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(54) Title: DETERGENT COMPOSITIONS COMPRISING POLYPEPTIDES HAVING XANTHAN DEGRADING ACTIVITY

(57) Abstract: The present invention relates to detergent compositions comprising polypeptides having xanthan degrading activity. The invention also relates to methods for producing said detergent compositions and to the use of said detergent compositions in cleaning applications.

## **DETERGENT COMPOSITIONS COMPRISING POLYPEPTIDES HAVING XANTHAN DEGRADING ACTIVITY**

### **Reference to a Sequence Listing**

This application contains a Sequence Listing in computer readable form, which is incorporated herein by reference.

### **Background of the invention**

#### **Field of the invention**

The present invention relates to detergent compositions comprising polypeptides having xanthan degrading activity. In particular the invention relates to such detergent compositions comprising polypeptides within the glycosyl hydrolase family 5 (GH5) having xanthan degrading activity. The invention also relates to methods for producing said detergent compositions and to the use of said detergent compositions in cleaning applications.

#### **Description of the related art**

Xanthan gum is a polysaccharide secreted by the bacterium *Xanthomonas campestris*. It is produced by the fermentation of glucose, sucrose, or lactose in an aqueous growth medium by *X. campestris*. After a fermentation period, the polysaccharide is precipitated from the growth medium with isopropyl alcohol, dried, and ground into a fine powder. Later, the powder is added to a liquid medium to form the gum.

Xanthan is composed of pentasaccharide subunits, forming a cellulose backbone with trisaccharide side chains composed of mannose-(beta1,4)-glucuronic-acid-(beta1,2)-mannose attached to alternate glucose residues in the backbone by alpha1,3 linkages. This biopolymer is of great commercial significance because of its superior pseudoplasticity, thixotropy, and viscosity.

In recent years xanthan gum has been widely used as an ingredient in many consumer products including foods (e.g., as thickening agent in salad dressings and dairy products) and cosmetics (e.g., as stabilizer and thickener in toothpaste and make-up to prevent ingredients from separating) and cosmetics (e.g., sun creams).

In addition, xanthan gum has found use in the oil industry where xanthan gum is used in large quantities to thicken drilling mud. These fluids serve to carry the solids cut by the drilling bit back to the surface. When the circulation stops, the solids still remain suspended in the drilling fluid. The widespread use of horizontal drilling has led to its expanded use. Xanthan gum is also added to self-consolidating concrete, including concrete poured underwater, to increase its viscosity.

The widespread use of xanthan gum has led to a desire to be able to degrade solutions or gels of xanthan gum. Complete enzymatic degradation of xanthan gum has till now required several enzymatic activities including xanthan lyase activity and endo-beta-1,4-glucanase activity. Xanthan lyases are enzymes that cleave the beta-D-mannosylalpha-beta-D-1,4-glucuronosyl bond of xanthan and have been described in the literature. Xanthan degrading enzymes are known in the art e.g., two xanthan lyases isolated from *Paenibacillus alginolyticus* XL-1.

Glycosyl hydrolases are enzymes that catalyze the hydrolysis of the glycosyl bond to release smaller sugars. There are over 100 classes of Glycosyl hydrolases which have been classified.. The glycosyl hydrolase family 5 (GH5) includes endo-glucanases (EC 3.2.1.4), endo-beta-1,4-xylanase (EC 3.2.1.8); beta-glucosidase (EC 3.2.1.21); beta-mannosidase (EC 3.2.1.25). However, until now identification of xanthan degrading enzymes have not been reported in glycosyl hydrolase family 5.

The mature peptide in SEQ ID NO: 2 is 45 % identical and the mature peptide in SEQ ID NO: 4 is 57 % identical to a predicted endoglucanase from the genome of *Echinicola vietnamensis* (UNIPROT: L0FVA9).

The mature peptide in SEQ ID NO: 6 is 47 % identical to an uncharacterized protein from the genome of *Barnesiella intestinhominis* (UNIPROT: K0WXE1).

The mature peptide in SEQ ID NO: 8 is 100 % identical to an uncharacterized protein from the genome of *Pseudomonas stutzeri* (UNIPROT: M2V1S3).

### Summary of the invention

The invention provides new and improved detergent compositions comprising enzymes for the degradation of xanthan gum and methods for producing said detergent compositions and to the use of said detergent compositions in cleaning applications.

The present inventors have surprisingly discovered a new group of enzymes that have xanthan degrading activity – and which do not belong to any glycosyl hydrolase family previously known to comprise this enzymatic activity. The enzymes have no significant sequence similarity to any known enzyme having xanthan degrading activity.

The present invention provides detergent compositions comprising polypeptides having xanthan degrading activity, i.e., having activity on xanthan gum and/or having activity on xanthan gum pretreated with xanthan lyase.

Accordingly, the present invention provides a detergent composition comprising polypeptide of glycosyl hydrolase family 5 having xanthan degrading activity. More particularly, the present invention provides a detergent composition comprising polypeptide of glycosyl hydrolase family 5 having xanthan degrading activity, selected from the group consisting of:

(a) a polypeptide having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at

least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the mature polypeptide of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8;

(b) a polypeptide encoded by a polynucleotide that hybridizes under medium stringency conditions with (i) the mature polypeptide coding sequence of any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7, (ii), or the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the mature polypeptide coding sequence of any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7;

(d) a variant of the mature polypeptide of any of SEQ ID NO: 2 SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 comprising a substitution, deletion, and/or insertion at one or more positions;

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has xanthan degrading activity; and

(f) a polypeptide comprising the polypeptide of (a), (b), (c), (d), or (e) and a N-terminal and/or C-terminal His-tag.

The present invention also relates to methods of degrading xanthan gum using the detergent composition comprising the polypeptides.

### Overview of Sequence Listing

SEQ ID NO: 1 is the DNA sequence of the EXa gene as isolated from an *Opiritaceae* sp.

SEQ ID NO: 2 is the amino acid sequence of the EXa GH5 polypeptide as deduced from SEQ ID NO: 1.

SEQ ID NO: 3 is the DNA sequence of the EXb gene as isolated from an environmental sample

SEQ ID NO: 4 is the amino acid sequence of the EXb GH5 polypeptide as deduced from SEQ ID NO: 3.

SEQ ID NO: 5 is the DNA sequence of the EXc gene as isolated from an environmental sample

SEQ ID NO: 6 is the amino acid sequence of the EXc GH5 polypeptide as deduced from SEQ ID NO: 5.

SEQ ID NO: 7 is the DNA sequence of the EXd gene as obtained from a public database (UNIPROT M2V1S3, originating from a strain of *Pseudomonas stutzeri* collected from a Galapagos Rift hydrothermal vent, Ecuador).

SEQ ID NO: 8 is the amino acid sequence of the EXd GH5 polypeptide as deduced from SEQ ID NO: 7.

SEQ ID NO:9 is synth codon optimized DNA encoding the EXa GH5 polypeptide.  
 SEQ ID NO:10 is synth codon optimized DNA encoding the EXb GH5 polypeptide.  
 SEQ ID NO:11 is synth codon optimized DNA encoding the EXc GH5 polypeptide.  
 SEQ ID NO:12 is synth codon optimized DNA encoding the EXd GH5 polypeptide.  
 SEQ ID NO:13 is the EXa GH5 polypeptide + His affinity tag expressed in *E.coli*.  
 SEQ ID NO:14 is the EXb GH5 polypeptide + His affinity tag expressed in *E.coli*.  
 SEQ ID NO:15 the EXc GH5 polypeptide + His affinity tag expressed in *E.coli*.  
 SEQ ID NO:16 is the EXb GH5 polypeptide + His affinity tag expressed in *B.subtilis*.  
 SEQ ID NO:17 is the EXc GH5 polypeptide + His affinity tag expressed in *B.subtilis*.  
 SEQ ID NO:18 is the EXd GH5 polypeptide + His affinity tag expressed in *B.subtilis*.  
 SEQ ID NO:19 is the His affinity tag sequence.

SEQ ID NO:20 is the amino acid sequence of the *Bacillus clausii* secretion signal .

SEQ ID NO:21 is the amino acid sequence of a xanthan lyase XLa from a *Paenibacillus* sp (SEQ ID NO: 8 from WO2013167581).

SEQ ID NO:22 is the amino acid sequence of a xanthan lyase XLb from a *Paenibacillus* sp (SEQ ID NO: 66 from WO2013167581).

SEQ ID NO:23 is the amino acid sequence of a xanthan lyase XLc from a *Paenibacillus* sp (SEQ ID NO: 68 from WO2013167581).

SEQ ID NO:24 is the amino acid sequence of a xanthan lyase XLd from a *Paenibacillus* sp (SEQ ID NO: 120 from WO2013167581).

Identity Matrix for mature peptides				
	SEQ ID NO:2 EXa	SEQ ID NO:4 EXb	SEQ ID NO:6 EXc	SEQ ID NO:8 EXd
SEQ ID NO:2 EXa		50	71	27
SEQ ID NO:4 EXb			47	31
SEQ ID NO:6 EXc				27
SEQ ID NO:8 EXd				

**Detailed Description of the Invention**

The present invention provides detergent composition comprising GH5 polypeptides having xanthan degrading activity. The polypeptides do not belong to a GH family known to comprise enzymes, which degrade xanthan. In addition, the detergent composition comprising a combination of xanthan lyase and an enzyme of the invention having xanthan degrading activity shows a synergistic improved wash performance over using a detergent composition comprising either a xanthan lyase or a GH5 polypeptide alone having xanthan degrading activity.

## Definitions

**Coding sequence:** The term "coding sequence" means a polynucleotide, which directly specifies the amino acid sequence of a polypeptide. The boundaries of the coding sequence are generally determined by an open reading frame, which begins with a start codon such as ATG, GTG, or TTG and ends with a stop codon such as TAA, TAG, or TGA. The coding sequence may be a genomic DNA, cDNA, synthetic DNA, or a combination thereof.

**Colour clarification:** During washing and wearing loose or broken fibers can accumulate on the surface of the fabrics. One consequence can be that the colours of the fabric appear less bright or less intense because of the surface contaminations. Removal of the loose or broken fibers from the textile will partly restore the original colours and looks of the textile. By the term "colour clarification", as used herein, is meant the partial restoration of the initial colours of textile.

### **Detergent Composition:**

The term "detergent composition", includes unless otherwise indicated, granular or powder-form all-purpose or heavy-duty washing agents, especially cleaning detergents; liquid, gel or paste-form all-purpose washing agents, especially the so-called heavy-duty liquid (HDL) types; liquid fine-fabric detergents; hand dishwashing agents or light duty dishwashing agents, especially those of the high-foaming type; machine dishwashing agents, including the various tablet, granular, liquid and rinse-aid types for household and institutional use; liquid cleaning and disinfecting agents, including antibacterial hand-wash types, cleaning bars, soap bars, mouthwashes, denture cleaners, car or carpet shampoos, bathroom cleaners; hair shampoos and hair-rinses; shower gels, foam baths; metal cleaners; as well as cleaning auxiliaries such as bleach additives and "stain-stick" or pre-treat types. The terms "detergent composition" and "detergent formulation" are used in reference to mixtures which are intended for use in a wash medium for the cleaning of soiled objects. In some embodiments, the term is used in reference to laundering fabrics and/or garments (e.g., "laundry detergents"). In alternative embodiments, the term refers to other detergents, such as those used to clean dishes, cutlery, etc. (e.g., "dishwashing detergents"). It is not intended that the present invention be limited to any particular detergent formulation or composition. The term "detergent composition" is not intended to be limited to compositions that contain surfactants. It is intended that in addition to the variants according to the invention, the term encompasses detergents that may contain, e.g., surfactants, builders, chelators or chelating agents, bleach system or bleach components, polymers, fabric conditioners, foam boosters, suds suppressors, dyes, perfume, tannin inhibitors, optical brighteners, bactericides, fungicides, soil suspending agents, anticorrosion agents, enzyme inhibitors or stabilizers, enzyme activators, transferase(s), hydrolytic enzymes, oxidoreductases, bluing agents and fluorescent dyes, antioxidants, and solubilizers.

