn Asp
305          310          315          320

Ile Ala Tyr Trp His Val Pro Phe Thr His Cys Pro Thr Gly Glu Lys
325          330          335

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Thr Arg Leu Asp Leu Lys Cys His Cys Asp Pro Lys Glu Asn Phe Asp  
340 345 350

Trp Lys Gly Tyr Ser Cys Thr Ser Arg Phe Phe Glu Met Asn Gly Met  
355 360 365

Asp Lys Pro Glu Gly Trp Glu Asn Gln Gln Asp  
370 375

<210> 174

<211> 8

<212> PRT

<213> Trichoderma reesei

<400> 174

Met Ala Ile Ala Arg Pro Val Arg  
1 5

<210> 175

<211> 15

<212> PRT

<213> Trichoderma reesei

<400> 175

Ala Leu Gly Gly Leu Ala Ala Ile Leu Trp Cys Phe Phe Leu Tyr  
1 5 10 15

<210> 176

<211> 371

<212> PRT

<213> Trichoderma reesei

<400> 176

Gln Leu Leu Arg Pro Ser Ser Ser Tyr Asn Ser Pro Gly Asp Arg Tyr  
1 5 10 15

Ile Asn Phe Glu Arg Asp Pro Asn Leu Asp Pro Thr Gly Glu Pro Glu  
20 25 30

Gly Ile Leu Val Arg Thr Ser Asp Arg Tyr Ala Pro Asp Ala Lys Asp  
35 40 45

Thr Asp Arg Ala Ser Ala Thr Leu Leu Ala Leu Val Arg Asn Glu Glu  
50 55 60

Val Asp Asp Met Val Ala Ser Met Val Asp Leu Glu Arg Thr Trp Asn  
65 70 75 80

Ser Lys Phe Asn Tyr Pro Trp Thr Phe Phe Asn Asp Lys Pro Phe Ser  
85 90 95

Glu Glu Phe Lys Lys Lys Thr Ser Ala Val Thr Asn Ala Thr Cys Asn  
100 105 110

Tyr Glu Leu Ile Pro Lys Glu His Trp Asp Ala Pro Ser Trp Ile Asp  
115 120 125

Pro Ala Ile Phe Glu Glu Ser Ala Ala Val Leu Lys Lys Asn Gly Val  
130 135 140

Gln Tyr Ala Asn Met Met Ser Tyr His Gln Met Cys Arg Trp Asn Ser  
145 150 155 160

Gly Met Phe Tyr Lys His Pro Ala Leu Lys Asp Val Arg Tyr Tyr Trp  
165 170 175

Arg Val Glu Pro Lys Val His Phe Phe Cys Asp Val Asp Tyr Asp Val  
180 185 190

Phe Arg Tyr Met Gln Asp Asn Asn Lys Thr Tyr Gly Phe Thr Ile Asn  
195 200 205

Leu Tyr Asp Asp Pro His Thr Leu Pro Thr Leu Trp Pro Gln Thr Ala  
210 215 220

Lys Phe Leu Ala Asp His Pro Asn Tyr Leu His Glu His Ser Ala Ile  
225 230 235 240

Lys Trp Val Ile Asp Asp Ala Arg Arg Pro Gln His Asn Arg Glu Ala  
245 250 255

Gln Gly Phe Ser Thr Cys His Phe Trp Ser Asn Phe Glu Val Ala Asp  
260 265 270

Met Glu Phe Trp Arg Ser Lys Val Tyr Glu Asp Tyr Phe Glu His Leu  
275 280 285

Asp Arg Ala Gly Gly Phe Phe Tyr Glu Arg Trp Gly Asp Ala Pro Val  
290 295 300

His Ser Ile Ala Leu Gly Leu Phe Glu Asp Ser Ser Lys Ile His Trp  
305 310 315 320

Phe Arg Asp Ile Gly Tyr Gln His Ile Pro Phe Phe Asn Cys Pro Asn  
325 330 335

Ser Pro Lys Cys Lys Gly Cys Val Thr Gly Arg Leu Thr Asp Gly Glu  
340 345 350

Pro Phe Leu His Arg Glu Asp Cys Arg Pro Asn Trp Phe Lys Tyr Ala  
355 360 365

Gly Met Gly  
370

<210> 177

<211> 6

<212> PRT

<213> Trichoderma reesei

<400> 177

Met Leu Asn Pro Arg Arg  
1 5

<210> 178

<211> 15

<212> PRT

<213> Trichoderma reesei

<400> 178

Ala Leu Ile Ala Ala Ala Phe Ile Leu Thr Val Phe Phe Leu Ile  
1 5 10 15

<210> 179

<211> 352

<212> PRT

<213> *Trichoderma reesei*

<400> 179

Ser Arg Ser His Asn Ser Glu Ser Ala Ser Thr Ser Glu Pro Lys Asp  
1 5 10 15

Ala Glu Ala Glu Ala Leu Ser Ala Ala Asn Ala Gln Gln Arg Ala Ala  
20 25 30

Pro Pro Pro Pro Pro Gln Lys Pro Met Ile Asp Met Ser Gly Met Ser  
35 40 45

Thr Tyr Asp Lys Leu Ala Tyr Ala Tyr Glu Tyr Asp Ile Glu Ser Lys  
50 55 60

Phe Pro Ala Tyr Ile Trp Gln Thr Trp Arg Lys Thr Pro Ser Glu Gly  
65 70 75 80

Asp Phe Glu Phe Arg Glu Gln Glu Ala Ser Trp Ser Ile Glu His Pro  
85 90 95

Gly Phe Ile His Glu Val Ile Thr Asp Ser Val Ala Asp Thr Leu Leu  
100 105 110

Gln Leu Leu Tyr Gly Ser Ile Pro Glu Val Leu Glu Ala Tyr His Ala  
115 120 125

Leu Pro Leu Pro Val Leu Lys Ala Asp Leu Phe Arg Tyr Leu Ile Leu  
130 135 140

Tyr Ala Arg Gly Gly Ile Tyr Ser Asp Ile Asp Thr Tyr Ala Ile Arg  
145 150 155 160

Ser Ala Leu Glu Trp Ile Pro Pro Gln Ile Pro Lys Glu Thr Val Gly  
165 170 175

Leu Val Ile Gly Ile Glu Ala Asp Pro Asp Arg Pro Asp Trp Ala Asp  
180 185 190

Trp Tyr Ser Arg Arg Ile Gln Phe Cys Gln Trp Thr Ile Gln Ser Lys  
195 200 205

Pro Gly His Pro Val Leu Arg Asp Ile Ile Ser Arg Ile Thr Asn Gln  
210 215 220

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Leu | Glu | Met | Lys | Lys | Ser | Gly | Lys | Leu | Ser | Ala | Phe | Gln | Gly | Asn |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Val | Val | Asp | Leu | Thr | Gly | Pro | Ala | Val | Trp | Thr | Asp | Thr | Ile | Met |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |

Asp Tyr Phe Asn Asp Glu Arg Tyr Phe Asp Met Glu Asn Ser Lys Gly  
260 265 270

Arg Ile Asp Tyr Arg Asn Phe Thr Gly Met Glu Thr Ser Lys Arg Val  
275 280 285

Gly Asp Val Val Val Leu Pro Ile Thr Ser Phe Ser Pro Gly Val Gly  
290 295 300

Gln Met Gly Ala Lys Asp Tyr Asp Asp Pro Met Ala Phe Val Lys His  
305 310 315 320

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000 1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1014 1015 1016 1017 1018 1019 1020 1021 1022 1023 1024 1025 1026 1027 1028 1029 1030 1031 1032 1033 1034 1035 1036 1037 1038 1039 1040 1

Asp Phe Glu Gly Thr Trp Lys Pro Glu Ser Glu Arg His Ile Gly Glu  
325 330 335

Ile Val Gln Glu Leu Gly Glu Gly Gln Gly Glu Ala Pro Lys Glu Gln  
340 345 350

<210> 180

<211> 22

<212> PRT

<213> Trichoderma reesei

<400> 180

Met Gly Met Gly Gln Cys Gln Trp Ser Pro Phe Arg Asn Lys Val Pro  
1 5 10 15

Thr Gln Met Arg Arg Cys  
20

<210> 181

<211> 15

<212> PRT

<213> Trichoderma reesei

<400> 181

Leu Pro Leu Tyr Ile Thr Val Val Cys Val Phe Leu Val Ile Val  
1 5 10 15

<210> 182

<211> 362

<212> PRT

<213> Trichoderma reesei

<400> 182

Asn Phe Asp Trp Ile Leu Ala Ile Pro Asn Pro Ala Ser Val Leu Arg  
1 5 10 15

Arg Glu Pro Lys Ala Pro Pro Leu Pro Gly Ser Thr Phe Pro Gln Lys  
20 25 30

Ile Trp Gln Thr Trp Lys Val Asp Pro Leu Asn Phe Asp Glu Arg Asp  
35 40 45

Leu Val Thr Ala Arg Thr Trp Thr Thr Ile Asn Pro Gly Met Arg Tyr  
50 55 60

Glu Val Val Thr Asp Ala Asn Glu Met Ala Tyr Ile Glu Asp Arg Tyr  
65 70 75 80

Gly Pro Asn Gly Phe Asp Arg Pro Asp Ile Val Glu Phe Tyr Lys Met  
85 90 95

Ile Asn Leu Pro Ile Ile Lys Ala Asp Leu Leu Arg Tyr Met Ile Met  
100 105 110

Tyr Ala Glu Gly Gly Ile Tyr Ala Asp Ile Asp Val Glu Thr Met Lys  
115 120 125

Pro Phe His Arg Phe Ile Pro Asp Arg Tyr Asp Glu Lys Asp Ile Asp  
130 135 140

Ile Ile Ile Gly Val Glu Ile Asp Gln Pro Asp Phe Lys Asp His Pro  
145 150 155 160

Ile Leu Gly Lys Lys Ser Met Ser Phe Cys Gln Trp Thr Phe Val Ala  
165 170 175

Arg Pro Gln Gln Pro Val Met Met Arg Leu Ile Glu Asn Ile Met Lys  
180 185 190

Trp Phe Lys Thr Val Ala Arg Asp Gln Gly Val Pro Leu Gly Glu Val  
195 200 205

Gln Leu Asp Phe Asp Gln Val Ile Ser Gly Thr Gly Pro Ser Ala Phe  
210 215 220

Thr Lys Ala Met Leu Glu Glu Met Asn Arg Lys Thr Lys Gly Pro Lys  
225 230 235 240

Val Thr Trp Asp Ala Phe His Asn Leu Asp Glu Ser Lys Leu Val Gly  
245 250 255

Gly Val Leu Val Leu Thr Val Glu Ala Phe Cys Ala Gly Gln Gly His  
260 265 270

Ser Asp Ser Gly Asn His Asn Ala Arg Asn Ala Leu Val Lys His His  
275 280 285

Phe His Ala Ser Asn Trp Pro Ser Arg His Pro Arg Tyr Lys His Pro  
290 295 300

Ala Tyr Gly Gln Val Glu Asp Cys Asn Trp Val Pro Glu Cys Val Arg  
305 310 315 320

Lys Trp Asp Glu Asp Thr Ser Asn Trp Asp Lys Tyr Ser Glu Asn Glu  
325 330 335

Gln Lys Lys Ile Leu Gln Asp Ile Glu Asn Ala Arg Leu Glu Arg Glu  
340 345 350

Arg Gln Gln Gln Ala Leu Ala Ala Leu Pro  
355 360

<210> 183

<211> 17

<212> PRT

<213> Trichoderma reesei

<400> 183

Met Ala Arg Pro Met Gly Ser Val Arg Leu Lys Lys Ala Asn Pro Ser  
1 5 10 15

Thr

<210> 184

<211> 16

<212> PRT

<213> Trichoderma reesei

<400> 184

Leu Ile Leu Gly Ala Val Leu Cys Ile Phe Ile Ile Ile Phe Leu Val  
1 5 10 15

<210> 185

<211> 339

<212> PRT



&lt;213&gt; Trichoderma reesei

&lt;400&gt; 185

Ser Pro Ser Ser Pro Ala Ser Ala Ser Arg Leu Ser Ile Val Ser Ala  
 1 5 10 15

Gln His His Leu Ser Pro Pro Thr Ser Pro Tyr Gln Ser Pro Arg Ser  
 20 25 30

Gly Ala Val Gln Gly Pro Pro Pro Val Thr Arg Tyr Asn Leu Asn Lys  
 35 40 45

Val Thr Val Thr Ser Asp Pro Val Arg Asn Gln Glu His Ile Leu Ile  
 50 55 60

Leu Thr Pro Met Ala Arg Phe Tyr Gln Glu Tyr Trp Asp Asn Leu Leu  
 65 70 75 80

Arg Leu Asn Tyr Pro His Glu Leu Ile Thr Leu Gly Phe Ile Leu Pro  
 85 90 95

Lys Thr Lys Glu Gly Asn Gln Ala Thr Ser Met Leu Gln Lys Gln Ile  
 100 105 110

Gln Lys Thr Gln Asn Tyr Gly Pro Glu Lys Asp Arg Phe Lys Ser Ile  
 115 120 125

Ile Ile Leu Arg Gln Asp Phe Asp Pro Ala Val Val Ser Gln Asp Glu  
 130 135 140

Ser Glu Arg His Lys Leu Ala Asn Gln Lys Ala Arg Arg Glu Val Met  
 145 150 155 160

Ala Lys Ala Arg Asn Ser Leu Leu Phe Thr Thr Leu Gly Pro Ser Thr  
 165 170 175

Ser Trp Val Leu Trp Leu Asp Ala Asp Ile Thr Glu Thr Ala Pro Thr  
 180 185 190

Leu Ile Gln Asp Leu Ala Ser His Asp Lys Pro Ile Ile Val Ala Asn  
 195 200 205

Cys Phe Gln Lys Tyr Tyr Asp Pro Glu Ser Lys Lys Met Ala Glu Arg  
 210 215 220

Pro Tyr Asp Phe Asn Ser Trp Gln Asp Ser Glu Thr Ala Leu Lys Met  
 225 230 235 240

Ala Glu Gln Met Gly Pro Asp Asp Ile Leu Leu Glu Gly Tyr Ala Glu  
 245 250 255

Met Ala Thr Tyr Arg Thr Leu Leu Ala Tyr Met Ser Thr Pro Gly Gly  
 260 265 270

Ser Lys Asp Leu Val Val Pro Leu Asp Gly Val Gly Gly Thr Ala Leu  
 275 280 285

Leu Val Lys Ala Asp Val His Arg Asp Gly Ala Met Phe Pro Pro Phe  
 290 295 300

Ala Phe Tyr His Leu Ile Glu Ser Glu Gly Phe Ala Lys Met Ala Lys  
 305 310 315 320

Arg Leu Gly Trp Gln Pro Tyr Gly Leu Pro Asn Tyr Lys Val Tyr His  
 325 330 335

Tyr Asn Glu

<210> 186

<211> 31

<212> PRT

<213> Trichoderma reesei

<400> 186

Met Leu Leu Pro Lys Gly Gly Leu Asp Trp Arg Ser Ala Arg Ala Gln  
1 5 10 15

Ile Pro Pro Thr Arg Ala Leu Trp Asn Ala Val Thr Arg Thr Arg  
20 25 30

<210> 187

<211> 15

<212> PRT

<213> Trichoderma reesei

<400> 187

Phe Ile Leu Leu Val Gly Ile Thr Gly Leu Ile Leu Leu Trp  
1 5 10 15

<210> 188

<211> 358

<212> PRT

<213> Trichoderma reesei

<400> 188

Arg Gly Val Ser Thr Ser Ala Ser Glu Met Gln Ser Phe Tyr Cys Trp  
1 5 10 15

Gly Pro Ala Lys Pro Pro Met Glu Met Ser Pro Asn Glu His Asn Arg  
20 25 30

Trp Asn Gly His Leu Gln Thr Pro Val Ile Phe Asn His His Ala Pro  
35 40 45

Val Glu Val Asn Ser Ser Thr Ile Glu His Val Asp Leu Asn Pro Ile  
50 55 60

Asn Ser Thr Lys Gln Ala Val Thr Lys Glu Glu Arg Ile Leu Ile Leu  
65 70 75 80

Thr Pro Leu Lys Asp Ala Ala Pro Tyr Leu Ser Lys Tyr Phe Glu Leu  
85 90 95

Leu Ala Glu Leu Thr Tyr Pro His Arg Leu Ile Asp Leu Ala Phe Leu  
100 105 110

Val Ser Asp Ser Thr Asp Asp Thr Leu Ala Val Leu Ala Ser Glu Leu  
115 120 125

Asp Arg Ile Gln Lys Arg Pro Asp Gln Ile Pro Phe His Ser Ala Thr  
130 135 140

Val Ile Glu Lys Asp Phe Gly Phe Lys Leu Ser Gln Asn Val Glu Glu  
145 150 155 160

Arg His Ser Phe Glu Ala Gln Gly Pro Arg Arg Lys Ala Met Gly Arg  
165 170 175

Ala Arg Asn Tyr Leu Leu Tyr Thr Ala Leu Lys Pro Glu His Ser Trp  
180 185 190

Val Tyr Trp Arg Asp Val Asp Ile Val Asp Ser Pro Thr Gly Ile Leu  
195 200 205

Glu Asp Phe Ile Ala His Asp Arg Asp Ile Leu Val Pro Asn Ile Trp  
210 215 220

Phe His Arg Tyr Arg Asp Gly Val Asp Ile Glu Gly Arg Phe Asp Tyr  
225 230 235 240

Asn Ser Trp Val Glu Ser Asp Lys Gly Arg Lys Leu Ala Asn Ser Leu  
245 250 255

Asp Lys Asp Val Val Leu Ala Glu Gly Tyr Lys Gln Tyr Asp Thr Gly  
260 265 270

Arg Thr Tyr Met Ala Lys Met Gly Asp Trp Arg Glu Asn Lys Asp Val  
275 280 285

Glu Leu Glu Leu Asp Gly Ile Gly Gly Val Asn Ile Leu Val Lys Ala  
290 295 300

Asp Val His Arg Ser Gly Ile Asn Phe Pro Cys Tyr Ala Phe Glu Asn  
305 310 315 320

Gln Ala Glu Thr Glu Gly Phe Ala Lys Met Ala Lys Arg Ala Gly Tyr  
325 330 335

Glu Val Tyr Gly Leu Pro Asn Tyr Val Val Trp His Ile Asp Thr Glu  
340 345 350

Glu Lys Gly Gly Asn Ala  
355

<210> 189

<211> 45

<212> PRT

<213> Trichoderma reesei

<400> 189

Met Met Pro Arg His His Ser Ser Gly Phe Ser Asn Gly Tyr Pro Arg  
1 5 10 15

Ala Asp Thr Phe Glu Ile Ser Pro His Arg Phe Gln Pro Arg Ala Thr  
20 25 30

Leu Pro Pro His Arg Lys Arg Lys Arg Thr Ala Ile Arg  
35 40 45

<210> 190

<211> 16

<212> PRT

<213> Trichoderma reesei

<400> 190

Val Gly Ile Ala Val Val Val Ile Leu Val Leu Val Leu Trp Phe Gly  
1 5 10 15

<210> 191

<211> 407

<212> PRT

&lt;213&gt; Trichoderma reesei

&lt;400&gt; 191

Gln Pro Arg Ser Val Ala Ser Leu Ile Ser Leu Gly Ile Leu Ser Gly  
 1 5 10 15

Tyr Asp Asp Leu Lys Leu Glu Thr Val Arg Tyr Tyr Asp Leu Ser Asn  
 20 25 30

Val Gln Gly Thr Ala Arg Gly Trp Glu Arg Glu Glu Arg Ile Leu Leu  
 35 40 45

Cys Val Pro Leu Arg Asp Ala Glu Gln His Leu Pro Met Phe Phe Ser  
 50 55 60

His Leu Lys Asn Phe Thr Tyr Pro His Asn Leu Ile Asp Leu Ala Phe  
 65 70 75 80

Leu Val Ser Asp Ser Lys Asp His Thr Leu Glu Ser Leu Thr Glu His  
 85 90 95

Leu Glu Ala Ile Gln Ala Asp Pro Asp Pro Lys Gln Pro Tyr Gly Glu  
 100 105 110

Ile Ser Ile Ile Glu Lys Asp Phe Gly Gln Lys Val Asn Gln Asp Val  
 115 120 125

Glu Ser Arg His Gly Phe Ala Ala Gln Ala Ser Arg Arg Lys Leu Met  
 130 135 140

Ala Gln Ala Arg Asn Trp Leu Leu Ser Ala Ala Leu Arg Pro Tyr His  
 145 150 155 160

Ser Trp Val Tyr Trp Arg Asp Val Asp Val Glu Thr Ala Pro Phe Thr  
 165 170 175

Ile Leu Glu Asp Leu Met Arg His Asn Lys Asp Val Ile Val Pro Asn  
 180 185 190

Val Trp Arg Pro Leu Pro Asp Trp Leu Gly Gly Glu Gln Pro Tyr Asp  
 195 200 205

Leu Asn Ser Trp Gln Glu Ser Glu Thr Ala Leu Ala Leu Ala Asp Thr  
 210 215 220

Leu Asp Glu Asp Ala Val Ile Val Glu Gly Tyr Ala Glu Tyr Ala Thr  
 225 230 235 240

Trp Arg Pro His Leu Ala Tyr Leu Arg Asp Pro Tyr Gly Asp Pro Asp  
 245 250 255

Met Glu Met Glu Ile Asp Gly Val Gly Gly Val Ser Ile Leu Ala Lys  
 260 265 270

Ala Lys Val Phe Arg Ala Gly Val His Phe Pro Ala Phe Ser Phe Glu  
 275 280 285

Lys His Ala Glu Thr Glu Gly Phe Gly Lys Met Ala Lys Arg Met His  
 290 295 300

Phe Ser Val Val Gly Leu Pro His Tyr Thr Ile Trp His Leu Tyr Glu  
 305 310 315 320

Pro Ser Val Asp Asp Ile Lys His Met Glu Glu Met Glu Arg Glu Arg  
 325 330 335

Ile Ala Arg Glu Lys Glu Glu Glu Arg Lys Lys Lys Glu Ala Gln  
340 345 350

Ile Lys Glu Glu Phe Gly Asp Ala Asn Ser Gln Trp Glu Gln Asp Lys  
355 360 365

Gln Gln Met Gln Asp Leu Lys Leu Gln Asp Arg Gly Gly Asp Lys Glu  
370 375 380

Ala Ala Ala Ala Gly Val Asn Gln Gly Ala Ala Ala Lys Ala Ala Gly  
385 390 395 400

Ala Met Glu Gly Gln Lys Asn  
405

<210> 192

<211> 119

<212> PRT

<213> Trichoderma reesei

<400> 192

Met Ser Leu Ser Arg Ser Pro Ser Pro Val Pro Gly Gly Gly Trp Ser  
1 5 10 15

Ser Pro Gly Leu Asn Ile Asn Ser Gly Arg Ser Ser Pro Ser Asn Ala  
20 25 30

Ala Gly Ser Ser Val Ser Trp Glu Ser Ala Lys Met Arg Lys Gln Gly  
35 40 45

Ala Asn Gly Tyr Pro Ser Phe Ser Thr Gln Asn Gln Gly Phe Phe Thr  
50 55 60

Arg His Met Arg Arg Ile Ser Ser Ser Leu Pro Arg Phe Ala Ala Gly  
65 70 75 80

Pro Gly Asn Thr Tyr Ala Glu Arg Glu Lys Tyr Glu Arg Gly Gly His  
85 90 95

Ser Pro His Ala Gly Gly Gly Arg Leu Arg Ala Phe Leu Ala Arg Ile  
100 105 110

Gly Arg Arg Leu Lys Trp Arg  
115

<210> 193

<211> 15

<212> PRT

<213> Trichoderma reesei

<400> 193

Ile Leu Leu Pro Leu Ile Ile Ile Cys Thr Ile Val Ala Tyr Tyr  
1 5 10 15

<210> 194

<211> 325

<212> PRT

<213> Trichoderma reesei

<400> 194

Gly Thr His Glu Ala Pro Gly Phe Val His Trp Trp Arg Arg Ile Ser

1

5

10

15

Met Gly Gly Gly Gly Glu Lys Phe Val Ile Ile Leu Gly Ala Asn Val  
 20 25 30  
 Gly Gly Gly Val Met Glu Trp Lys Gly Ala Arg Glu Trp Ala Ile Glu  
 35 40 45  
 Arg Asp Ser Val Arg Asn Lys Arg Lys Tyr Ala Thr Arg Trp Gly Tyr  
 50 55 60  
 Asp Leu Glu Ile Val Asp Met Lys Thr Lys Lys Arg Tyr Ala His Glu  
 65 70 75 80  
 Trp Arg Glu Ser Trp Glu Lys Val Asp Phe Ile Arg Ala Ala Met Arg  
 85 90 95  
 Lys Tyr Pro Lys Ala Glu Trp Phe Trp Trp Leu Asp Leu Asn Thr Tyr  
 100 105 110  
 Val Met Glu Pro Ser Tyr Ser Leu Gln Arg His Leu Phe Asn His Leu  
 115 120 125  
 Asp Arg His Val Tyr Arg Asp Ile Asn Val Phe Asn Pro Leu Asn Ile  
 130 135 140  
 Thr His Pro Pro Thr Glu Glu Tyr Leu Asp Ala Glu Ala Arg Ser Pro  
 145 150 155 160  
 Val Gly Asp Gly Asn Ile Asn Ser Val Asn Leu Met Leu Thr Gln Asp  
 165 170 175  
 Cys Ser Gly Phe Asn Leu Gly Ser Phe Phe Ile Arg Arg Ser Ala Trp  
 180 185 190  
 Thr Glu Gln Leu Leu Asp Ile Trp Trp Asp Pro Val Leu Tyr Glu Gln  
 195 200 205  
 Lys His Met Glu Trp Glu His Lys Glu Gln Asp Ala Leu Glu Gln Leu  
 210 215 220  
 Tyr Arg Thr Gln Pro Trp Ile Arg Gln His Thr Gly Phe Leu Pro Gln  
 225 230 235 240  
 Arg Leu Ile Asn Ser Phe Pro Pro Ala Ala Cys Ala Asp Glu Ser Gly  
 245 250 255  
 Leu Asn Asn Thr Arg Ile His Tyr Asn Glu Lys Asp Arg Asp Phe Val  
 260 265 270  
 Val Asn Met Ala Gly Cys Glu Trp Gly Arg Asp Cys Trp Gly Glu Met  
 275 280 285  
 Tyr His Tyr Arg Glu Phe Ser Tyr Trp Leu Asn Arg Asn Pro Trp Glu  
 290 295 300  
 Leu Phe Lys Glu Glu Ile Val Ala Val Ile Trp Tyr Lys Leu Thr Gly  
 305 310 315 320  
 Gln Arg Val Lys Leu  
 325

&lt;210&gt; 195

&lt;211&gt; 33

&lt;212&gt; PRT

&lt;213&gt; Trichoderma reesei

&lt;400&gt; 195

Met His Phe Ala Tyr Pro Ser Arg Lys Ser Ser Asn Pro Pro Pro Phe  
 1 5 10 15

Arg Pro Arg Ser Thr Arg Leu Pro Gly Leu Arg Arg Ser Arg Ile Lys  
 20 25 30

Thr

&lt;210&gt; 196

&lt;211&gt; 15

&lt;212&gt; PRT

&lt;213&gt; Trichoderma reesei

&lt;400&gt; 196

Ile Gly Ile Val Leu Phe Leu Val Leu Ala Thr Leu Trp Phe Phe  
 1 5 10 15

&lt;210&gt; 197

&lt;211&gt; 262

&lt;212&gt; PRT

&lt;213&gt; Trichoderma reesei

&lt;400&gt; 197

Ser Asn Pro Arg Val Pro Arg Pro Asp Pro Glu Arg Val Pro Ser Gly  
 1 5 10 15

Arg Pro Pro Val Val Leu Val Thr Val Ile Asp Pro Thr Gln Tyr Pro  
 20 25 30

Asn Ala Tyr Leu Lys Thr Ile Lys Glu Asn Arg Glu Gln Tyr Ala Ala  
 35 40 45

Lys His Gly Tyr Glu Ala Phe Ile Val Lys Ala Tyr Asp Tyr Asp Thr  
 50 55 60

Gln Gly Ala Pro Gln Ser Trp Ser Lys Leu Met Ala Met Arg His Ala  
 65 70 75 80

Leu Thr Lys Phe Pro Glu Cys Arg Phe Val Trp Tyr Leu Asp Gln Asp  
 85 90 95

Ala Tyr Ile Met Asp Met Ser Lys Ser Leu Glu Glu Gln Leu Leu Asn  
 100 105 110

Arg Gln Lys Leu Glu Ser Leu Met Ile Lys Asn Tyr Pro Val Val Pro  
 115 120 125

Pro Asp Ser Ile Ile Lys Thr Phe Ser His Leu Arg Pro Asp Glu Val  
 130 135 140

Asp Leu Ile Val Ser Gln Asp Ser Ser Gly Leu Val Ala Gly Ser Val  
 145 150 155 160

Val Val Arg Asn Ser Gln Trp Ser Lys Phe Leu Leu Glu Thr Trp Met  
 165 170 175

Asp Pro Leu Tyr Arg Ser Tyr Asn Phe Gln Lys Ala Glu Arg His Ala  
 180 185 190

Leu Glu His Ile Val Gln Trp His Pro Thr Ile Leu Ser Lys Leu Ala  
 195 200 205

Leu Val Pro Gln Arg Thr Leu Gly Pro Tyr Thr Arg Thr Asp Gln Gly  
210 215 220

Asp Ala Tyr Gln Asp Gly Asp Phe Val Val Met Phe Thr Gly Cys Thr  
225 230 235 240