**Dish wash:** The term "dish wash" refers to all forms of washing dishes, e.g., by hand or automatic dish wash. Washing dishes includes, but is not limited to, the cleaning of all forms of crockery such as plates, cups, glasses, bowls, all forms of cutlery such as spoons, knives, forks and serving utensils as well as ceramics, plastics, metals, china, glass and acrylics.

**Dish washing composition:** The term “dish washing composition” refers to all forms of compositions for cleaning hard surfaces. The present invention is not restricted to any particular type of dish wash composition or any particular detergent.

**Enzyme Detergency benefit:** The term “enzyme detergency benefit” is defined herein as the advantageous effect an enzyme may add to a detergent compared to the same detergent without the enzyme. Important detergency benefits which can be provided by enzymes are stain removal with no or very little visible soils after washing and or cleaning, prevention or reduction of redeposition of soils released in the washing process an effect that also is termed anti-redeposition, restoring fully or partly the whiteness of textiles, which originally were white but after repeated use and wash have obtained a greyish or yellowish appearance an effect that also is termed whitening. Textile care benefits, which are not directly related to catalytic stain removal or prevention of redeposition of soils are also important for enzyme detergency benefits. Examples of such textile care benefits are prevention or reduction of dye transfer from one fabric to another fabric or another part of the same fabric an effect that is also termed dye transfer inhibition or anti-backstaining, removal of protruding or broken fibers from a fabric surface to decrease pilling tendencies or remove already existing pills or fuzz an effect that also is termed anti-pilling, improvement of the fabric-softness, colour clarification of the fabric and removal of particulate soils which are trapped in the fibers of the fabric or garment. Enzymatic bleaching is a further enzyme detergency benefit where the catalytic activity generally is used to catalyze the formation of bleaching component such as hydrogen peroxide or other peroxides.

**Fragment:** The term “fragment” means a polypeptide having one or more (e.g., several) amino acids absent from the amino and/or carboxyl terminus of a mature polypeptide or domain; wherein the fragment has xanthan degrading activity.

**Hard surface cleaning:** The term “Hard surface cleaning” is defined herein as cleaning of hard surfaces wherein hard surfaces may include floors, tables, walls, roofs etc. as well as surfaces of hard objects such as cars (car wash) and dishes (dish wash). Dish washing includes but are not limited to cleaning of plates, cups, glasses, bowls, and cutlery such as spoons, knives, forks, serving utensils, ceramics, plastics, metals, china, glass and acrylics.

**Improved wash performance:** The term “improved wash performance” is defined herein as a (variant) enzyme (also a blend of enzymes, not necessarily only variants but also backbones, and in combination with certain cleaning composition etc.) displaying an alteration of the wash performance of a protease variant relative to the wash performance of the parent protease variant e.g. by increased stain removal. The term “wash performance” includes wash performance in laundry but also e.g. in dish wash.

**Isolated:** The term “isolated” means a substance in a form or environment that does not occur in nature. Non-limiting examples of isolated substances include (1) any non-naturally occurring substance, (2) any substance including, but not limited to, any enzyme, variant, nucleic acid, protein, peptide or cofactor, that is at least partially removed from one or more or all of the naturally occurring constituents with which it is associated in nature; (3) any substance modified by the hand of man

relative to that substance found in nature; or (4) any substance modified by increasing the amount of the substance relative to other components with which it is naturally associated (e.g., recombinant production in a host cell; multiple copies of a gene encoding the substance; and use of a stronger promoter than the promoter naturally associated with the gene encoding the substance). An isolated substance may be present in a fermentation broth sample; e.g. a host cell may be genetically modified to express the polypeptide of the invention. The fermentation broth from that host cell will comprise the isolated polypeptide.

**Mature polypeptide:** The term "mature polypeptide" means a polypeptide in its final form following translation and any post-translational modifications, such as N-terminal processing, C-terminal truncation, glycosylation, phosphorylation, etc. In one aspect, the mature polypeptide is amino acids 1 to 802 of SEQ ID NO: 2. In a second aspect, the mature polypeptide is amino acids 1 to 808 of SEQ ID NO: 4. In a third aspect, the mature polypeptide is amino acids 1 to 800 of SEQ ID NO: 6. In a fourth aspect, the mature polypeptide is amino acids 1 to 657 of SEQ ID NO: 8. It is known in the art that a host cell may produce a mixture of two or more different mature polypeptides (*i.e.*, with a different C-terminal and/or N-terminal amino acid) expressed by the same polynucleotide. It is also known in the art that different host cells process polypeptides differently, and thus, one host cell expressing a polynucleotide may produce a different mature polypeptide (*e.g.*, having a different C-terminal and/or N-terminal amino acid) as compared to another host cell expressing the same polynucleotide.

**Mature polypeptide coding sequence:** The term "mature polypeptide coding sequence" means a polynucleotide that encodes a mature polypeptide having xanthan degrading activity. In one aspect, the mature polypeptide coding sequence is nucleotides 109 to 2514 of SEQ ID NO: 1. Nucleotides 1 to 108 of SEQ ID NO: 1 encode a signal peptide. In one aspect, the mature polypeptide coding sequence is nucleotides 112 to 2493 of SEQ ID NO: 3. Nucleotides 1 to 111 of SEQ ID NO: 3 encode a signal peptide. In one aspect, the mature polypeptide coding sequence is nucleotides 106 to 2505 of SEQ ID NO: 5. Nucleotides 1 to 105 of SEQ ID NO: 5 encode a signal peptide. In one aspect, the mature polypeptide coding sequence is nucleotides 109 to 2079 of SEQ ID NO: 7. Nucleotides 1 to 108 of SEQ ID NO: 7 encode a signal peptide.

**Nucleic acid construct:** The term "nucleic acid construct" means a nucleic acid molecule, either single- or double-stranded, which is isolated from a naturally occurring gene or is modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature or which is synthetic, which comprises one or more control sequences.

**Operably linked:** The term "operably linked" means a configuration in which a control sequence is placed at an appropriate position relative to the coding sequence of a polynucleotide such that the control sequence directs expression of the coding sequence.

**Sequence identity:** The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter "sequence identity".

For purposes of the present invention, the sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970,



*J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the `-nobrief` option) is used as the percent identity and is calculated as follows:

$$(\text{Identical Residues} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment})$$

For purposes of the present invention, the sequence identity between two deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *supra*) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *supra*), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labeled "longest identity" (obtained using the `-nobrief` option) is used as the percent identity and is calculated as follows:

$$(\text{Identical Deoxyribonucleotides} \times 100) / (\text{Length of Alignment} - \text{Total Number of Gaps in Alignment})$$

**Textile:** The term "textile" means any textile material including yarns, yarn intermediates, fibers, non-woven materials, natural materials, synthetic materials, and any other textile material, fabrics made of these materials and products made from fabrics (e.g., garments and other articles). The textile or fabric may be in the form of knits, wovens, denims, non-wovens, felts, yarns, and towelling. The textile may be cellulose based such as natural cellulose, including cotton, flax/linen, jute, ramie, sisal or coir or manmade cellulose (e.g. originating from wood pulp) including viscose/rayon, ramie, cellulose acetate fibers (tricell), lyocell or blends thereof. The textile or fabric may also be non-cellulose based such as natural polyamides including wool, camel, cashmere, mohair, rabbit and silk or synthetic polymer such as nylon, aramid, polyester, acrylic, polypropylene and spandex/elastane, or blends thereof as well as blend of cellulose based and non-cellulose based fibers. Examples of blends are blends of cotton and/or rayon/viscose with one or more companion material such as wool, synthetic fibers (e.g. polyamide fibers, acrylic fibers, polyester fibers, polyvinyl alcohol fibers, polyvinyl chloride fibers, polyurethane fibers, polyurea fibers, aramid fibers), and cellulose-containing fibers (e.g. rayon/viscose, ramie, flax/linen, jute, cellulose acetate fibers, lyocell). Fabric may be conventional washable laundry, for example stained household laundry. When the term fabric or garment is used it is intended to include the broader term textiles as well.

**Textile care benefit:** "Textile care benefits", which are not directly related to catalytic stain removal or prevention of redeposition of soils, are also important for enzyme detergency benefits. Examples of such textile care benefits are prevention or reduction of dye transfer from one textile to another textile or another part of the same textile an effect that is also termed dye transfer inhibition or anti-backstaining, removal of protruding or broken fibers from a textile surface to decrease pilling tendencies or remove already existing pills or fuzz an effect that also is termed anti-pilling,

improvement of the textile-softness, colour clarification of the textile and removal of particulate soils which are trapped in the fibers of the textile. Enzymatic bleaching is a further enzyme detergency benefit where the catalytic activity generally is used to catalyze the formation of bleaching component such as hydrogen peroxide or other peroxides or other bleaching species.

**Wash performance:** The term “wash performance” is used as an enzyme’s ability to remove stains present on the object to be cleaned during e.g. wash or hard surface cleaning. The improvement in the wash performance may be quantified by calculating the so-called intensity value (Int) as defined in ‘Automatic Mechanical Stress Assay (AMSA) for laundry’ herein. See also the wash performance test in Example 18 herein.

**Whiteness:** The term “Whiteness” is defined herein as a broad term with different meanings in different regions and for different customers. Loss of whiteness can e.g. be due to greying, yellowing, or removal of optical brighteners/hueing agents. Greying and yellowing can be due to soil redeposition, body soils, colouring from e.g. iron and copper ions or dye transfer. Whiteness might include one or several issues from the list below: colorant or dye effects; incomplete stain removal (e.g. body soils, sebum ect.); re-deposition (greying, yellowing or other discolorations of the object) (removed soils re-associates with other part of textile, soiled or unsoiled); chemical changes in textile during application; and clarification or brightening of colours.

**Xanthan Lyase:** The term “xanthan lyase” is defined herein as an enzyme that cleaves the beta-D-mannosyl-beta-D-1,4-glucuronosyl bonds in xanthan gum (EC 4.2.2.12). For purposes of the present invention, xanthan lyase activity is determined according to the procedure described in the Examples in the ‘Xanthan lyase activity assay’.

**Xanthan degrading activity:** The term “xanthan degrading activity” is defined herein as ability to cause viscosity reduction of a xanthan solution. Xanthan solution is highly viscous even at low polymer concentrations, and this viscosity is associated with the polymer degree of xanthan. Therefore, viscosity reduction can be used to monitor xanthan degradation. The viscosity reduction may be detected using the viscosity pressure assay described in Example 6.