Lys Ser Gly Glu Gln Ser Cys Glu Thr Val Ser Ala Ser Tyr Tyr Gln  
245 250 255

Lys Trp Ser Ser Ser Leu  
260

<210> 198

<211> 90

<212> PRT

<213> Trichoderma reesei

<400> 198

Met Ile Arg Asp Pro Phe Gly Ile His Ser Lys Asn Ala Phe Lys Ala  
1 5 10 15

Thr Ala Leu Arg Ala Ala Arg Asp Ile Lys Glu Ala Ala Thr Gln Ala  
20 25 30

Gly Ala Asn Ala Leu Glu Met Ser Phe Ser Leu Pro Lys His Val Pro  
35 40 45

Asp Phe Gly Asp Pro Ser Arg Ala Leu Glu Asp Arg Ala Trp Ala Ala  
50 55 60

Leu Leu Pro Met Tyr Lys Asp Lys Pro Tyr Ala Tyr Ala Pro Ser Met  
65 70 75 80

Arg Leu Arg Pro Trp Trp Arg Arg Arg Lys  
85 90

<210> 199

<211> 19

<212> **PRT**

<213> Trichoderma reesei

<400> 199

Val Leu Gly Met Ile Ala Ala Ala Val Met Phe Val Leu Tyr Val Thr  
1 5 10 15

Gly Phe Phe

<210> 200

<211> 565

<212> PRT

<213> Trichoderma reesei

<400> 200

Ser Ser Gly Gln Thr Glu Glu Ala Lys Lys Lys Ala Ser Gly Ser Ala  
1 5 10 15

Phe Ser Trp Leu Gly Leu Ser Gln Glu Arg Gly Gly Val Asp Trp Asp  
20 25 30

Glu Arg Arg Lys Ser Val Val Glu Ala Phe Glu Val Trp Asp Ala Tyr  
35 40 45

Glu Arg Tyr Ala Trp Gly Lys Asp Glu Phe His Pro Ile Ser Lys Asn  
50 55 60



Gly Arg Asn Met Ala Pro Lys Gly Leu Gly Trp Ile Ile Ile Asp Ser  
 65 70 75 80  
 Leu Asp Thr Met Met Leu Met Asn Gln Thr Thr Arg Leu Gln His Ala  
 85 90 95  
 Arg Glu Trp Ile Ser Thr Ser Leu Thr Trp Asp Gln Asp Gln Asp Val  
 100 105 110  
 Asn Thr Phe Glu Thr Thr Ile Arg Met Leu Gly Gly Leu Leu Ser Ala  
 115 120 125  
 His Tyr Leu Ser Thr Glu Phe Pro Glu Leu Ala Pro Leu Thr Glu Asp  
 130 135 140  
 Asp Glu Gly Ala Pro Gly Glu Asp Leu Tyr Leu Glu Lys Ala Lys Asp  
 145 150 155 160  
 Leu Ala Asp Arg Leu Leu Ser Ala Phe Glu Ser Glu Ser Gly Ile Pro  
 165 170 175  
 Tyr Ala Ser Val Asn Ile Gly Glu Tyr Lys Gly Pro Ser His Ser Asp  
 180 185 190  
 Asn Gly Ala Ser Ser Thr Ala Glu Ala Thr Thr Leu Gln Leu Glu Phe  
 195 200 205  
 Lys Tyr Leu Ala Lys Leu Thr Gly Glu Lys Asn Phe Trp Asp Lys Val  
 210 215 220  
 Glu Lys Val Met Glu Val Val Asp Asp Asn Gln Pro Glu Asp Gly Leu  
 225 230 235 240  
 Val Pro Ile Tyr Ile Tyr Ala Thr Thr Gly Glu Phe Arg Gly Gln Asn  
 245 250 255  
 Ile Arg Leu Gly Ser Arg Gly Asp Ser Tyr Tyr Glu Tyr Leu Ile Lys  
 260 265 270  
 Gln Tyr Leu Gln Thr Asn Lys Gln Glu Pro Ile Tyr Glu Glu Met Trp  
 275 280 285  
 Asp Glu Ala Leu Ala Gly Val Arg Lys His Leu Val Thr Tyr Thr Glu  
 290 295 300  
 Pro Ser Glu Phe Thr Ile Ile Ala Glu Arg Pro Asp Gly Leu Glu His  
 305 310 315 320  
 Pro Met Ser Pro Lys Met Asp His Leu Val Cys Phe Met Pro Gly Thr  
 325 330 335  
 Ile Ala Leu Ala Ala Thr Gly Gly Leu Thr Glu Ala Glu Ala Arg Lys  
 340 345 350  
 Leu Ser Thr Trp Asn Lys Lys Lys Asp Asp Asp Met Gln Leu Ala Arg  
 355 360 365  
 Glu Leu Met His Thr Cys Trp Gly Met Tyr Lys Tyr Met Lys Thr Gly  
 370 375 380  
 Leu Ala Pro Glu Ile Met Tyr Phe Asn Ile Pro Asn Pro Pro Pro Glu  
 385 390 395 400  
 Ser Ser Ala Pro His Gln Ala Pro Ala Ala Phe Asp Glu Asp Pro His  
 405 410 415

Ala Glu Trp Arg Lys Asp Phe Val Val His Ser Asn Asp Val His Asn  
420 425 430

Leu Gln Arg Pro Glu Thr Val Glu Ser Leu Phe Tyr Met Trp Arg Ile  
435 440 445

Thr Gly Asp Val Lys Tyr Arg Glu Trp Gly Trp Asp Met Phe Lys Ser  
450 455 460

Phe Val Asn Tyr Thr Ala Val Glu Asp Gln Gly Gly Phe Thr Ser Leu  
465 470 475 480

Leu Asp Ala Asn Ser Ile Pro Pro Thr Pro Lys Asp Asn Met Glu Ser  
485 490 495

Phe Trp Leu Ala Glu Thr Leu Lys Tyr Met Tyr Leu Leu Phe Ser Pro  
500 505 510

Asn Asp Val Leu Pro Leu His Lys Ile Val Leu Asn Thr Glu Ala His  
515 520 525

Pro Phe Pro Arg Phe Asp Met Gly Pro Leu Phe Ser Thr Gly Trp Lys  
530 535 540

Arg Lys Pro Arg Asp Gly Ser Ala Lys Lys Lys Ala Thr Thr Ala Ala

545 550 555 560

Thr Thr Asp Ala Glu  
565

<210> 201

<211> 7

<212> PRT

<213> Trichoderma reesei

<400> 201

Met Ala Arg Arg Arg Tyr Arg  
1 5

<210> 202

<211> 15

<212> PRT

<213> Trichoderma reesei

<400> 202

Leu Phe Met Ile Cys Ala Ala Val Ile Leu Phe Leu Leu Tyr Arg  
1 5 10 15

<210> 203

<211> 871

<212> PRT

<213> Trichoderma reesei

<400> 203

Val Ser Gln Asn Thr Trp Asp Asp Ser Ala His Tyr Ala Thr Leu Arg  
1 5 10 15

His Pro Pro Ala Ser Asn Pro Pro Ala Ala Gly Gly Glu Ser Pro Leu  
20 25 30

Lys Pro Ala Ala Lys Pro Glu His Glu His Glu His Glu Asn Gly Tyr  
35 40 45

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Ala Pro Glu Ser Lys Pro Lys Pro Gln Ser Glu Pro Lys Pro Glu Ser
50                      55                      60

Lys Pro Ala Pro Glu His Ala Ala Gly Gly Gln Lys Ser Gln Gly Lys
65                      70                      75                      80

Pro Ser Tyr Glu Asp Asp Glu Glu Thr Gly Lys Asn Pro Pro Lys Ser
85                      90                      95

Ala Val Ile Pro Ser Asp Thr Arg Leu Pro Pro Asp Asn Lys Val His
100                     105                     110

Trp Arg Pro Val Lys Glu His Phe Pro Val Pro Ser Glu Ser Val Ile
115                     120                     125

Ser Leu Pro Thr Gly Lys Pro Leu Lys Val Pro Arg Val Gln His Glu
130                     135                     140

Phe Gly Val Glu Ser Pro Glu Ala Lys Ser Arg Arg Val Ala Arg Gln
145                     150                     155                     160

Glu Arg Val Gly Lys Glu Ile Glu Arg Ala Trp Ser Gly Tyr Lys Lys
165                     170                     175

Phe Ala Trp Met His Asp Glu Leu Ser Pro Val Ser Ala Lys His Arg
180                     185                     190

Asp Pro Phe Cys Gly Trp Ala Ala Thr Leu Val Asp Ser Leu Asp Thr
195                     200                     205

Leu Trp Ile Ala Gly Leu Lys Glu Gln Phe Asp Glu Ala Ala Arg Ala
210                     215                     220

Val Glu Gln Ile Asp Phe Thr Thr Thr Pro Arg Asn Asn Ile Pro Val
225                     230                     235                     240

Phe Glu Thr Thr Ile Arg Tyr Leu Gly Gly Leu Leu Gly Ala Phe Asp
245                     250                     255

Val Ser Gly Gly His Asp Gly Gly Tyr Pro Met Leu Leu Thr Lys Ala
260                     265                     270

Val Glu Leu Ala Glu Ile Leu Met Gly Ile Phe Asp Thr Pro Asn Arg
275                     280                     285

Met Pro Ile Leu Tyr Tyr Gln Trp Gln Pro Glu Tyr Ala Ser Gln Pro
290                     295                     300

His Arg Ala Gly Ser Val Gly Ile Ala Glu Leu Gly Thr Leu Ser Met
305                     310                     315                     320

Glu Phe Thr Arg Leu Ala Gln Leu Thr Ser Gln Tyr Lys Tyr Tyr Asp
325                     330                     335

Ala Val Asp Arg Ile Thr Asp Ala Leu Ile Glu Leu Gln Lys Gln Gly
340                     345                     350

Thr Ser Ile Pro Gly Leu Phe Pro Glu Asn Leu Asp Ala Ser Gly Cys
355                     360                     365

Asn His Thr Ala Thr Ala Leu Arg Ser Ser Leu Ser Glu Ala Ala Gln
370                     375                     380

Lys Gln Met Asp Glu Asp Leu Ser Asn Lys Pro Glu Asn Tyr Arg Pro
385                     390                     395                     400

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Gly Lys Asn Ser Lys Ala Asp Pro Gln Thr Val Glu Lys Gln Pro Ala  
 405 410 415  
 Lys Lys Gln Asn Glu Pro Val Glu Lys Ala Lys Gln Val Pro Thr Gln  
 420 425 430  
 Gln Thr Ala Lys Arg Gly Lys Pro Pro Phe Gly Ala Asn Gly Phe Thr  
 435 440 445  
 Ala Asn Trp Asp Cys Val Pro Gln Gly Leu Val Val Gly Gly Tyr Gly  
 450 455 460  
 Phe Gln Gln Tyr His Met Gly Gly Gly Gln Asp Ser Ala Tyr Glu Tyr  
 465 470 475 480  
 Phe Pro Lys Glu Tyr Leu Leu Leu Gly Gly Leu Glu Ser Lys Tyr Gln  
 485 490 495  
 Lys Leu Tyr Val Asp Ala Val Glu Ala Ile Asn Glu Trp Leu Leu Tyr  
 500 505 510  
 Arg Pro Met Thr Asp Gly Asp Trp Asp Ile Leu Phe Pro Ala Lys Val  
 515 520 525  
 Ser Thr Ala Gly Asn Pro Ser Gln Asp Leu Val Ala Thr Phe Glu Val  
 530 535 540  
 Thr His Leu Thr Cys Phe Ile Gly Gly Met Tyr Gly Leu Gly Gly Lys  
 545 550 555 560  
 Ile Phe Gly Arg Glu Lys Asp Leu Glu Thr Ala Lys Arg Leu Thr Asp  
 565 570 575  
 Gly Cys Val Trp Ala Tyr Gln Ser Thr Val Ser Gly Ile Met Pro Glu  
 580 585 590  
 Gly Ser Gln Val Leu Ala Cys Pro Thr Leu Glu Lys Cys Asp Phe Asn  
 595 600 605  
 Glu Thr Leu Trp Trp Glu Lys Leu Asp Pro Ala Lys Asp Trp Arg Asp  
 610 615 620  
 Lys Gln Val Ala Asp Asp Lys Asp Lys Ala Thr Val Gly Glu Ala Leu  
 625 630 635 640  
 Lys Glu Thr Ala Asn Ser His Asp Ala Ala Gly Gly Ser Lys Ala Val  
 645 650 655  
 His Lys Arg Ala Ala Val Pro Leu Pro Lys Pro Gly Ala Asp Asp Asp  
 660 665 670  
 Val Gly Ser Glu Leu Pro Gln Ser Leu Lys Asp Lys Ile Gly Phe Lys  
 675 680 685  
 Asn Gly Glu Gln Lys Lys Pro Thr Gly Ser Ser Val Gly Ile Gln Arg  
 690 695 700  
 Asp Pro Asp Ala Pro Val Asp Ser Val Leu Glu Ala His Arg Leu Pro  
 705 710 715 720  
 Pro Gln Glu Pro Glu Glu Gln Gln Val Ile Leu Pro Asp Lys Pro Gln  
 725 730 735

Thr His Glu Glu Phe Val Lys Gln Arg Ile Ala Glu Met Gly Phe Ala  
740 745 750

Pro Gly Val Val His Ile Gln Ser Arg Gln Tyr Ile Leu Arg Pro Glu  
755 760 765

Ala Ile Glu Ser Val Trp Tyr Met Tyr Arg Ile Thr Gly Asp Pro Ile  
770 775 780

Trp Met Glu Lys Gly Trp Lys Met Phe Glu Ala Thr Ile Arg Ala Thr  
785 790 795 800

Arg Thr Glu Ile Ala Asn Ser Ala Ile Asp Asp Val Asn Ser Glu Glu  
805 810 815

Pro Gly Leu Lys Asp Glu Met Glu Ser Phe Trp Leu Ala Glu Thr Leu  
820 825 830

Lys Tyr Tyr Tyr Leu Leu Phe Ser Glu Pro Ser Val Ile Ser Leu Asp  
835 840 845

Glu Trp Val Leu Asn Thr Glu Ala His Pro Phe Lys Arg Pro Gly Gly  
850 855 860

Ser Val Ile Gly His Ser Ile  
865 870

<210> 204

<211> 13

<212> PRT

<213> Trichoderma reesei

<400> 204

Met Leu Asn Gln Leu Gln Gly Arg Val Pro Arg Arg Tyr  
1 5 10

<210> 205

<211> 15

<212> PRT

<213> Trichoderma reesei

<400> 205

Ile Ala Leu Val Ala Phe Ala Phe Phe Val Ala Phe Leu Leu Trp  
1 5 10 15

<210> 206

<211> 542

<212> PRT

<213> Trichoderma reesei

<400> 206

Ser Gly Tyr Asp Phe Val Pro Arg Thr Ala Thr Val Gly Arg Phe Lys  
1 5 10 15

Tyr Val Pro Ser Ser Tyr Asp Trp Ser Lys Ala Lys Val Tyr Tyr Pro  
20 25 30

Val Lys Asp Met Lys Thr Leu Pro Gln Gly Thr Pro Val Thr Phe Pro  
35 40 45

Arg Leu Gln Leu Arg Asn Gln Ser Glu Ala Gln Asp Asp Thr Thr Lys  
50 55 60

Ala Arg Lys Gln Ala Val Lys Asp Ala Phe Val Lys Ser Trp Glu Ala  
65 70 75 80

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      60              70              80              90
Tyr Lys Thr Tyr Ala Trp Thr Lys Asp Gln Leu Gln Pro Leu Ser Leu
      85              90              95

Ser Gly Lys Glu Thr Phe Ser Gly Trp Ser Ala Gln Leu Val Asp Ala
      100              105              110

Leu Asp Thr Leu Trp Ile Met Asp Leu Lys Asp Asp Phe Phe Leu Ala
      115              120              125

Val Lys Glu Val Ala Val Ile Asp Trp Ser Lys Thr Lys Asp Asn Lys
      130              135              140

Val Ile Asn Leu Phe Glu Val Thr Ile Arg Tyr Leu Gly Gly Leu Ile
      145              150              155              160

Ala Ala Tyr Asp Leu Ser Gln Glu Pro Val Leu Arg Ala Lys Ala Ile
      165              170              175

Glu Leu Gly Asp Thr Leu Tyr Ala Thr Phe Asp Thr Pro Asn Arg Leu
      180              185              190

Pro Ser His Trp Leu Asp Tyr Ser Lys Ala Lys Lys Gly Thr Gln Arg
      195              200              205

Ala Asp Asp Ser Met Ser Gly Ala Ala Gly Gly Thr Leu Cys Met Glu
      210              215              220

Phe Thr Arg Leu Ser Gln Ile Thr Gly Asp Pro Lys Tyr Tyr Asp Ala
      225              230              235              240

Thr Glu Arg Ile Lys Gln Phe Phe Tyr Arg Phe Gln Asn Glu Thr Thr
      245              250              255

Leu Pro Gly Met Trp Pro Val Met Met Asn Tyr Arg Glu Glu Thr Met
      260              265              270

Val Glu Ser Arg Tyr Ser Met Gly Gly Ser Ala Asp Ser Leu Tyr Glu
      275              280              285

Tyr Leu Val Lys Met Pro Ala Leu Leu Gly Gly Leu Asp Pro Gln Tyr
      290              295              300

Pro Glu Met Ala Ile Arg Ala Leu Asp Thr Ala Arg Asp Asn Leu Leu
      305              310              315              320

Phe Arg Pro Met Thr Glu Lys Gly Asp Asn Ile Leu Ala Leu Gly Asn
      325              330              335

Ala Leu Val Asp His Gly Asn Val Gln Arg Ile Thr Glu Met Gln His
      340              345              350

Leu Thr Cys Phe Ala Gly Gly Met Tyr Ala Met Ala Gly Lys Leu Phe
      355              360              365

Lys Arg Asp Asp Tyr Val Asp Leu Gly Ser Arg Ile Ser Ser Gly Cys
      370              375              380

Val Trp Ala Tyr Asp Ser Phe Pro Ser Gly Ile Met Pro Glu Ser Ala
      385              390              395              400

Asp Met Ala Ala Cys Ala Lys Leu Asp Gly Pro Cys Pro Tyr Asp Glu
      405              410              415

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Val Lys Ala Pro Val Asp Pro Asp Gly Arg Arg Pro His Gly Phe Ile  
420 425 430

His Val Lys Ser Arg His Tyr Leu Leu Arg Pro Glu Ala Ile Glu Ser  
435 440 445

Val Phe Tyr Met Trp Arg Ile Thr Gly Asp Gln Val Trp Arg Asp Thr  
450 455 460

Ala Trp Arg Met Trp Glu Asn Ile Val Arg Glu Ala Glu Thr Glu His  
465 470 475 480

Ala Phe Ala Ile Val Glu Asp Val Thr Arg Thr Ala Ser Lys Leu Thr  
485 490 495

Asn Asn Tyr Leu Leu Gln Thr Phe Trp Leu Ala Glu Thr Leu Lys Tyr  
500 505 510

Phe Tyr Leu Ile Phe Asp Asp Glu Ser Ala Ile Asp Leu Asp Lys Trp  
515 520 525

Val Phe Asn Thr Glu Ala His Pro Phe Lys Arg Pro Ala Val  
530 535 540

<210> 207

<211> 13

<212> PRT

<213> Trichoderma reesei

<400> 207

Met Leu Val Val Gly Arg Pro Arg Leu Val Arg Asn Ser  
1 5 10

<210> 208

<211> 16

<212> PRT

<213> Trichoderma reesei

<400> 208

Ile Ile Leu Thr Leu Ala Ile Leu Ser Ile Trp His Leu Gly Leu Leu  
1 5 10 15

<210> 209

<211> 576

<212> PRT

<213> Trichoderma reesei

<400> 209

Ser Arg Thr Pro Thr Ser Ala Ser Ala Leu Val Ser Ala Ser Val Ser  
1 5 10 15

Ala Ser Ser Glu Trp Ser Arg Leu Glu Arg Leu Met Asn Arg Gly Ala  
20 25 30

Pro Leu Thr Pro Tyr Pro Asp Ser Asn Ser Ser Phe Asp Trp Ser Ala  
35 40 45

Ile Pro Phe Arg Tyr Pro Pro His Asn Thr Thr His Leu Pro Pro Arg  
50 55 60

His Lys Gln Pro Pro Leu Pro Arg Ile Gln His Arg Phe Gly Pro Glu  
65 70 75 80

Ser Pro Ala Ala Ala Lys Glu Arg Ile Lys Arg Leu Lys Ala Val Lys  
 85 90 95  
 Gln Val Phe Leu Arg Ala Trp Gln Ala Tyr Lys Gly Tyr Ala Trp Lys  
 100 105 110  
 Gln Asp Ala Leu Leu Pro Ile Ser Gly Gly Gly Arg Glu Gln Phe Ser  
 115 120 125  
 Gly Trp Ala Ala Thr Leu Val Asp Ala Leu Asp Thr Leu Trp Ile Met  
 130 135 140  
 Gly Leu Arg Glu Glu Phe Asp Glu Ala Val Ala Ala Val Ala Glu Ile  
 145 150 155 160  
 Asp Phe Gly Ser Ser Thr Ser Ser Arg Val Asn Ile Phe Glu Thr Asn  
 165 170 175  
 Ile Arg Tyr Leu Gly Gly Leu Leu Ala Ala Tyr Asp Leu Ser Gly Arg  
 180 185 190  
 Glu Val Leu Leu Lys Lys Ala Val Glu Leu Gly Asp Leu Ile Tyr Ala  
 195 200 205  
 Gly Phe Asn Thr Glu Asn Gly Met Pro Val Asp Phe Leu Asn Phe Tyr  
 210 215 220  
 Ser Ala Lys Ser Gly Glu Gly Leu Val Val Glu Ser Ser Val Val Ser  
 225 230 235 240  
 Ala Ser Pro Gly Thr Leu Ser Leu Glu Leu Ala His Leu Ser Gln Val  
 245 250 255  
 Thr Gly Asp Asp Lys Tyr Tyr Ser Ala Val Ser Gln Val Met Asp Val  
 260 265 270  
 Phe Tyr Gln Gly Gln Asn Lys Thr Arg Leu Pro Gly Val Trp Pro Ile  
 275 280 285  
 Asp Val Asn Met Arg Ala Lys Asp Val Val Ser Gly Ser Arg Phe Thr  
 290 295 300  
 Leu Gly Gly Cys Ala Asp Ser Leu Tyr Glu Tyr Leu Pro Lys Met His  
 305 310 315 320  
 Gln Leu Leu Gly Gly Gly Glu Pro Lys Tyr Glu Thr Met Ser Arg Thr  
 325 330 335  
 Phe Leu Gln Ala Ala Asp Arg His Phe Val Phe Arg Pro Met Leu Pro  
 340 345 350  
 Gly Ala Glu Glu Asp Val Leu Met Pro Gly Asn Val Asn Val Asp Glu  
 355 360 365  
 Asp Ser Gly Glu Ala Val Leu Asp Pro Glu Thr Glu His Leu Ala Cys  
 370 375 380  
 Phe Val Gly Gly Met Phe Gly Leu Ala Gly Arg Leu Phe Ser Arg Pro  
 385 390 395 400  
 Asp Asp Val Glu Thr Gly Val Arg Leu Thr Asn Gly Cys Val Tyr Ala  
 405 410 415  
 Tyr Arg Ala Phe Pro Thr Gly Met Met Pro Glu Arg Leu Asp Leu Ala  
 420 425 430



Pro Cys Arg Asp Arg Ser Ser Arg Cys Pro Trp Asp Glu Glu His Trp  
435 440 445

Leu Glu Glu Arg Ala Lys Arg Pro Glu Trp Glu Pro His Leu Pro Arg  
450 455 460

Gly Phe Thr Ser Ala Lys Asp Pro Arg Tyr Leu Leu Arg Pro Glu Ala  
465 470 475 480

Ile Glu Ser Val Phe Tyr Ser Tyr Arg Ile Thr Gly Arg Gln Glu Phe  
485 490 495

Gln Thr Ala Ala Trp Asp Met Phe Thr Ala Val Glu Lys Gly Thr Arg  
500 505 510

Thr Gln Phe Ala Asn Ala Ala Val Leu Asp Val Thr Arg Ala Ala Asp  
515 520 525

Glu Leu Pro Gln Glu Asp Tyr Met Glu Ser Phe Trp Leu Ala Glu Thr  
530 535 540

Leu Lys Tyr Phe Tyr Leu Met Phe Thr Thr Pro Asp Ile Ile Ser Leu  
545 550 555 560

Asp Asp Tyr Val Leu Asn Thr Glu Ala His Pro Phe Lys Leu Val Gly  
565 570 575

<210> 210

<211> 17

<212> PRT

<213> Trichoderma reesei

<400> 210

Met Val Met Leu Val Ala Ile Ala Leu Ala Trp Leu Gly Cys Ser Leu  
1 5 10 15

Leu

<210> 211

<211> 1053

<212> PRT

<213> Trichoderma reesei

<400> 211

Arg Pro Val Asp Ala Met Arg Ala Asp Tyr Leu Ala Gln Leu Arg Gln  
1 5 10 15

Glu Thr Val Asp Met Phe Tyr His Gly Tyr Ser Asn Tyr Met Glu His  
20 25 30

Ala Phe Pro Glu Asp Glu Leu Arg Pro Ile Ser Cys Thr Pro Leu Thr  
35 40 45

Arg Asp Arg Asp Asn Pro Gly Arg Ile Ser Leu Asn Asp Ala Leu Gly  
50 55 60

Asn Tyr Ser Leu Thr Leu Ile Asp Ser Leu Ser Thr Leu Ala Ile Leu  
65 70 75 80

Ala Gly Gly Pro Gln Asn Gly Pro Tyr Thr Gly Pro Gln Ala Leu Ser  
85 90 95

Asp Phe Gln Asp Gly Val Ala Glu Phe Val Arg His Tyr Gly Asp Gly  
 100 105 110  
 Arg Ser Gly Pro Ser Gly Ala Gly Ile Arg Ala Arg Gly Phe Asp Leu  
 115 120 125  
 Asp Ser Lys Val Gln Val Phe Glu Thr Val Ile Arg Gly Val Gly Gly  
 130 135 140  
 Leu Leu Ser Ala His Leu Phe Ala Ile Gly Glu Leu Pro Ile Thr Gly  
 145 150 155 160  
 Tyr Val Pro Arg Pro Glu Gly Val Ala Gly Asp Asp Pro Leu Glu Leu  
 165 170 175  
 Ala Pro Ile Pro Trp Pro Asn Gly Phe Arg Tyr Asp Gly Gln Leu Leu  
 180 185 190  
 Arg Leu Ala Leu Asp Leu Ser Glu Arg Leu Leu Pro Ala Phe Tyr Thr  
 195 200 205  
 Pro Thr Gly Ile Pro Tyr Pro Arg Val Asn Leu Arg Ser Gly Ile Pro  
 210 215 220  
 Phe Tyr Val Asn Ser Pro Leu His Gln Asn Leu Gly Glu Ala Val Glu  
 225 230 235 240  
 Glu Gln Ser Gly Arg Pro Glu Ile Thr Glu Thr Cys Ser Ala Gly Ala  
 245 250 255  
 Gly Ser Leu Val Leu Glu Phe Thr Val Leu Ser Arg Leu Thr Gly Asp  
 260 265 270  
 Ala Arg Phe Glu Gln Ala Ala Lys Arg Ala Phe Trp Glu Val Trp His  
 275 280 285  
 Arg Arg Ser Glu Ile Gly Leu Ile Gly Asn Gly Ile Asp Ala Glu Arg  
 290 295 300  
 Gly Leu Trp Ile Gly Pro His Ala Gly Ile Gly Ala Gly Met Asp Ser  
 305 310 315 320  
 Phe Phe Glu Tyr Ala Leu Lys Ser His Ile Leu Leu Ser Gly Leu Gly  
 325 330 335  
 Met Pro Asn Ala Ser Thr Ser Arg Arg Gln Ser Thr Thr Ser Trp Leu  
 340 345 350  
 Asp Pro Asn Ser Leu His Pro Pro Leu Pro Pro Glu Met His Thr Ser  
 355 360 365  
 Asp Ala Phe Leu Gln Ala Trp His Gln Ala His Ala Ser Val Lys Arg  
 370 375 380  
 Tyr Leu Tyr Thr Asp Arg Ser His Phe Pro Tyr Tyr Ser Asn Asn His  
 385 390 395 400  
 Arg Ala Thr Gly Gln Pro Tyr Ala Met Trp Ile Asp Ser Leu Gly Ala  
 405 410 415  
 Phe Tyr Pro Gly Leu Leu Ala Leu Ala Gly Glu Val Glu Glu Ala Ile  
 420 425 430  
 Glu Ala Asn Leu Val Tyr Thr Ala Leu Trp Thr Arg Tyr Ser Ala Leu  
 435 440 445

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Pro Glu Arg Trp Ser Val Arg Glu Gly Asn Val Glu Ala Gly Ile Gly
450          455          460

Trp Trp Pro Gly Arg Pro Glu Phe Ile Glu Ser Thr Tyr His Ile Tyr
465          470          475          480

Arg Ala Thr Arg Asp Pro Trp Tyr Leu His Val Gly Glu Met Val Leu
485          490          495

Arg Asp Ile Arg Arg Arg Cys Tyr Ala Glu Cys Gly Trp Ala Gly Leu
500          505          510

Gln Asp Val Gln Thr Gly Glu Lys Gln Asp Arg Met Glu Ser Phe Phe
515          520          525

Leu Gly Glu Thr Ala Lys Tyr Met Tyr Leu Leu Phe Asp Pro Asp His
530          535          540

Pro Leu Asn Lys Leu Asp Ala Ala Tyr Val Phe Thr Thr Glu Gly His
545          550          555          560

Pro Leu Ile Ile Pro Lys Ser Lys Arg Gly Ser Gly Ser His Asn Arg
565          570          575

Gln Asp Arg Ala Arg Lys Ala Lys Lys Ser Arg Asp Val Ala Val Tyr
580          585          590

Thr Tyr Tyr Asp Glu Ser Phe Thr Asn Ser Cys Pro Ala Pro Arg Pro
595          600          605

Pro Ser Glu His His Leu Ile Gly Ser Ala Thr Ala Ala Arg Pro Asp
610          615          620

Leu Phe Ser Val Ser Arg Phe Thr Asp Leu Tyr Arg Thr Pro Asn Val
625          630          635          640

His Gly Pro Leu Glu Lys Val Glu Met Arg Asp Lys Lys Lys Gly Arg
645          650          655

Val Val Arg Tyr Arg Ala Thr Ser Asn His Thr Ile Phe Pro Trp Thr
660          665          670

Leu Pro Pro Ala Met Leu Pro Glu Asn Gly Thr Cys Ala Ala Pro Pro
675          680          685

Glu Arg Ile Ile Ser Leu Ile Glu Phe Pro Ala Asn Asp Ile Thr Ser
690          695          700

Gly Ile Thr Ser Arg Phe Gly Asn His Leu Ser Trp Gln Thr His Leu
705          710          715          720

Gly Pro Thr Val Asn Ile Leu Glu Gly Leu Arg Leu Gln Leu Glu Gln
725          730          735

Val Ser Asp Pro Ala Thr Gly Glu Asp Lys Trp Arg Ile Thr His Ile
740          745          750

Gly Asn Thr Gln Leu Gly Arg His Glu Thr Val Phe Phe His Ala Glu
755          760          765

His Val Arg His Leu Lys Asp Glu Val Phe Ser Cys Arg Arg Arg Arg
770          775          780

Asp Ala Val Glu Ile Glu Leu Leu Val Asp Lys Pro Ser Asp Thr Asn
785          790          795          800

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Asn Asn Asn Thr Leu Ala Ser Ser Asp Asp Asp Val Val Val Asp Ala  
 805 810 815  
 Lys Ala Glu Glu Gln Asp Gly Met Leu Ala Asp Asp Asp Gly Asp Thr  
 820 825 830  
 Leu Asn Ala Glu Thr Leu Ser Ser Asn Ser Leu Phe Gln Ser Leu Leu  
 835 840 845  
 Arg Ala Val Ser Ser Val Phe Glu Pro Val Tyr Thr Ala Ile Pro Glu  
 850 855 860  
 Ser Asp Pro Ser Ala Gly Thr Ala Lys Val Tyr Ser Phe Asp Ala Tyr  
 865 870 875 880  
 Thr Ser Thr Gly Pro Gly Ala Tyr Pro Met Pro Ser Ile Ser Asp Thr  
 885 890 895  
 Pro Ile Pro Gly Asn Pro Phe Tyr Asn Phe Arg Asn Pro Ala Ser Asn  
 900 905 910  
 Phe Pro Trp Ser Thr Val Phe Leu Ala Gly Gln Ala Cys Glu Gly Pro  
 915 920 925  
 Leu Pro Ala Ser Ala Pro Arg Glu His Gln Val Ile Val Met Leu Arg  
 930 935 940  
 Gly Gly Cys Ser Phe Ser Arg Lys Leu Asp Asn Ile Pro Ser Phe Ser  
 945 950 955 960  
 Pro His Asp Arg Ala Leu Gln Leu Val Val Val Leu Asp Glu Pro Pro  
 965 970 975  
 Pro Pro Pro Pro Pro Pro Pro Ala Asn Asp Arg Arg Asp Val Thr Arg  
 980 985 990  
 Pro Leu Leu Asp Thr Glu Gln Thr Thr Pro Lys Gly Met Lys Arg Leu  
 995 1000 1005  
 His Gly Ile Pro Met Val Leu Val Arg Ala Ala Arg Gly Asp Tyr  
 1010 1015 1020  
 Glu Leu Phe Gly His Ala Ile Gly Val Gly Met Arg Arg Lys Tyr  
 1025 1030 1035  
 Arg Val Glu Ser Gln Gly Leu Val Val Glu Asn Ala Val Val Leu  
 1040 1045 1050

&lt;210&gt; 212

&lt;211&gt; 45

&lt;212&gt; PRT

&lt;213&gt; Trichoderma reesei

&lt;400&gt; 212

Met Met Pro Arg His His Ser Ser Gly Phe Ser Asn Gly Tyr Pro Arg  
 1 5 10 15  
 Ala Asp Thr Phe Glu Ile Ser Pro His Arg Phe Gln Pro Arg Ala Thr  
 20 25 30  
 Leu Pro Pro His Arg Lys Arg Lys Arg Thr Ala Ile Arg  
 35 40 45

&lt;210&gt; 213

&lt;211&gt; 16

&lt;212&gt; PRT

&lt;213&gt; Trichoderma reesei

&lt;400&gt; 213

Val Gly Ile Ala Val Val Val Ile Leu Val Leu Val Leu Trp Phe Gly  
 1 5 10 15

&lt;210&gt; 214

&lt;211&gt; 407

&lt;212&gt; PRT

&lt;213&gt; Trichoderma reesei

&lt;400&gt; 214

Gln Pro Arg Ser Val Ala Ser Leu Ile Ser Leu Gly Ile Leu Ser Gly  
 1 5 10 15

Tyr Asp Asp Leu Lys Leu Glu Thr Val Arg Tyr Tyr Asp Leu Ser Asn  
 20 25 30

Val Gln Gly Thr Ala Arg Gly Trp Glu Arg Glu Glu Arg Ile Leu Leu  
 35 40 45

Cys Val Pro Leu Arg Asp Ala Glu Gln His Leu Pro Met Phe Phe Ser  
 50 55 60

His Leu Lys Asn Phe Thr Tyr Pro His Asn Leu Ile Asp Leu Ala Phe  
 65 70 75 80

Leu Val Ser Asp Ser Lys Asp His Thr Leu Glu Ser Leu Thr Glu His  
 85 90 95

Leu Glu Ala Ile Gln Ala Asp Pro Asp Pro Lys Gln Pro Tyr Gly Glu  
 100 105 110

Ile Ser Ile Ile Glu Lys Asp Phe Gly Gln Lys Val Asn Gln Asp Val  
 115 120 125

Glu Ser Arg His Gly Phe Ala Ala Gln Ala Ser Arg Arg Lys Leu Met  
 130 135 140

Ala Gln Ala Arg Asn Trp Leu Leu Ser Ala Ala Leu Arg Pro Tyr His  
 145 150 155 160

Ser Trp Val Tyr Trp Arg Asp Val Asp Val Glu Thr Ala Pro Phe Thr  
 165 170 175

Ile Leu Glu Asp Leu Met Arg His Asn Lys Asp Val Ile Val Pro Asn  
 180 185 190

Val Trp Arg Pro Leu Pro Asp Trp Leu Gly Gly Glu Gln Pro Tyr Asp  
 195 200 205

Leu Asn Ser Trp Gln Glu Ser Glu Thr Ala Leu Ala Leu Ala Asp Thr  
 210 215 220

Leu Asp Glu Asp Ala Val Ile Val Glu Gly Tyr Ala Glu Tyr Ala Thr  
 225 230 235 240

Trp Arg Pro His Leu Ala Tyr Leu Arg Asp Pro Tyr Gly Asp Pro Asp  
 245 250 255

Met Glu Met Glu Ile Asp Gly Val Gly Gly Val Ser Ile Leu Ala Lys  
 260 265 270

Ala Lys Val Phe Arg Ala Gly Val His Phe Pro Ala Phe Ser Phe Glu  
275 280 285

Lys His Ala Glu Thr Glu Gly Phe Gly Lys Met Ala Lys Arg Met His  
290 295 300

Phe Ser Val Val Gly Leu Pro His Tyr Thr Ile Trp His Leu Tyr Glu  
305 310 315 320

Pro Ser Val Asp Asp Ile Lys His Met Glu Glu Met Glu Arg Glu Arg  
325 330 335

Ile Ala Arg Glu Lys Glu Glu Glu Glu Arg Lys Lys Lys Glu Ala Gln  
340 345 350

Ile Lys Glu Glu Phe Gly Asp Ala Asn Ser Gln Trp Glu Gln Asp Lys  
355 360 365

Gln Gln Met Gln Asp Leu Lys Leu Gln Asp Arg Gly Gly Asp Lys Glu  
370 375 380

Ala Ala Ala Ala Gly Val Asn Gln Gly Ala Ala Ala Lys Ala Ala Gly  
385 390 395 400

Ala Met Glu Gly Gln Lys Asn  
405

<210> 215

<211> 31

<212> PRT

<213> Trichoderma reesei

<400> 215

Met Leu Leu Pro Lys Gly Gly Leu Asp Trp Arg Ser Ala Arg Ala Gln  
1 5 10 15

Ile Pro Pro Thr Arg Ala Leu Trp Asn Ala Val Thr Arg Thr Arg  
20 25 30

<210> 216

<211> 15

<212> PRT

<213> Trichoderma reesei

<400> 216

Phe Ile Leu Leu Val Gly Ile Thr Gly Leu Ile Leu Leu Trp  
1 5 10 15

<210> 217

<211> 358

<212> PRT

<213> Trichoderma reesei

<400> 217

Arg Gly Val Ser Thr Ser Ala Ser Glu Met Gln Ser Phe Tyr Cys Trp  
1 5 10 15

Gly Pro Ala Lys Pro Pro Met Glu Met Ser Pro Asn Glu His Asn Arg  
20 25 30

Trp Asn Gly His Leu Gln Thr Pro Val Ile Phe Asn His His Ala Pro  
35 40 45

Val Glu Val Asn Ser Ser Thr Ile Glu His Val Asn Leu Asn Pro Ile

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Val Glu Val Asn Ser Ser Thr Thr Lys Glu His Val Asp Leu Asn Phe Ile
50                               55                               60

Asn Ser Thr Lys Gln Ala Val Thr Lys Glu Glu Arg Ile Leu Ile Leu

65                               70                               75                               80

Thr Pro Leu Lys Asp Ala Ala Pro Tyr Leu Ser Lys Tyr Phe Glu Leu
85                               90                               95

Leu Ala Glu Leu Thr Tyr Pro His Arg Leu Ile Asp Leu Ala Phe Leu
100                              105                              110

Val Ser Asp Ser Thr Asp Asp Thr Leu Ala Val Leu Ala Ser Glu Leu
115                              120                              125

Asp Arg Ile Gln Lys Arg Pro Asp Gln Ile Pro Phe His Ser Ala Thr
130                              135                              140

Val Ile Glu Lys Asp Phe Gly Phe Lys Leu Ser Gln Asn Val Glu Glu
145                              150                              155                              160

Arg His Ser Phe Glu Ala Gln Gly Pro Arg Arg Lys Ala Met Gly Arg
165                              170                              175

Ala Arg Asn Tyr Leu Leu Tyr Thr Ala Leu Lys Pro Glu His Ser Trp
180                              185                              190

Val Tyr Trp Arg Asp Val Asp Ile Val Asp Ser Pro Thr Gly Ile Leu
195                              200                              205

Glu Asp Phe Ile Ala His Asp Arg Asp Ile Leu Val Pro Asn Ile Trp
210                              215                              220

Phe His Arg Tyr Arg Asp Gly Val Asp Ile Glu Gly Arg Phe Asp Tyr
225                              230                              235                              240

Asn Ser Trp Val Glu Ser Asp Lys Gly Arg Lys Leu Ala Asn Ser Leu
245                              250                              255

Asp Lys Asp Val Val Leu Ala Glu Gly Tyr Lys Gln Tyr Asp Thr Gly
260                              265                              270

Arg Thr Tyr Met Ala Lys Met Gly Asp Trp Arg Glu Asn Lys Asp Val
275                              280                              285

Glu Leu Glu Leu Asp Gly Ile Gly Gly Val Asn Ile Leu Val Lys Ala
290                              295                              300

Asp Val His Arg Ser Gly Ile Asn Phe Pro Cys Tyr Ala Phe Glu Asn
305                              310                              315                              320

Gln Ala Glu Thr Glu Gly Phe Ala Lys Met Ala Lys Arg Ala Gly Tyr
325                              330                              335

Glu Val Tyr Gly Leu Pro Asn Tyr Val Val Trp His Ile Asp Thr Glu
340                              345                              350

Glu Lys Gly Gly Asn Ala
355

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&lt;210&gt; 218

&lt;211&gt; 17

&lt;212&gt; PRT

&lt;213&gt; Trichoderma reesei

&lt;400&gt; 218

Met Ala Arg Pro Met Gly Ser Val Arg Leu Lys Lys Ala Asn Pro Ser  
 1 5 10 15

Thr

&lt;210&gt; 219

&lt;211&gt; 16

&lt;212&gt; PRT

&lt;213&gt; Trichoderma reesei

&lt;400&gt; 219

Leu Ile Leu Gly Ala Val Leu Cys Ile Phe Ile Ile Ile Phe Leu Val  
 1 5 10 15

&lt;210&gt; 220

&lt;211&gt; 339

&lt;212&gt; PRT

&lt;213&gt; Trichoderma reesei

&lt;400&gt; 220

Ser Pro Ser Ser Pro Ala Ser Ala Ser Arg Leu Ser Ile Val Ser Ala  
 1 5 10 15

Gln His His Leu Ser Pro Pro Thr Ser Pro Tyr Gln Ser Pro Arg Ser  
 20 25 30

Gly Ala Val Gln Gly Pro Pro Pro Val Thr Arg Tyr Asn Leu Asn Lys  
 35 40 45

Val Thr Val Thr Ser Asp Pro Val Arg Asn Gln Glu His Ile Leu Ile  
 50 55 60

Leu Thr Pro Met Ala Arg Phe Tyr Gln Glu Tyr Trp Asp Asn Leu Leu  
 65 70 75 80

Arg Leu Asn Tyr Pro His Glu Leu Ile Thr Leu Gly Phe Ile Leu Pro  
 85 90 95

Lys Thr Lys Glu Gly Asn Gln Ala Thr Ser Met Leu Gln Lys Gln Ile  
 100 105 110

Gln Lys Thr Gln Asn Tyr Gly Pro Glu Lys Asp Arg Phe Lys Ser Ile  
 115 120 125

Ile Ile Leu Arg Gln Asp Phe Asp Pro Ala Val Val Ser Gln Asp Glu  
 130 135 140

Ser Glu Arg His Lys Leu Ala Asn Gln Lys Ala Arg Arg Glu Val Met  
 145 150 155 160

Ala Lys Ala Arg Asn Ser Leu Leu Phe Thr Thr Leu Gly Pro Ser Thr  
 165 170 175

Ser Trp Val Leu Trp Leu Asp Ala Asp Ile Thr Glu Thr Ala Pro Thr  
 180 185 190

Leu Ile Gln Asp Leu Ala Ser His Asp Lys Pro Ile Ile Val Ala Asn  
 195 200 205

Cys Phe Gln Lys Tyr Tyr Asp Pro Glu Ser Lys Lys Met Ala Glu Arg  
 210 215 220

Pro Thr Ser Phe Ser Ser Thr Gln Asp Ser Glu Thr Ala Leu Lys Met



Phe Lys Asp Phe Asn Ser Lys Gln Asp Ser Glu Thr Ala Leu Lys Met  
 225 230 235 240

Ala Glu Gln Met Gly Pro Asp Asp Ile Leu Leu Glu Gly Tyr Ala Glu  
 245 250 255

Met Ala Thr Tyr Arg Thr Leu Leu Ala Tyr Met Ser Thr Pro Gly Gly  
 260 265 270

Ser Lys Asp Leu Val Val Pro Leu Asp Gly Val Gly Gly Thr Ala Leu  
 275 280 285

Leu Val Lys Ala Asp Val His Arg Asp Gly Ala Met Phe Pro Pro Phe  
 290 295 300

Ala Phe Tyr His Leu Ile Glu Ser Glu Gly Phe Ala Lys Met Ala Lys  
 305 310 315 320

Arg Leu Gly Trp Gln Pro Tyr Gly Leu Pro Asn Tyr Lys Val Tyr His  
 325 330 335

Tyr Asn Glu

<210> 221

<211> 33

<212> PRT

<213> Trichoderma reesei

<400> 221

Met His Phe Ala Tyr Pro Ser Arg Lys Ser Ser Asn Pro Pro Pro Phe  
 1 5 10 15

Arg Pro Arg Ser Thr Arg Leu Pro Gly Leu Arg Arg Ser Arg Ile Lys  
 20 25 30

Thr

<210> 222

<211> 15

<212> PRT

<213> Trichoderma reesei

<400> 222

Ile Gly Ile Val Leu Phe Leu Val Leu Ala Thr Leu Trp Phe Phe  
 1 5 10 15

<210> 223

<211> 262

<212> PRT

<213> Trichoderma reesei

<400> 223

Ser Asn Pro Arg Val Pro Arg Pro Asp Pro Glu Arg Val Pro Ser Gly  
 1 5 10 15

Arg Pro Pro Val Val Leu Val Thr Val Ile Asp Pro Thr Gln Tyr Pro  
 20 25 30

Asn Ala Tyr Leu Lys Thr Ile Lys Glu Asn Arg Glu Gln Tyr Ala Ala  
 35 40 45

Lys His Gly Tyr Glu Ala Phe Ile Val Lys Ala Tyr Asp Tyr Asp Thr  
 50 55 60

50                      55                      60  
 Gln Gly Ala Pro Gln Ser Trp Ser Lys Leu Met Ala Met Arg His Ala  
 65                      70                      75                      80  
 Leu Thr Lys Phe Pro Glu Cys Arg Phe Val Trp Tyr Leu Asp Gln Asp  
                     85                      90                      95  
 Ala Tyr Ile Met Asp Met Ser Lys Ser Leu Glu Glu Gln Leu Leu Asn  
                     100                      105                      110  
 Arg Gln Lys Leu Glu Ser Leu Met Ile Lys Asn Tyr Pro Val Val Pro  
                     115                      120                      125  
 Pro Asp Ser Ile Ile Lys Thr Phe Ser His Leu Arg Pro Asp Glu Val  
                     130                      135                      140  
 Asp Leu Ile Val Ser Gln Asp Ser Ser Gly Leu Val Ala Gly Ser Val  
 145                      150                      155                      160  
 Val Val Arg Asn Ser Gln Trp Ser Lys Phe Leu Leu Glu Thr Trp Met  
                     165                      170                      175  
 Asp Pro Leu Tyr Arg Ser Tyr Asn Phe Gln Lys Ala Glu Arg His Ala  
                     180                      185                      190  
 Leu Glu His Ile Val Gln Trp His Pro Thr Ile Leu Ser Lys Leu Ala  
                     195                      200                      205  
 Leu Val Pro Gln Arg Thr Leu Gly Pro Tyr Thr Arg Thr Asp Gln Gly  
                     210                      215                      220  
 Asp Ala Tyr Gln Asp Gly Asp Phe Val Val Met Phe Thr Gly Cys Thr  
 225                      230                      235                      240  
 Lys Ser Gly Glu Gln Ser Cys Glu Thr Val Ser Ala Ser Tyr Tyr Gln  
                     245                      250                      255  
 Lys Trp Ser Ser Ser Leu  
                     260  
 <210> 224  
 <211> 119  
 <212> PRT  
 <213> Trichoderma reesei  
 <400> 224  
 Met Ser Leu Ser Arg Ser Pro Ser Pro Val Pro Gly Gly Gly Trp Ser  
 1                      5                      10                      15  
 Ser Pro Gly Leu Asn Ile Asn Ser Gly Arg Ser Ser Pro Ser Asn Ala  
                     20                      25                      30  
 Ala Gly Ser Ser Val Ser Trp Glu Ser Ala Lys Met Arg Lys Gln Gly  
                     35                      40                      45  
 Ala Asn Gly Tyr Pro Ser Phe Ser Thr Gln Asn Gln Gly Phe Phe Thr  
                     50                      55                      60  
 Arg His Met Arg Arg Ile Ser Ser Ser Leu Pro Arg Phe Ala Ala Gly  
 65                      70                      75                      80  
 Pro Gly Asn Thr Tyr Ala Glu Arg Glu Lys Tyr Glu Arg Gly Gly His  
                     85                      90                      95

Ser Pro His Ala Gly Gly Gly Arg Leu Arg Ala Phe Leu Ala Arg Ile  
 100 105 110

Gly Arg Arg Leu Lys Trp Arg  
 115

<210> 225

<211> 16

<212> PRT

<213> Trichoderma reesei

<400> 225

Ile Leu Leu Pro Leu Ile Ile Ile Cys Thr Ile Val Ala Tyr Tyr Gly  
 1 5 10 15

<210> 226

<211> 324

<212> PRT

<213> Trichoderma reesei

<400> 226

Thr His Glu Ala Pro Gly Phe Val His Trp Trp Arg Arg Ile Ser Met  
 1 5 10 15

Gly Gly Gly Gly Glu Lys Phe Val Ile Ile Leu Gly Ala Asn Val Gly  
 20 25 30

Gly Gly Val Met Glu Trp Lys Gly Ala Arg Glu Trp Ala Ile Glu Arg  
 35 40 45

Asp Ser Val Arg Asn Lys Arg Lys Tyr Ala Thr Arg Trp Gly Tyr Asp  
 50 55 60

Leu Glu Ile Val Asp Met Lys Thr Lys Lys Arg Tyr Ala His Glu Trp  
 65 70 75 80

Arg Glu Ser Trp Glu Lys Val Asp Phe Ile Arg Ala Ala Met Arg Lys  
 85 90 95

Tyr Pro Lys Ala Glu Trp Phe Trp Trp Leu Asp Leu Asn Thr Tyr Val  
 100 105 110

Met Glu Pro Ser Tyr Ser Leu Gln Arg His Leu Phe Asn His Leu Asp  
 115 120 125

Arg His Val Tyr Arg Asp Ile Asn Val Phe Asn Pro Leu Asn Ile Thr  
 130 135 140

His Pro Pro Thr Glu Glu Tyr Leu Asp Ala Glu Ala Arg Ser Pro Val  
 145 150 155 160

Gly Asp Gly Asn Ile Asn Ser Val Asn Leu Met Leu Thr Gln Asp Cys  
 165 170 175

Ser Gly Phe Asn Leu Gly Ser Phe Phe Ile Arg Arg Ser Ala Trp Thr  
 180 185 190

Glu Gln Leu Leu Asp Ile Trp Trp Asp Pro Val Leu Tyr Glu Gln Lys  
 195 200 205

His Met Glu Trp Glu His Lys Glu Gln Asp Ala Leu Glu Gln Leu Tyr  
 210 215 220

Arg Thr Gln Pro Trp Ile Arg Gln His Thr Gly Phe Leu Pro Gln Arg  
 225 230 235 240

Leu Ile Asn Ser Phe Pro Pro Ala Ala Cys Ala Asp Glu Ser Gly Leu  
245 250 255

Asn Asn Thr Arg Ile His Tyr Asn Glu Lys Asp Arg Asp Phe Val Val  
260 265 270

Asn Met Ala Gly Cys Glu Trp Gly Arg Asp Cys Trp Gly Glu Met Tyr  
275 280 285

His Tyr Arg Glu Phe Ser Tyr Trp Leu Asn Arg Asn Pro Trp Glu Leu  
290 295 300

Phe Lys Glu Glu Ile Val Ala Val Ile Trp Tyr Lys Leu Thr Gly Gln  
305 310 315 320

Arg Val Lys Leu

<210> 227

<211> 339

<212> PRT

<213> *Saccharomyces cerevisiae*

<400> 227

Met Ala Gly Ser Thr Ser Ser Leu Val Ile Cys Ala Ile Gly Ile Tyr  
1 5 10 15

Ala Thr Phe Leu Thr Trp Ala Leu Val Gln Glu Pro Leu Ala Thr Arg  
20 25 30

Thr Trp Pro Asn Ser Met Gly Lys Phe Gln Phe Pro Asn Val Ile Ser  
35 40 45

Leu Ile Gln Ala Ser Val Ala Met Met Met Gly Tyr Leu Tyr Leu Asn  
50 55 60

Trp Lys Lys Val Glu Tyr Pro Pro Arg Lys Met Ile Lys Asp His Trp  
65 70 75 80

Lys Gln Leu Met Leu Ile Ser Phe Thr Gln Ser Ser Ser Gly Pro Leu  
85 90 95

Ala Thr Thr Ser Leu Lys His Val Asp Tyr Leu Thr Tyr Met Leu Ala  
100 105 110

Lys Ser Cys Lys Met Ile Pro Val Leu Leu Val His Leu Leu Leu Tyr  
115 120 125

Arg Thr Pro Ile Ala Ser Gln Lys Lys Val Val Ala Leu Leu Val Ser  
130 135 140

Leu Gly Val Thr Ile Phe Thr Ile Gly Gly Asn Asp Gly Lys Lys Leu  
145 150 155 160

Lys Arg Ser Phe Asn Glu Ser Gly Asn Asp Asn Lys Leu Gln Gly Phe  
165 170 175

Gly Leu Leu Phe Ser Ser Leu Phe Leu Asp Gly Leu Thr Asn Ala Thr  
180 185 190

Gln Asp Lys Leu Leu Lys Ala Asn Lys Ala Lys Glu Lys Gly Lys Gln  
195 200 205

Thr Leu Ile Thr Gly Ala His Leu Met Phe Thr Leu Asn Leu Phe Val

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210                               215                               220

Ile Leu Trp Asn Ile Leu Tyr Phe Ile Val Ile Asp Cys Lys Gln Trp
225                               230                               235                               240

Asp Asn Ala Val Ser Val Leu Thr Met Asp Pro Gln Val Trp Gly Tyr
245                               250                               255

Leu Met Leu Tyr Ser Phe Cys Gly Ala Met Gly Gln Cys Phe Ile Phe
260                               265                               270

Tyr Thr Leu Glu Gln Phe Gly Ser Leu Val Leu Ile Met Ile Thr Val
275                               280                               285

Thr Arg Lys Met Val Ser Met Ile Leu Ser Ile Ile Val Phe Gly Lys
290                               295                               300

Ser Val Arg Phe Gln Gln Trp Val Gly Met Phe Ile Val Phe Gly Gly
305                               310                               315                               320

Ile Thr Trp Glu Ala Leu Asn Lys Lys Lys Ala Asn Ile Pro Lys Ala
325                               330                               335

Lys Ser Ala

<210> 228
<211> 348
<212> PRT
<213> Homo sapiens

<400> 228
Met Ala Glu Lys Val Leu Val Thr Gly Gly Ala Gly Tyr Ile Gly Ser
1                               5                               10                               15

His Thr Val Leu Glu Leu Leu Glu Ala Gly Tyr Leu Pro Val Val Ile
20                               25                               30

Asp Asn Phe His Asn Ala Phe Arg Gly Gly Gly Ser Leu Pro Glu Ser
35                               40                               45

Leu Arg Arg Val Gln Glu Leu Thr Gly Arg Ser Val Glu Phe Glu Glu
50                               55                               60

Met Asp Ile Leu Asp Gln Gly Ala Leu Gln Arg Leu Phe Lys Lys Tyr
65                               70                               75                               80

Ser Phe Met Ala Val Ile His Phe Ala Gly Leu Lys Ala Val Gly Glu
85                               90                               95

Ser Val Gln Lys Pro Leu Asp Tyr Tyr Arg Val Asn Leu Thr Gly Thr
100                              105                              110

Ile Gln Leu Leu Glu Ile Met Lys Ala His Gly Val Lys Asn Leu Val
115                              120                              125

Phe Ser Ser Ser Ala Thr Val Tyr Gly Asn Pro Gln Tyr Leu Pro Leu
130                              135                              140

Asp Glu Ala His Pro Thr Gly Gly Cys Thr Asn Pro Tyr Gly Lys Ser
145                              150                              155                              160

Lys Phe Phe Ile Glu Glu Met Ile Arg Asp Leu Cys Gln Ala Asp Lys
165                              170                              175

Thr Tro Asn Val Val Leu Leu Arg Trp Phe Asn Pro Thr Glv Ala His

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180              185              190
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Ala Ser Gly Cys Ile Gly Glu Asp Pro Gln Gly Ile Pro Asn Asn Leu
195              200              205

Met Pro Tyr Val Ser Gln Val Ala Ile Gly Arg Arg Glu Ala Leu Asn
210              215              220

Val Phe Gly Asn Asp Tyr Asp Thr Glu Asp Gly Thr Gly Val Arg Asp
225              230              235              240

Tyr Ile His Val Val Asp Leu Ala Lys Gly His Ile Ala Ala Leu Arg
245              250              255

Lys Leu Lys Glu Gln Cys Gly Cys Arg Ile Tyr Asn Leu Gly Thr Gly
260              265              270

Thr Gly Tyr Ser Val Leu Gln Met Val Gln Ala Met Glu Lys Ala Ser
275              280              285

Gly Lys Lys Ile Pro Tyr Lys Val Val Ala Arg Arg Glu Gly Asp Val
290              295              300

Ala Ala Cys Tyr Ala Asn Pro Ser Leu Ala Gln Glu Glu Leu Gly Trp
305              310              315              320

Thr Ala Ala Leu Gly Leu Asp Arg Met Cys Glu Asp Leu Trp Arg Trp
325              330              335

Gln Lys Gln Asn Pro Ser Gly Phe Gly Thr Gln Ala
340              345

<210> 229
<211> 106
<212> PRT
<213> Trichoderma reesei

<400> 229
Met Ala Ser Thr Asn Ala Arg Tyr Val Arg Tyr Leu Leu Ile Ala Phe
1      5      10      15

Phe Thr Ile Leu Val Phe Tyr Phe Val Ser Asn Ser Lys Tyr Glu Gly
20     25     30

Val Asp Leu Asn Lys Gly Thr Phe Thr Ala Pro Asp Ser Thr Lys Thr
35     40     45

Thr Pro Lys Pro Pro Ala Thr Gly Asp Ala Lys Asp Phe Pro Leu Ala
50     55     60

Leu Thr Pro Asn Asp Pro Gly Phe Asn Asp Leu Val Gly Ile Ala Pro
65     70     75     80

Gly Pro Arg Met Asn Ala Thr Phe Val Thr Leu Ala Arg Asn Ser Asp
85     90     95

Val Trp Asp Ile Ala Arg Ser Ile Arg Gln
100    105

<210> 230
<211> 83
<212> PRT
<213> Trichoderma reesei

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&lt;400&gt; 230

Met Ala Ser Thr Asn Ala Arg Tyr Val Arg Tyr Leu Leu Ile Ala Phe  
 1 5 10 15

Phe Thr Ile Leu Val Phe Tyr Phe Val Ser Asn Ser Lys Tyr Glu Gly  
 20 25 30

Val Asp Leu Asn Lys Gly Thr Phe Thr Ala Pro Asp Ser Thr Lys Thr  
 35 40 45

Thr Pro Lys Pro Pro Ala Thr Gly Asp Ala Lys Asp Phe Pro Leu Ala  
 50 55 60

Leu Thr Pro Asn Asp Pro Gly Phe Asn Asp Leu Val Gly Ile Ala Pro  
 65 70 75 80

Gly Pro Arg

&lt;210&gt; 231

&lt;211&gt; 51

&lt;212&gt; PRT

<213> *Trichoderma reesei*

&lt;400&gt; 231

Met Ala Ser Thr Asn Ala Arg Tyr Val Arg Tyr Leu Leu Ile Ala Phe  
 1 5 10 15

Phe Thr Ile Leu Val Phe Tyr Phe Val Ser Asn Ser Lys Tyr Glu Gly  
 20 25 30

Val Asp Leu Asn Lys Gly Thr Phe Thr Ala Pro Asp Ser Thr Lys Thr  
 35 40 45

Thr Pro Lys  
 50

&lt;210&gt; 232

&lt;211&gt; 722

&lt;212&gt; PRT

<213> *Homo sapiens*

&lt;400&gt; 232

Met Glu Lys Asn Gly Asn Asn Arg Lys Leu Arg Val Cys Val Ala Thr  
 1 5 10 15

Cys Asn Arg Ala Asp Tyr Ser Lys Leu Ala Pro Ile Met Phe Gly Ile  
 20 25 30

Lys Thr Glu Pro Glu Phe Phe Glu Leu Asp Val Val Val Leu Gly Ser  
 35 40 45

His Leu Ile Asp Asp Tyr Gly Asn Thr Tyr Arg Met Ile Glu Gln Asp  
 50 55 60

Asp Phe Asp Ile Asn Thr Arg Leu His Thr Ile Val Arg Gly Glu Asp  
 65 70 75 80

Glu Ala Ala Met Val Glu Ser Val Gly Leu Ala Leu Val Lys Leu Pro  
 85 90 95

Asp Val Leu Asn Arg Leu Lys Pro Asp Ile Met Ile Val His Gly Asp  
 100 105 110

Arg Phe Asp Ala Leu Ala Leu Ala Thr Ser Ala Ala Leu Met Asn Ile

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115              120              125

Arg Ile Leu His Ile Glu Gly Gly Glu Val Ser Gly Thr Ile Asp Asp
130              135              140

Ser Ile Arg His Ala Ile Thr Lys Leu Ala His Tyr His Val Cys Cys
145              150              155              160

Thr Arg Ser Ala Glu Gln His Leu Ile Ser Met Cys Glu Asp His Asp
165              170              175

Arg Ile Leu Leu Ala Gly Cys Pro Ser Tyr Asp Lys Leu Leu Ser Ala
180              185              190

Lys Asn Lys Asp Tyr Met Ser Ile Ile Arg Met Trp Leu Gly Asp Asp
195              200              205

Val Lys Ser Lys Asp Tyr Ile Val Ala Leu Gln His Pro Val Thr Thr
210              215              220

Asp Ile Lys His Ser Ile Lys Met Phe Glu Leu Thr Leu Asp Ala Leu
225              230              235              240

Ile Ser Phe Asn Lys Arg Thr Leu Val Leu Phe Pro Asn Ile Asp Ala
245              250              255

Gly Ser Lys Glu Met Val Arg Val Met Arg Lys Lys Gly Ile Glu His
260              265              270

His Pro Asn Phe Arg Ala Val Lys His Val Pro Phe Asp Gln Phe Ile
275              280              285

Gln Leu Val Ala His Ala Gly Cys Met Ile Gly Asn Ser Ser Cys Gly
290              295              300

Val Arg Glu Val Gly Ala Phe Gly Thr Pro Val Ile Asn Leu Gly Thr
305              310              315              320

Arg Gln Ile Gly Arg Glu Thr Gly Glu Asn Val Leu His Val Arg Asp
325              330              335

Ala Asp Thr Gln Asp Lys Ile Leu Gln Ala Leu His Leu Gln Phe Gly
340              345              350

Lys Gln Tyr Pro Cys Ser Lys Ile Tyr Gly Asp Gly Asn Ala Val Pro
355              360              365

Arg Ile Leu Lys Phe Leu Lys Ser Ile Asp Leu Gln Glu Pro Leu Gln
370              375              380

Lys Lys Phe Cys Phe Pro Pro Val Lys Glu Asn Ile Ser Gln Asp Ile
385              390              395              400

Asp His Ile Leu Glu Thr Leu Ser Ala Leu Ala Val Asp Leu Gly Gly
405              410              415

Thr Asn Leu Arg Val Ala Ile Val Ser Met Lys Gly Glu Ile Val Lys
420              425              430

Lys Tyr Thr Gln Phe Asn Pro Lys Thr Tyr Glu Glu Arg Ile Asn Leu
435              440              445

Ile Leu Gln Met Cys Val Glu Ala Ala Ala Glu Ala Val Lys Leu Asn
450              455              460

Cys Arg Ile Leu Gly Val Gly Ile Ser Thr Gly Gly Arg Val Asn Pro
465              470              475              480

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Arg Glu Gly Ile Val Leu His Ser Thr Lys Leu Ile Gln Glu Trp Asn  
485 490 495