Xanthan degrading activity includes activity towards intact xanthan as well as activity towards xanthan pretreated with xanthan lyase (modified xanthan gum – see Example 8).

**Activity on xanthan gum:** The term “GH5 polypeptide having activity on xanthan gum” or a “polypeptide having activity on xanthan gum and belonging to the GH5 class of glycosyl hydrolases” is defined as a polypeptide comprising a domain belonging to the GH5 class of glycosyl hydrolases, and having significant activity on xanthan gum. In one aspect of the invention a GH5 polypeptide having activity on xanthan gum may be a polypeptide having a sequence selected among SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, and SEQ ID NO: 8.

**Activity on xanthan gum pretreated with xanthan lyase:** The term “GH5 polypeptide having activity on xanthan gum pretreated with xanthan lyase” or a “polypeptide having activity on xanthan gum pretreated with xanthan lyase and belonging to the GH5 class of glycosyl hydrolases” is defined as a polypeptide comprising a domain belonging to the GH5 class of glycosyl hydrolases, and having significant activity on xanthan gum pretreated with xanthan lyase (modified xanthan gum

– see Example 8). In one aspect of the invention a GH5 polypeptide having activity on xanthan gum pretreated with xanthan lyase may be a polypeptide having a sequence selected among SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, and SEQ ID NO: 8.

#### **Detergent compositions comprising Polypeptides having xanthan degrading activity**

In an embodiment, the present invention relates to detergent compositions comprising polypeptides having a sequence identity to the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%, which have xanthan degrading activity. In one aspect, the polypeptides differ by up to 10 amino acids, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, from the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8.

In a particular embodiment the invention relates to detergent compositions comprising polypeptides having a sequence identity to the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%, and wherein the polypeptide has at least at least 70% of the xanthan degrading activity of the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8.

In a particular embodiment the invention relates to detergent compositions comprising polypeptides having a sequence identity to the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%, and wherein the polypeptide has at least at least 75% of the xanthan degrading activity of the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8.

In a particular embodiment the invention relates to detergent compositions comprising polypeptides having a sequence identity to the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%, and wherein the polypeptide has at least at least 80% of the xanthan degrading activity of the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8.

In a particular embodiment the invention relates to detergent compositions comprising polypeptides having a sequence identity to the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%, and wherein the polypeptide has at least at least 85% of the xanthan degrading activity of the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8.

In a particular embodiment the invention relates to detergent compositions comprising polypeptides having a sequence identity to the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least

90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%, and wherein the polypeptide has at least at least 90% of the xanthan degrading activity of the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8.

In a particular embodiment the invention relates to detergent compositions comprising polypeptides having a sequence identity to the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%, and wherein the polypeptide has at least at least 95% of the xanthan degrading activity of the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8.

In a particular embodiment the invention relates to detergent compositions comprising polypeptides having a sequence identity to the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%, and wherein the polypeptide has at least at least 100% of the xanthan degrading activity of the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8.

In an embodiment, the polypeptide comprised in the detergent composition of present invention has been isolated. A polypeptide preferably comprises or consists of the amino acid sequence of any of SEQ ID NO: 2, 4, 6 and 8 or an allelic variant thereof; or is a fragment thereof having xanthan degrading activity. In another aspect, the polypeptide comprises or consists of the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8. In another aspect, the polypeptide comprises or consists of amino acids 1 to 802 of SEQ ID NO: 2, amino acids 1 to 808 of SEQ ID NO: 4, amino acids 1 to 800 of SEQ ID NO: 6, or amino acids 1 to 657 of SEQ ID NO: 8.

In another embodiment, the present invention relates to a detergent compositions comprising a polypeptide having xanthan degrading activity encoded by a polynucleotide that hybridizes under very low stringency conditions, low stringency conditions, medium stringency conditions, medium-high stringency conditions, high stringency conditions, or very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, (ii), or (iii) the full-length complement of (i) or (ii). In an embodiment, the polypeptide comprised in the detergent composition has been isolated.

For purposes of the present invention, hybridization indicates that the polynucleotide hybridizes to a labeled nucleic acid probe corresponding to (i) any of SEQ ID NO: 1, 3, 5, or 7; (ii) the mature polypeptide coding sequence of any of SEQ ID NO: 1, 3, 5, or 7; (iii) the full-length complement thereof; or (iv) a subsequence thereof; under very low to very high stringency conditions. Molecules to which the nucleic acid probe hybridizes under these conditions can be detected using, for example, X-ray film or any other detection means known in the art.

In another embodiment, the present invention relates to a detergent compositions comprising a polypeptide having xanthan degrading activity encoded by a polynucleotide having a sequence identity to the mature polypeptide coding sequence of any of SEQ ID NO: 1, 3, 5, or 7 of at least 60%, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least

91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. In a further embodiment, the polypeptide has been isolated.

In another embodiment, the present invention relates to detergent compositions comprising variants of the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8 comprising a substitution, deletion, and/or insertion at one or more (*e.g.*, several) positions. In an embodiment, the number of amino acid substitutions, deletions and/or insertions introduced into the mature polypeptide of any of SEQ ID NO: 2, 4, 6 and 8 is up to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. The amino acid changes may be of a minor nature, that is conservative amino acid substitutions or insertions that do not significantly affect the folding and/or activity of the protein; small deletions, typically of 1-30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue; a small linker peptide of up to 20-25 residues; or a small extension that facilitates purification by changing net charge or another function, such as a poly-histidine tag, an antigenic epitope or a binding domain. SEQ ID NO: 13, 14 and 15 show the polypeptides of the invention (SEQ ID NO: 2, 4 and 6) with an N-terminal poly histidine tag (His-tag). SEQ ID NO: 16, 17 and 18 show the polypeptides of the invention (SEQ ID NO: 4, 6 and 8) with an N-terminal poly histidine tag.

Examples of conservative substitutions are within the groups of basic amino acids (arginine, lysine and histidine), acidic amino acids (glutamic acid and aspartic acid), polar amino acids (glutamine and asparagine), hydrophobic amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tryptophan and tyrosine), and small amino acids (glycine, alanine, serine, threonine and methionine). Amino acid substitutions that do not generally alter specific activity are known in the art and are described, for example, by H. Neurath and R.L. Hill, 1979, *In, The Proteins*, Academic Press, New York. Common substitutions are Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, and Asp/Gly.

### **Sources of polypeptides having xanthan degrading activity**

A polypeptide having xanthan degrading activity as comprised in the detergent composition of the present invention may be obtained from microorganisms of any genus. For purposes of the present invention, the term "obtained from" as used herein in connection with a given source shall mean that the polypeptide encoded by a polynucleotide is produced by the source or by a strain in which the polynucleotide from the source has been inserted.

In an aspect, the polypeptide is a polypeptide obtained from an *Opitutaceae* species.

### **Polynucleotides**

The present invention also relates to polynucleotides encoding a polypeptide, as described herein. In an embodiment, the polynucleotide encoding the polypeptide of the present invention has been isolated.

**Detergent composition**

In one embodiment of the present invention, the polypeptide of the present invention may be added to a detergent composition in an amount corresponding to 0.0001-200 mg of enzyme protein, such as 0.0005-100 mg of enzyme protein, preferably 0.001-30 mg of enzyme protein, more preferably 0.005-8 mg of enzyme protein, even more preferably 0.01-2 mg of enzyme protein per litre of wash liquor.

A composition for use in automatic dishwash (ADW), for example, may include 0.0001%-50%, such as 0.001%-20%, such as 0.01%-10%, such as 0.05%-5% of enzyme protein by weight of the composition.

A composition for use in laundry powder, for example, may include 0.0001%-50%, such as 0.001%-20%, such as 0.01%-10%, such as 0.05%-5% of enzyme protein by weight of the composition.

A composition for use in laundry liquid, for example, may include 0.0001%-10%, such as 0.001-7%, such as 0.1%-5% of enzyme protein by weight of the composition.

The enzyme(s) of the detergent composition of the invention may be stabilized using conventional stabilizing agents, e.g., a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative, e.g., an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid, and the composition may be formulated as described in, for example, WO92/19709 and WO92/19708.

In certain markets different wash conditions and, as such, different types of detergents are used. This is disclosed in e.g. EP 1 025 240. For example, In Asia (Japan) a low detergent concentration system is used, while the United States uses a medium detergent concentration system, and Europe uses a high detergent concentration system.

In one embodiment, the invention is directed to detergent compositions comprising an enzyme of the present invention in combination with one or more additional cleaning composition components. The choice of additional components is within the skill of the artisan and includes conventional ingredients, including the exemplary non-limiting components set forth below.

The choice of components may include, for textile care, the consideration of the type of textile to be cleaned, the type and/or degree of soiling, the temperature at which cleaning is to take place, and the formulation of the detergent product. Although components mentioned below are categorized by general header according to a particular functionality, this is not to be construed as a limitation, as a component may comprise additional functionalities as will be appreciated by the skilled artisan.

In one embodiment, the invention is directed to an ADW (Automatic Dish Wash) composition comprising an enzyme of the present invention in combination with one or more additional ADW composition components. The choice of additional components is within the skill of the artisan and includes conventional ingredients, including the exemplary non-limiting components set forth below.

In one embodiment the detergent composition of present invention comprises up to

### Surfactants

The detergent composition may comprise one or more surfactants, which may be anionic and/or cationic and/or non-ionic and/or semi-polar and/or zwitterionic, or a mixture thereof. In a particular embodiment, the detergent composition includes a mixture of one or more nonionic surfactants and one or more anionic surfactants. The surfactant(s) is typically present at a level of from about 0.1% to 60% by weight, such as about 1% to about 40%, or about 3% to about 20%, or about 3% to about 10%. The surfactant(s) is chosen based on the desired cleaning application, and may include any conventional surfactant(s) known in the art.

When included therein the detergent will usually contain from about 1% to about 40% by weight of an anionic surfactant, such as from about 5% to about 30%, including from about 5% to about 15%, or from about 15% to about 20%, or from about 20% to about 25% of an anionic surfactant. Non-limiting examples of anionic surfactants include sulfates and sulfonates, in particular, linear alkylbenzenesulfonates (LAS), isomers of LAS, branched alkylbenzenesulfonates (BABS), phenylalkanesulfonates, alpha-olefinsulfonates (AOS), olefin sulfonates, alkene sulfonates, alkane-2,3-diylbis(sulfates), hydroxyalkanesulfonates and disulfonates, alkyl sulfates (AS) such as sodium dodecyl sulfate (SDS), fatty alcohol sulfates (FAS), primary alcohol sulfates (PAS), alcohol ethersulfates (AES or AEOS or FES, also known as alcohol ethoxysulfates or fatty alcohol ether sulfates), secondary alkanesulfonates (SAS), paraffin sulfonates (PS), ester sulfonates, sulfonated fatty acid glycerol esters, alpha-sulfo fatty acid methyl esters (alpha-SFMe or SES) including methyl ester sulfonate (MES), alkyl- or alkenylsuccinic acid, dodecenylolefin/tetradecenylolefin succinic acid (DTSA), fatty acid derivatives of amino acids, diesters and monoesters of sulfo-succinic acid or salt of fatty acids (soap), and combinations thereof.

When included therein the detergent will usually contain from about 1% to about 40% by weight of a cationic surfactant, for example from about 0.5% to about 30%, in particular from about 1% to about 20%, from about 3% to about 10%, such as from about 3% to about 5%, from about 8% to about 12% or from about 10% to about 12%. Non-limiting examples of cationic surfactants include alkyldimethylethanolamine quat (ADMEAQ), cetyltrimethylammonium bromide (CTAB), dimethyldistearylammonium chloride (DSDMAC), and alkylbenzyltrimethylammonium, alkyl quaternary ammonium compounds, alkoxyated quaternary ammonium (AQA) compounds, ester quats, and combinations thereof.

When included therein the detergent will usually contain from about 0.2% to about 40% by weight of a nonionic surfactant, for example from about 0.5% to about 30%, in particular from about 1% to about 20%, from about 3% to about 10%, such as from about 3% to about 5%, from about 8% to about 12%, or from about 10% to about 12%. Non-limiting examples of nonionic surfactants include alcohol ethoxylates (AE or AEO), alcohol propoxylates, propoxylated fatty alcohols (PFA), alkoxyated fatty acid alkyl esters, such as ethoxylated and/or propoxylated fatty acid alkyl esters, alkylphenol ethoxylates (APE), nonylphenol ethoxylates (NPE), alkylpolyglycosides (APG), alkoxyated amines, fatty acid monoethanolamides (FAM), fatty acid diethanolamides (FADA), ethoxylated fatty acid monoethanolamides (EFAM), propoxylated fatty acid monoethanolamides (PFAM), polyhydroxyalkyl

fatty acid amides, or *N*-acyl *N*-alkyl derivatives of glucosamine (glucamides, GA, or fatty acid glucamides, FAGA), as well as products available under the trade names SPAN and TWEEN, and combinations thereof.

When included therein the detergent will usually contain from about 0% to about 10% by weight of a semipolar surfactant. Non-limiting examples of semipolar surfactants include amine oxides (AO) such as alkyldimethylamineoxide, *N*-(coco alkyl)-*N,N*-dimethylamine oxide and *N*-(tallow-alkyl)-*N,N*-bis(2-hydroxyethyl)amine oxide, , and combinations thereof.

When included therein the detergent will usually contain from about 0% to about 10% by weight of a zwitterionic surfactant. Non-limiting examples of zwitterionic surfactants include betaines such as alkyldimethylbetaines, sulfobetaines, and combinations thereof.

### **Hydrotropes**

A hydrotrope is a compound that solubilises hydrophobic compounds in aqueous solutions (or oppositely, polar substances in a non-polar environment). Typically, hydrotropes have both hydrophilic and a hydrophobic character (so-called amphiphilic properties as known from surfactants); however the molecular structure of hydrotropes generally do not favor spontaneous self-aggregation, see e.g. review by Hodgdon and Kaler (2007), *Current Opinion in Colloid & Interface Science* 12: 121-128. Hydrotropes do not display a critical concentration above which self-aggregation occurs as found for surfactants and lipids forming micellar, lamellar or other well defined meso-phases. Instead, many hydrotropes show a continuous-type aggregation process where the sizes of aggregates grow as concentration increases. However, many hydrotropes alter the phase behavior, stability, and colloidal properties of systems containing substances of polar and non-polar character, including mixtures of water, oil, surfactants, and polymers. Hydrotropes are classically used across industries from pharma, personal care, food, to technical applications. Use of hydrotropes in detergent compositions allow for example more concentrated formulations of surfactants (as in the process of compacting liquid detergents by removing water) without inducing undesired phenomena such as phase separation or high viscosity.

The detergent may contain 0-10% by weight, for example 0-5% by weight, such as about 0.5 to about 5%, or about 3% to about 5%, of a hydrotrope. Any hydrotrope known in the art for use in detergents may be utilized. Non-limiting examples of hydrotropes include sodium benzenesulfonate, sodium *p*-toluene sulfonate (STS), sodium xylene sulfonate (SXS), sodium cumene sulfonate (SCS), sodium cymene sulfonate, amine oxides, alcohols and polyglycolethers, sodium hydroxynaphthoate, sodium hydroxynaphthalene sulfonate, sodium ethylhexyl sulfate, and combinations thereof.

### **Builders and Co-Builders**

The detergent composition may contain about 0-65% by weight, such as about 5% to about 50% of a detergent builder or co-builder, or a mixture thereof. In a dish wash detergent, the level of builder is typically 40-65%, particularly 50-65%. The builder and/or co-builder may particularly be a chelating agent that forms water-soluble complexes with Ca and Mg. Any builder and/or co-builder



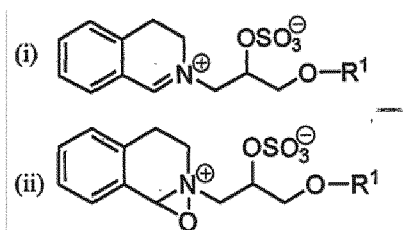
known in the art for use in detergents may be utilized. Non-limiting examples of builders include zeolites, diphosphates (pyrophosphates), triphosphates such as sodium triphosphate (STP or STPP), carbonates such as sodium carbonate, soluble silicates such as sodium metasilicate, layered silicates (e.g., SKS-6 from Hoechst), ethanolamines such as 2-aminoethan-1-ol (MEA), diethanolamine (DEA, also known as 2,2'-iminodiethan-1-ol), triethanolamine (TEA, also known as 2,2',2''-nitrilotriethan-1-ol), and (carboxymethyl)inulin (CMI), and combinations thereof.

The detergent composition may also contain 0-50% by weight, such as about 5% to about 30%, of a detergent co-builder. The detergent composition may include a co-builder alone, or in combination with a builder, for example a zeolite builder. Non-limiting examples of co-builders include homopolymers of polyacrylates or copolymers thereof, such as poly(acrylic acid) (PAA) or copoly(acrylic acid/maleic acid) (PAA/PMA). Further non-limiting examples include citrate, chelators such as aminocarboxylates, aminopolycarboxylates and phosphonates, and alkyl- or alkenylsuccinic acid. Additional specific examples include 2,2',2''-nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), iminodisuccinic acid (IDS), ethylenediamine-*N,N*-disuccinic acid (EDDS), methylglycinediacetic acid (MGDA), glutamic acid-*N,N*-diacetic acid (GLDA), 1-hydroxyethane-1,1-diphosphonic acid (HEDP), ethylenediaminetetra(methylenephosphonic acid) (EDTMPA), diethylenetriaminepentakis(methylenephosphonic acid) (DTMPA or DTPMPA), *N*-(2-hydroxyethyl)iminodiacetic acid (EDG), aspartic acid-*N*-monoacetic acid (ASMA), aspartic acid-*N,N*-diacetic acid (ASDA), aspartic acid-*N*-monopropionic acid (ASMP), iminodisuccinic acid (IDA), *N*-(2-sulfomethyl)-aspartic acid (SMAS), *N*-(2-sulfoethyl)-aspartic acid (SEAS), *N*-(2-sulfomethyl)-glutamic acid (SMGL), *N*-(2-sulfoethyl)-glutamic acid (SEGL), *N*-methyliminodiacetic acid (MIDA), alpha-alanine-*N,N*-diacetic acid ( $\alpha$ -ALDA), serine-*N,N*-diacetic acid (SEDA), isoserine-*N,N*-diacetic acid (ISDA), phenylalanine-*N,N*-diacetic acid (PHDA), anthranilic acid-*N,N*-diacetic acid (ANDA), sulfanilic acid-*N,N*-diacetic acid (SLDA), taurine-*N,N*-diacetic acid (TUDA) and sulfomethyl-*N,N*-diacetic acid (SMDA), *N*-(2-hydroxyethyl)ethylenediamine-*N,N',N''*-triacetic acid (HEDTA), diethanolglycine (DEG), diethylenetriamine penta(methylenephosphonic acid) (DTPMP), aminotris(methylenephosphonic acid) (ATMP), and combinations and salts thereof. Further exemplary builders and/or co-builders are described in, e.g., WO 09/102854, US 5977053

### **Bleaching Systems**

The detergent may contain 0-30% by weight, such as about 1% to about 20%, of a bleaching system. Any bleaching system known in the art for use in detergents may be utilized. Suitable bleaching system components include bleaching catalysts, photobleaches, bleach activators, sources of hydrogen peroxide such as sodium percarbonate, sodium perborates and hydrogen peroxide—urea (1:1), preformed peracids and mixtures thereof. Suitable preformed peracids include, but are not limited to, peroxydicarboxylic acids and salts, diperoxydicarboxylic acids, perimidic acids and salts, peroxymonosulfuric acids and salts, for example, Oxone (R), and mixtures thereof. Non-limiting examples of bleaching systems include peroxide-based bleaching systems, which may comprise, for example, an inorganic salt, including alkali metal salts such as sodium salts of perborate (usually

mono- or tetra-hydrate), percarbonate, persulfate, perphosphate, persilicate salts, in combination with a peracid-forming bleach activator. The term bleach activator is meant herein as a compound which reacts with hydrogen peroxide to form a peracid via perhydrolysis. The peracid thus formed constitutes the activated bleach. Suitable bleach activators to be used herein include those belonging to the class of esters, amides, imides or anhydrides. Suitable examples are tetraacetylenediamine (TAED), sodium 4-[(3,5,5-trimethylhexanoyl)oxy]benzene-1-sulfonate (ISONOBS), 4-(dodecanoyloxy)benzene-1-sulfonate (LOBS), 4-(decanoyloxy)benzene-1-sulfonate, 4-(decanoyloxy)benzoate (DOBS or DOBA), 4-(nonanoyloxy)benzene-1-sulfonate (NOBS), and/or those disclosed in WO98/17767. A particular family of bleach activators of interest was disclosed in EP624154 and particularly preferred in that family is acetyl triethyl citrate (ATC). ATC or a short chain triglyceride like triacetin has the advantage that it is environmentally friendly. Furthermore acetyl triethyl citrate and triacetin have good hydrolytical stability in the product upon storage and are efficient bleach activators. Finally ATC is multifunctional, as the citrate released in the perhydrolysis reaction may function as a builder. Alternatively, the bleaching system may comprise peroxyacids of, for example, the amide, imide, or sulfone type. The bleaching system may also comprise peracids such as 6-(phthalimido)peroxyhexanoic acid (PAP). The bleaching system may also include a bleach catalyst. In some embodiments the bleach component may be an organic catalyst selected from the group consisting of organic catalysts having the following formulae:



(iii) and mixtures thereof;

wherein each R<sup>1</sup> is independently a branched alkyl group containing from 9 to 24 carbons or linear alkyl group containing from 11 to 24 carbons, preferably each R<sup>1</sup> is independently a branched alkyl group containing from 9 to 18 carbons or linear alkyl group containing from 11 to 18 carbons, more preferably each R<sup>1</sup> is independently selected from the group consisting of 2-propylheptyl, 2-butyloctyl, 2-pentylonyl, 2-hexyldecyl, dodecyl, tetradecyl, hexadecyl, octadecyl, isononyl, isodecyl, isotridecyl and isopentadecyl. Other exemplary bleaching systems are described, e.g. in WO2007/087258, WO2007/087244, WO2007/087259, EP1867708 (Vitamin K) and WO2007/087242. Suitable photobleaches may for example be sulfonated zinc or aluminium phthalocyanines.