Ser Val Asp Leu Arg Thr Pro Leu Ser Asp Thr Leu His Leu Pro Val  
500 505 510

Trp Val Asp Asn Asp Gly Asn Cys Ala Ala Leu Ala Glu Arg Lys Phe  
515 520 525

Gly Gln Gly Lys Gly Leu Glu Asn Phe Val Thr Leu Ile Thr Gly Thr  
530 535 540

Gly Ile Gly Gly Gly Ile Ile His Gln His Glu Leu Ile His Arg Ser  
545 550 555 560

Ser Phe Cys Ala Ala Glu Leu Gly His Leu Val Val Ser Leu Asp Gly  
565 570 575

Pro Asp Cys Ser Cys Gly Ser His Gly Cys Ile Glu Ala Tyr Ala Ser  
580 585 590

Gly Met Ala Leu Gln Arg Glu Ala Lys Lys Leu His Asp Glu Asp Leu  
595 600 605

Leu Leu Val Glu Gly Met Ser Val Pro Lys Asp Glu Ala Val Gly Ala  
610 615 620

Leu His Leu Ile Gln Ala Ala Lys Leu Gly Asn Ala Lys Ala Gln Ser  
625 630 635 640

Ile Leu Arg Thr Ala Gly Thr Ala Leu Gly Leu Gly Val Val Asn Ile  
645 650 655

Leu His Thr Met Asn Pro Ser Leu Val Ile Leu Ser Gly Val Leu Ala  
660 665 670

Ser His Tyr Ile His Ile Val Lys Asp Val Ile Arg Gln Gln Ala Leu  
675 680 685

Ser Ser Val Gln Asp Val Asp Val Val Val Ser Asp Leu Val Asp Pro  
690 695 700

Ala Leu Leu Gly Ala Ala Ser Met Val Leu Asp Tyr Thr Thr Arg Arg  
705 710 715 720

Ile Tyr

<210> 233

<211> 753

<212> PRT

<213> Homo sapiens

<400> 233

Met Glu Thr Tyr Gly Tyr Leu Gln Arg Glu Ser Cys Phe Gln Gly Pro  
1 5 10 15

His Glu Leu Tyr Phe Lys Asn Leu Ser Lys Arg Asn Lys Gln Ile Met  
20 25 30

Glu Lys Asn Gly Asn Asn Arg Lys Leu Arg Val Cys Val Ala Thr Cys  
35 40 45

Asn Arg Ala Asp Tyr Ser Lys Leu Ala Pro Ile Met Phe Gly Ile Lys  
50 55 60

Thr Glu Pro Glu Phe Phe Glu Leu Asp Val Val Val Leu Gly Ser His  
 65 70 75 80  
 Leu Ile Asp Asp Tyr Gly Asn Thr Tyr Arg Met Ile Glu Gln Asp Asp  
 85 90 95  
 Phe Asp Ile Asn Thr Arg Leu His Thr Ile Val Arg Gly Glu Asp Glu  
 100 105 110  
 Ala Ala Met Val Glu Ser Val Gly Leu Ala Leu Val Lys Leu Pro Asp  
 115 120 125  
 Val Leu Asn Arg Leu Lys Pro Asp Ile Met Ile Val His Gly Asp Arg  
 130 135 140  
 Phe Asp Ala Leu Ala Leu Ala Thr Ser Ala Ala Leu Met Asn Ile Arg  
 145 150 155 160  
 Ile Leu His Ile Glu Gly Gly Glu Val Ser Gly Thr Ile Asp Asp Ser  
 165 170 175  
 Ile Arg His Ala Ile Thr Lys Leu Ala His Tyr His Val Cys Cys Thr  
 180 185 190  
 Arg Ser Ala Glu Gln His Leu Ile Ser Met Cys Glu Asp His Asp Arg  
 195 200 205  
 Ile Leu Leu Ala Gly Cys Pro Ser Tyr Asp Lys Leu Leu Ser Ala Lys  
 210 215 220  
 Asn Lys Asp Tyr Met Ser Ile Ile Arg Met Trp Leu Gly Asp Asp Val  
 225 230 235 240  
 Lys Ser Lys Asp Tyr Ile Val Ala Leu Gln His Pro Val Thr Thr Asp  
 245 250 255  
 Ile Lys His Ser Ile Lys Met Phe Glu Leu Thr Leu Asp Ala Leu Ile  
 260 265 270  
 Ser Phe Asn Lys Arg Thr Leu Val Leu Phe Pro Asn Ile Asp Ala Gly  
 275 280 285  
 Ser Lys Glu Met Val Arg Val Met Arg Lys Lys Gly Ile Glu His His  
 290 295 300  
 Pro Asn Phe Arg Ala Val Lys His Val Pro Phe Asp Gln Phe Ile Gln  
 305 310 315 320  
 Leu Val Ala His Ala Gly Cys Met Ile Gly Asn Ser Ser Cys Gly Val  
 325 330 335  
 Arg Glu Val Gly Ala Phe Gly Thr Pro Val Ile Asn Leu Gly Thr Arg  
 340 345 350  
 Gln Ile Gly Arg Glu Thr Gly Glu Asn Val Leu His Val Arg Asp Ala  
 355 360 365  
 Asp Thr Gln Asp Lys Ile Leu Gln Ala Leu His Leu Gln Phe Gly Lys  
 370 375 380  
 Gln Tyr Pro Cys Ser Lys Ile Tyr Gly Asp Gly Asn Ala Val Pro Arg  
 385 390 395 400

Ile Leu Lys Phe Leu Lys Ser Ile Asp Leu Gln Glu Pro Leu Gln Lys  
 405 410 415  
 Lys Phe Cys Phe Pro Pro Val Lys Glu Asn Ile Ser Gln Asp Ile Asp  
 420 425 430  
 His Ile Leu Glu Thr Leu Ser Ala Leu Ala Val Asp Leu Gly Gly Thr  
 435 440 445  
 Asn Leu Arg Val Ala Ile Val Ser Met Lys Gly Glu Ile Val Lys Lys  
 450 455 460  
 Tyr Thr Gln Phe Asn Pro Lys Thr Tyr Glu Glu Arg Ile Asn Leu Ile  
 465 470 475 480  
 Leu Gln Met Cys Val Glu Ala Ala Ala Glu Ala Val Lys Leu Asn Cys  
 485 490 495  
 Arg Ile Leu Gly Val Gly Ile Ser Thr Gly Gly Arg Val Asn Pro Arg  
 500 505 510  
 Glu Gly Ile Val Leu His Ser Thr Lys Leu Ile Gln Glu Trp Asn Ser  
 515 520 525  
 Val Asp Leu Arg Thr Pro Leu Ser Asp Thr Leu His Leu Pro Val Trp  
 530 535 540  
 Val Asp Asn Asp Gly Asn Cys Ala Ala Leu Ala Glu Arg Lys Phe Gly  
 545 550 555 560  
 Gln Gly Lys Gly Leu Glu Asn Phe Val Thr Leu Ile Thr Gly Thr Gly  
 565 570 575  
 Ile Gly Gly Gly Ile Ile His Gln His Glu Leu Ile His Gly Ser Ser  
 580 585 590  
 Phe Cys Ala Ala Glu Leu Gly His Leu Val Val Ser Leu Asp Gly Pro  
 595 600 605  
 Asp Cys Ser Cys Gly Ser His Gly Cys Ile Glu Ala Tyr Ala Ser Gly  
 610 615 620  
 Met Ala Leu Gln Arg Glu Ala Lys Lys Leu His Asp Glu Asp Leu Leu  
 625 630 635 640  
 Leu Val Glu Gly Met Ser Val Pro Lys Asp Glu Ala Val Gly Ala Leu  
 645 650 655  
 His Leu Ile Gln Ala Ala Lys Leu Gly Asn Ala Lys Ala Gln Ser Ile  
 660 665 670  
 Leu Arg Thr Ala Gly Thr Ala Leu Gly Leu Gly Val Val Asn Ile Leu  
 675 680 685  
 His Thr Met Asn Pro Ser Leu Val Ile Leu Ser Gly Val Leu Ala Ser  
 690 695 700  
 His Tyr Ile His Ile Val Lys Asp Val Ile Arg Gln Gln Ala Leu Ser  
 705 710 715 720  
 Ser Val Gln Asp Val Asp Val Val Val Ser Asp Leu Val Asp Pro Ala  
 725 730 735  
 Leu Leu Gly Ala Ala Ser Met Val Leu Asp Tyr Thr Thr Arg Arg Ile  
 740 745 750

Tyr

&lt;210&gt; 234

&lt;211&gt; 359

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 234

Met Pro Leu Glu Leu Glu Leu Cys Pro Gly Arg Trp Val Gly Gly Gln  
 1 5 10 15

His Pro Cys Phe Ile Ile Ala Glu Ile Gly Gln Asn His Gln Gly Asp  
 20 25 30

Leu Asp Val Ala Lys Arg Met Ile Arg Met Ala Lys Glu Cys Gly Ala  
 35 40 45

Asp Cys Ala Lys Phe Gln Lys Ser Glu Leu Glu Phe Lys Phe Asn Arg  
 50 55 60

Lys Ala Leu Glu Arg Pro Tyr Thr Ser Lys His Ser Trp Gly Lys Thr  
 65 70 75 80

Tyr Gly Glu His Lys Arg His Leu Glu Phe Ser His Asp Gln Tyr Arg  
 85 90 95

Glu Leu Gln Arg Tyr Ala Glu Glu Val Gly Ile Phe Phe Thr Ala Ser  
 100 105 110

Gly Met Asp Glu Met Ala Val Glu Phe Leu His Glu Leu Asn Val Pro  
 115 120 125

Phe Phe Lys Val Gly Ser Gly Asp Thr Asn Asn Phe Pro Tyr Leu Glu  
 130 135 140

Lys Thr Ala Lys Lys Gly Arg Pro Met Val Ile Ser Ser Gly Met Gln  
 145 150 155 160

Ser Met Asp Thr Met Lys Gln Val Tyr Gln Ile Val Lys Pro Leu Asn  
 165 170 175

Pro Asn Phe Cys Phe Leu Gln Cys Thr Ser Ala Tyr Pro Leu Gln Pro  
 180 185 190

Glu Asp Val Asn Leu Arg Val Ile Ser Glu Tyr Gln Lys Leu Phe Pro  
 195 200 205

Asp Ile Pro Ile Gly Tyr Ser Gly His Glu Thr Gly Ile Ala Ile Ser  
 210 215 220

Val Ala Ala Val Ala Leu Gly Ala Lys Val Leu Glu Arg His Ile Thr  
 225 230 235 240

Leu Asp Lys Thr Trp Lys Gly Ser Asp His Ser Ala Ser Leu Glu Pro  
 245 250 255

Gly Glu Leu Ala Glu Leu Val Arg Ser Val Arg Leu Val Glu Arg Ala  
 260 265 270

Leu Gly Ser Pro Thr Lys Gln Leu Leu Pro Cys Glu Met Ala Cys Asn  
 275 280 285

Glu Lys Leu Gly Lys Ser Val Val Ala Lys Val Lys Ile Pro Glu Gly  
 290 295 300

Thr Ile Leu Thr Met Asp Met Leu Thr Val Lys Val Gly Glu Pro Lys  
305 310 315 320

Gly Tyr Pro Pro Glu Asp Ile Phe Asn Leu Val Gly Lys Lys Val Leu  
325 330 335

Val Thr Val Glu Glu Asp Asp Thr Ile Met Glu Glu Leu Val Asp Asn  
340 345 350

His Gly Lys Lys Ile Lys Ser  
355

<210> 235

<211> 248

<212> PRT

<213> Homo sapiens

<400> 235

Met Gly Leu Ser Arg Val Arg Ala Val Phe Phe Asp Leu Asp Asn Thr  
1 5 10 15

Leu Ile Asp Thr Ala Gly Ala Ser Arg Arg Gly Met Leu Glu Val Ile  
20 25 30

Lys Leu Leu Gln Ser Lys Tyr His Tyr Lys Glu Glu Ala Glu Ile Ile  
35 40 45

Cys Asp Lys Val Gln Val Lys Leu Ser Lys Glu Cys Phe His Pro Tyr  
50 55 60

Asn Thr Cys Ile Thr Asp Leu Arg Thr Ser His Trp Glu Glu Ala Ile  
65 70 75 80

Gln Glu Thr Lys Gly Gly Ala Ala Asn Arg Lys Leu Ala Glu Glu Cys  
85 90 95

Tyr Phe Leu Trp Lys Ser Thr Arg Leu Gln His Met Thr Leu Ala Glu  
100 105 110

Asp Val Lys Ala Met Leu Thr Glu Leu Arg Lys Glu Val Arg Leu Leu  
115 120 125

Leu Leu Thr Asn Gly Asp Arg Gln Thr Gln Arg Glu Lys Ile Glu Ala  
130 135 140

Cys Ala Cys Gln Ser Tyr Phe Asp Ala Val Val Val Gly Gly Glu Gln  
145 150 155 160

Arg Glu Glu Lys Pro Ala Pro Ser Ile Phe Tyr Tyr Cys Cys Asn Leu  
165 170 175

Leu Gly Val Gln Pro Gly Asp Cys Val Met Val Gly Asp Thr Leu Glu  
180 185 190

Thr Asp Ile Gln Gly Gly Leu Asn Ala Gly Leu Lys Ala Thr Val Trp  
195 200 205

Ile Asn Lys Asn Gly Ile Val Pro Leu Lys Ser Ser Pro Val Pro His  
210 215 220

Tyr Met Val Ser Ser Val Leu Glu Leu Pro Ala Leu Leu Gln Ser Ile  
225 230 235 240

Asp Cys Lys Val Ser Met Ser Thr  
245

&lt;210&gt; 236

&lt;211&gt; 434

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 236

Met Asp Ser Val Glu Lys Gly Ala Ala Thr Ser Val Ser Asn Pro Arg  
 1 5 10 15

Gly Arg Pro Ser Arg Gly Arg Pro Pro Lys Leu Gln Arg Asn Ser Arg  
 20 25 30

Gly Gly Gln Gly Arg Gly Val Glu Lys Pro Pro His Leu Ala Ala Leu  
 35 40 45

Ile Leu Ala Arg Gly Gly Ser Lys Gly Ile Pro Leu Lys Asn Ile Lys  
 50 55 60

His Leu Ala Gly Val Pro Leu Ile Gly Trp Val Leu Arg Ala Ala Leu  
 65 70 75 80

Asp Ser Gly Ala Phe Gln Ser Val Trp Val Ser Thr Asp His Asp Glu  
 85 90 95

Ile Glu Asn Val Ala Lys Gln Phe Gly Ala Gln Val His Arg Arg Ser  
 100 105 110

Ser Glu Val Ser Lys Asp Ser Ser Thr Ser Leu Asp Ala Ile Ile Glu  
 115 120 125

Phe Leu Asn Tyr His Asn Glu Val Asp Ile Val Gly Asn Ile Gln Ala  
 130 135 140

Thr Ser Pro Cys Leu His Pro Thr Asp Leu Gln Lys Val Ala Glu Met  
 145 150 155 160

Ile Arg Glu Glu Gly Tyr Asp Ser Val Phe Ser Val Val Arg Arg His  
 165 170 175

Gln Phe Arg Trp Ser Glu Ile Gln Lys Gly Val Arg Glu Val Thr Glu  
 180 185 190

Pro Leu Asn Leu Asn Pro Ala Lys Arg Pro Arg Arg Gln Asp Trp Asp  
 195 200 205

Gly Glu Leu Tyr Glu Asn Gly Ser Phe Tyr Phe Ala Lys Arg His Leu  
 210 215 220

Ile Glu Met Gly Tyr Leu Gln Gly Gly Lys Met Ala Tyr Tyr Glu Met  
 225 230 235 240

Arg Ala Glu His Ser Val Asp Ile Asp Val Asp Ile Asp Trp Pro Ile  
 245 250 255

Ala Glu Gln Arg Val Leu Arg Tyr Gly Tyr Phe Gly Lys Glu Lys Leu  
 260 265 270

Lys Glu Ile Lys Leu Leu Val Cys Asn Ile Asp Gly Cys Leu Thr Asn  
 275 280 285

Gly His Ile Tyr Val Ser Gly Asp Gln Lys Glu Ile Ile Ser Tyr Asp  
 290 295 300

Val Lys Asp Ala Ile Gly Ile Ser Leu Leu Lys Lys Ser Gly Ile Glu

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305              310              315              320
Val Arg Leu Ile Ser Glu Arg Ala Cys Ser Lys Gln Thr Leu Ser Ser
      325              330              335

Leu Lys Leu Asp Cys Lys Met Glu Val Ser Val Ser Asp Lys Leu Ala
      340              345              350

Val Val Asp Glu Trp Arg Lys Glu Met Gly Leu Cys Trp Lys Glu Val
      355              360              365

Ala Tyr Leu Gly Asn Glu Val Ser Asp Glu Glu Cys Leu Lys Arg Val
      370              375              380

Gly Leu Ser Gly Ala Pro Ala Asp Ala Cys Ser Thr Ala Gln Lys Ala
      385              390              395              400

Val Gly Tyr Ile Cys Lys Cys Asn Gly Gly Arg Gly Ala Ile Arg Glu
      405              410              415

Phe Ala Glu His Ile Cys Leu Leu Met Glu Lys Val Asn Asn Ser Cys
      420              425              430

Gln Lys

<210> 237
<211> 336
<212> PRT
<213> Mus musculus

<400> 237
Met Ala Pro Ala Arg Glu Asn Val Ser Leu Phe Phe Lys Leu Tyr Cys
 1              5              10              15

Leu Thr Val Met Thr Leu Val Ala Ala Ala Tyr Thr Val Ala Leu Arg
      20              25              30

Tyr Thr Arg Thr Thr Ala Glu Glu Leu Tyr Phe Ser Thr Thr Ala Val
      35              40              45

Cys Ile Thr Glu Val Ile Lys Leu Leu Ile Ser Val Gly Leu Leu Ala
      50              55              60

Lys Glu Thr Gly Ser Leu Gly Arg Phe Lys Ala Ser Leu Ser Glu Asn
      65              70              75              80

Val Leu Gly Ser Pro Lys Glu Leu Ala Lys Leu Ser Val Pro Ser Leu
      85              90              95

Val Tyr Ala Val Gln Asn Asn Met Ala Phe Leu Ala Leu Ser Asn Leu
      100             105             110

Asp Ala Ala Val Tyr Gln Val Thr Tyr Gln Leu Lys Ile Pro Cys Thr
      115             120             125

Ala Leu Cys Thr Val Leu Met Leu Asn Arg Thr Leu Ser Lys Leu Gln
      130             135             140

Trp Ile Ser Val Phe Met Leu Cys Gly Gly Val Thr Leu Val Gln Trp
      145             150             155             160

Lys Pro Ala Gln Ala Thr Lys Val Val Val Ala Gln Asn Pro Leu Leu
      165             170             175

Gly Phe Gly Ala Ile Ala Ile Ala Val Leu Cys Ser Gly Phe Ala Gly

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|                    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 180                |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |     |     |
| Val                | Tyr | Phe | Glu | Lys | Val | Leu | Lys | Ser | Ser | Asp | Thr | Ser | Leu | Trp | Val |
|                    |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Arg                | Asn | Ile | Gln | Met | Tyr | Leu | Ser | Gly | Ile | Val | Val | Thr | Leu | Ala | Gly |
|                    | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| Thr                | Tyr | Leu | Ser | Asp | Gly | Ala | Glu | Ile | Gln | Glu | Lys | Gly | Phe | Phe | Tyr |
| 225                |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Gly                | Tyr | Thr | Tyr | Tyr | Val | Trp | Phe | Val | Ile | Phe | Leu | Ala | Ser | Val | Gly |
|                    |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Gly                | Leu | Tyr | Thr | Ser | Val | Val | Val | Lys | Tyr | Thr | Asp | Asn | Ile | Met | Lys |
|                    |     |     |     | 260 |     |     |     | 265 |     |     |     |     | 270 |     |     |
| Gly                | Phe | Ser | Ala | Ala | Ala | Ala | Ile | Val | Leu | Ser | Thr | Ile | Ala | Ser | Val |
|                    |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| Leu                | Leu | Phe | Gly | Leu | Gln | Ile | Thr | Leu | Ser | Phe | Ala | Leu | Gly | Ala | Leu |
|                    | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
| Leu                | Val | Cys | Val | Ser | Ile | Tyr | Leu | Tyr | Gly | Leu | Pro | Arg | Gln | Asp | Thr |
| 305                |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
| Thr                | Ser | Ile | Gln | Gln | Glu | Ala | Thr | Ser | Lys | Glu | Arg | Ile | Ile | Gly | Val |
|                    |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |
| <210> 238          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <211> 403          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <212> PRT          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <213> Mus musculus |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| <400> 238          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Met                | Ile | His | Thr | Asn | Leu | Lys | Arg | Lys | Phe | Ser | Cys | Phe | Val | Leu | Val |
| 1                  |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |
| Phe                | Leu | Leu | Phe | Ala | Ile | Ile | Cys | Val | Trp | Lys | Lys | Gly | Ser | Asp | Tyr |
|                    |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| Glu                | Ala | Leu | Thr | Leu | Gln | Ala | Lys | Val | Phe | Gln | Met | Pro | Lys | Ser | Gln |
|                    |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
| Glu                | Lys | Val | Ala | Val | Gly | Pro | Ala | Pro | Gln | Ala | Val | Phe | Ser | Asn | Ser |
|                    | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| Lys                | Gln | Asp | Pro | Lys | Glu | Gly | Val | Gln | Ile | Leu | Ser | Tyr | Pro | Arg | Val |
| 65                 |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |
| Thr                | Ala | Lys | Val | Lys | Pro | Gln | Pro | Ser | Leu | Gln | Val | Trp | Asp | Lys | Asp |
|                    |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |
| Ser                | Thr | Tyr | Ser | Lys | Leu | Asn | Pro | Arg | Leu | Leu | Lys | Ile | Trp | Arg | Asn |
|                    |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Tyr                | Leu | Asn | Met | Asn | Lys | Tyr | Lys | Val | Ser | Tyr | Lys | Gly | Pro | Gly | Pro |
|                    |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| Gly                | Val | Lys | Phe | Ser | Val | Glu | Ala | Leu | Arg | Cys | His | Leu | Arg | Asp | His |
|                    | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Val                | Asn | Val | Ser | Met | Ile | Glu | Ala | Thr | Asp | Phe | Pro | Phe | Asn | Thr | Thr |
| 145                |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |



Glu Trp Glu Gly Tyr Leu Pro Lys Glu Asn Phe Arg Thr Lys Ala Gly  
 165 170 175  
 Pro Trp His Lys Cys Ala Val Val Ser Ser Ala Gly Ser Leu Lys Asn  
 180 185 190  
 Ser Gln Leu Gly Arg Glu Ile Asp Asn His Asp Ala Val Leu Arg Phe  
 195 200 205  
 Asn Gly Ala Pro Thr Asp Asn Phe Gln Gln Asp Val Gly Thr Lys Thr  
 210 215 220  
 Thr Ile Arg Leu Val Asn Ser Gln Leu Val Thr Thr Glu Lys Arg Phe  
 225 230 235 240  
 Leu Lys Asp Ser Leu Tyr Thr Glu Gly Ile Leu Ile Leu Trp Asp Pro  
 245 250 255  
 Ser Val Tyr His Ala Asp Ile Pro Gln Trp Tyr Gln Lys Pro Asp Tyr  
 260 265 270  
 Asn Phe Phe Glu Thr Tyr Lys Ser Tyr Arg Arg Leu His Pro Ser Gln  
 275 280 285  
 Pro Phe Tyr Ile Leu Lys Pro Gln Met Pro Trp Glu Leu Trp Asp Ile  
 290 295 300  
 Ile Gln Glu Ile Ser Pro Asp Leu Ile Gln Pro Asn Pro Pro Ser Ser  
 305 310 315 320  
 Gly Met Leu Gly Ile Ile Ile Met Met Thr Leu Cys Asp Gln Val Asp  
 325 330 335  
 Ile Tyr Glu Phe Leu Pro Ser Lys Arg Lys Thr Asp Val Cys Tyr Tyr  
 340 345 350  
 His Gln Lys Phe Phe Asp Ser Ala Cys Thr Met Gly Ala Tyr His Pro  
 355 360 365  
 Leu Leu Phe Glu Lys Asn Met Val Lys His Leu Asn Glu Gly Thr Asp  
 370 375 380  
 Glu Asp Ile Tyr Leu Phe Gly Lys Ala Thr Leu Ser Gly Phe Arg Asn  
 385 390 395 400

Asn Arg Cys

<210> 239

<211> 329

<212> PRT

<213> Homo sapiens

<400> 239

Met Val Ser Lys Ser Arg Trp Lys Leu Leu Ala Met Leu Ala Leu Val  
 1 5 10 15  
 Leu Val Val Met Val Trp Tyr Ser Ile Ser Arg Glu Asp Ser Phe Tyr  
 20 25 30  
 Phe Pro Ile Pro Glu Lys Lys Glu Pro Cys Leu Gln Gly Glu Ala Glu  
 35 40 45  
 Ser Lys Ala Ser Lys Leu Phe Gly Asn Tyr Ser Arg Asn Gln Pro Ile

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Ser Arg Met Ser Arg Ser Lys Gly Met Lys Ser Arg Asp Ser Lys Lys
50                               55                               60

Phe Leu Arg Leu Glu Asp Tyr Phe Trp Val Lys Thr Pro Ser Ala Tyr
65                               70                               75                               80

Glu Leu Pro Tyr Gly Thr Lys Gly Ser Glu Asp Leu Leu Leu Arg Val
85                               90                               95

Leu Ala Ile Thr Ser Ser Ser Ile Pro Lys Asn Ile Gln Ser Leu Arg
100                              105                              110

Cys Arg Arg Cys Val Val Val Gly Asn Gly His Arg Leu Arg Asn Ser
115                               120                               125

Ser Leu Gly Asp Ala Ile Asn Lys Tyr Asp Val Val Ile Arg Leu Asn
130                              135                              140

Asn Ala Pro Val Ala Gly Tyr Glu Gly Asp Val Gly Ser Lys Thr Thr
145                              150                              155                              160

Met Arg Leu Phe Tyr Pro Glu Ser Ala His Phe Asp Pro Lys Val Glu
165                              170                              175

Asn Asn Pro Asp Thr Leu Leu Val Leu Val Ala Phe Lys Ala Met Asp
180                              185                              190

Phe His Trp Ile Glu Thr Ile Leu Ser Asp Lys Lys Arg Val Arg Lys
195                              200                              205

Gly Phe Trp Lys Gln Pro Pro Leu Ile Trp Asp Val Asn Pro Lys Gln
210                              215                              220

Ile Arg Ile Leu Asn Pro Phe Phe Met Glu Ile Ala Ala Asp Lys Leu
225                              230                              235                              240

Leu Ser Leu Pro Met Gln Gln Pro Arg Lys Ile Lys Gln Lys Pro Thr
245                              250                              255

Thr Gly Leu Leu Ala Ile Thr Leu Ala Leu His Leu Cys Asp Leu Val
260                              265                              270

His Ile Ala Gly Phe Gly Tyr Pro Asp Ala Tyr Asn Lys Lys Gln Thr
275                              280                              285

Ile His Tyr Tyr Glu Gln Ile Thr Leu Lys Ser Met Ala Gly Ser Gly
290                              295                              300

His Asn Val Ser Gln Glu Ala Leu Ala Ile Lys Arg Met Leu Glu Met
305                              310                              315                              320

Gly Ala Ile Lys Asn Leu Thr Ser Phe
325

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&lt;210&gt; 240

&lt;211&gt; 863

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 240

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Met Arg Phe Arg Ile Tyr Lys Arg Lys Val Leu Ile Leu Thr Leu Val
1          5          10          15

Val Ala Ala Cys Gly Phe Val Leu Trp Ser Ser Asn Gly Arg Gln Arg
20          25          30

Lys Asn Glu Ala Leu Ala Pro Pro Leu Leu Asp Ala Glu Pro Ala Arg
35          40          45

Gly Ala Gly Gly Arg Gly Gly Asp His Pro Ser Val Ala Val Gly Ile
50          55          60

Arg Arg Val Ser Asn Val Ser Ala Ala Ser Leu Val Pro Ala Val Pro
65          70          75          80

Gln Pro Glu Ala Asp Asn Leu Thr Leu Arg Tyr Arg Ser Leu Val Tyr
85          90          95

Gln Leu Asn Phe Asp Gln Thr Leu Arg Asn Val Asp Lys Ala Gly Thr
100         105         110

Trp Ala Pro Arg Glu Leu Val Leu Val Val Gln Val His Asn Arg Pro
115         120         125

Glu Tyr Leu Arg Leu Leu Leu Asp Ser Leu Arg Lys Ala Gln Gly Ile
130         135         140

Asp Asn Val Leu Val Ile Phe Ser His Asp Phe Trp Ser Thr Glu Ile
145         150         155         160

Asn Gln Leu Ile Ala Gly Val Asn Phe Cys Pro Val Leu Gln Val Phe
165         170         175

Phe Pro Phe Ser Ile Gln Leu Tyr Pro Asn Glu Phe Pro Gly Ser Asp
180         185         190

Pro Arg Asp Cys Pro Arg Asp Leu Pro Lys Asn Ala Ala Leu Lys Leu
195         200         205

Gly Cys Ile Asn Ala Glu Tyr Pro Asp Ser Phe Gly His Tyr Arg Glu
210         215         220

Ala Lys Phe Ser Gln Thr Lys His His Trp Trp Trp Lys Leu His Phe
225         230         235         240

Val Trp Glu Arg Val Lys Ile Leu Arg Asp Tyr Ala Gly Leu Ile Leu
245         250         255

Phe Leu Glu Glu Asp His Tyr Leu Ala Pro Asp Phe Tyr His Val Phe
260         265         270

Lys Lys Met Trp Lys Leu Lys Gln Gln Glu Cys Pro Glu Cys Asp Val
275         280         285

Leu Ser Leu Gly Thr Tyr Ser Ala Ser Arg Ser Phe Tyr Gly Met Ala
290         295         300

Asp Lys Val Asp Val Lys Thr Trp Lys Ser Thr Glu His Asn Met Gly
305         310         315         320

Leu Ala Leu Thr Arg Asn Ala Tyr Gln Lys Leu Ile Glu Cys Thr Asp
325         330         335

Thr Phe Cys Thr Tyr Asp Asp Tyr Asn Trp Asp Trp Thr Leu Gln Tyr
340         345         350

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Leu Thr Val Ser Cys Leu Pro Lys Phe Trp Lys Val Leu Val Pro Gln
355                               360                               365

Ile Pro Arg Ile Phe His Ala Gly Asp Cys Gly Met His His Lys Lys
370                               375                               380

Thr Cys Arg Pro Ser Thr Gln Ser Ala Gln Ile Glu Ser Leu Leu Asn
385                               390                               395                               400

Asn Asn Lys Gln Tyr Met Phe Pro Glu Thr Leu Thr Ile Ser Glu Lys
405                               410                               415

Phe Thr Val Val Ala Ile Ser Pro Pro Arg Lys Asn Gly Gly Trp Gly
420                               425                               430

Asp Ile Arg Asp His Glu Leu Cys Lys Ser Tyr Arg Arg Leu Gln Thr
435                               440                               445

Arg Pro Ala Pro Gly Arg Pro Pro Ser Val Ser Ala Leu Asp Gly Asp
450                               455                               460

Pro Ala Ser Leu Thr Arg Glu Val Ile Arg Leu Ala Gln Asp Ala Glu
465                               470                               475                               480

Val Glu Leu Glu Arg Gln Arg Gly Leu Leu Gln Gln Ile Gly Asp Ala
485                               490                               495

Leu Ser Ser Gln Arg Gly Arg Val Pro Thr Ala Ala Pro Pro Ala Gln
500                               505                               510

Pro Arg Val Pro Val Thr Pro Ala Pro Ala Val Ile Pro Ile Leu Val
515                               520                               525