Preferably the bleach component comprises a source of peracid in addition to bleach catalyst, particularly organic bleach catalyst. The source of peracid may be selected from (a) pre-formed peracid; (b) percarbonate, perborate or persulfate salt (hydrogen peroxide source) preferably in combination with a bleach activator; and (c) perhydrolyase enzyme and an ester for forming peracid in situ in the presence of water in a textile or hard surface treatment step.

### **Polymers**

The detergent may contain 0-10% by weight, such as 0.5-5%, 2-5%, 0.5-2% or 0.2-1% of a polymer. Any polymer known in the art for use in detergents may be utilized. The polymer may function as a co-builder as mentioned above, or may provide antiredeposition, fiber protection, soil release, dye transfer inhibition, grease cleaning and/or anti-foaming properties. Some polymers may have more than one of the above-mentioned properties and/or more than one of the below-mentioned motifs. Exemplary polymers include (carboxymethyl)cellulose (CMC), poly(vinyl alcohol) (PVA), poly(vinylpyrrolidone) (PVP), poly(ethyleneglycol) or poly(ethylene oxide) (PEG), ethoxylated poly(ethyleneimine), carboxymethyl inulin (CMI), and polycarboxylates such as PAA, PAA/PMA, poly-aspartic acid, and lauryl methacrylate/acrylic acid copolymers, hydrophobically modified CMC (HM-CMC) and silicones, copolymers of terephthalic acid and oligomeric glycols, copolymers of poly(ethylene terephthalate) and poly(oxyethene terephthalate) (PET-POET), PVP, poly(vinylimidazole) (PVI), poly(vinylpyridine-*N*-oxide) (PVPO or PVPNO) and polyvinylpyrrolidone-vinylimidazole (PVPVI). Further exemplary polymers include sulfonated polycarboxylates, polyethylene oxide and polypropylene oxide (PEO-PPO) and diquaternium ethoxy sulfate. Other exemplary polymers are disclosed in, e.g., WO 2006/130575. Salts of the above-mentioned polymers are also contemplated.

### **Fabric hueing agents**

The detergent compositions of the present invention may also include fabric hueing agents such as dyes or pigments, which when formulated in detergent compositions can deposit onto a fabric when said fabric is contacted with a wash liquor comprising said detergent compositions and thus altering the tint of said fabric through absorption/reflection of visible light. Fluorescent whitening agents emit at least some visible light. In contrast, fabric hueing agents alter the tint of a surface as they absorb at least a portion of the visible light spectrum. Suitable fabric hueing agents include dyes and dye-clay conjugates, and may also include pigments. Suitable dyes include small molecule dyes and polymeric dyes. Suitable small molecule dyes include small molecule dyes selected from the group consisting of dyes falling into the Colour Index (C.I.) classifications of Direct Blue, Direct Red, Direct Violet, Acid Blue, Acid Red, Acid Violet, Basic Blue, Basic Violet and Basic Red, or mixtures thereof, for example as described in WO2005/03274, WO2005/03275, WO2005/03276 and EP1876226 (hereby incorporated by reference). The detergent composition preferably comprises from about 0.00003 wt% to about 0.2 wt%, from about 0.00008 wt% to about 0.05 wt%, or even from about 0.0001 wt% to about 0.04 wt% fabric hueing agent. The composition may comprise from 0.0001 wt% to 0.2 wt% fabric hueing agent, this may be especially preferred when the composition is in the form of a unit dose pouch. Suitable hueing agents are also disclosed in, e.g. WO 2007/087257 and WO2007/087243.

### **Additional enzymes**

The detergent additive as well as the detergent composition may comprise one or more additional enzymes such as a protease, a lipase, a cutinase, an amylase, a carbohydrase, a cellulase,

a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, an oxidase, e.g., a laccase, and/or a peroxidase and/or a xanthan lyase.

In general the properties of the selected enzyme(s) should be compatible with the selected detergent, (i.e., pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, etc.), and the enzyme(s) should be present in effective amounts.

### **Cellulases**

Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases from the genera *Bacillus*, *Pseudomonas*, *Humicola*, *Fusarium*, *Thielavia*, *Acremonium*, e.g., the fungal cellulases produced from *Humicola insolens*, *Myceliophthora thermophila* and *Fusarium oxysporum* disclosed in US 4,435,307, US 5,648,263, US 5,691,178, US 5,776,757 and WO 89/09259.

Especially suitable cellulases are the alkaline or neutral cellulases having colour care benefits. Examples of such cellulases are cellulases described in EP 0 495 257, EP 0 531 372, WO 96/11262, WO 96/29397, WO 98/08940. Other examples are cellulase variants such as those described in WO 94/07998, EP 0 531 315, US 5,457,046, US 5,686,593, US 5,763,254, WO 95/24471, WO 98/12307 and WO99/001544.

Other cellulases are endo-beta-1,4-glucanase enzyme having a sequence of at least 97% identity to the amino acid sequence of position 1 to position 773 of SEQ ID NO:2 of WO 2002/099091 or a family 44 xyloglucanase, which a xyloglucanase enzyme having a sequence of at least 60% identity to positions 40-559 of SEQ ID NO: 2 of WO 2001/062903.

Commercially available cellulases include Celluzyme™, and Carezyme™ (Novozymes A/S) Carezyme Premium™ (Novozymes A/S), Celluclean™ (Novozymes A/S), Celluclean Classic™ (Novozymes A/S), Cellusoft™ (Novozymes A/S), Whitezyme™ (Novozymes A/S), Clazinase™, and Puradax HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao Corporation).

### **Mannanases**

Suitable mannanases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included. The mannanase may be an alkaline mannanase of Family 5 or 26. It may be a wild-type from *Bacillus* or *Humicola*, particularly *B. agaradhaerens*, *B. licheniformis*, *B. halodurans*, *B. clausii*, or *H. insolens*. Suitable mannanases are described in WO 1999/064619. A commercially available mannanase is Mannaway (Novozymes A/S).

### **Xanthan lyases**

Suitable xanthan lyases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful enzymes include the xanthan lyases disclosed in WO2013167581 and shown herein as SEQ ID NO:21, 22, 23 and 24.

### Proteases

Suitable proteases include those of bacterial, fungal, plant, viral or animal origin e.g. vegetable or microbial origin. Microbial origin is preferred. Chemically modified or protein engineered mutants are included. It may be an alkaline protease, such as a serine protease or a metalloprotease. A serine protease may for example be of the S1 family, such as trypsin, or the S8 family such as subtilisin. A metalloproteases protease may for example be a thermolysin from e.g. family M4 or other metalloprotease such as those from M5, M7 or M8 families.

The term "subtilases" refers to a sub-group of serine protease according to Siezen et al., Protein Engng. 4 (1991) 719-737 and Siezen et al. Protein Science 6 (1997) 501-523. Serine proteases are a subgroup of proteases characterized by having a serine in the active site, which forms a covalent adduct with the substrate. The subtilases may be divided into 6 sub-divisions, i.e. the Subtilisin family, the Thermitase family, the Proteinase K family, the Lantibiotic peptidase family, the Kexin family and the Pyrolysin family.

Examples of subtilases are those derived from *Bacillus* such as *Bacillus lentus*, *B. alkalophilus*, *B. subtilis*, *B. amyloliquefaciens*, *Bacillus pumilus* and *Bacillus gibsonii* described in; US7262042 and WO9/021867, and *subtilisin lentus*, *subtilisin Novo*, *subtilisin Carlsberg*, *Bacillus licheniformis*, *subtilisin BPN'*, *subtilisin 309*, *subtilisin 147* and *subtilisin 168* described in WO89/06279 and protease PD138 described in (WO93/18140). Other useful proteases may be those described in WO92/175177, WO01/016285, WO02/026024 and WO02/016547. Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the *Fusarium* protease described in WO89/06270, WO94/25583 and WO05/040372, and the chymotrypsin proteases derived from *Cellumonas* described in WO05/052161 and WO05/052146.

A further preferred protease is the alkaline protease from *Bacillus lentus* DSM 5483, as described for example in WO95/23221, and variants thereof which are described in WO92/21760, WO95/23221, EP1921147 and EP1921148.

Examples of metalloproteases are the neutral metalloprotease as described in WO07/044993 (Genencor Int.) such as those derived from *Bacillus amyloliquefaciens*.

Examples of useful proteases are the variants described in: WO92/19729, WO96/034946, WO98/20115, WO98/20116, WO99/011768, WO01/44452, WO03/006602, WO04/03186, WO04/041979, WO07/006305, WO11/036263, WO11/036264, especially the variants with substitutions in one or more of the following positions: 3, 4, 9, 15, 27, 36, 57, 68, 76, 87, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 106, 118, 120, 123, 128, 129, 130, 160, 167, 170, 194, 195, 199, 205, 206, 217, 218, 222, 224, 232, 235, 236, 245, 248, 252 and 274 using the BPN' numbering. More preferred the subtilase variants may comprise the mutations: S3T, V4I, S9R, A15T, K27R, \*36D, V68A, N76D, N87S,R, \*97E, A98S, S99G,D,A, S99AD, S101G,M,R S103A, V104I,Y,N, S106A, G118V,R, H120D,N, N123S, S128L, P129Q, S130A, G160D, Y167A, R170S, A194P, G195E, V199M, V205I, L217D, N218D, M222S, A232V, K235L, Q236H, Q245R, N252K, T274A (using BPN' numbering).

Suitable commercially available protease enzymes include those sold under the trade names Alcalase®, Duralase™, Durazym™, Release®, Release® Ultra, Savinase®, Savinase® Ultra, Primase®, Polarzyme®, Kannase®, Liquease®, Liquease® Ultra, Ovozyme®, Coronase®, Coronase® Ultra, Blaze®, Neutrase®, Everlase® and Esperase® (Novozymes A/S), those sold under the tradename Maxatase®, Maxacal®, Maxapem®, Purafect®, Purafect Prime®, , Purafect MA®, Purafect Ox®, Purafect OxP®, Puramax®, Properase®, , FN2®, FN3®, FN4®, Excellase®, Eraser®, Opticlean® and Optimase® (Danisco/DuPont), Axapem™ (Gist-Brocades N.V.), BLAP (sequence shown in Figure 29 of US5352604) and variants hereof (Henkel AG) and KAP (*Bacillus alkalophilus subtilisin*) from Kao.

### Lipases and cutinases

Suitable lipases and cutinases include those of bacterial or fungal origin. Chemically modified or protein engineered mutant enzymes are included. Examples include lipase from *Thermomyces*, e.g. from *T. lanuginosus* (previously named *Humicola lanuginosa*) as described in EP258068 and EP305216, cutinase from *Humicola*, e.g. *H. insolens* (WO96/13580), lipase from strains of *Pseudomonas* (some of these now renamed to *Burkholderia*), e.g. *P. alcaligenes* or *P. pseudoalcaligenes* (EP218272), *P. cepacia* (EP331376), *P. sp.* strain SD705 (WO95/06720 & WO96/27002), *P. wisconsinensis* (WO96/12012), GDSL-type *Streptomyces* lipases (WO10/065455), cutinase from *Magnaporthe grisea* (WO10/107560), cutinase from *Pseudomonas mendocina* (US5,389,536), lipase from *Thermobifida fusca* (WO11/084412), *Geobacillus stearothermophilus* lipase (WO11/084417), lipase from *Bacillus subtilis* (WO11/084599), and lipase from *Streptomyces griseus* (WO11/150157) and *S. pristinaespiralis* (WO12/137147).

Other examples are lipase variants such as those described in EP407225, WO92/05249, WO94/01541, WO94/25578, WO95/14783, WO95/30744, WO95/35381, WO95/22615, WO96/00292, WO97/04079, WO97/07202, WO00/34450, WO00/60063, WO01/92502, WO07/87508 and WO09/109500.

Preferred commercial lipase products include include Lipolase™, Lipex™; Lipolex™ and Lipoclean™ (Novozymes A/S), Lumafast (originally from Genencor) and Lipomax (originally from Gist-Brocades).

Still other examples are lipases sometimes referred to as acyltransferases or perhydrolases, e.g. acyltransferases with homology to *Candida antarctica* lipase A (WO10/111143), acyltransferase from *Mycobacterium smegmatis* (WO05/56782), perhydrolases from the CE 7 family (WO09/67279), and variants of the *M. smegmatis* perhydrolase in particular the S54V variant used in the commercial product Gentle Power Bleach from Huntsman Textile Effects Pte Ltd (WO10/100028).

### Amylases

Suitable amylases which can be used together with the enzyme of the invention may be an alpha-amylase or a glucoamylase and may be of bacterial or fungal origin. Chemically modified or

protein engineered mutants are included. Amylases include, for example, alpha-amylases obtained from *Bacillus*, e.g., a special strain of *Bacillus licheniformis*, described in more detail in GB 1,296,839.

Suitable amylases include amylases having SEQ ID NO: 2 in WO 95/10603 or variants having 90% sequence identity to SEQ ID NO: 3 thereof. Preferred variants are described in WO 94/02597, WO 94/18314, WO 97/43424 and SEQ ID NO: 4 of WO 99/019467, such as variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 178, 179, 181, 188, 190, 197, 201, 202, 207, 208, 209, 211, 243, 264, 304, 305, 391, 408, and 444.

Different suitable amylases include amylases having SEQ ID NO: 6 in WO 02/010355 or variants thereof having 90% sequence identity to SEQ ID NO: 6. Preferred variants of SEQ ID NO: 6 are those having a deletion in positions 181 and 182 and a substitution in position 193.

Other amylases which are suitable are hybrid alpha-amylase comprising residues 1-33 of the alpha-amylase derived from *B. amyloliquefaciens* shown in SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of the *B. licheniformis* alpha-amylase shown in SEQ ID NO: 4 of WO 2006/066594 or variants having 90% sequence identity thereof. Preferred variants of this hybrid alpha-amylase are those having a substitution, a deletion or an insertion in one or more of the following positions: G48, T49, G107, H156, A181, N190, M197, I201, A209 and Q264. Most preferred variants of the hybrid alpha-amylase comprising residues 1-33 of the alpha-amylase derived from *B. amyloliquefaciens* shown in SEQ ID NO: 6 of WO 2006/066594 and residues 36-483 of SEQ ID NO: 4 are those having the substitutions:

M197T;

H156Y+A181T+N190F+A209V+Q264S; or

G48A+T49I+G107A+H156Y+A181T+N190F+I201F+A209V+Q264S.

Further amylases which are suitable are amylases having SEQ ID NO: 6 in WO 99/019467 or variants thereof having 90% sequence identity to SEQ ID NO: 6. Preferred variants of SEQ ID NO: 6 are those having a substitution, a deletion or an insertion in one or more of the following positions: R181, G182, H183, G184, N195, I206, E212, E216 and K269. Particularly preferred amylases are those having deletion in positions R181 and G182, or positions H183 and G184.

Additional amylases which can be used are those having SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 2 or SEQ ID NO: 7 of WO 96/023873 or variants thereof having 90% sequence identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 7. Preferred variants of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 7 are those having a substitution, a deletion or an insertion in one or more of the following positions: 140, 181, 182, 183, 184, 195, 206, 212, 243, 260, 269, 304 and 476, using SEQ ID 2 of WO 96/023873 for numbering. More preferred variants are those having a deletion in two positions selected from 181, 182, 183 and 184, such as 181 and 182, 182 and 183, or positions 183 and 184. Most preferred amylase variants of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 7 are those having a deletion in positions 183 and 184 and a substitution in one or more of positions 140, 195, 206, 243, 260, 304 and 476.

Other amylases which can be used are amylases having SEQ ID NO: 2 of WO 08/153815, SEQ ID NO: 10 in WO 01/66712 or variants thereof having 90% sequence identity to SEQ ID NO: 2

of WO 08/153815 or 90% sequence identity to SEQ ID NO: 10 in WO 01/66712. Preferred variants of SEQ ID NO: 10 in WO 01/66712 are those having a substitution, a deletion or an insertion in one of more of the following positions: 176, 177, 178, 179, 190, 201, 207, 211 and 264.

Further suitable amylases are amylases having SEQ ID NO: 2 of WO 09/061380 or variants having 90% sequence identity to SEQ ID NO: 2 thereof. Preferred variants of SEQ ID NO: 2 are those having a truncation of the C-terminus and/or a substitution, a deletion or an insertion in one of more of the following positions: Q87, Q98, S125, N128, T131, T165, K178, R180, S181, T182, G183, M201, F202, N225, S243, N272, N282, Y305, R309, D319, Q320, Q359, K444 and G475. More preferred variants of SEQ ID NO: 2 are those having the substitution in one of more of the following positions: Q87E,R, Q98R, S125A, N128C, T131I, T165I, K178L, T182G, M201L, F202Y, N225E,R, N272E,R, S243Q,A,E,D, Y305R, R309A, Q320R, Q359E, K444E and G475K and/or deletion in position R180 and/or S181 or of T182 and/or G183. Most preferred amylase variants of SEQ ID NO: 2 are those having the substitutions:

N128C+K178L+T182G+Y305R+G475K;

N128C+K178L+T182G+F202Y+Y305R+D319T+G475K;

S125A+N128C+K178L+T182G+Y305R+G475K; or

S125A+N128C+T131I+T165I+K178L+T182G+Y305R+G475K wherein the variants are C-terminally truncated and optionally further comprises a substitution at position 243 and/or a deletion at position 180 and/or position 181.

Further suitable amylases are amylases having SEQ ID NO: 1 of WO13184577 or variants having 90% sequence identity to SEQ ID NO: 1 thereof. Preferred variants of SEQ ID NO: 1 are those having a substitution, a deletion or an insertion in one of more of the following positions: K176, R178, G179, T180, G181, E187, N192, M199, I203, S241, R458, T459, D460, G476 and G477. More preferred variants of SEQ ID NO: 1 are those having the substitution in one of more of the following positions: K176L, E187P, N192FYH, M199L, I203YF, S241QADN, R458N, T459S, D460T, G476K and G477K and/or deletion in position R178 and/or S179 or of T180 and/or G181. Most preferred amylase variants of SEQ ID NO: 1 are those having the substitutions:

E187P+I203Y+G476K

E187P+I203Y+R458N+T459S+D460T+G476K

wherein the variants optionally further comprises a substitution at position 241 and/or a deletion at position 178 and/or position 179.

Further suitable amylases are amylases having SEQ ID NO: 1 of WO10104675 or variants having 90% sequence identity to SEQ ID NO: 1 thereof. Preferred variants of SEQ ID NO: 1 are those having a substitution, a deletion or an insertion in one of more of the following positions: N21, D97, V128, K177, R179, S180, I181, G182, M200, L204, E242, G477 and G478. More preferred variants of SEQ ID NO: 1 are those having the substitution in one of more of the following positions: N21D, D97N, V128I, K177L, M200L, L204YF, E242QA, G477K and G478K and/or deletion in position R179 and/or S180 or of I181 and/or G182. Most preferred amylase variants of SEQ ID NO: 1 are those having the substitutions:



N21D+D97N+V128I

wherein the variants optionally further comprises a substitution at position 200 and/or a deletion at position 180 and/or position 181.

Other suitable amylases are the alpha-amylase having SEQ ID NO: 12 in WO01/66712 or a variant having at least 90% sequence identity to SEQ ID NO: 12. Preferred amylase variants are those having a substitution, a deletion or an insertion in one or more of the following positions of SEQ ID NO: 12 in WO01/66712: R28, R118, N174; R181, G182, D183, G184, G186, W189, N195, M202, Y298, N299, K302, S303, N306, R310, N314; R320, H324, E345, Y396, R400, W439, R444, N445, K446, Q449, R458, N471, N484. Particular preferred amylases include variants having a deletion of D183 and G184 and having the substitutions R118K, N195F, R320K and R458K, and a variant additionally having substitutions in one or more position selected from the group: M9, G149, G182, G186, M202, T257, Y295, N299, M323, E345 and A339, most preferred a variant that additionally has substitutions in all these positions.

Other examples are amylase variants such as those described in WO2011/098531, WO2013/001078 and WO2013/001087.

Commercially available amylases are Duramyl™, Termamyl™, Fungamyl™, Stainzyme™, Stainzyme Plus™, Natalase™, Liquozyme X and BAN™ (from Novozymes A/S), and Rapidase™, Purastar™/Effectenz™, Powerase, Preferenz S1000, Preferenz S100 and Preferenz S110 (from Genencor International Inc./DuPont).

### **Peroxidases/Oxidases**

A peroxidase according to the invention is a peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, as set out by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB), or any fragment derived therefrom, exhibiting peroxidase activity.

Suitable peroxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinopsis*, e.g., from *C. cinerea* (EP 179,486), and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257.

A peroxidase according to the invention also include a haloperoxidase enzyme, such as chloroperoxidase, bromoperoxidase and compounds exhibiting chloroperoxidase or bromoperoxidase activity. Haloperoxidases are classified according to their specificity for halide ions. Chloroperoxidases (E.C. 1.11.1.10) catalyze formation of hypochlorite from chloride ions.

In an embodiment, the haloperoxidase of the invention is a chloroperoxidase. Preferably, the haloperoxidase is a vanadium haloperoxidase, i.e., a vanadate-containing haloperoxidase. In a preferred method of the present invention the vanadate-containing haloperoxidase is combined with a source of chloride ion.

Haloperoxidases have been isolated from many different fungi, in particular from the fungus group dematiaceous hyphomycetes, such as *Caldariomyces*, e.g., *C. fumago*, *Alternaria*, *Curvularia*, e.g., *C. verruculosa* and *C. inaequalis*, *Drechslera*, *Ulocladium* and *Botrytis*.

Haloperoxidases have also been isolated from bacteria such as *Pseudomonas*, e.g., *P. pyrocinia* and *Streptomyces*, e.g., *S. aureofaciens*.