Ile Ala Cys Asp Arg Ser Thr Val Arg Arg Cys Leu Asp Lys Leu Leu
530                               535                               540

His Tyr Arg Pro Ser Ala Glu Leu Phe Pro Ile Ile Val Ser Gln Asp
545                               550                               555                               560

Cys Gly His Glu Glu Thr Ala Gln Ala Ile Ala Ser Tyr Gly Ser Ala
565                               570                               575

Val Thr His Ile Arg Gln Pro Asp Leu Ser Ser Ile Ala Val Pro Pro
580                               585                               590

Asp His Arg Lys Phe Gln Gly Tyr Tyr Lys Ile Ala Arg His Tyr Arg
595                               600                               605

Trp Ala Leu Gly Gln Val Phe Arg Gln Phe Arg Phe Pro Ala Ala Val
610                               615                               620

Val Val Glu Asp Asp Leu Glu Val Ala Pro Asp Phe Phe Glu Tyr Phe
625                               630                               635                               640

Arg Ala Thr Tyr Pro Leu Leu Lys Ala Asp Pro Ser Leu Trp Cys Val
645                               650                               655

Ser Ala Trp Asn Asp Asn Gly Lys Glu Gln Met Val Asp Ala Ser Arg
660                               665                               670

Pro Glu Leu Leu Tyr Arg Thr Asp Phe Phe Pro Gly Leu Gly Trp Leu
675                               680                               685

Leu Leu Ala Glu Leu Trp Ala Glu Leu Glu Pro Lys Trp Pro Lys Ala
690                               695                               700

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Phe Trp Asp Asp Trp Met Arg Arg Pro Glu Gln Arg Gln Gly Arg Ala  
705 710 715 720

Cys Ile Arg Pro Glu Ile Ser Arg Thr Met Thr Phe Gly Arg Lys Gly  
725 730 735

Val Ser His Gly Gln Phe Phe Asp Gln His Leu Lys Phe Ile Lys Leu  
740 745 750

Asn Gln Gln Phe Val His Phe Thr Gln Leu Asp Leu Ser Tyr Leu Gln  
755 760 765

Arg Glu Ala Tyr Asp Arg Asp Phe Leu Ala Arg Val Tyr Gly Ala Pro  
770 775 780

Gln Leu Gln Val Glu Lys Val Arg Thr Asn Asp Arg Lys Glu Leu Gly  
785 790 795 800

Glu Val Arg Val Gln Tyr Thr Gly Arg Asp Ser Phe Lys Ala Phe Ala  
805 810 815

Lys Ala Leu Gly Val Met Asp Asp Leu Lys Ser Gly Val Pro Arg Ala  
820 825 830

Gly Tyr Arg Gly Ile Val Thr Phe Gln Phe Arg Gly Arg Arg Val His  
835 840 845

Leu Ala Pro Pro Pro Thr Trp Glu Gly Tyr Asp Pro Ser Trp Asn  
850 855 860

<210> 241

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 241

gtaacgccag ggtttccca gtcacgacgg tttaaacgta ttgcgatgag cagcaga 57

<210> 242

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 242

atccacttaa cggtactgaa atctggtctc ctaaccacc aag 43

<210> 243

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 243  
 ctccttcaat atcatcttct gtctgtgaaa tgaggtcct tcc 43

<210> 244  
 <211> 57  
 <212> DNA  
 <213> Artificial Sequence

<220>  
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<400> 244  
 gcggataaca atttcacaca ggaaacagcg tttaaacc aa acgcagcaga aaccata 57

<210> 245  
 <211> 51  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer

<400> 245  
 gatttcagta acgtaagtg gatgcggccg cgacagaaga tgatattgaa g 51

<210> 246  
 <211> 50  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer

<400> 246  
 gacagaagat gatattgaag gaggcggccg ctaaagtgga tcccggtgac 50

<210> 247  
 <211> 68  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer

<400> 247  
 ggtgggttag gagaccagat ttcagtaacg ttaagtggat gcggccgcct agcatcgact 60  
 actgctgc 68

<210> 248  
 <211> 46  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer

<400> 248

gcagcagtag tcgatgctag gcgcgccatg caaagataca catcaa 46

<210> 249

<211> 46

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 249

ttgatgtgta tctttgcatg gcgcgcctag catcgactac tgctgc 46

<210> 250

<211> 68

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 250

agggacctca tttcacagac agaagatgat attgaaggag gcggccgcgg ctgatgaggc 60

tgagagag 68

<210> 251

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 251

tcgctgtaac gaacttctgt 20

<210> 252

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 252

cattgttgac ctccactagc 20

<210> 253

<211> 20

<212> DNA

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<223> Primer

<400> 253

gctgctgac ggacatttt 20

<210> 254

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gtttctggca gctggact 18

<210> 255

<211> 20

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tcgctgaac gaacttctgt 20

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<223> Primer

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tgcgtagccg tctcgctct 20

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<210> 258

<211> 20

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<220>

<223> Primer

<400> 258

ccggtagcgt tagagagacg 20



<210> 259  
 <211> 58  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer

<400> 259  
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<210> 260  
 <211> 69  
 <212> DNA  
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<220>  
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<400> 260  
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 ctccagacg 69

<210> 261  
 <211> 69  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer

<400> 261  
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 aataagcag 69

<210> 262  
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 <212> DNA  
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<220>  
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<400> 262  
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<210> 263  
 <211> 20  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Primer

<400> 263  
 gatcgacaaa gggtccagcg 20

<210> 264  
<211> 20  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Primer  
  
<400> 264  
aattgtatca ttccgaggct 20  
  
<210> 265  
<211> 19  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Primer  
  
<400> 265  
ctgtttggcc ctcgaaact 19  
  
<210> 266  
<211> 20  
<212> DNA  
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<220>  
<223> Primer  
  
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tgcgtcgccg tctcgctcct 20  
  
<210> 267  
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<212> DNA  
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ttcgccatcc aaatttctc 20  
  
<210> 268  
<211> 19  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Primer  
  
<400> 268  
catcctcaag gcctcagac 19  
  
<210> 269

<211> 20  
 <212> DNA  
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 <220>  
 <223> Primer  
  
 <400> 269  
 cccaagtcgt ctcagctctc 20  
  
 <210> 270  
 <211> 21  
 <212> DNA  
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 <220>  
 <223> Primer  
  
 <400> 270  
 tcgaaggctt cagtgagga a 21  
  
 <210> 271  
 <211> 57  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Primer  
  
 <400> 271  
 gtaacgccag ggttttccca gtcacgacgg tttaaaccatc tcggagtgat gcttcct 57  
  
 <210> 272  
 <211> 68  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Primer  
  
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 gcgctggcaa cgagagcaga gcagcagtag tcgatgctag gcggccgcac cagacgaaac 60  
 cagacgag 68  
  
 <210> 273  
 <211> 68  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Primer  
  
 <400> 273  
 caaccagccg cagcctcagc ctctctcagc ctcatcagcc gcggccgcgc gaatcgagtt 60  
 gatgattc 68  
  
 <210> 274

<211> 57  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer

<400> 274  
gcggataaca atttcacaca ggaaacagcg tttaaacctg gttgggatct gaccact 57

<210> 275  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer

<400> 275  
ctgtttggcc ctcgaaact 19

<210> 276  
<211> 20  
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<213> Artificial Sequence

<220>  
<223> Primer

<400> 276  
ccatgagctt gaacaggtaa 20

<210> 277  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer

<400> 277  
ttcgccatcc aaatttcttc 20

<210> 278  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer

<400> 278  
catcctcaag gcctcagac 19

<210> 279  
<211> 20

<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer

<400> 279  
gcagacaaac agagcaacga        20

<210> 280  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer

<400> 280  
ccatgagctt gaacaggtaa        20

<210> 281  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer

<400> 281  
tagagggtgt cgatggaagc        20

<210> 282  
<211> 19  
<212> DNA  
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<220>  
<223> Primer

<400> 282  
catcctcaag gcctcagac        19

<210> 283  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer

<400> 283  
ggtctcttct ttgccagcac        20

<210> 284  
<211> 20  
<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 284

gtcgcctgaa ctgaattgc 20

<210> 285

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 285

gattaagttg ggtaacgcca gggttttccc agtcacgacg gtttaaacac ctcatgaggg 60

actatgg 67

<210> 286

<211> 69

<212> DNA

<213> Artificial Sequence

<220>

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<220>

<223> Primer

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gtaacgccag gggtttcca gtacgacgg tttaaagcc tcctggat tcagaca 57

<210> 401

<211> 68

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 401

gcgctggcaa cgagagcaga gcagcagtag tcgatgctag gcggccgcga cgccagaaag 60

aaatgctc 68

<210> 402

<211> 68

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 402

caaccagccg cagcctcagc ctctctcagc ctcctcagcc gcggccgcga cctggtcagc 60

tgctcttt 68

<210> 403

<211> 57

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<213> Artificial Sequence

<220>

<223> Primer

<400> 403

gcggataaca attcacaca ggaacagcg tttaactgg aaccacatcg acttcac 57

<210> 404

<211> 20

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<213> Artificial Sequence

<220>

<223> Primer

<400> 404

aaccaccttg tcaccgtctc 20

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<220>

<223> Primer

<400> 405

ccatgagctt gaacaggtaa 20

<210> 406

<211> 20

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<220>

<223> Primer

<400> 406

gtcgtcgagg ctgctttatc 20

<210> 407

<211> 19

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<223> Primer

<400> 407

catcctcaag gcctcagac 19

<210> 408

<211> 20

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<213> Artificial Sequence

<220>

<223> Primer

<400> 408

gatctcgaat ccgaggacaa 20

<210> 409

<211> 398

<212> PRT

<213> Homo sapiens

<400> 409

Met Arg Leu Arg Glu Pro Leu Leu Ser Gly Ser Ala Ala Met Pro Gly  
1 5 10 15

Ala Ser Leu Gln Arg Ala Cys Arg Leu Leu Val Ala Val Cys Ala Leu  
 20 25 30

His Leu Gly Val Thr Leu Val Tyr Tyr Leu Ala Gly Arg Asp Leu Ser  
 35 40 45

Arg Leu Pro Gln Leu Val Gly Val Ser Thr Pro Leu Gln Gly Gly Ser  
 50 55 60

Asn Ser Ala Ala Ala Ile Gly Gln Ser Ser Gly Glu Leu Arg Thr Gly  
 65 70 75 80

Gly Ala Arg Pro Pro Pro Pro Leu Gly Ala Ser Ser Gln Pro Arg Pro  
 85 90 95

Gly Gly Asp Ser Ser Pro Val Val Asp Ser Gly Pro Gly Pro Ala Ser  
 100 105 110

Asn Leu Thr Ser Val Pro Val Pro His Thr Thr Ala Leu Ser Leu Pro  
 115 120 125

Ala Cys Pro Glu Glu Ser Pro Leu Leu Val Gly Pro Met Leu Ile Glu  
 130 135 140

Phe Asn Met Pro Val Asp Leu Glu Leu Val Ala Lys Gln Asn Pro Asn  
 145 150 155 160

Val Lys Met Gly Gly Arg Tyr Ala Pro Arg Asp Cys Val Ser Pro His  
 165 170 175

Lys Val Ala Ile Ile Ile Pro Phe Arg Asn Arg Gln Glu His Leu Lys  
 180 185 190

Tyr Trp Leu Tyr Tyr Leu His Pro Val Leu Gln Arg Gln Gln Leu Asp  
 195 200 205

Tyr Gly Ile Tyr Val Ile Asn Gln Ala Gly Asp Thr Ile Phe Asn Arg  
 210 215 220

Ala Lys Leu Leu Asn Val Gly Phe Gln Glu Ala Leu Lys Asp Tyr Asp  
 225 230 235 240

Tyr Thr Cys Phe Val Phe Ser Asp Val Asp Leu Ile Pro Met Asn Asp  
 245 250 255

His Asn Ala Tyr Arg Cys Phe Ser Gln Pro Arg His Ile Ser Val Ala  
 260 265 270

Met Asp Lys Phe Gly Phe Ser Leu Pro Tyr Val Gln Tyr Phe Gly Gly  
 275 280 285

Val Ser Ala Leu Ser Lys Gln Gln Phe Leu Thr Ile Asn Gly Phe Pro  
 290 295 300

Asn Asn Tyr Trp Gly Trp Gly Gly Glu Asp Asp Asp Ile Phe Asn Arg  
 305 310 315 320

Leu Val Phe Arg Gly Met Ser Ile Ser Arg Pro Asn Ala Val Val Gly  
 325 330 335

Arg Cys Arg Met Ile Arg His Ser Arg Asp Lys Lys Asn Glu Pro Asn  
 340 345 350

Pro Gln Arg Phe Asn Arg Ile Ala His Thr Lys Glu Thr Met Leu Ser

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355          360          365

Asp Gly Leu Asn Ser Leu Thr Tyr Gln Val Leu Asp Val Gln Arg Tyr
370          375          380

Pro Leu Tyr Thr Gln Ile Thr Val Asp Ile Gly Thr Pro Ser
385          390          395

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<211> 488
<212> PRT
<213> trichoderma reesei

<400> 410
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Ala Gln Ala Gln Val Val Gln Phe Asp Ile Glu Lys Arg His Ala Pro
20          25          30

Arg Leu Ser Arg Arg Asp Gly Thr Ile Asp Gly Thr Leu Ser Asn Gln
35          40          45

Arg Val Gln Gly Gly Tyr Phe Ile Asn Val Gln Val Gly Ser Pro Gly
50          55          60

Gln Asn Ile Thr Leu Gln Leu Asp Thr Gly Ser Ser Asp Val Trp Val
65          70          75          80

Pro Ser Ser Thr Ala Ala Ile Cys Thr Gln Val Ser Glu Arg Asn Pro
85          90          95

Gly Cys Gln Phe Gly Ser Phe Asn Pro Asp Asp Ser Asp Thr Phe Asp
100         105         110

Glu Val Gly Gln Gly Leu Phe Asp Ile Thr Tyr Val Asp Gly Ser Ser
115         120         125

Ser Lys Gly Asp Tyr Phe Gln Asp Asn Phe Gln Ile Asn Gly Val Thr
130         135         140

Val Lys Asn Leu Thr Met Gly Leu Gly Leu Ser Ser Ser Ile Pro Asn
145         150         155         160

Gly Leu Ile Gly Val Gly Tyr Met Asn Asp Glu Ala Ser Val Ser Thr
165         170         175

Thr Arg Ser Thr Tyr Pro Asn Leu Pro Ile Val Leu Gln Gln Gln Lys
180         185         190

Leu Ile Asn Ser Val Ala Phe Ser Leu Trp Leu Asn Asp Leu Asp Ala
195         200         205

Ser Thr Gly Ser Ile Leu Phe Gly Gly Ile Asp Thr Glu Lys Tyr His
210         215         220

Gly Asp Leu Thr Ser Ile Asp Ile Ile Ser Pro Asn Gly Gly Lys Thr
225         230         235         240

Phe Thr Glu Phe Ala Val Asn Leu Tyr Ser Val Gln Ala Thr Ser Pro
245         250         255

Ser Gly Thr Asp Thr Leu Ser Thr Ser Glu Asp Thr Leu Ile Ala Val

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|                                                                                    |     |     |
|------------------------------------------------------------------------------------|-----|-----|
| 260                                                                                | 265 | 270 |
| Leu Asp Ser Gly Thr Thr Leu Thr Tyr Leu Pro Gln Asp Met Ala Glu<br>275 280 285     |     |     |
| Glu Ala Trp Asn Glu Val Gly Ala Glu Tyr Ser Asn Glu Leu Gly Leu<br>290 295 300     |     |     |
| Ala Val Val Pro Cys Ser Val Gly Asn Thr Asn Gly Phe Phe Ser Phe<br>305 310 315 320 |     |     |
| Thr Phe Ala Gly Thr Asp Gly Pro Thr Ile Asn Val Thr Leu Ser Glu<br>325 330 335     |     |     |
| Leu Val Leu Asp Leu Phe Ser Gly Gly Pro Ala Pro Arg Phe Ser Ser<br>340 345 350     |     |     |
| Gly Pro Asn Lys Gly Gln Ser Ile Cys Glu Phe Gly Ile Gln Asn Gly<br>355 360 365     |     |     |
| Thr Gly Ser Pro Phe Leu Leu Gly Asp Thr Phe Leu Arg Ser Ala Phe<br>370 375 380     |     |     |
| Val Val Tyr Asp Leu Val Asn Asn Gln Ile Ala Ile Ala Pro Thr Asn<br>385 390 395 400 |     |     |
| Phe Asn Ser Thr Arg Thr Asn Val Val Ala Phe Ala Ser Ser Gly Ala<br>405 410 415     |     |     |
| Pro Ile Pro Ser Ala Thr Ala Ala Pro Asn Gln Ser Arg Thr Gly His<br>420 425 430     |     |     |
| Ser Ser Ser Thr His Ser Gly Leu Ser Ala Ala Ser Gly Phe His Asp<br>435 440 445     |     |     |
| Gly Asp Asp Glu Asn Ala Gly Ser Leu Thr Ser Val Phe Ser Gly Pro<br>450 455 460     |     |     |
| Gly Met Ala Val Val Gly Met Thr Ile Cys Tyr Thr Leu Leu Gly Ser<br>465 470 475 480 |     |     |
| Ala Ile Phe Gly Ile Gly Trp Leu<br>485                                             |     |     |

$\langle 210 \rangle$  411

<211> 761

&lt;212&gt; PRT

<213> trichoderma reesei

<400> 411

Met Arg Ser Thr Leu Tyr Gly Leu Ala Ala Leu Pro Leu Ala Ala Gln  
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Ala Leu Glu Phe Ile Asp Asp Thr Val Ala Gln Gln Asn Gly Ile Met  
20 25 30

Arg Tyr Thr Leu Thr Thr Thr Lys Gly Ala Thr Ser Lys His Leu His  
35 40 45

Arg Arg Gln Asp Ser Ala Asp Leu Met Ser Gln Gln Thr Gly Tyr Phe  
50 55 60

Tyr Ser Ile Gln Leu Glu Ile Gly Thr Pro Pro Gln Ala Val Ser Val  
65 70 75 80

Asn Phe Asp Thr Gly Ser Ser Glu Leu Trp Val Asn Pro Val Cys Ser  
 85 90 95  
 Lys Ala Thr Asp Pro Ala Phe Cys Lys Thr Phe Gly Gln Tyr Asn His  
 100 105 110  
 Ser Thr Thr Phe Val Asp Ala Lys Ala Pro Gly Gly Ile Lys Tyr Gly  
 115 120 125  
 Thr Gly Phe Val Asp Phe Asn Tyr Gly Tyr Asp Tyr Val Gln Leu Gly  
 130 135 140  
 Ser Leu Arg Ile Asn Gln Gln Val Phe Gly Val Ala Thr Asp Ser Glu  
 145 150 155 160  
 Phe Ala Ser Val Gly Ile Leu Gly Ala Gly Pro Asp Leu Ser Gly Trp  
 165 170 175  
 Thr Ser Pro Tyr Pro Phe Val Ile Asp Asn Leu Val Lys Gln Gly Phe  
 180 185 190  
 Ile Lys Ser Arg Ala Phe Ser Leu Asp Ile Arg Gly Leu Asp Ser Asp  
 195 200 205  
 Arg Gly Ser Val Thr Tyr Gly Gly Ile Asp Ile Lys Lys Phe Ser Gly  
 210 215 220  
 Pro Leu Ala Lys Lys Pro Ile Ile Pro Ala Ala Gln Ser Pro Asp Gly  
 225 230 235 240  
 Tyr Thr Arg Tyr Trp Val His Met Asp Gly Met Ser Ile Thr Lys Glu  
 245 250 255  
 Asp Gly Ser Lys Phe Glu Ile Phe Asp Lys Pro Asn Gly Gln Pro Val  
 260 265 270  
 Leu Leu Asp Ser Gly Tyr Thr Val Ser Thr Leu Pro Gly Pro Leu Met  
 275 280 285  
 Asp Lys Ile Leu Glu Ala Phe Pro Ser Ala Arg Leu Glu Ser Thr Ser  
 290 295 300  
 Gly Asp Tyr Ile Val Asp Cys Asp Ile Ile Asp Thr Pro Gly Arg Val  
 305 310 315 320  
 Asn Phe Lys Phe Gly Asn Val Val Val Asp Val Glu Tyr Lys Asp Phe  
 325 330 335  
 Ile Trp Gln Gln Pro Asp Leu Gly Ile Cys Lys Leu Gly Val Ser Gln  
 340 345 350  
 Asp Asp Asn Phe Pro Val Leu Gly Asp Thr Phe Leu Arg Ala Ala Tyr  
 355 360 365  
 Val Val Phe Asp Trp Asp Asn Gln Glu Val His Ile Ala Ala Asn Glu  
 370 375 380  
 Asp Cys Gly Asp Glu Leu Ile Pro Ile Gly Ser Gly Pro Asp Ala Ile  
 385 390 395 400  
 Pro Ala Ser Ala Ile Gly Lys Cys Ser Pro Ser Val Lys Thr Asp Thr  
 405 410 415  
 Thr Thr Ser Val Ala Glu Thr Thr Ala Thr Ser Ala Ala Ala Ser Thr  
 420 425 430  
 Ser Glu Leu Ala Ala Thr Thr Ser Glu Ala Ala Thr Thr Ser Ser Glu

435                      440                      445  
 Ala Ala Thr Thr Ser Ala Ala Ala Glu Thr Thr Ser Val Pro Leu Asn  
 450                      455                      460  
 Thr Ala Pro Ala Thr Thr Gly Leu Leu Pro Thr Thr Ser His Arg Phe  
 465                      470                      475                      480  
 Ser Asn Gly Thr Ala Pro Tyr Pro Ile Pro Ser Leu Ser Ser Val Ala  
 485                      490                      495  
 Ala Ala Ala Gly Ser Ser Thr Val Pro Ser Glu Ser Ser Thr Gly Ala  
 500                      505                      510  
 Ala Ala Ala Gly Thr Thr Ser Ala Ala Thr Gly Ser Gly Ser Gly Ser  
 515                      520                      525  
 Gly Ser Gly Asp Ala Thr Thr Ala Ser Ala Thr Tyr Thr Ser Thr Phe  
 530                      535                      540  
 Thr Thr Thr Asn Val Tyr Thr Val Thr Ser Cys Pro Pro Ser Val Thr  
 545                      550                      555                      560  
 Asn Cys Pro Val Gly His Val Thr Thr Glu Val Val Val Ala Tyr Thr  
 565                      570                      575  
 Thr Trp Cys Pro Val Glu Asn Gly Pro His Pro Thr Ala Pro Pro Lys  
 580                      585                      590  
 Pro Ala Ala Pro Glu Ile Thr Ala Thr Phe Thr Leu Pro Asn Thr Tyr  
 595                      600                      605  
 Thr Cys Ser Gln Gly Lys Asn Thr Cys Ser Asn Pro Lys Thr Ala Pro  
 610                      615                      620  
 Asn Val Ile Val Val Thr Pro Ile Val Thr Gln Thr Ala Pro Val Val  
 625                      630                      635                      640  
 Ile Pro Gly Ile Ala Ala Pro Thr Pro Thr Pro Ser Val Ala Ala Ser  
 645                      650                      655  
 Ser Pro Ala Ser Pro Ser Val Val Pro Ser Pro Thr Ala Pro Val Ala  
 660                      665                      670  
 Thr Ser Pro Ala Gln Ser Ala Tyr Tyr Pro Pro Pro Pro Pro Pro Glu  
 675                      680                      685  
 His Ala Val Ser Thr Pro Val Ala Asn Pro Pro Ala Val Thr Pro Ala  
 690                      695                      700  
 Pro Ala Pro Phe Pro Ser Gly Gly Leu Thr Thr Val Ile Ala Pro Gly  
 705                      710                      715                      720  
 Ser Thr Gly Val Pro Ser Gln Pro Ala Gln Ser Gly Leu Pro Pro Val  
 725                      730                      735  
 Pro Ala Gly Ala Ala Gly Phe Arg Ala Pro Ala Ala Val Ala Leu Leu  
 740                      745                      750  
 Ala Gly Ala Val Ala Ala Ala Leu Leu  
 755                      760

&lt;210&gt; 412

&lt;211&gt; 477

&lt;212&gt; PRT

&lt;213&gt; trichoderma reesei

&lt;400&gt; 412

Met Arg Phe Val Gln Tyr Val Ser Leu Ala Gly Leu Phe Ala Ala Ala  
 1 5 10 15

Thr Val Ser Ala Gly Val Val Thr Val Pro Phe Glu Lys Arg Asn Leu  
 20 25 30

Asn Pro Asp Phe Ala Pro Ser Leu Leu Arg Arg Asp Gly Ser Val Ser  
 35 40 45

Leu Asp Ala Ile Asn Asn Leu Thr Gly Gly Gly Tyr Tyr Ala Gln Phe  
 50 55 60

Ser Val Gly Thr Pro Pro Gln Lys Leu Ser Phe Leu Leu Asp Thr Gly  
 65 70 75 80

Ser Ser Asp Thr Trp Val Asn Ser Val Thr Ala Asp Leu Cys Thr Asp  
 85 90 95

Glu Phe Thr Gln Gln Thr Val Gly Glu Tyr Cys Phe Arg Gln Phe Asn  
 100 105 110

Pro Arg Arg Ser Ser Ser Tyr Lys Ala Ser Thr Glu Val Phe Asp Ile  
 115 120 125

Thr Tyr Leu Asp Gly Arg Arg Ile Arg Gly Asn Tyr Phe Thr Asp Thr  
 130 135 140

Val Thr Ile Asn Gln Ala Asn Ile Thr Gly Gln Lys Ile Gly Leu Ala  
 145 150 155 160

Leu Gln Ser Val Arg Gly Thr Gly Ile Leu Gly Leu Gly Phe Arg Glu  
 165 170 175

Asn Glu Ala Ala Asp Thr Lys Tyr Pro Thr Val Ile Asp Asn Leu Val  
 180 185 190

Ser Gln Lys Val Ile Pro Val Pro Ala Phe Ser Leu Tyr Leu Asn Asp  
 195 200 205

Leu Gln Thr Ser Gln Gly Ile Leu Leu Phe Gly Gly Val Asp Thr Asp  
 210 215 220

Lys Phe His Gly Gly Leu Ala Thr Leu Pro Leu Gln Ser Leu Pro Pro  
 225 230 235 240

Ser Ile Ala Glu Thr Gln Asp Ile Val Met Tyr Ser Val Asn Leu Asp  
 245 250 255

Gly Phe Ser Ala Ser Asp Val Asp Thr Pro Asp Val Ser Ala Lys Ala  
 260 265 270

Val Leu Asp Ser Gly Ser Thr Ile Thr Leu Leu Pro Asp Ala Val Val  
 275 280 285

Gln Glu Leu Phe Asp Glu Tyr Asp Val Leu Asn Ile Gln Gly Leu Pro  
 290 295 300

Val Pro Phe Ile Asp Cys Ala Lys Ala Asn Ile Lys Asp Ala Thr Phe

305 310 315 320

Asn Phe Lys Phe Asp Gly Lys Thr Ile Lys Val Pro Ile Asp Glu Met  
 325 330 335

Val Leu Asn Asn Leu Ala Ala Ala Ser Asp Glu Ile Met Ser Asp Pro  
 340 345 350  
 Ser Leu Ser Lys Phe Phe Lys Gly Trp Ser Gly Val Cys Thr Phe Gly  
 355 360 365  
 Met Gly Ser Thr Lys Thr Phe Gly Ile Gln Ser Asp Glu Phe Val Leu  
 370 375 380  
 Leu Gly Asp Thr Phe Leu Arg Ser Ala Tyr Val Val Tyr Asp Leu Gln  
 385 390 395 400  
 Asn Lys Gln Ile Gly Ile Ala Gln Ala Thr Leu Asn Ser Thr Ser Ser  
 405 410 415  
 Thr Ile Val Glu Phe Lys Ala Gly Ser Lys Thr Ile Pro Gly Pro Ala  
 420 425 430  
 Ser Thr Gly Asp Asp Ser Asp Asp Ser Ser Asp Asp Ser Asp Glu Asp  
 435 440 445  
 Ser Ala Gly Ala Ala Leu His Pro Thr Phe Ser Ile Ala Leu Ala Gly  
 450 455 460  
 Thr Leu Phe Thr Ala Val Ser Met Met Met Ser Val Leu  
 465 470 475

&lt;210&gt; 413

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 413

cctatgagtc gttaccag a 21

&lt;210&gt; 414

&lt;211&gt; 18

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 414

tggccgagtc tatgcgta 18

&lt;210&gt; 415

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 415

ggagttgctt taatgtcggg 20

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<211> 20

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<223> Primer

<400> 416

ttaggcgacc tcttttcca 20

<210> 417

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<212> DNA

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gtaagacgcc ccgtctc 17

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tggatcatgt tggcgacg 18

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<400> 420

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 <223> Primer  
  
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 <212> DNA  
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 agccagca 68  
  
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 agccagca 68

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<400> 434  
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<210> 435  
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<400> 435  
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agcagtccac ctgctcaaaa 20

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<213> Artificial Sequence

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ttaggcgacc tcttttcca 20

<210> 443

<211> 20

<212> DNA

<213> Artificial Sequence

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<210> 444

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 444

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<210> 445

<211> 20

<212> DNA

<213> Artificial Sequence

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<223> Primer

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<210> 446

<211> 20

<212> DNA

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&lt;223&gt; Primer

&lt;400&gt; 446

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&lt;210&gt; 447

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 447

ttgttgagcc agagtcgaga 20

&lt;210&gt; 448

&lt;211&gt; 69

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 448

gattaagtgt ggtaacgcca ggggttttccc agtcacgacg gtttaaaccg gttgtccatt 60

tcataccttc 69

&lt;210&gt; 449

&lt;211&gt; 68

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 449

gcgctggcaa cgagagcaga gcagcagtag tcgatgctag gcggccgcgg ggaagcaagt 60

ttcgaagt 68

&lt;210&gt; 450

&lt;211&gt; 66

<212> **DNA**

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 450

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ggccac 66

&lt;210&gt; 451

&lt;211&gt; 68

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 451

gtcattaagt ccatcattcc acgtccttca gaccgaattc ggcgcgcgg ggaagcaagt 60

ttcgaagt 68

&lt;210&gt; 452

&lt;211&gt; 68

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 452

atgatgcctt tgcagaaatg gcttgctcgc tgactgatac ggcgcgccta tcgcgaaagt 60

agccaata 68

&lt;210&gt; 453

&lt;211&gt; 58

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 453

agcggataac aatttcacac aggaaacagc gtttaaacca tcctttcct caccacga 58

&lt;210&gt; 454

&lt;211&gt; 68

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Primer

&lt;400&gt; 454

tgattgtacc ccagctgcga ttgatgtgta tctttgcatg atttaaattc tccttagctc 60

tgtacagt 68

&lt;210&gt; 455

&lt;211&gt; 28

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## REFERENCES CITED IN THE DESCRIPTION

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## Patentkrav

1. Filamentøs svampecelle til produktion af glycoproteiner med pattedyrlignende fucosylerede N-glycaner omfattende mindst en deletions- eller  
5 mutation reducerer sådan endogen proteaseaktivitet sammenlignet med en filamentøs modersvampecelle, der ikke har mutationen, endvidere omfattende et polynukleotid, der koder for et polypeptid, der har  $\alpha 1,6$  fucosyltransferaseaktivitet, og et eller flere polynukleotider, der koder for et polypeptid, der har GDP-fucosesynteseaktivitet, hvor GDP-  
10 fucosesynteseaktivitet omfatter a. GMD-polynukleotid eller et funktionelt variantpolynukleotid, der koder for et polypeptid, der har GDP-mannose-dehydrataseaktivitet, og b. FX-polynukleotid eller et funktionelt variantpolynukleotid, der koder for et polypeptid, der har både GDP-keto-deoxy-mannose-epimerase- og GDP-keto-deoxy-galactose-reductaseaktivitet og  
15 eventuelt GDP-fucosetransporteraktivitet, og hvor den filamentøse svampecelle er *Trichoderma*-celle.
2. Filamentøs svampecelle ifølge krav 1, hvor polynukleotidet, der koder for polypeptidet, der har  $\alpha 1,6$  fucosyltransferaseaktivitet, omfatter enten  
20 polynukleotidet fra SEQ ID NO:1 eller et funktionelt variantpolynukleotid, der koder for et polypeptid, der har mindst 50 %, mindst 60 %, mindst 70 %, mindst 90 % eller mindst 95 % identitet med SEQ ID NO:6, hvor polypeptidet har  $\alpha 1,6$  fucosyltransferaseaktivitet.
3. Filamentøs svampecelle ifølge krav 1 eller 2, hvor det ene eller de flere polynukleotider, der koder for polypeptidet, der har GDP-fucosesynteseaktivitet  
25 omfatter a. *C. elegans* GMD-polynukleotid fra SEQ ID NO:2 eller et funktionelt variantpolynukleotid, der koder for et polypeptid, der har mindst 50 %, mindst 60 %, mindst 70 %, mindst 90 % eller mindst 95 % identitet med SEQ ID NO:7, hvor polypeptidet har GDP-mannose-dehydrataseaktivitet; og b. *C. elegans* FX-polynukleotid fra SEQ ID NO:3 eller et funktionelt variantpolynukleotid, der  
30 koder for et polypeptid, der har mindst 50 %, mindst 60 %, mindst 70 %, mindst 90 % eller mindst 95 % identitet med SEQ ID NO:8, hvor polypeptidet har både

GDP-keto-deoxy-mannose-epimerase- og GDP-keto-deoxy-galactose-reductaseaktivitet;

og, eventuelt, hvor polynukleotidet, der koder for GDP-fucosetransporter er C. elegans GFT<sub>r</sub> fra SEQ ID NO:4 eller et funktionelt variantpolynukleotid, der

- 5 koder for et polypeptid, der har mindst 50 %, mindst 60 %, mindst 70 %, mindst 90 % eller mindst 95 % identitet med SEQ ID NO:9, hvor polypeptidet har GDP-fucosetransporteraktivitet.