In an preferred embodiment, the haloperoxidase is derivable from *Curvularia* sp., in particular *Curvularia verruculosa* or *Curvularia inaequalis*, such as *C. inaequalis* CBS 102.42 as described in WO 95/27046; or *C. verruculosa* CBS 147.63 or *C. verruculosa* CBS 444.70 as described in WO 97/04102; or from *Drechslera hartlebii* as described in WO 01/79459, *Dendryphiella salina* as described in WO 01/79458, *Phaeotrichoconis crotalarie* as described in WO 01/79461, or *Geniculosporium* sp. as described in WO 01/79460.

An oxidase according to the invention include, in particular, any laccase enzyme comprised by the enzyme classification EC 1.10.3.2, or any fragment derived therefrom exhibiting laccase activity, or a compound exhibiting a similar activity, such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5).

Preferred laccase enzymes are enzymes of microbial origin. The enzymes may be derived from plants, bacteria or fungi (including filamentous fungi and yeasts).

Suitable examples from fungi include a laccase derivable from a strain of *Aspergillus*, *Neurospora*, e.g., *N. crassa*, *Podospora*, *Botrytis*, *Collybia*, *Fomes*, *Lentinus*, *Pleurotus*, *Trametes*, e.g., *T. villosa* and *T. versicolor*, *Rhizoctonia*, e.g., *R. solani*, *Coprinopsis*, e.g., *C. cinerea*, *C. comatus*, *C. friesii*, and *C. plicatilis*, *Psathyrella*, e.g., *P. condelleana*, *Panaeolus*, e.g., *P. papilionaceus*, *Myceliophthora*, e.g., *M. thermophila*, *Schytalidium*, e.g., *S. thermophilum*, *Polyporus*, e.g., *P. pinsitus*, *Phlebia*, e.g., *P. radiata* (WO 92/01046), or *Coriolus*, e.g., *C. hirsutus* (JP 2238885).

Suitable examples from bacteria include a laccase derivable from a strain of *Bacillus*.

A laccase derived from *Coprinopsis* or *Myceliophthora* is preferred; in particular a laccase derived from *Coprinopsis cinerea*, as disclosed in WO 97/08325; or from *Myceliophthora thermophila*, as disclosed in WO 95/33836.

The detergent enzyme(s) may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of these enzymes. A detergent additive of the invention, i.e., a separate additive or a combined additive, can be formulated, for example, as a granulate, liquid, slurry, etc. Preferred detergent additive formulations are granulates, in particular non-dusting granulates, liquids, in particular stabilized liquids, or slurries.

Non-dusting granulates may be produced, e.g. as disclosed in US 4,106,991 and 4,661,452 and may optionally be coated by methods known in the art. Examples of waxy coating materials are polyethyleneglycol (PEG) with mean molar weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application

by fluid bed techniques are given in GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

### **Adjunct materials**

Any detergent components known in the art for use in detergents may also be utilized. Other optional detergent components include anti-corrosion agents, anti-shrink agents, anti-soil redeposition agents, anti-wrinkling agents, bactericides, binders, corrosion inhibitors, disintegrants/disintegration agents, dyes, enzyme stabilizers (including boric acid, borates, CMC, and/or polyols such as propylene glycol), fabric conditioners including clays, fillers/processing aids, fluorescent whitening agents/optical brighteners, foam boosters, foam (suds) regulators, perfumes, soil-suspending agents, softeners, suds suppressors, tarnish inhibitors, and wicking agents, either alone or in combination. Any ingredient known in the art for use in detergents may be utilized. The choice of such ingredients is well within the skill of the artisan.

### **Dispersants**

The detergent compositions of the present invention can also contain dispersants. In particular powdered detergents may comprise dispersants. Suitable water-soluble organic materials include the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms. Suitable dispersants are for example described in Powdered Detergents, Surfactant science series volume 71, Marcel Dekker, Inc.

### **Dye transfer inhibiting agents**

The detergent compositions of the present invention may also include one or more dye transfer inhibiting agents. Suitable polymeric dye transfer inhibiting agents include, but are not limited to, polyvinylpyrrolidone polymers, polyamine *N*-oxide polymers, copolymers of *N*-vinylpyrrolidone and *N*-vinylimidazole, polyvinylloxazolidones and polyvinylimidazoles or mixtures thereof. When present in a subject composition, the dye transfer inhibiting agents may be present at levels from about 0.0001 % to about 10%, from about 0.01% to about 5% or even from about 0.1% to about 3% by weight of the composition.

### **Fluorescent whitening agent**

The detergent compositions of the present invention will preferably also contain additional components that may tint articles being cleaned, such as fluorescent whitening agent or optical brighteners. Where present the brightener is preferably at a level of about 0.01% to about 0.5%. Any fluorescent whitening agent suitable for use in a laundry detergent composition may be used in the composition of the present invention. The most commonly used fluorescent whitening agents are

those belonging to the classes of diaminostilbene-sulfonic acid derivatives, diarylpyrazoline derivatives and bisphenyl-distyryl derivatives. Examples of the diaminostilbene-sulfonic acid derivative type of fluorescent whitening agents include the sodium salts of: 4,4'-bis-(2-diethanolamino-4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(2,4-dianilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(2-anilino-4-(*N*-methyl-*N*-2-hydroxy-ethylamino)-s-triazin-6-ylamino) stilbene-2,2'-disulfonate, 4,4'-bis-(4-phenyl-1,2,3-triazol-2-yl)stilbene-2,2'-disulfonate and sodium 5-(2*H*-naphtho[1,2-*d*][1,2,3]triazol-2-yl)-2-[(*E*)-2-phenylvinyl]benzenesulfonate. Preferred fluorescent whitening agents are Tinopal DMS and Tinopal CBS available from Ciba-Geigy AG, Basel, Switzerland. Tinopal DMS is the disodium salt of 4,4'-bis-(2-morpholino-4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulfonate. Tinopal CBS is the disodium salt of 2,2'-bis-(phenyl-styryl)-disulfonate. Also preferred are fluorescent whitening agents is the commercially available Parawhite KX, supplied by Paramount Minerals and Chemicals, Mumbai, India. Other fluorescers suitable for use in the invention include the 1-3-diaryl pyrazolines and the 7-alkylaminocoumarins.

Suitable fluorescent brightener levels include lower levels of from about 0.01, from 0.05, from about 0.1 or even from about 0.2 wt % to upper levels of 0.5 or even 0.75 wt%.

#### **Soil release polymers**

The detergent compositions of the present invention may also include one or more soil release polymers which aid the removal of soils from fabrics such as cotton and polyester based fabrics, in particular the removal of hydrophobic soils from polyester based fabrics. The soil release polymers may for example be nonionic or anionic terephthalate based polymers, polyvinyl caprolactam and related copolymers, vinyl graft copolymers, polyester polyamides see for example Chapter 7 in Powdered Detergents, Surfactant science series volume 71, Marcel Dekker, Inc. Another type of soil release polymers are amphiphilic alkoxyated grease cleaning polymers comprising a core structure and a plurality of alkoxyate groups attached to that core structure. The core structure may comprise a polyalkylenimine structure or a polyalkanolamine structure as described in detail in WO 2009/087523 (hereby incorporated by reference). Furthermore random graft co-polymers are suitable soil release polymers. Suitable graft co-polymers are described in more detail in WO 2007/138054, WO 2006/108856 and WO 2006/113314 (hereby incorporated by reference). Other soil release polymers are substituted polysaccharide structures especially substituted cellulosic structures such as modified cellulose derivatives such as those described in EP 1867808 or WO 2003/040279 (both are hereby incorporated by reference). Suitable cellulosic polymers include cellulose, cellulose ethers, cellulose esters, cellulose amides and mixtures thereof. Suitable cellulosic polymers include anionically modified cellulose, nonionically modified cellulose, cationically modified cellulose, zwitterionically modified cellulose, and mixtures thereof. Suitable cellulosic polymers include methyl cellulose, carboxy methyl cellulose, ethyl cellulose, hydroxyl ethyl cellulose, hydroxyl propyl methyl cellulose, ester carboxy methyl cellulose, and mixtures thereof.

**Anti-redeposition agents**

The detergent compositions of the present invention may also include one or more anti-redeposition agents such as carboxymethylcellulose (CMC), polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), polyoxyethylene and/or polyethyleneglycol (PEG), homopolymers of acrylic acid, copolymers of acrylic acid and maleic acid, and ethoxylated polyethyleneimines. The cellulose based polymers described under soil release polymers above may also function as anti-redeposition agents.

**Rheology Modifiers**

The detergent compositions of the present invention may also include one or more rheology modifiers, structurants or thickeners, as distinct from viscosity reducing agents. The rheology modifiers are selected from the group consisting of non-polymeric crystalline, hydroxy-functional materials, polymeric rheology modifiers which impart shear thinning characteristics to the aqueous liquid matrix of a liquid detergent composition. The rheology and viscosity of the detergent can be modified and adjusted by methods known in the art, for example as shown in EP 2169040.

**Formulation of detergent products**

The detergent composition of the invention may be in any convenient form, e.g., a bar, a homogenous tablet, a tablet having two or more layers, a pouch having one or more compartments, a regular or compact powder, a granule, a paste, a gel, or a regular, compact or concentrated liquid.

Pouches can be configured as single or multicompartments. It can be of any form, shape and material which is suitable for hold the composition, e.g. without allowing the release of the composition to release of the composition from the pouch prior to water contact. The pouch is made from water soluble film which encloses an inner volume. Said inner volume can be divided into compartments of the pouch. Preferred films are polymeric materials preferably polymers which are formed into a film or sheet. Preferred polymers, copolymers or derivatives thereof are selected polyacrylates, and water soluble acrylate copolymers, methyl cellulose, carboxy methyl cellulose, sodium dextrin, ethyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose, malto dextrin, poly methacrylates, most preferably polyvinyl alcohol copolymers and, hydroxypropyl methyl cellulose (HPMC). Preferably the level of polymer in the film for example PVA is at least about 60%. Preferred average molecular weight will typically be about 20,000 to about 150,000. Films can also be of blended compositions comprising hydrolytically degradable and water soluble polymer blends such as polylactide and polyvinyl alcohol (known under the Trade reference M8630 as sold by MonoSol LLC, Indiana, USA) plus plasticisers like glycerol, ethylene glycerol, propylene glycol, sorbitol and mixtures thereof. The pouches can comprise a solid laundry cleaning composition or part components and/or a liquid cleaning composition or part components separated by the water soluble film. The compartment for liquid components can be different in composition than compartments containing solids: US2009/0011970 A1.

Detergent ingredients can be separated physically from each other by compartments in water dissolvable pouches or in different layers of tablets. Thereby negative storage interaction between components can be avoided. Different dissolution profiles of each of the compartments can also give rise to delayed dissolution of selected components in the wash solution.

A liquid or gel detergent, which is not unit dosed, may be aqueous, typically containing at least 20% by weight and up to 95% water, such as up to about 70% water, up to about 65% water, up to about 55% water, up to about 45% water, up to about 35% water. Other types of liquids, including without limitation, alkanols, amines, diols, ethers and polyols may be included in an aqueous liquid or gel. An aqueous liquid or gel detergent may contain from 0-30% organic solvent.

A liquid or gel detergent may be non-aqueous.