4. Filamentøs svampecelle ifølge et hvilket som helst af de foregående krav, hvor polypeptidet, der har fucosyltransferaseaktivitet, endvidere omfatter en
- 10 Golgi-mårettet sekvens til målretning af ekspression af polypeptidet i den filamentøse svampecelles Golgi-compartment, såsom en Golgi-mårettet sekvens omfattende den N-terminale del af polypeptidet fra SEQ ID NO: 10.

5. Filamentøs svampecelle ifølge et hvilket som helst af de foregående krav, hvor cellen har en mutation, der nedsætter ekspressionsniveauet af et ALG3-gen sammenlignet med ekspressionsniveauet i en modercelle, der ikke har en
- 15 sådan mutation.

6. Filamentøs svampecelle ifølge et hvilket som helst af kravene 1-4, der endvidere omfatter et eller flere polynukleotider, der koder for et polypeptid udvalgt fra gruppen bestående af:

- 20 i.  $\alpha$ 1,2 mannosidase,  
ii. N-acetylglucosaminyltransferase I katalytisk domæne,  
iii.  $\alpha$  mannosidase II,  
iv. N-acetylglucosaminyltransferase II katalytisk domæne og, eventuelt,  
v. endvidere omfattende et polynukleotid, der koder for  $\beta$ 1,4
- 25 galactosyltransferase.

7. Filamentøs svampecelle ifølge et hvilket som helst af de foregående krav, hvor et fucosyltransferase, et N-acetylglucosaminyltransferase I, et  $\alpha$  mannosidase II, et N-acetylglucosaminyltransferase II og/eller et  $\beta$ 1,4 galactosyltransferase omfatter Golgi-mårettet sekvens udvalgt fra gruppen af

N-terminal del af GnTI, fortrinsvis human GnTI, N-terminal del af GnTII, fortrinsvis human GnTII og N-terminal del af Kre2, fortrinsvis *T. reesei* Kre2.

8. Filamentøs svampecelle ifølge et hvilket som helst af de foregående krav, der endvidere omfatter et eller flere polynukleotider, der koder for et polypeptid

5 udvalgt fra gruppen bestående af:

i) glucosamin UDP-N-acetylglucosamin-2-epimerase/N-acetylmannosaminkinase,

ii) N-acetylneuraminsyresynthase,

iii) N-acetylneuraminsyrephosphatase,

10 iv) cytidinmonophosphat-N-acetylneuraminsyresynthetase,

v) CMP-sialinsyretransporter og

vi) sialyltransferase.

9. Filamentøs svampecelle ifølge et hvilket som helst af de foregående krav, hvor mutationen reducerer proteaseaktiviteten af en endogen protease udvalgt

15 fra gruppen bestående af *Trichoderma reesei* pep1 (SEQ ID NO:17),

*Trichoderma reesei* pep2 (SEQ ID NO:18), *Trichoderma reesei* pep3 (SEQ ID

NO:19), *Trichoderma reesei* pep4 (SEQ ID NO:20), *Trichoderma reesei* pep5

(SEQ ID NO:21), *Trichoderma reesei* pep7 (SEQ ID NO:23), *Trichoderma*

*reesei* pep8 (SEQ ID NO:410), *Trichoderma reesei* pep11 (SEQ ID NO:411),

20 *Trichoderma reesei* pep12 (SEQ ID NO:412), *Trichoderma reesei* tpp1 (SEQ ID

NO:34), *Trichoderma reesei* tsp1 (SEQ ID NO:24), *Trichoderma reesei* slp1

(SEQ ID NO:25), *Trichoderma reesei* slp2 (SEQ ID NO:26), *Trichoderma reesei*

slp3 (SEQ ID NO:27), *Trichoderma reesei* slp5 (SEQ ID NO:28), *Trichoderma*

*reesei* slp6 (SEQ ID NO:29), *Trichoderma reesei* slp7 (SEQ ID NO:30),

25 *Trichoderma reesei* slp8 (SEQ ID NO:31), *Trichoderma reesei* gap1 (SEQ ID

NO:32) og *Trichoderma reesei* gap2 (SEQ ID NO:33).

10. Filamentøs svampecelle ifølge krav 9, hvor cellen omfatter mutationer, der reducerer eller eliminerer aktiviteten af de

a. tre endogene proteaser pep1, tsp1 og slp1 hos *Trichoderma reesei*;

30 b. tre endogene proteaser gap1, slp1 og pep1 hos *Trichoderma reesei*;

c. tre endogene proteaser udvalgt fra gruppen bestående af pep1, pep2, pep3, pep4, pep5, pep8, pep11, pep12, tsp1, slp1, slp2, slp3, slp7, gap1 og gap2 hos *Trichoderma reesei*;

d. tre til seks proteaser udvalgt fra gruppen bestående af pep1, pep2, pep3,

5 pep4, pep5, tsp1, slp1, slp2, slp3, gap1 og gap2 hos *Trichoderma reesei*; eller

e. syv til ti proteaser udvalgt fra gruppen bestående af pep1, pep2, pep3, pep4, pep5, pep7, pep8, tsp1, slp1, slp2, slp3, slp5, slp6, slp7, slp8, tpp1, gap1 og gap2 hos *Trichoderma reesei*.

11. Filamentøs svampecelle ifølge et hvilket som helst af de foregående krav,

10 hvor cellen endvidere omfatter mutationer i et eller flere gener, der koder for glycosylhydrolaser, hvor mutationerne eliminerer eller reducerer aktiviteten af de tilsvarende hydrolaser, og hvor hydrolaserne er udvalgt fra gruppen bestående af xylanase, cellobiohydrolase og endoglucanase.

12. Fremgangsmåde til produktion af et glycoprotein eller antistof med

15 fucosyleret N-glycan omfattende:

a. tilvejebringelse af en filamentøs svampecelle ifølge et hvilket som helst af de foregående krav, hvilken celle endvidere omfatter et polynukleotid, der koder for et glycoprotein eller et antistof, og

b. dyrkning af cellen for at producere glycoproteinet eller antistoffet med

20 fucosyleret N-glycan.

13. Fremgangsmåde ifølge krav 12, hvor det fucosylerede N-glycan er udvalgt

fra gruppen bestående af  $\text{Man}_3\text{GlcNAc}_2(\text{Fuc})$ ,  $\text{GlcNAcMan}_3\text{GlcNAc}_2(\text{Fuc})$ ,

$\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2(\text{Fuc})$ ,  $\text{Gal}_{1-2}\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2(\text{Fuc})$ ,  $\text{Neu}_5\text{Ac}_{1-2}\text{Gal}_{1-}$

$_2\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2(\text{Fuc})$ , og eventuelt hvor mindst 5 mol%, mindst 10 mol%

25 eller mindst 15 mol% af de samlede secererede N-glycaner består af

$\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2(\text{Fuc})$ .

14. Fremgangsmåde ifølge et hvilket som helst af kravene 12 og 13, hvor

polynukleotidet, der koder for et glycoprotein eller et antistof er et rekombinant

polynukleotid, der koder for et heterologt glycoprotein eller heterologt antistof.



## DRAWINGS

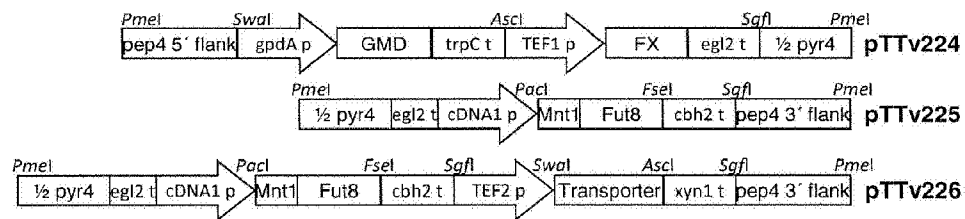
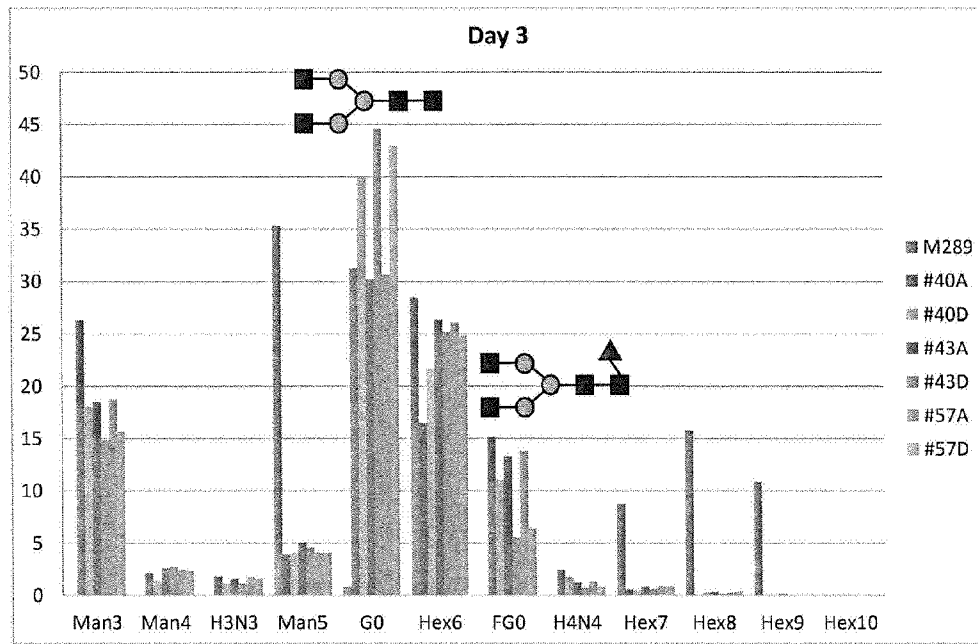


Figure 1

**Figure 2A**

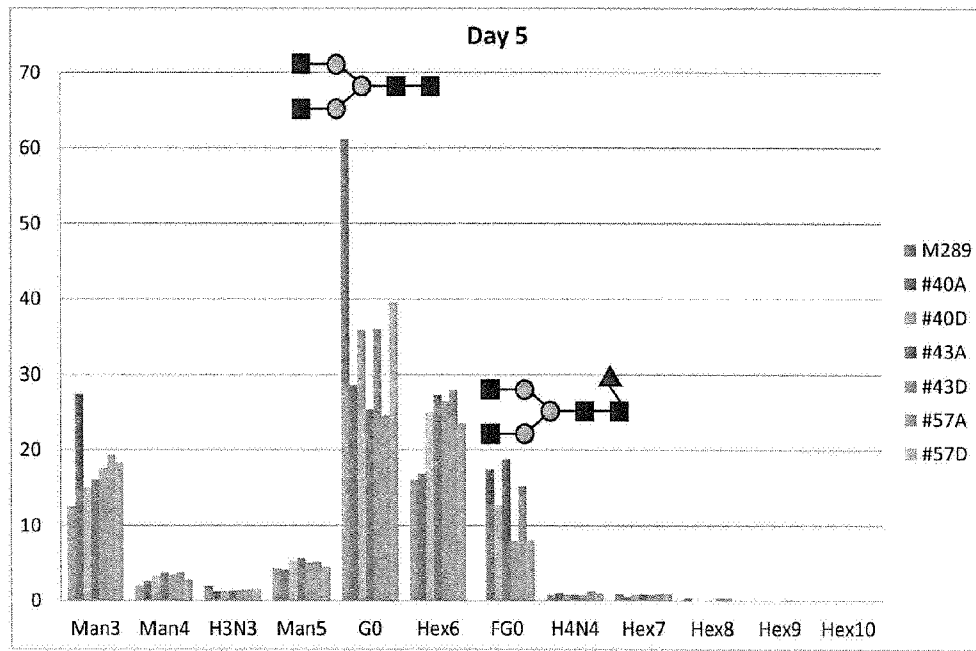


Figure 2B

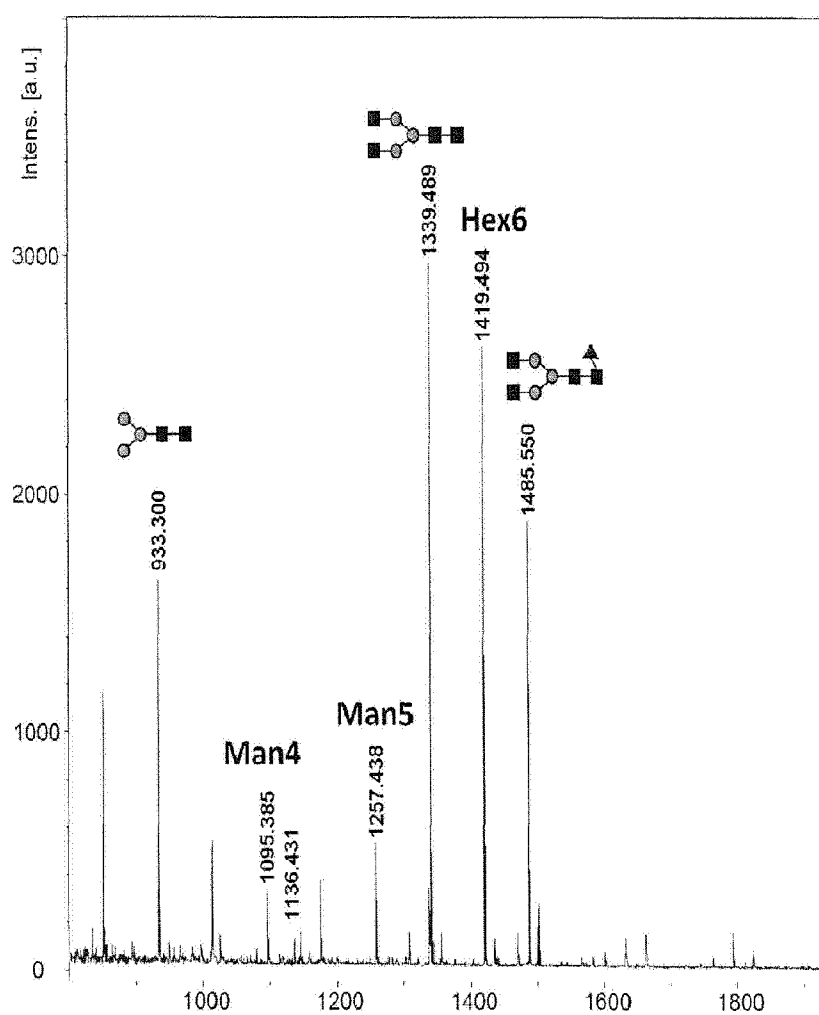


Figure 3

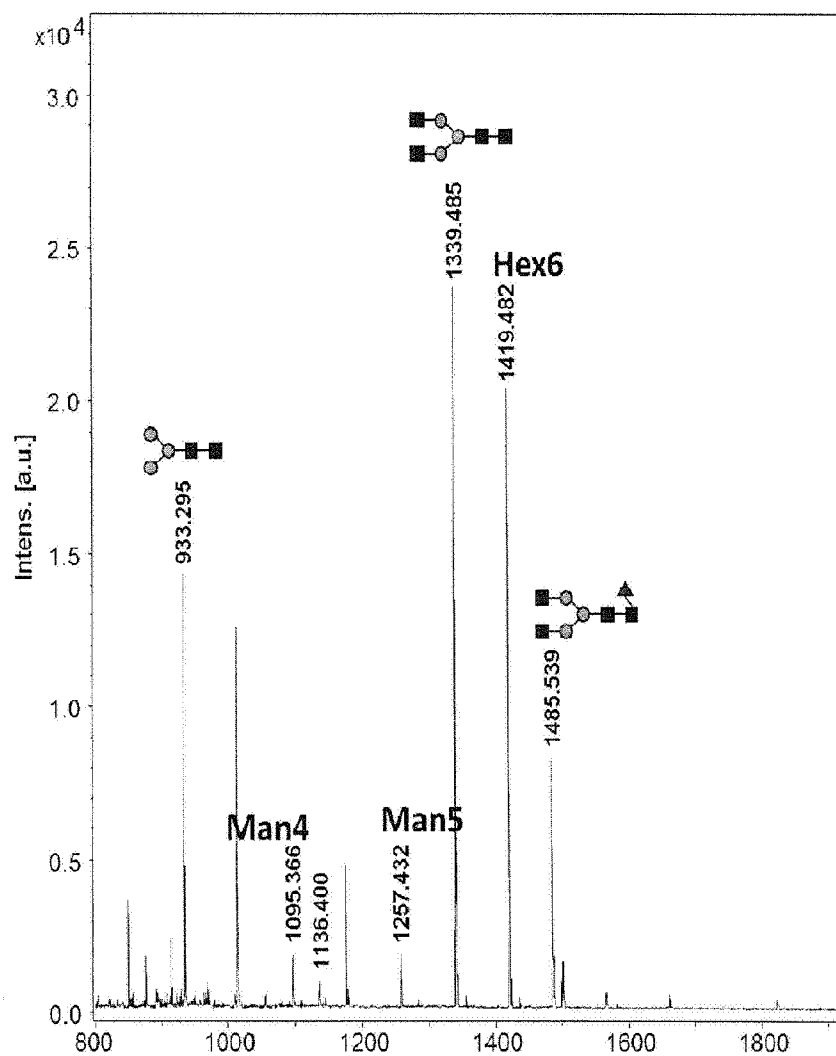


Figure 4

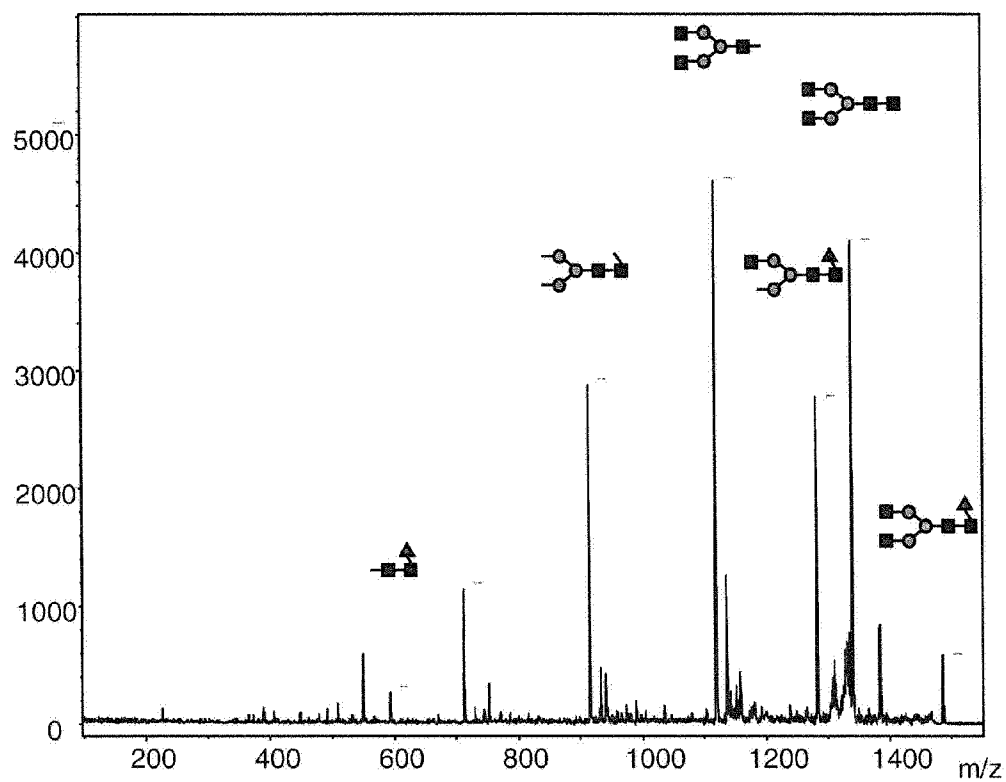


Figure 5

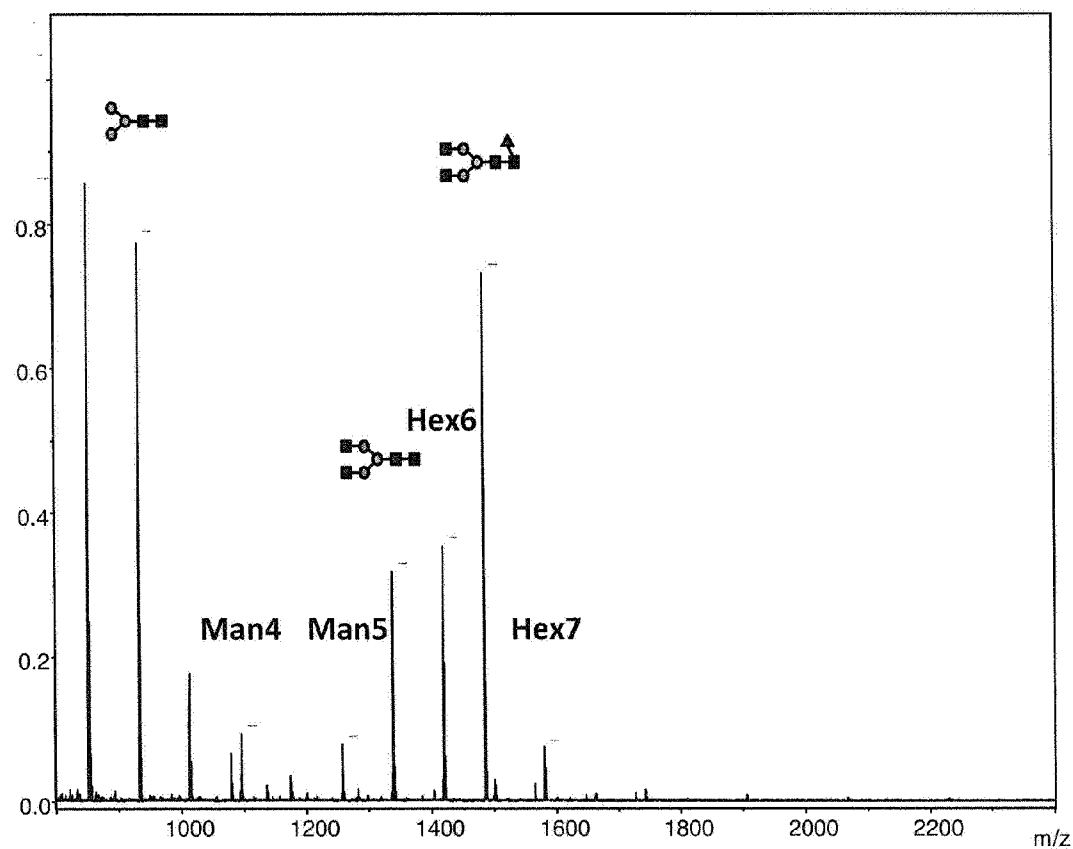


Figure 6

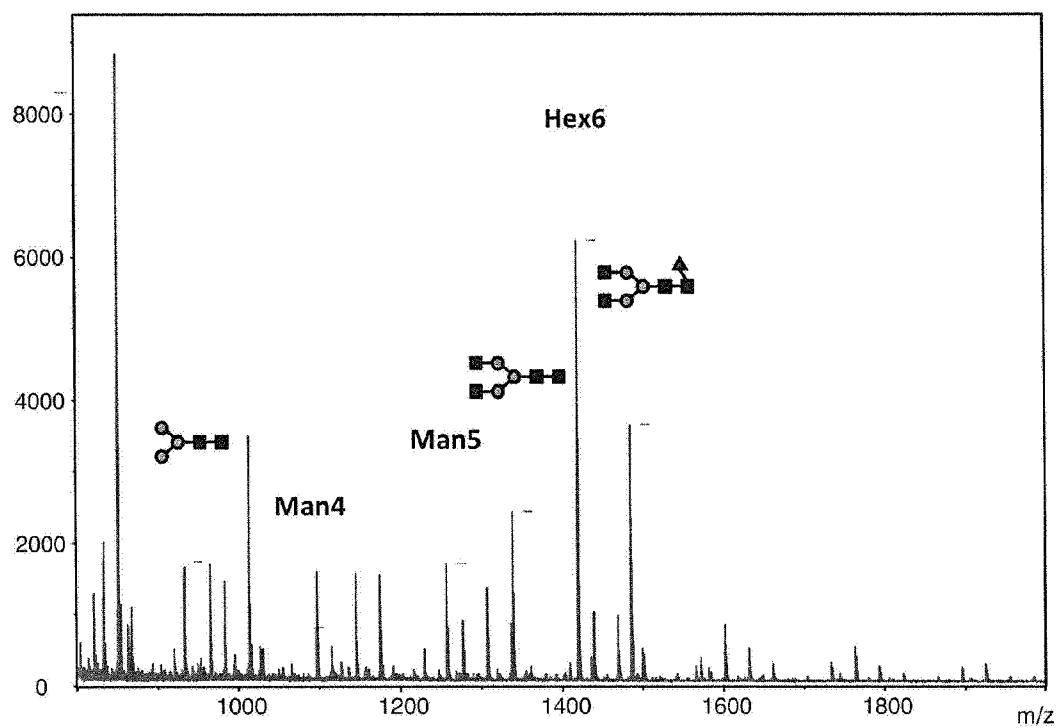


Figure 7



## FX alignment

CLUSTAL 2.1 multiple sequence alignment [overall sequence homology &gt;62%]

```

EcaballusFX      -----
ClupusFX         -----
HsapiensFX       -----
RnorvegicusFX    -----
MmusculusFX      -----
CgriseusFX       -----
BtaurusFX        -----
GgallusFX        -----
XlaevisFX        -----
DrerioFX         -----
AsumFX           -----
BmalayiFX        MASSRRRLKKELTDIQSSDSRTFCCVEFDENLLHWTGLLVDPKPEPYNKGAFKVAIDFPV 60
CelegansFX       -----

EcaballusFX      -----
ClupusFX         -----
HsapiensFX       -----
RnorvegicusFX    -----
MmusculusFX      -----
CgriseusFX       -----
BtaurusFX        -----
GgallusFX        -----
XlaevisFX        -----
DrerioFX         -----
AsumFX           -----
BmalayiFX        EYPFKPPKITFLTKIYHPNVDEKGQVCLPIISPDNWKPATKTEQVMNALLGLITEPEPDH 120
CelegansFX       -----

EcaballusFX      -----MGEPPQGS MRILVTGSSGL 18
ClupusFX         -----MSEPPGGS VRILVTGSSGL 18
HsapiensFX       -----MGEPPQGS MRILVTGSSGL 18
RnorvegicusFX    -----MGEPPHGS MRILVTGSSGL 18
MmusculusFX      -----MGEPPHGS MRILVTGSSGL 18
CgriseusFX       -----MGEPPQGS RRILVTGSSGL 18
BtaurusFX        -----MGDPGRG TRRILVTGSSGL 18
GgallusFX        -----MTEALGAKPKRILVTGGTGL 20
XlaevisFX        -----MEGKRILVTGSSGL 14
DrerioFX         -----MNGTVEE PMRVLVTGSSGL 18
AsumFX           -----MASEKQMI VLVTTGGTGL 17
BmalayiFX        PLRADLAE EPTKDRKKFNKTAEDYTKYAVKRPDGFETRHKIMDREQSMTVLVTGGTGL 180
CelegansFX       -----MKTILVTGGTGL 12
                      :****:

EcaballusFX      VGRAIQKV VADGAGLPGEDWVFVSSKDADLTDA AQTRALFEKVRPTHVIHLAAMVGG LFR 78
ClupusFX         VGRAIQKV VADGAGLPGEDWVFVSSKDADLTDA AQTRALFEKVRPTHVIHLAAMVGG LFR 78
HsapiensFX       VGKAIQKV VADGAGLPGEDWVFVSSKDADLTDA AQTRALFEKVQPTHVIHLAAMVGG LFR 78
RnorvegicusFX    VGRAIQKV VADGAGLPGEEWVFVSSKDADLTDA AQTQALFQKVQPTHVIHLAAMVGG LFR 78
MmusculusFX      VGRAIQKV VADGAGLPGEEWVFVSSKDADLTDA AQTQALFQKVQPTHVIHLAAMVGG LFR 78
CgriseusFX       VGRAIQKV VADGAGLPGEEWVFVSSKDADLTDA AQTQALFQKVQPTHVIHLAAMVGG LFR 78
BtaurusFX        VGRAIQKV VEDGAGLPGEDWVFVSSKDADLTDA AQTRALFQQVQPTHVIHLAAMVGG LFR 78
GgallusFX        VGRAIQEV VANGEGRPDEEWVFVSSRDADLTSA VETKALFEKHKPTHVIHLAAMVGG LFK 80
XlaevisFX        VGKAI EKVVADGEGRPDEQWIFISSKDADLTNA ADTKCLFEKHKPTHVIHLAAMVGG LFR 74
DrerioFX         VGRAIER VVKD-EGREGEEWTFLLSSKDANLLS AEETRAIFQKYRPTHVIHLAAMVGG LFR 77
AsumFX           VGKAI EKIVKTEESRPNERWIFIGSKDCDLS DLEATRKLFAKHKPTHVIHLAAMVGG LFR 77
BmalayiFX        VGRSIEK IITTEEARPNETWIFVGRNDCDLTD IEATRKLFMKCRPSHVIHLAAMVGG LFR 240
CelegansFX       VGSIAKK VVETTEKRDDEKWFIFGSKDCDLEN LEETRELFESVKPTHVIHLAAMVGG LFR 72
** :*::::      . * * *: . . * : * . * : * . :*:*****:

```

Figure 8

```

EcaballusFX      NIKYNLDFWRKNVHINDNVLSAFEVVGARKVVSCLSTCIPFDKTTYPIDETMIHNGPPHS 138
ClupusFX         NIKYNLDFWRKNVHINDNVLSAFEVVGVRKVVSCSLSTCIPFDKTTYPIDETMIHNGPPHN 138
HsapiensFX       NIKYNLDFWRKNVHMNDNVLSAFEVVGARKVVSCLSTCIPFDKTTYPIDETMIHNGPPHN 138
RnorvegicusFX    NIKYNLDFWRKNVHINDNVLSAFEVVGTRKVVSCSLSTCIPFDKTTYPIDETMIHNGPPHS 138
MmusculusFX      NIKYNLDFWRKNVHINDNVLSAFEVVGARKVVSCLSTCIPFDKTTYPIDETMIHNGPPHS 138
CgriseusFX       NIKYNLDFWRKNVHINDNVLSAFEVVGTRKVVSCSLSTCIPFDKTTYPIDETMIHNGPPHS 138
BtaurusFX        NIKYNLDFWRKNVHINDNVLSAFEVVGVRKVVSCSLSTCIPFDKTTYPIDETMIHNGPPHS 138
GgallusFX        NIRCNLDFWRNRNIHINDNVLSAYECGVQKVVSCSLSTCIPFDKTTYPIDETMIHNGPPHS 140
XlaevisFX        NMKYNLDFLRNNLHINDNVLSAYEMGVQKVVSCSLSTCIPFDKTTYPIDETMIHNGPPHT 134
DrerioFX         NMRQNLDFWRNRNVFINDNVLQTANEFVGVKVVSCSLSTCIPFDKTTYPIDETMIHNGPPHD 137
AuumFX           NLQHNLQFFRKNNMQINDNVLAVCDENDIEKCVSCLSTCIPFDKTTYPIDETMVHNGPPHD 137
BmalayiFX        NLHCNLQFFRKNNMQINDNVLMACNEFDVVKCISCLSTCVFPDKTTYPIDETMVHNGPPHS 300
CelegansFX       NLAHNLQFFRKNNMAINDNVLALCHEFDVLCVSCSLSTCIPFDKTSYPIDETMVHLGPPHD 132
*:  **:* *.*: :***** . * . * :*****:*****:*****:* ****

EcaballusFX      SNFGYSYAKRMIDVQNRAVFQQHGCTFTAVIPTNVFGPHDNFNIEDGHVLPGLIHKVHLA 198
ClupusFX         SNFGYSYAKRMIDVQNRAVFQQHGCTFTAVIPTNVFGPHDNFNIEDGHVLPGLIHKVHLA 198
HsapiensFX       SNFGYSYAKRMIDVQNRAVFQQYVGCTFTAVIPTNVFGPHDNFNIEDGHVLPGLIHKVHLA 198
RnorvegicusFX    SNFGYSYAKRMIDVQNRAVFQQHGCTFTSVIPTNVFGPYDNFNIEDGHVLPGLIHKVHLA 198
MmusculusFX      SNFGYSYAKRMIDVQNRAVFQQHGCTFTAVIPTNVFGPYDNFNIEDGHVLPGLIHKVHLA 198
CgriseusFX       SNFGYSYAKRMIDVQNRAVFQQHGCTFTAVIPTNVFGPHDNFNIEDGHVLPGLIHKVHLA 198
BtaurusFX        SNFGYSYAKRMIDVQNRAVFQQHGCTFTAVIPTNVFGPHDNFNSIEDGHVLPGLIHKVHLA 198
GgallusFX        SNFGYSYAKRMIDVQNRGYFEQHGCRFTAVIPTNVFGPHDNFNIEDGHVLPGLIHKVYLA 200
XlaevisFX        SNFGYSYAKRMIDVQNRAVYEQHGCKFTSVIPTNVFGPHDNFNIEDGHVLPGLIHKVYSA 194
DrerioFX         SNFGYAFAKRMIDVQNRCTCFKQYGRRYTSVPIPTNVFGAHDNFNIEDGHVLPGLIHKTYLA 197
AuumFX           SNFGYSYAKRMIDVNLNRGYAQEGRKYTSVPCNVFGPYDNVNLVEYGHVLPALIHKTFIA 197
BmalayiFX        SNFGYSYAKRMIDVNLNRGYAQEGRKYTSVPCNVFGPHDNVNLKDGHVLPALIHKTYIA 360
CelegansFX       SNFGYSYAKRMIDVNLNGYAEHGRKYTSVPCNVFGPHDNVNLQSGHVLPALIHKAYVA 192
*****:***** *:  :*: * :*: * ****:***:.. ***:* ***** *

EcaballusFX      KSSGSALTVMWGTGKPRRQFIYSLDLARLFIWVLRREYNEVEPIILSVGEEDEVSIQEAAEA 258
ClupusFX         KSSGSALTVMWGTGKPRRQFIYSLDLARLFIWVLRREYNEVEPIILSVGEEDEVSIQEAAEA 258
HsapiensFX       KSSGSALTVMWGTGNPRRQFIYSLDLAQLFIWVLRREYNEVEPIILSVGEEDEVSIKEAAEA 258
RnorvegicusFX    KSSGSALTVMWGTGKPRRQFIYSLDLARLFIWVLRREYNEVEPIILSVGEEDEVSIKEAAEA 258
MmusculusFX      KSSDSALTVMWGTGKPRRQFIYSLDLARLFIWVLRREYSEVEPIILSVGEEDEVSIKEAAEA 258
CgriseusFX       KSSGSALTVMWGTGKPRRQFIYSLDLARLFIWVLRREYNEVEPIILSVGEEDEVSIKEAAEA 258
BtaurusFX        KSSGSALTVMWGTGRPRRQFIYSLDLARLFIWVLRREYDEVEPIILSVGEEDEVSVQEAAEA 258
GgallusFX        KQNGSALTVMWGTGKPRRQFIYSLDLARLFWVLRREYEEVEPIILSVGEEDEVSIKEAAEA 260
XlaevisFX        KQNGTALSIWGTGQPRRQFIYSLDLARLFIWVLRREYNEVDPIILSVGEEDEVSIKEAESA 254
DrerioFX         KKEGKPLQVWGSCKPLRQFIYSLDLARLFWVLRREYDEVDPIILSVGEEDELSTKDCADA 257
AuumFX           KRDGKPLEVFGSGAPLRQFIYSLDLARLFWVLRREYEEVEPIILSVGEEDEVSIMDAVNA 257
BmalayiFX        KHEGTPLEVFGSGTPLRQFIYSLDLARLFIWVARSYEEIDPIILSVGEEDEVSIMDAVHA 420
CelegansFX       QRDGTPLQVYGSCTPLRQFIYSIDLARLFIWVREYEDVEPIILSVNESDEVSTIRDAVSA 252
:  ...* :*: * * *****:***:***: . *.*: :*****:***:***: :.. :

EcaballusFX      VVEAMDFHGDVTFDFTKSDGQFKKTASNGKLRLAYLPDFRFTPFKQAVKETCAWFTDNYEQ 318
ClupusFX         VVEAMDFHGEVTFDFTKSDGQFKKTASNGKLRTYLPDFRFTPFKQAVKETCAWFTDNYEQ 318
HsapiensFX       VVEAMDFHGEVTFDFTKSDGQFKKTASNSKLRTYLPDFRFTPFKQAVKETCAWFTDNYEQ 318
RnorvegicusFX    VVEAMDFSGEVTFDSTKSDGQYKKTASNGKLRSYLPDFCFPTPFKQAVKETCAWFTDNYEQ 318
MmusculusFX      VVEAMDFNGEVTFDSTKSDGQYKKTASNGKLRSYLPDFRFTPFKQAVKETCTWFTDNYEQ 318
CgriseusFX       VVEAMDFCGEVTFDSTKSDGQYKKTASNGKLRLAYLPDFRFTPFKQAVKETCAWFTDNYEQ 318
BtaurusFX        VVEAMDFHGEVTFDFTKSDGQFKKTASNAKLRLAYLPDFRFTPFKQAVKETCAWFTDNYEQ 318
GgallusFX        IVEAMDFRGELIFDFTKADGQFKKTASNAKLRLHYLPNFQFTPFKQAVKETCTWFTDNYAS 320
XlaevisFX        IVAAMEFKGELIFDSTKSDGQFKKTASNAKLRLKYLPDFQFTPFKQAVKETCNWFNSNYAQ 314
DrerioFX         VVDALGFKGDVIYDTSKADGQFKKTASNAKLRLQYLPDFQFTPFREAIKETCDWFWVANYDI 317
AuumFX           IVKAFDFKGGIVQDKSKADGQYKKTASNAKLRLKYLPDFKFTPFDAIKESVDWFWVANYED 317
BmalayiFX        VVRAFDFKGEIVHDKTKADGQYKKTASNAKLRLKYLPNFKFTPFETAIKESVDWFIIDNYDN 480
CelegansFX       VVKAFIDFTGDVEYDTSKADGQFKKTASNEKLLKLPDFQFTPFQEQAIQESVQWFWVDNYET 312
*:  *:  * * :  *.*:***:***** ** :*: * **** *:*: * ** **

```

Figure 8

|               |         |
|---------------|---------|
| EcaballusFX   | ARK 321 |
| ClupusFX      | ARK 321 |
| HsapiensFX    | ARK 321 |
| RnorvegicusFX | ARK 321 |
| MmusculusFX   | ARK 321 |
| CgriseusFX    | ARK 321 |
| BtaurusFX     | ARK 321 |
| GgallusFX     | ARK 323 |
| XlaevisFX     | ARK 317 |
| DrerioFX      | ARK 320 |
| AsuumFX       | ARK 320 |
| BmalayiFX     | ARK 483 |
| CelegansFX    | ARK 315 |
|               | ***     |

**Figure 8**

## GMD alignment, eukaryotic

CLUSTAL 2.1 multiple sequence alignment [overall sequence homology &gt;59%]

```

MmusculusGMD      -----MAQAPAKCPS-----YPGSGDGEMG 20
RnorvegicusGMD    -----MAHAPASCRR-----YPGSGDGEMG 20
CgriseusGMD       -----MAHAPASCPS-----SRNSGDGDKG 20
HsapiensGMD       -----MAHAPARCP-----ARGSGDGEMG 20
BtaurusGMD        -----MAQAAAHYPG-----ACGAGDAETG 20
XlaevisGMF        -----MAQNQGNCS-----SPSNSS---G 17
DrerioGMD         -----MAQCTATTTC-----GGMNGD--SK 18
SsalarGMD         -----MAQCTEPSTS-----TGANGE--LK 18
CintestinalisGMD -----MADSSCNGKRR-----NGDSCDTETK 21
AgambiaeGMD       -----MEGEAAVTNP-----MEGEAAVTNP 10
AaegyptiGMD       -----MSLENGSNKKRRLSLEN-----HLATESEGSSSD 30
DmelanogasterGMD  -----MLNTRLIAMSTSDGAPETKKQRPESSSNGSKDQNGTEAGAEGD 43
AmelliferaGMD     -----MKMATD-----MKMATD 6
LloaGMD           -----MNTEALEDCLGNRHS-----MKPISEVEKCKF 27
BmalayiGMD        -----MTINATKMNTTETQENCLGNRSS-----MKTISEVERYKC 34
CelegansGMD       MPTGKSESSDISEVVGNMBSKVEGLEACIGMSHE-----VSTTPAAELAAF 47

MmusculusGMD      KLRKVALITGITGQDGSYLAEFLLKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 79
RnorvegicusGMD    KLRKVALITGITGQDGSYLAEFLLKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 79
CgriseusGMD       KPRKVALITGITGQDGSYLAEFLLKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 79
HsapiensGMD       KPRNVALITGITGQDGSYLAEFLLKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 79
BtaurusGMD        KPRKVALITGITGQDGSYLAEFLLKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 79
XlaevisGMF        KPRKVALITGITGQDGSYLAEFLLKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 76
DrerioGMD         RKRKVALITGITGQDGSYLAELLAKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 77
SsalarGMD         KPRKVALITGITGQDGSYLAEFLLKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 77
CintestinalisGMD KPRKVALITGITGQDGSYLAELLAKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 80
AgambiaeGMD       VDRKVALITGITGQDGSYLAEFLLKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 69
AaegyptiGMD       PNRRVALITGITGQDGSYLAEFLLKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 89
DmelanogasterGMD SRDKVALITGITGQDGSYLAEFLLKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 102
AmelliferaGMD     RRRVALITGITGQDGSYLAEFLLKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 65
LloaGMD           RTFKIALITGISGQDGSYLAELLKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 87
BmalayiGMD        RARKIALITGISGQDGSYLAELLKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 94
CelegansGMD       RARKVALITGISGQDGSYLAELLKGYEVHGIIVRRSSSFNTGRIEHLKYNPQAH-EGN 107

      . : : ** : ***** : . : . : ** : ** : * * * : ** : *

MmusculusGMD      MKLHYGDLTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDLAEYADVDGVGTLRLLDAL 139
RnorvegicusGMD    MKLHYGDLTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDLAEYADVDGVGTLRLLDAL 139
CgriseusGMD       MKLHYGDLTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDLAEYADVDGVGTLRLLDAL 139
HsapiensGMD       MKLHYGDLTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDLAEYADVDGVGTLRLLDAL 139
BtaurusGMD        MKLHYGDLTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDLAEYADVDGVGTLRLLDAL 139
XlaevisGMF        MKLHYGDLTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDLAEYADVDGLGTLRLLDAL 136
DrerioGMD         MKLHYGDLTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDLAEYADVDGVGTLRLLDAL 137
SsalarGMD         MKLHYGDLTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDLAEYADVDGVGTLRLLDAL 137
CintestinalisGMD MKLHYGDLTDSSCLVKIINEVKPTEIYNLGAQSHVKISFDLAEYADVDGVGTLRLLDAL 140
AgambiaeGMD       MKLHYGDMTDSSALVKIINQVRPSEIYNLGAQSHVKISFDLAEYADVDGVGTLRLLDAL 129
AaegyptiGMD       MKLHYGDMTDSSCLVKIINQVRPSEIYNLGAQSHVKISFDLAEYADVDGVGTLRLLDAL 149
DmelanogasterGMD MKLHYGDMTDSSCLVKIINQVRPSEIYNLGAQSHVKISFDLAEYADVDGVGTLRLLDAL 162
AmelliferaGMD     MKLHYGDMTDSSCLVKIINQVRPSEIYNLGAQSHVKISFDLAEYADVDGVGTLRLLDAL 125
LloaGMD           FTLHYGDMTDSSCLIKLVSIQPTVEVYHLAAQSHVKISFDLAEYADVDGVGTLRLLDAL 147
BmalayiGMD        FTLHYGDMTDSSCLIKLVSIQPTVEVYHLAAQSHVKISFDLAEYADVDGVGTLRLLDAL 154
CelegansGMD       FSLHYGDMTDSSCLIKLVSIQPTVEVYHLAAQSHVKISFDLAEYADVDGVGTLRLLDAL 167

      . : ***** : ** : . : : . : * : * : * * * : ** : . : ***** : ** : * : ** :

```

Figure 9

|                  |                                                                                 |     |
|------------------|---------------------------------------------------------------------------------|-----|
| MmusculusGMD     | KTCGLINSVKFYQASTSELYGKVQEIPQKETTPFYPSPYGAAKLYAYWIVVNFREAYNL                     | 199 |
| RnorvegicusGMD   | KTCGLINSVKFYQASTSELYGKVQEIPQKETTPFYPSPYGAAKLYAYWIVVNFREAYNL                     | 199 |
| CgriseusGMD      | KTCGLINSVKFYQASTSELYGKVQEIPQKETTPFYPSPYGAAKLYAYWIVVNFREAYNL                     | 199 |
| HsapiansGMD      | KTCGLINSVKFYQASTSELYGKVQEIPQKETTPFYPSPYGAAKLYAYWIVVNFREAYNL                     | 199 |
| BtaurusGMD       | KTCGLISSVRFYQASTSELYGKVQEIPQKETTPFYPSPYGAAKLYAYWIVVNFREAYNL                     | 199 |
| XlaevisGMF       | KTCGLINTVKFYQASTSELYGKVQEIPQKETTPFYPSPYGAAKLYAYWIVVNFREAYNL                     | 196 |
| DrerioGMD        | KTCGLTDTVFYQASTSELYGKVQEIPQKETTPFYPSPYGAAKLYAYWIVVINFREAYNL                     | 197 |
| SsalarGMD        | KTCGLTSNVKIFYQASTSELYGKVQEIPQKETTPFYPSPYGAAKLYAYWIVVNFREAYNL                    | 197 |
| CintestinalisGMD | RTCGMSDSVRFYQASTSEMFGKVQEIPQKETTPFYPSPYAAKLYAYWIVVNYREAYNM                      | 200 |
| AgambiaeGMD      | RTVGQERTVRFYQASTSELYGKVAETPQNEKTTPFYPSPYACAKMYGYWIVINRYEAYDM                    | 189 |
| AaegyptiGMD      | RTCGLEKSVRFYQASTSELYGKVVEETPQNEKTTPFYPSPYACAKMYGYWIVINRYEAYNM                   | 209 |
| DmelanogasterGMD | RTCGMEKNRVFYQASTSELYGKVVEETPQNEQTTPFYPSPYACAKMYGFWIVINRYEAYNM                   | 222 |
| AmelliferaGMD    | RTCGLEKSVMFYHASTSELYGRVTQVPQNEKTTPFYPSPYACAKLYSFWIVINRYEAYNM                    | 185 |
| LloaGMD          | HACGLTNKRVFYQASTSELYGKVQEVQPQKETTPFYPSPYAAKLYSYWIVVNYREAYNM                     | 207 |
| BmalayiGMD       | HACGLTNKVKFYQASTSELYGKVQEVQPQKETTPFYPSPYAAKLYSYWIVVNYREAYSM                     | 214 |
| CelegansGMD      | HACRLTEKVRFYQASTSELYGKVQEIPQSEKTTPFYPSPYAVAKMYGYWIVVNYREAYNM                    | 227 |
| :                | : : . * : * : * * * * : : * : * : * * . * * * * * * * . * : * : * * * : * * * : |     |
| MmusculusGMD     | FAVNGILFNHESPRRGANFVTRKISRSAKIYLGQLCEFSLGNLDKARDWGHAHDYVEAM                     | 259 |
| RnorvegicusGMD   | FAVNGILFNHESPRRGANFVTRKISRSAKIYLGQLCEFSLGNLDKARDWGHAHDYVEAM                     | 259 |
| CgriseusGMD      | FAVNGILFNHESPRRGANFVTRKISRSAKIYLGQLCEFSLGNLDKARDWGHAHDYVEAM                     | 259 |
| HsapiansGMD      | FAVNGILFNHESPRRGANFVTRKISRSAKIYLGQLCEFSLGNLDKARDWGHAHDYVEAM                     | 259 |
| BtaurusGMD       | FAVNGILFNHESPRRGANFVTRKISRSAKIHLGQLCEFSLGNLDKARDWGHAHDYVEAM                     | 259 |
| XlaevisGMF       | FAVNGILFNHESPRRGANFVTRKISRSAKIHLGQMESFSLGNLDKARDWGHAHDYVEAM                     | 256 |
| DrerioGMD        | FAVNGILFNHESPRRGSNFVTRKISRSAKIHLGQLCEFSLGNLDSMRDWGHAKDYVEAM                     | 257 |
| SsalarGMD        | FAVNGILFNHESPRRGANFVTRKISRSAKIHLGQLESFSLGNLDSKRWDGHAKDYVEAM                     | 257 |
| CintestinalisGMD | HASNGILFNHESPRRGNFVTRKITRSVAKIHLGLQELITLGNLDSKRWDGHAKDYVKGEM                    | 260 |
| AgambiaeGMD      | FACNGILFNHESPRRGENFVTRKITRSVAKISLQQEQYLELGNLDSKRWDGHAKDYVEAM                    | 249 |
| AaegyptiGMD      | PACNGILFNHESPRRGENFVTRKITRSVAKISLNQMDCELGNLDSKRWDGHAGDYVEAM                     | 269 |
| DmelanogasterGMD | YACNGILFNHESPRRGENFVTRKITRSVAKIYHKQMEYFELGNLDSKRWDGHASDYVEAM                    | 282 |
| AmelliferaGMD    | FACNGILFNHESPRRGENFVTRKITRSVAKIHLGLQDILELGNLDAKRWDGHAKDYVEAM                    | 245 |
| LloaGMD          | FACNGILFNHESPRRGETFVTRKITRAVAKIALGQQEILELGNLSSSRDWGHAKDYVEAM                    | 267 |
| BmalayiGMD       | FACNGILFNHESPRRGETFVTRKITRAVAKIALGQQEVLDELGNLSSSRDWGHAKDYVEAM                   | 274 |
| CelegansGMD      | FACNGILFNHESPRRGETFVTRKITRSVAKISLQQESIIEGNLSALRDWGHAREYVEAM                     | 287 |
| :                | . * * * * * * * * * * . * * * * : : * : * * * : : * * * : * * * * : * * *       |     |
| MmusculusGMD     | WLMLQNDEPEDFVIATGEVHSVREFVEKSFMHIGKTIWEGKNENEVGRCKETGKHVHTV                     | 319 |
| RnorvegicusGMD   | WLMLQNDEPEDFVIATGEVHSVREFVEKSFMHIGKTIWEGKNENEVGRCKETGKHVHTV                     | 319 |
| CgriseusGMD      | WLMLQNDEPEDFVIATGEVHSVREFVEKSFMHIGKTIWEGKNENEVGRCKETGKHVHTV                     | 319 |
| HsapiansGMD      | WLMLQNDEPEDFVIATGEVHSVREFVEKSFHIGKTIWEGKNENEVGRCKETGKHVHTV                      | 319 |
| BtaurusGMD       | WLMLQKDEPEDFVIATGEVHSVREFVEKSFPHIGKTIWEGKNENEVGRCKETGKHVHTV                     | 319 |
| XlaevisGMF       | WLMLQTDPEDEPFVISTGEVHSVREFVEKAFKHIGKTIWEGKNENEVGRCSSETGKHVHV                    | 316 |
| DrerioGMD        | WLMLQQUEEPDFVIATGEVHSVREFVEKAPFHVGTIWEGKDENEVGRQCETGKHVHV                       | 317 |
| SsalarGMD        | WLMLQQUEEPEDLVIIATGEVHSVREFVEKSPKHVGTIWEGKDENEVGRQCETGKHVHV                     | 317 |
| CintestinalisGMD | WMMLQHDEPGDYVLSTNETHSVREFVEKSPKHIGVELEVGGEGVNEIGVDKSGTVKRVQI                    | 320 |
| AgambiaeGMD      | WLMMQOERPBDFVIATGETHSVREFVEQAFFYIGREIEWRGVGVDEBVEKGTDTVVRRI                     | 309 |
| AaegyptiGMD      | WMMLQPPQPDYVIATGECHSVREFVEHSFRHIGREIEWRGEGVNEVGEKGTDTVVRRI                      | 329 |
| DmelanogasterGMD | WMMLQRESPPDYVIATGETHSVREFVEAAPPKHIDREITWKGVGVDEBVGENGTVGTVVRRI                  | 342 |
| AmelliferaGMD    | WLMLQQPTADDYVIATGETHSVREFVEAAPQYVGRITIKWESEGINEGDVQGTGVLVKV                     | 305 |
| LloaGMD          | WKILQYDPDDFVIATGKSYTVRRFAELAFEIEGKTIWEGEGVHEVGKEBDTGIVRVV                       | 327 |
| BmalayiGMD       | WRILQYDKPDDFVIATGKSYTVRRFAELAFEIEGKKTIWEGEGVHEVGKEBDTGIVRVV                     | 334 |
| CelegansGMD      | WRILQHSDPDDFVIATGKFVSREFCNLAFAIEGVELQWEGVEEVEGKNKD-GVIRVKV                      | 346 |
| :                | * * * * * * * * * * . * * * * : : * : * * * : : * * * : * * * * : * * *         |     |

Figure 9

|                  |                                                         |     |
|------------------|---------------------------------------------------------|-----|
| MmusculusGMD     | DLKYYRPTTEVDLQGDSCSAQQKLNWKPRVAFDELVRMVFQADVELMRTNPNA   | 372 |
| RnorvegicusGMD   | DLKYYRPTTEVDLQGDSCSAQQKLNWKPRVAFDELVRMVFQADVELMRTNPNA   | 372 |
| CgriseusGMD      | DLKYYRPTTEVDLQGDSCSAQQKLNWKPRVAFDELVRMVFQADVELMRTNPNA   | 372 |
| HsapiensGMD      | DLKYYRPTTEVDLQGDCTKAKQKLNWKPRVAFDELVRMVFHADVELMRTNPNA   | 372 |
| BtaurusGMD       | DLKYYRPTTEVDLQGDSCSKARQKLSWKPRVAFDELVRMVFADVELMRTNPNA   | 372 |
| XlaevisGMF       | DLKYYRPTTEVDLQGDSCSAKNKLGWTPKVSFDELVKEMVESDVKLMATNPNA   | 369 |
| DrerioGMD        | DPKYYRPTTEVDYLQGDSSKAFKVLGWKPRVTFEELVKEMVDADIKLMQNNPNA  | 370 |
| SsalarGMD        | DSKYYRPTTEVEYLQGDSTKALTKLGWKAKITFEELVKEMVDADIHLMKNNPNA  | 370 |
| CintestinalisGMD | SERYRPTTEVEFLLGDYSKAKKVLGWSPDIKFNELVKEMVEADINLMKKTNPNA  | 373 |
| AgambiaeGMD      | NPKFFRPTTEVDLLGDASKAKAQLGWSPKVTFFLELIADMMAADIQLMKNNPSA  | 362 |
| AegyptiGMD       | NPKFFRPTTEVDLLGDASKAKRELGWTPKVSFLQLVSDMMVADIELMKKNPNA   | 382 |
| DmelanogasterGMD | NPKYFRPTTEVDLLQGDASKANRELNWTPKVTFVELVSDMMKADIELMRKNPIA  | 395 |
| AmelliferaGMD    | NPKYFRPTTEVDVLLGDATKAKEKIGWKPTITFENLVKDMDSDELMSKNPNA    | 358 |
| LloaGMD          | SPKYYRPTTEVDLLIGDPTKAKQKLNWEAKITLLEELVKEMVASDLQLMKSNPMA | 380 |
| BmalayiGMD       | SSKYYRPTTEVDLLIGDSTKAKQKLNWEAKITLLEELVKEMVASDLQLMKSNPMA | 387 |
| CelegansGMD      | SPKYYRPTTEVETLLGNAEKAKKTLGWEAKVTVPELVKEMVASDIILMKSNPMA  | 399 |

. :\*:\*\*\*\*\*: \* \*: :\* :.\* . . :\*: :\*: :\*: \*\* ..\* \*

Figure 9

## FUT8 alignment

CLUSTAL 2.1 multiple sequence alignment [overall sequence homology &gt;77%]

```

MmusculusFUT8      LEPEYNVSSSLSSRKIEDRVETLKMRAWTGSWRWIMLILFAWGTLFLFYIGGHLVRDNDHP 60
RnorvegicusFUT8    -----MRAWTGSWRWIMLILFAWGTLFLFYIGGHLVRDNDHP 36
CgriseusFUT8       -----MRAWTGSWRWIMLILFAWGTLFLFYIGGHLVRDNDHP 36
BtaurusFUT8        -----MRPWTGSWRWIMLILFAWGTLFLFYIGGHLVRDNDHP 36
ClupusFUT8         -----MRPWTGSWRWIMLILFAWGTLFLFYIGGHLVRDNDHP 36
HsapiensFUT8       -----MRPWTGSWRWIMLILFAWGTLFLFYIGGHLVRDNDHP 36
GgallusFUT8        -----MRPWTGSWRWIMLILFAWGTLFLFYIGGHLVRDSEHP 36
StropicalisFUT8    -----MRPWTGSWRWIMLILFAWGTLFLFYIGGHLVRDNEHP 36
DrerioFUT8         -----MRPWTGSWRWIALVLLAWGTLLFYIGGHLVKDSEHA 36
                    **.*.***** **.*.*****:*.:.

MmusculusFUT8      DHSSRELSKILAKLERLKKQNEDLRMAESLRIPEGPIDQGTATGRVRVLEEQLVKAKEQ 120
RnorvegicusFUT8    DHSSRELSKILAKLERLKKQNEDLRMAESLRIPEGPIDQGTATGRVRVLEEQLVKAKEQ 96
CgriseusFUT8       DHSSRELSKILAKLERLKKQNEDLRMAESLRIPEGPIDQGTATGRVRVLEEQLVKAKEQ 96
BtaurusFUT8        DHSSRELSKILAKLERLKKQNEDLRMAESLRIPEGPIDQGPASGRIRALEEQLVKAKEQ 96
ClupusFUT8         DHSSRELSKILAKLERLKKQNEDLRMAESLRIPEGPIDQAPASGRVRALEEQLVKAKEQ 96
HsapiensFUT8       DHSSRELSKILAKLERLKKQNEDLRMAESLRIPEGPIDQGPALGRVRVLEEQLVKAKEQ 96
GgallusFUT8        DHSSRELSKILAKLERLKKQNEDLRMAESLRIPEGPIDQGPAGKVHALEEQLVKAKEQ 96
StropicalisFUT8    DHSSRELSKILAKLERLKKQNEDLRMAESLRIPEGPIEQGAAAGRIRALEEQLVKAKEQ 96
DrerioFUT8         PRSSRELAKILTKLERLKKQNEDLRMAESLRIPEGQSDGPISGRRLSLEEQLSRAKQK 96
                    :****.*.***:*****:****.*. : : : : ****.*.***:

MmusculusFUT8      IENYKKQARN---GLGKDHEILRRRIENGAKELWFFLQSELKKLKHLEGNELQRHADEIL 177
RnorvegicusFUT8    IENYKKQARN---GLGKDHEILRRRIENGAKELWFFLQSELKKLKHLEGNELQRHADEIL 153
CgriseusFUT8       IENYKKQARN---DLGKDHEILRRRIENGAKELWFFLQSELKKLKHLEGNELQRHADEIL 153
BtaurusFUT8        IENYKKQTRN---GLGKDHEILRRRIENGAKELWFFLQSELKKLKHLEGNELQRHADEFL 153
ClupusFUT8         IENYKKQTRN---GLGKDHEILRRRIENGAKELWFFLQSELKKLKHLEGNELQRHADEFL 153
HsapiensFUT8       IENYKKQTRN---GLGKDHEILRRRIENGAKELWFFLQSELKKLKHLEGNELQRHADEFL 153
GgallusFUT8        IENYKKQTGD---GLGKDHEILRRRIENGAKELWFFLQSELKKLKHLEGNELQRHADEFL 153
StropicalisFUT8    IEMVQQSSNAVSGLGKDHEILRRRIENGAKELWFFVQSEVKKLKHLEGNELQRHADEIT 156
DrerioFUT8         IQSFQRLSGE---CPGKDHEILRRRIENGAKELWFFVQSEVKKLKHLEGNELQRHADEIT 153
                    *: : : : : . ***** **.*.***:****.*.***:****.*.***: : : : : * : :

MmusculusFUT8      LDLGHHERSIMTDLYYLSQTDGAGDWREKEAKDLTELVRQRRITYLQNPKDCSKARKLVCN 237
RnorvegicusFUT8    LDLGHHERSIMTDLYYLSQTDGAGDWREKEAKDLTELVRQRRITYLQNPKDCSKARKLVCN 213
CgriseusFUT8       LDLGHHERSIMTDLYYLSQTDGAGDWREKEAKDLTELVRQRRITYLQNPKDCSKARKLVCN 213
BtaurusFUT8        SDLGHHERSIMTDLYYLSQTDGAGDWREKEAKDLTELVRQRRITYLQNPKDCSKARKLVCN 213
ClupusFUT8         SDLGHHERSIMTDLYYLSQTDGAGDWREKEAKDLTELVRQRRITYLQNPKDCSKARKLVCN 213
HsapiensFUT8       LDLGHHERSIMTDLYYLSQTDGAGDWREKEAKDLTELVRQRRITYLQNPKDCSKARKLVCN 213
GgallusFUT8        SDLGHQERSIMTDLYYLSQTDGAGDWREKEAKDLTELVRQRRITYLQNPKDCSKARKLVCN 213
StropicalisFUT8    IDMGHQQRSMVTDLYYLSQTDGAGDWREKEAKDLTELVRQRRITYLQNPKDCSKARKLVCN 216
DrerioFUT8         QDLGHQQRSMVTDLYYLSQADGAGDWREKEAKDLTELVRQRRITYLQNPKDCSKARKLVCN 213
                    *.**.*.***:*****:****.*.***:****.*.***:****.*.***:****.*.***:

MmusculusFUT8      INKGCYGCQLHHVVYCFMIAVGTQRTLILESQNWRVATGGWETVFRPVSETCTDRSGLS 297
RnorvegicusFUT8    INKGCYGCQLHHVVYCFMIAVGTQRTLILESQNWRVATGGWETVFRPVSETCTDRSGLS 273
CgriseusFUT8       INKGCYGCQLHHVVYCFMIAVGTQRTLILESQNWRVATGGWETVFRPVSETCTDRSGLS 273
BtaurusFUT8        INKGCYGCQLHHVVYCFMIAVGTQRTLILESQNWRVATGGWETVFRPVSETCTDRSGVS 273
ClupusFUT8         INKGCYGCQLHHVVYCFMIAVGTQRTLILESQNWRVATGGWETVFRPVNETCTDRSGTS 273
HsapiensFUT8       INKGCYGCQLHHVVYCFMIAVGTQRTLILESQNWRVATGGWETVFRPVSETCTDRSGIS 273
GgallusFUT8        INKGCYGCQLHHVVYCFMIAVGTQRTLILESQNWRVATGGWETVFRPVSETCTDRSGT 273
StropicalisFUT8    INKGCYGCQLHHVVYCFMIAVGTQRTLILESQNWRVATGGWETVFKPVSETCTDRSGSS 276
DrerioFUT8         INKGCYGCQLHHVVYCFMIAVGTQRTLILESQNWRVATGGWETVFKPVSDTCTDRGTAS 273
                    *****:*****:****.*.***:****.*.***:****.*.***:

```

Figure 10

|                                                             |                                                                 |     |
|-------------------------------------------------------------|-----------------------------------------------------------------|-----|
| MmusculusFUT8                                               | TGHWSGEVNDKNIQVVLPVIVDSLHPRPPYLPPLAVPEDLADRLLRVHGDPVAVWVWSQFVK  | 357 |
| RnorvegicusFUT8                                             | TGHWSGEVNDKNIQVVLPVIVDSLHPRPPYLPPLAVPEDLADRLLRVHGDPVAVWVWSQFVK  | 333 |
| CgriseusFUT8                                                | TGHWSGEVKDKNVQVVLPVIVDSLHPRPPYLPPLAVPEDLADRLLRVHGDPVAVWVWSQFVK  | 333 |
| BtaurusFUT8                                                 | TGHWSGEIKDKNVQVVLPVIVDSLHPRPPYLPPLAVPEDLADRLLRVHGDPVAVWVWSQFVK  | 333 |
| ClupusFUT8                                                  | TGHWSGEVKDKNVQVVLPVIVDSLHPRPPYLPPLAVPEDLADRLLRVHGDPVAVWVWSQFVK  | 333 |
| HsapiensFUT8                                                | TGHWSGEVKDKNVQVVLPVIVDSLHPRPPYLPPLAVPEDLADRLLRVHGDPVAVWVWSQFVK  | 333 |
| GgallusFUT8                                                 | TGHWSGETNDKDVQVVLPVIVDSLHPRPPYLPPLAVPEDLADRLLRVHGDPVAVWVWSQFVK  | 333 |
| StropicalisFUT8                                             | TGHWSGEANDKNVQVVLPVIVDSLHPRPPYLPPLGVPEDLADRLLRVHGDPVAVWVWSQFVK  | 336 |
| DrerioFUT8                                                  | TGHWSGEAHRDQVVLPVIVDSLHPRPPYLPPLAVPEDLAPRLQRLHGDPVAVWVWSQFVK    | 333 |
| ***** :*:::*****.***** ** *:***:*****                       |                                                                 |     |
| MmusculusFUT8                                               | YLIRPQPWLEKEIEEATKKLGFKHPVIGVHVRRTDKVGTEAAAFHPIEEYMVHVEEHFQLL   | 417 |
| RnorvegicusFUT8                                             | YLIRPQPWLEKEIEEATKKLGFKHPVIGVHVRRTDKVGTEAAAFHPIEEYMVHVEEHFQLL   | 393 |
| CgriseusFUT8                                                | YLIRPQPWLEKEIEEATKKLGFKHPVIGVHVRRTDKVGTEAAAFHPIEEYMVHVEEHFQLL   | 393 |
| BtaurusFUT8                                                 | YLIRPQPWLEKEIEEATKKLGFKHPVIGVHVRRTDKVGTEAAAFHPIEEYMVHVEEHFQLL   | 393 |
| ClupusFUT8                                                  | YLIRPQPWLEKEIEEATKKLGFKHPVIGVHVRRTDKVGTEAAAFHPIEEYMVHVEEHFQLL   | 393 |
| HsapiensFUT8                                                | YLIRPQPWLEKEIEEATKKLGFKHPVIGVHVRRTDKVGTEAAAFHPIEEYMVHVEEHFQLL   | 393 |
| GgallusFUT8                                                 | YLIRPQPWLEKEIEEATKKLGFKHPVIGVHVRRTDKVGTEAAAFHPIEEYMVHVEEHFQLL   | 393 |
| StropicalisFUT8                                             | YLIRPQPWLEKEIEEATKKLGFKHPVIGVHVRRTDKVGTEAAAFHPIEEYMVHVEEHFQLL   | 396 |
| DrerioFUT8                                                  | FLVRPQAWLEKEIQETCLKGFKHPVIGVHVRRTDKVGTEAAAFHPIEEYMVHVEDHYQSL    | 393 |
| *:***.***:*:*: ***** *:*****:*****:*****:*****              |                                                                 |     |
| MmusculusFUT8                                               | ARRMQVDKKRVYLATDDPTLLKEAKTKYSNYEFISDNSISWSAGLHNRVTENSLRGVILD    | 477 |
| RnorvegicusFUT8                                             | ARRMQVDKKRVYLATDDPALLKEAKTKYSNYEFISDNSISWSAGLHNRVTENSLRGVILD    | 453 |
| CgriseusFUT8                                                | ERRMKVDKKRVYLATDDPSLLKEAKTKYSNYEFISDNSISWSAGLHNRVTENSLRGVILD    | 453 |
| BtaurusFUT8                                                 | ARRMQVDKKRVYLATDDPSLLKEAKTKYPHYEFISDNSISWSAGLHNRVTENSLRGVILD    | 453 |
| ClupusFUT8                                                  | ARRMQVDKKRVYLATDDPSLLKEAKTKYPTYEFISDNSISWSAGLHNRVTENSLRGVILD    | 453 |
| HsapiensFUT8                                                | ARRMQVDKKRVYLATDDPSLLKEAKTKYPNYEFISDNSISWSAGLHNRVTENSLRGVILD    | 453 |
| GgallusFUT8                                                 | ARRMHVDKKRVYLATDDPSLLQEAQSKYPNYEFISDNSISWSAGLHNRVTENSLRGVILD    | 453 |
| StropicalisFUT8                                             | ARRMQVDKKRVYLATDDPTLLQEAQAKYPQYEFISDNSISWSAGLHNRVTENSLRGVILD    | 456 |
| DrerioFUT8                                                  | AQRMHVDKKRVYLATDDPSLLQEAQTKYPDYEFISDNSISWSAGLHNRVTENSLRGVILD    | 453 |
| :*:*:*:*****:*:*:*: *****:*****:*****:*****:*****           |                                                                 |     |
| MmusculusFUT8                                               | IHFLSQADFLVCTFSSQVCRVAYEIMQTLHPDASANFHSLLDDIYFPGGQNAHNQIAVYPH   | 537 |
| RnorvegicusFUT8                                             | IHFLSQADFLVCTFSSQVCRVAYEIMQTLHPDASANFHSLLDDIYFPGGQNAHNQIAVYPH   | 513 |
| CgriseusFUT8                                                | IHFLSQADFLVCTFSSQVCRVAYEIMQTLHPDASANFHSLLDDIYFPGGQNAHNQIAVYPH   | 513 |
| BtaurusFUT8                                                 | IHFLSQADFLVCTFSSQVCRVAYEIMQTLHPDASANFHSLLDDIYFPGGQNAHNQIAVYPH   | 513 |
| ClupusFUT8                                                  | IHFLSQADFLVCTFSSQVCRVAYEIMQTLHPDASANFHSLLDDIYFPGGQNAHNQIAVYPH   | 513 |
| HsapiensFUT8                                                | IHFLSQADFLVCTFSSQVCRVAYEIMQTLHPDASANFHSLLDDIYFPGGQNAHNQIAVYPH   | 513 |
| GgallusFUT8                                                 | IHFLSQADFLVCTFSSQVCRVAYEIMQTLHPDASAYFHSLLDDIYFPGGQNAHNQIAVYPH   | 513 |
| StropicalisFUT8                                             | IHFLSQADFLVCTFSSQVCRVAYEIMQTLHPDASANFHSLLDDIYFPGGQNAHNQIAVYPH   | 516 |
| DrerioFUT8                                                  | IHFLSRTNYLVCTFSSQVCRVAYEIMQTLHPDASSYFSLDDIYFPGGQNAHNQIAVYPH     | 513 |
| *****:*****:*****:*****:*****:*****:*****:*****:*****:***** |                                                                 |     |
| MmusculusFUT8                                               | KPRTEBEEIPMEPGDIIGVAGNHWDGYSGKGNRKLKGTGLYPSYKVKREKIEITVKYPTYPEA | 597 |
| RnorvegicusFUT8                                             | KPRTEBEEIPMEPGDIIGVAGNHWDGYSGKGNRKLKGTGLYPSYKVKREKIEITVKYPTYPEA | 573 |
| CgriseusFUT8                                                | QPRTEBEEIPMEPGDIIGVAGNHWDGYSGKGNRKLKGTGLYPSYKVKREKIEITVKYPTYPEA | 573 |
| BtaurusFUT8                                                 | EPRTADEIPMEPGDIIGVAGNHWDGYSGKGNRKLKGTGLYPSYKVKREKIEITVKYPTYPEA  | 573 |
| ClupusFUT8                                                  | QPRTEBEEIPMEPGDIIGVAGNHWDGYSGKGNRKLKGTGLYPSYKVKREKIEITVKYPTYPEA | 573 |
| HsapiensFUT8                                                | QPRTEBEEIPMEPGDIIGVAGNHWDGYSGKGNRKLKGTGLYPSYKVKREKIEITVKYPTYPEA | 573 |
| GgallusFUT8                                                 | HPRTADEIPMEPGDIIGVAGNHWDGYSGKGNRKLKGTGLYPSYKVKREKIEITVKYPTYPEA  | 573 |
| StropicalisFUT8                                             | QPRNAEBEIPMEPGDIIGVAGNHWDGYSGKGNRKLKGTGLYPSYKVKREKIEITVKYPTYPEA | 576 |
| DrerioFUT8                                                  | QPRNSDDIPLPBGDIIGVAGNHWDGYSGKGNRKLKGTGLYPSYKVKREKIEITVKYPTYPEA  | 573 |
| .*.*:.*:*:*****:*****:*****:*****:*****:*****:*****:*****   |                                                                 |     |
| MmusculusFUT8                                               | EK----- 599                                                     |     |
| RnorvegicusFUT8                                             | EK----- 575                                                     |     |
| CgriseusFUT8                                                | EK----- 575                                                     |     |
| BtaurusFUT8                                                 | EK----- 575                                                     |     |
| ClupusFUT8                                                  | EK----- 575                                                     |     |
| HsapiensFUT8                                                | EK----- 575                                                     |     |
| GgallusFUT8                                                 | EK----- 575                                                     |     |
| StropicalisFUT8                                             | EK----- 578                                                     |     |
| DrerioFUT8                                                  | DKLLKKP 580                                                     |     |
| :*                                                          |                                                                 |     |

Figure 10



## FucT8 alignment

CLUSTAL 2.1 multiple sequence alignment [overall sequence homology &gt;46%]

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HsapiensFucT      ----MNRAPLKRSRILHMAITGASDPSAEAEA--NGEKPFLLRALQIALVVSLEYWVTSIS 54
HsapiensFucT2     -----MALTGASDPSAEAEA--NGEKPFLLRALQIALVVSLEYWVTSIS 41
ClupusFucT        ----MNRAPLKRSRILHMAITGASDPLGEAEA--IKEKPFLLRALQITLVVSLEYWVTSIS 54
BtaurusFucT       ----MNRASLKRSKILHMAITGTSDFSGEAEA--SQEKPFVLRALQIALVVSLEYWVTSIS 54
MmusculusFucT     ----MNRAPLKRSRILRMALTGVSASVSEESE--SGNKPFLLRALQIALVVSLEYWVTSIS 53
MmusculusFucT2    -----MALTGVSAVSEESE--SGNKPFLLRALQIALVVSLEYWVTSIS 40
RnorvegicusFucT   ----MNRVPLKRSRILRMALTGASAVSEED--SENKPFLLRALQIALVVSLEYWVTSIS 53
CgriseusFucT      ----MNRAPLKRSRILRMALTGSGTASEEDED--SRNKPFLLRALQIALVVSLEYWVTSIS 55
GgallusFucT       ----MSRSQLTRTGILRMALGGAADPLLPAEGAGGRRTPFVLRALRIALVVSLEYWVTSIS 56
XtropicalisFucT   ----MN--FKRSSILRMALMGAGEGDEKVS--RESFLVRAVKIALVVTLYWFISIT 49
DrerioFucT        ----MAFTDSTRPGDKEE-----PFFMRATKIALVVTLYWFISIS 36
AuumFucT          MHHDSKITPVATIHLLPKGLGRGLLQQFAMKNGRQHETLAQSKIKIVLAWSAYWICSIG 60
LloaFucT          -----
CelegansFucT      -----MKLHEENKALFEAMRNRENPTKWESYKQVITAVSAYWVFSIG 43

HsapiensFucT      MVFLNKYLLDSPS--LRLDTPIFVTFYQCLVTTLLCKGLSALAACCPG-AVDFFSLRLDL 111
HsapiensFucT2     MVFLNKYLLDSPS--LRLDTPIFVTFYQCLVTTLLCKGLSALAACCPG-AVDFFSLRLDL 98
ClupusFucT        MVFLNKYLLDSPS--LQLDTPIFVTFYQCLVTTLLCKGLSLTAAFCPG-AMDFPTLRDL 111
BtaurusFucT       MVFLNKYLLDSPS--LRLDTPIFVTFYQCLVTVLLCKGLSSLATCCPG-TVDFPALHDL 111
MmusculusFucT     MVFLNKYLLDSPS--LQLDTPIFVTFYQCLVTSLLCKGLSLTATCCPG-MVDFPTLNLDV 110
MmusculusFucT2    MVFLNKYLLDSPS--LQLDTPIFVTFYQCLVTSLLCKGLSLTATCCPG-MVDFPTLNLDL 97
RnorvegicusFucT   MVFLNKYLLDSPS--LQLDTPIFVTFYQCLVTSLLCKGLSLTATCCPG-MVDFPTLNLDL 110
CgriseusFucT      MVFLNKYLLDSPS--LQLDTPIFVTFYQCLVTSLLCKGLSLTATCCPG-TVDFPTLNLDL 112
GgallusFucT       MVFLNKYLLDSPS--LRLDAPLFVTFYQCAVTAALCLGLSLGAACGP--CAALPALRLDL 112
XtropicalisFucT   MVFLNKYLLDSPS--LKLDAPLFVTFYQCVTVVLCGLSLLTHVVPSHILEPFLRLFDL 107
DrerioFucT        MVFLNNYLLDSK---ELDAPVITFFQCVSVGLCLLMSFLSSLCPG-SVDFPSLKFDL 91
AuumFucT          LVFLNKYLLSS--ENLKLNAPLFITWYQCLVTVVLCYTCYLSRIFPS-RFSFPSIAFDH 117
LloaFucT          ---MSKYSLSSTYTGKLLNAPLFVTFYQCFVTVLLCCVFCWVSKQYPS-LVTFPFVGFHD 56
CelegansFucT      LVFLNKYLLSS---VQLDAPLFITWYQCLVTVFLCLFLSKTSKAYG--LFKFPSPMIDA 97
      :.* *.*      *::*:*:*:*: *  *  .  :      :* : :.*

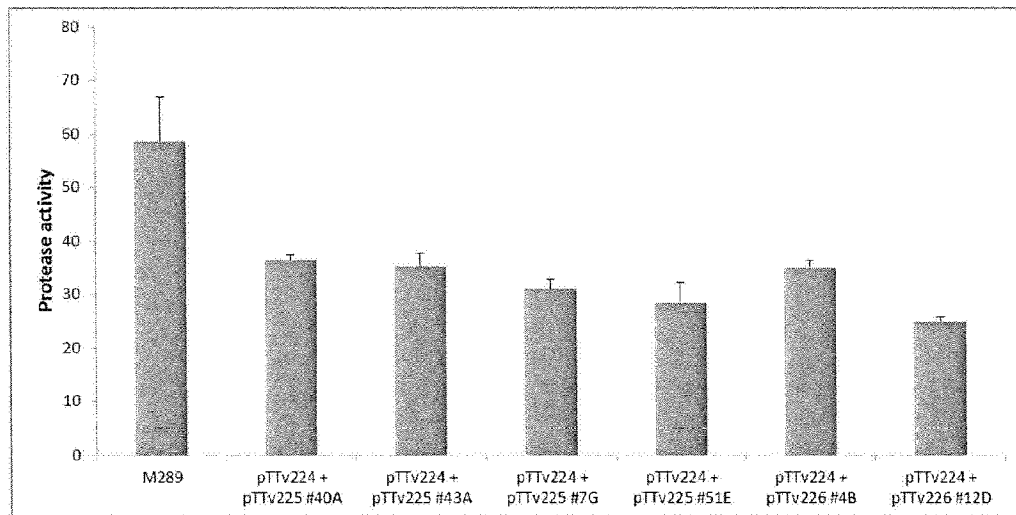
HsapiensFucT      RVARSVLPLSVVFIGMITFNNLCLKYVGVAFYNVGRSLTTFVNVLLSYLLLKQTTSFYAL 171
HsapiensFucT2     RVARSVLPLSVVFIGMITFNNLCLKYVGVAFYNVGRSLTTFVNVLLSYLLLKQTTSFYAL 158
ClupusFucT        RVARSVLPLSVVFIGMITFNNLCLKYVGVAFYNVGRSLTTFVNVLLSYLLLKQTTSFYAL 171
BtaurusFucT       KVARSVLPLSVVFIGMITFNNLCLKYVGVAFYNVGRSLTTFVNVLLSYLLLKQTTSFYAL 171
MmusculusFucT     KVARSVLPLSVVFIGMITFNNLCLKYVGVPFNVGRSLTTFVNVLLSYLLLKQTTSFYAL 170
MmusculusFucT2    KVARSVLPLSVVFIGMITFNNLCLKYVGVPFNVGRSLTTFVNVLLSYLLLKQTTSFYAL 157
RnorvegicusFucT   KVARSVLPLSVVFIGMITFNNLCLKYVGVAFYNVGRSLTTFVNVLLSYLLLKQTTSFYAL 170
CgriseusFucT      KVARSVLPLSVVFIGMISFNNLCLKYVGVAFYNVGRSLTTFVNVLLSYLLLKQTTSFYAL 172
GgallusFucT       KVSRSVLPLSVVFIGMVTNNLCLKHVGVAFYNVGRSLTTFVNVLLSYLLLKQTTSLYAL 172
XtropicalisFucT   KVLRTVPLSIVFIGMITFNNLCLKYLGVAFYTVGRCLSTFVNVLLSYIMLKQTTSMYAL 167
DrerioFucT        RVSREILPLTIVFISMITFNNLCLKYVGVAFYTVGRSLSTFVNVILSYVVLKQTTSLYAV 151
AuumFucT          RISREVLPLSFVFMVAMITFNNLCLKYVGVSFYVYVGRSLTTFVNVVCSYLILGQGTSWRAL 177
LloaFucT          RISREVLPLSFVFMVAMITFNNLCLKYVGVSFYVYVGRSLTTFVNVVCSYLILGQGTSLKTI 116
CelegansFucT      KISREVLPLSVVFMVAMISFNNLCLKYVGVSFYVYVGRSLTTFVNVVCTYLILGQGTSGQAI 157
      : : * :*:*:*.*: :*: :*:*:*:*: :*: :*:*:*: :*: :* * * : :

```

Figure 11

|                 |                                                                |     |
|-----------------|----------------------------------------------------------------|-----|
| HsapiensFucT    | LTCGIIIGGFWLGVDDQEGAE-----GTLSSLGTGTFGVLASLCVSLNAIYTKKVL       | 221 |
| HsapiensFucT2   | LTCGIIIGGFWLGVDDQEGAE-----GTLSSLGTGTFGVLASLCVSLNAIYTKKVL       | 208 |
| ClupusFucT      | LTCGIIIGGFWLGVDDQEGAE-----GTLSSLGTGTFGVLASLCVSLNAIYTKKVL       | 221 |
| BtaurusFucT     | LTCGIIIGGFWLGVDDQEGAE-----GTLSSLGTGTFGVLASLCVSLNAIYTKKVL       | 221 |
| MmusculusFucT   | LTCGIIIGGFWLGVDDQEGAE-----GTLSSLGTGTFGVLASLCVSLNAIYTKKVL       | 220 |
| MmusculusFucT2  | LTCGIIIGGFWLGVDDQEGAE-----GTLSSLGTGTFGVLASLCVSLNAIYTKKVL       | 207 |
| RnorvegicusFucT | LTCGIIIGGFWLGVDDQEGAE-----GTLSSLGTGTFGVLASLCVSLNAIYTKKVL       | 220 |
| CgriseusFucT    | LTCGIIIGGFWLGVDDQEGAE-----GTLSSLGTGTFGVLASLCVSLNAIYTKKVL       | 222 |
| GgallusFucT     | LTCGIIIGGFWLGVDDQEGAE-----GTLSSLGTGTFGVLASLCVSLNAIYTKKVL       | 222 |
| XtropicalisFucT | LTCGIIIGGFWLGVDDQEGAE-----GTLSSLGTGTFGVLASLCVSLNAIYTKKVL       | 217 |
| DrerioFucT      | LTCGIIIGGFWLGVDDQEGAE-----GTLSSLGTGTFGVLASLCVSLNAIYTKKVL       | 201 |
| AsuumFucT       | LTCGIIIGGFWLGVDDQEGAE-----GTLSSLGTGTFGVLASLCVSLNAIYTKKVL       | 227 |
| LloaFucT        | LTCGIIIGGFWLGVDDQEGAE-----GTLSSLGTGTFGVLASLCVSLNAIYTKKVL       | 176 |
| CelegansFucT    | LTCGIIIGGFWLGVDDQEGAE-----GTLSSLGTGTFGVLASLCVSLNAIYTKKVL       | 207 |
|                 | *.:*: ** **:* ** . :*: ** * :*: ** :*:***:* :.*                |     |
| HsapiensFucT    | AVDGSIWRLTFYNNVNACILFLPLLLLLLGEQLQALRDFALQLSAHFWGMMTLGGFLGFAIG | 281 |
| HsapiensFucT2   | AVDGSIWRLTFYNNVNACILFLPLLLLLLGEQLQALRDFALQLSAHFWGMMTLGGFLGFAIG | 268 |
| ClupusFucT      | AVDGSIWRLTFYNNVNACILFLPLLLLLLGEQLQALRDFALQLSAHFWGMMTLGGFLGFAIG | 281 |
| BtaurusFucT     | AVDGSIWRLTFYNNVNACILFLPLLLLLLGEQLQALRDFALQLSAHFWGMMTLGGFLGFAIG | 281 |
| MmusculusFucT   | AVDGSIWRLTFYNNVNACILFLPLLLLLLGEQLQALRDFALQLSAHFWGMMTLGGFLGFAIG | 280 |
| MmusculusFucT2  | AVDGSIWRLTFYNNVNACILFLPLLLLLLGEQLQALRDFALQLSAHFWGMMTLGGFLGFAIG | 267 |
| RnorvegicusFucT | AVDGSIWRLTFYNNVNACILFLPLLLLLLGEQLQALRDFALQLSAHFWGMMTLGGFLGFAIG | 280 |
| CgriseusFucT    | AVDGSIWRLTFYNNVNACILFLPLLLLLLGEQLQALRDFALQLSAHFWGMMTLGGFLGFAIG | 282 |
| GgallusFucT     | AVDGSIWRLTFYNNVNACILFLPLLLLLLGEQLQALRDFALQLSAHFWGMMTLGGFLGFAIG | 282 |
| XtropicalisFucT | AVDGSIWRLTFYNNVNACILFLPLLLLLLGEQLQALRDFALQLSAHFWGMMTLGGFLGFAIG | 277 |
| DrerioFucT      | AVDGSIWRLTFYNNVNACILFLPLLLLLLGEQLQALRDFALQLSAHFWGMMTLGGFLGFAIG | 261 |
| AsuumFucT       | AVDGSIWRLTFYNNVNACILFLPLLLLLLGEQLQALRDFALQLSAHFWGMMTLGGFLGFAIG | 287 |
| LloaFucT        | AVDGSIWRLTFYNNVNACILFLPLLLLLLGEQLQALRDFALQLSAHFWGMMTLGGFLGFAIG | 236 |
| CelegansFucT    | AVDGSIWRLTFYNNVNACILFLPLLLLLLGEQLQALRDFALQLSAHFWGMMTLGGFLGFAIG | 267 |
|                 | *. : :*: ** ** .** *:.. *: . : * ** :*:*.**:*** :.             |     |
| HsapiensFucT    | YVTGLQIKFTSPLTHNVSGTAKACAQTVLAVLYEETKSFLWWTSNMMLVGGSSAYTWVR    | 341 |
| HsapiensFucT2   | YVTGLQIKFTSPLTHNVSGTAKACAQTVLAVLYEETKSFLWWTSNMMLVGGSSAYTWVR    | 328 |
| ClupusFucT      | YVTGLQIKFTSPLTHNVSGTAKACAQTVLAVLYEETKSFLWWTSNMMLVGGSSAYTWVR    | 341 |
| BtaurusFucT     | YVTGLQIKFTSPLTHNVSGTAKACAQTVLAVLYEETKSFLWWTSNMMLVGGSSAYTWVR    | 341 |
| MmusculusFucT   | YVTGLQIKFTSPLTHNVSGTAKACAQTVLAVLYEETKSFLWWTSNMMLVGGSSAYTWVR    | 340 |
| MmusculusFucT2  | YVTGLQIKFTSPLTHNVSGTAKACAQTVLAVLYEETKSFLWWTSNMMLVGGSSAYTWVR    | 327 |
| RnorvegicusFucT | YVTGLQIKFTSPLTHNVSGTAKACAQTVLAVLYEETKSFLWWTSNMMLVGGSSAYTWVR    | 340 |
| CgriseusFucT    | YVTGLQIKFTSPLTHNVSGTAKACAQTVLAVLYEETKSFLWWTSNMMLVGGSSAYTWVR    | 342 |
| GgallusFucT     | YVTGLQIKFTSPLTHNVSGTAKACAQTVLAVLYEETKSFLWWTSNMMLVGGSSAYTWVR    | 342 |
| XtropicalisFucT | YVTGLQIKFTSPLTHNVSGTAKACAQTVLAVLYEETKSFLWWTSNMMLVGGSSAYTWVR    | 337 |
| DrerioFucT      | YVTGLQIKFTSPLTHNVSGTAKACAQTVLAVLYEETKSFLWWTSNMMLVGGSSAYTWVR    | 321 |
| AsuumFucT       | YVTGLQIKFTSPLTHNVSGTAKACAQTVLAVLYEETKSFLWWTSNMMLVGGSSAYTWVR    | 347 |
| LloaFucT        | YVTGLQIKFTSPLTHNVSGTAKACAQTVLAVLYEETKSFLWWTSNMMLVGGSSAYTWVR    | 296 |
| CelegansFucT    | YVTGLQIKFTSPLTHNVSGTAKACAQTVLAVLYEETKSFLWWTSNMMLVGGSSAYTWVR    | 327 |
|                 | **** *: ** :***:****:****:*** :. : * : ** ** :*:** ** ** :     |     |
| HsapiensFucT    | GWEMKKTPEEP--SPKDSEKS-----AMGV                                 | 364 |
| HsapiensFucT2   | GWEMKKTPEEP--SPKDSEKS-----AMGV                                 | 351 |
| ClupusFucT      | GWEMKKTPEEP--SPKDSEKS-----AMGV                                 | 364 |
| BtaurusFucT     | GWEMKKTPEEP--SPKDSEKS-----AMGV                                 | 364 |
| MmusculusFucT   | GWEMKKTPEEP--SPKDSEKS-----AMGV                                 | 363 |
| MmusculusFucT2  | GWEMKKTPEEP--SPKDSEKS-----AMGV                                 | 350 |
| RnorvegicusFucT | GWEMKKTPEEP--SPKDSEKS-----AMGV                                 | 363 |
| CgriseusFucT    | GWEMKKTPEEP--SPKDSEKS-----AMGV                                 | 365 |
| GgallusFucT     | GWEMKKTPEEP--SPKDSEKS-----AMGV                                 | 365 |
| XtropicalisFucT | GWEMKKTPEEP--SPKDSEKS-----AMGV                                 | 362 |
| DrerioFucT      | GWEMKKTPEEP--SPKDSEKS-----AMGV                                 | 348 |
| AsuumFucT       | GWEMKKTPEEP--SPKDSEKS-----AMGV                                 | 385 |
| LloaFucT        | GWEMKKTPEEP--SPKDSEKS-----AMGV                                 | 331 |
| CelegansFucT    | GWEMKKTPEEP--SPKDSEKS-----AMGV                                 | 363 |
|                 | : . . *                                                        |     |

Figure 11

**Figure 12**

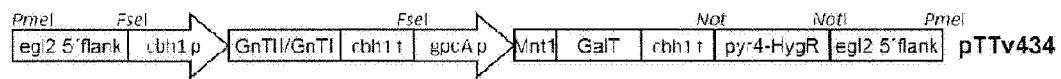
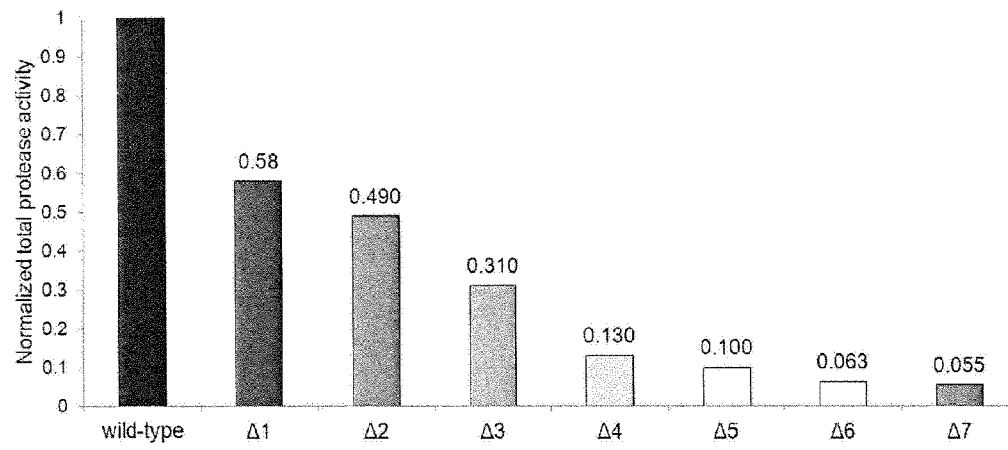


Figure 13

**Figure 14**