### **Laundry soap bars**

The enzymes of the invention may be added to laundry soap bars and used for hand washing laundry, fabrics and/or textiles. The term laundry soap bar includes laundry bars, soap bars, combo bars, syndet bars and detergent bars. The types of bar usually differ in the type of surfactant they contain, and the term laundry soap bar includes those containing soaps from fatty acids and/or synthetic soaps. The laundry soap bar has a physical form which is solid and not a liquid, gel or a powder at room temperature. The term solid is defined as a physical form which does not significantly change over time, i.e. if a solid object (e.g. laundry soap bar) is placed inside a container, the solid object does not change to fill the container it is placed in. The bar is a solid typically in bar form but can be in other solid shapes such as round or oval.

The laundry soap bar may contain one or more additional enzymes, protease inhibitors such as peptide aldehydes (or hydrosulfite adduct or hemiacetal adduct), boric acid, borate, borax and/or phenylboronic acid derivatives such as 4-formylphenylboronic acid, one or more soaps or synthetic surfactants, polyols such as glycerine, pH controlling compounds such as fatty acids, citric acid, acetic acid and/or formic acid, and/or a salt of a monovalent cation and an organic anion wherein the monovalent cation may be for example  $\text{Na}^+$ ,  $\text{K}^+$  or  $\text{NH}_4^+$  and the organic anion may be for example formate, acetate, citrate or lactate such that the salt of a monovalent cation and an organic anion may be, for example, sodium formate.

The laundry soap bar may also contain complexing agents like EDTA and HEDP, perfumes and/or different type of fillers, surfactants e.g. anionic synthetic surfactants, builders, polymeric soil release agents, detergent chelators, stabilizing agents, fillers, dyes, colorants, dye transfer inhibitors, alkoxyated polycarbonates, suds suppressers, structurants, binders, leaching agents, bleaching activators, clay soil removal agents, anti-redeposition agents, polymeric dispersing agents, brighteners, fabric softeners, perfumes and/or other compounds known in the art.

The laundry soap bar may be processed in conventional laundry soap bar making equipment such as but not limited to: mixers, plodders, e.g. a two stage vacuum plodder, extruders, cutters, logo-stampers, cooling tunnels and wrappers. The invention is not limited to preparing the laundry soap bars by any single method. The premix of the invention may be added to the soap at different stages of the

process. For example, the premix containing a soap, the enzyme of the invention, optionally one or more additional enzymes, a protease inhibitor, and a salt of a monovalent cation and an organic anion may be prepared and the mixture is then plodded. The enzyme of the invention and optional additional enzymes may be added at the same time as the protease inhibitor for example in liquid form. Besides the mixing step and the plodding step, the process may further comprise the steps of milling, extruding, cutting, stamping, cooling and/or wrapping.

#### **Formulation of enzyme in co-granule**

The enzyme comprised in the detergent compositions of the invention may be formulated as a granule for example as a co-granule that combines one or more enzymes. Each enzyme will then be present in more granules securing a more uniform distribution of enzymes in the detergent. This also reduces the physical segregation of different enzymes due to different particle sizes. Methods for producing multi-enzyme co-granulates for the detergent industry are disclosed in the IP.com disclosure IPCOM000200739D.

Another example of formulation of enzymes by the use of co-granulates are disclosed in WO 2013/188331, which relates to a detergent composition comprising (a) a multi-enzyme co-granule; (b) less than 10 wt zeolite (anhydrous basis); and (c) less than 10 wt phosphate salt (anhydrous basis), wherein said enzyme co-granule comprises from 10 to 98 wt% moisture sink component and the composition additionally comprises from 20 to 80 wt% detergent moisture sink component. WO 2013/188331 also relates to a method of treating and/or cleaning a surface, preferably a fabric surface comprising the steps of (i) contacting said surface with the detergent composition as claimed and described herein in an aqueous wash liquor, (ii) rinsing and/or drying the surface.

The multi-enzyme co-granule may comprise an enzyme of the invention and (a) one or more enzymes selected from the group consisting of first-wash lipases, cleaning cellulases, xyloglucanases, perhydrolases, peroxidases, lipoxygenases, laccases and mixtures thereof; and (b) one or more enzymes selected from the group consisting of hemicellulases, proteases, care cellulases, cellobiose dehydrogenases, xylanases, phospholipases, esterases, cutinases, pectinases, mannanases, pectate lyases, keratinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, tannases, pentosanases, lichenases glucanases, arabinosidases, hyaluronidase, chondroitinase, amylases, and mixtures thereof.

#### **Use in degrading xanthan gum**

Xanthan gum is used as an ingredient in many consumer products including foods and cosmetics as well as in the oil and drilling industry. Therefore, enzymes having xanthan degrading activity can be applied in improved cleaning processes, such as the easier removal of stains containing xanthan gum, as well as the degradation of xanthan gum, which is often used in the oil and drilling industry. Thus, the present invention is directed to the use of the detergent composition of the invention to degrade xanthan gum. The detergent composition of present invention can also comprise a combination of an enzyme as described herein and a xanthan lyase. The use of such a

detergent composition to degrade xanthan gum is also envisaged.

Degradation of xanthan gum may be measured using the viscosity reduction assay as described herein on xanthan gum. Xanthan degrading activity may alternatively be measured as reducing ends on xanthan gum using the colorimetric assay developed by Lever (1972), *Anal. Biochem.* 47: 273-279, 1972.

### **Use in detergents**

The present invention is directed to the use of the detergent compositions of the invention in cleaning processes such as the laundering of textiles and fabrics (e.g. household laundry washing and industrial laundry washing), as well as household and industrial hard surface cleaning, such as dish wash.

An embodiment is the use of a detergent composition comprising a combination of the enzymes as described herein together with xanthan lyases in cleaning processes such as the laundering of textiles and fabrics (e.g. household laundry washing and industrial laundry washing), as well as household and industrial hard surface cleaning, such as dish wash.

The invention also relates to methods for degrading xanthan gum on the surface of a textile or hard surface, such as dish wash, comprising applying a detergent composition comprising one or more enzymes as described herein to xanthan gum. The invention further relates to methods for degrading xanthan gum on the surface of a textile or hard surface, such as dish wash, comprising applying a detergent composition comprising one or more xanthan lyases to xanthan gum. An embodiment is a method for degrading xanthan gum on the surface of a textile or hard surface, such as dish wash, comprising applying a detergent composition comprising one or more enzymes as described herein together with one or more xanthan lyase to xanthan gum. An embodiment is the detergent composition comprising one or more detergent components as described above.

The present invention is further described by the following examples that should not be construed as limiting the scope of the invention.

## **Examples**

### **Activity assays**

#### **Xanthan lyase activity assay**

0.8 mL 100 mM HEPES buffer, pH 6.0 was mixed with 0.2 mL Xanthan gum (5 mg/mL) dissolved in water in a 1 mL 1 cm cuvette. The cuvette was inserted into a spectrophotometer (Agilent G1103A 8453A, CA, USA) with temperature control set at 40 °C. The solution was pre-incubated for 10 min and 0.1 mL sample was added and the solution was mixed by aspirating and dispensing the solution for at least 5 times using a pipette. Total reaction volume was 1.1 mL. Absorbance at 235



nm was collected for 10 min using a 30 sec measuring interval. Initial activity was calculated by using the software (UV-Visible Chemstation Rev A.10.01 [81], Agilent).

#### **Example 1: Strain and DNA**

The DNA in SEQ ID NO: 1 encoding the GH5 polypeptide EXa of SEQ ID NO: 2 was obtained from an *Opitutaceae* species isolated from an environmental soil sample collected in Denmark.

The DNA SEQ ID NO: 3 encoding the GH5 polypeptide EXb of SEQ ID NO: 4 was isolated from an environmental sample collected in Denmark.

The DNA SEQ ID NO: 5 encoding the GH5 polypeptide EXc of SEQ ID NO: 5 was isolated from an environmental sample collected in Denmark.

The DNA SEQ ID NO: 7 encoding the GH5 polypeptide EXd of SEQ ID NO: 8 was obtained from the public database (UNIPROT M2V1S3) but originates from a strain of *Pseudomonas stutzeri* collected from a Galapagos Rift hydrothermal vent, Ecuador.

Codon optimized synthetic DNA encoding the mature peptide sequences of the four polypeptides were prepared (SEQ ID NO: 9; SEQ ID NO: 10, SEQ ID NO: 11; SEQ ID NO: 12).

#### **Example 2: Cloning and expression of GH5 polypeptides**

The GH5 encoding genes were either cloned by conventional techniques from the strains indicated above or from the synthetic DNA and inserted into a suitable plasmid as described below.

##### **Example 2a: Cloning and expression of GH5 polypeptides in *E.coli***

The mature peptide encoding part of the GH5 endo-glucanase genes, SEQ ID NO: 1, 3, 5 and 7 was inserted with an N-terminal poly histidine tag with an extra proline and arginine (HHHHHHP) (SEQ ID NO: 19) after the methionine in the *E.coli* pET-32a(+) vector from Novagen with standard recombinant techniques. The expression plasmid containing the insert was purified from an *E.coli* transformant harboring the plasmid and transformed into *E.coli* Xjb (DE3) host cells (from Zymo Research). A fresh clone of *E.coli* Xjb (DE3) containing the pET32-GH5 vector, was grown overnight in Terrific Broth containing 100 ug/ml ampicillin. Next day, a fresh 500 ml culture was inoculated with 1 ml overnight culture and cells were cultured (37 °C, 250 rpm) to an optical density (OD600) between 6-8. Protein expression was induced by 1 mM isopropylthio-D-galactosidase (IPTG) and 6 mM arabinose for 4.5 hours at 20 °C. After continued culture, cells were harvested by centrifugation and lysed by Bugbuster® (Novagen). The soluble fraction was used for polyhistidine tag purification of the GH5 polypeptides SEQ ID NO: 13, 14 and 15 as described in example 4.

##### **Example 2b: Cloning and expression of GH5 polypeptides in *Bacillus subtilis***

The synthetic codon optimized genes SEQ ID NO: 10, 11 and 12 were cloned into the *Bacillus* expression vector described in WO 2012/025577. The genes were expressed by replacing the native secretion signal sequence with the *Bacillus clausii* secretion signal MKKPLGKIVASTALLISVAFSSSIASA (SEQ ID NO: 20) with an extra affinity tag sequence (HHHHHHP) (SEQ ID NO: 19) at the C-terminal of the signal peptide, to facilitate the purification process. This resulted in a recombinant mature polypeptide with a His tag at the front of the N-terminal of the mature wild type sequence (SEQ ID NO: 16, 17 and 18).

One clone with the correct recombinant gene sequence was selected and the corresponding plasmid was integrated by homologous recombination into the *Bacillus subtilis* host cell genome (pectate lyase locus) and the gene construct was expressed under the control of a triple promoter system as described in WO99/43835. The gene coding for chloramphenicol acetyltransferase was used as a marker (as described in Diderichsen *et al.*, 1993, Plasmid 30:312-315).

Chloramphenicol resistant transformants were analyzed by PCR to verify the correct size of the amplified fragment. A recombinant *B. subtilis* clone containing the integrated expression construct was selected and cultivated on a rotary shaking table in 500 mL baffled Erlenmeyer flasks each containing 100 ml yeast extract-based media. The clone was cultivated for 5 days at 30°C. The enzyme containing supernatants were harvested and the enzyme purified as described in Example 5.

### **Example 3: Purification of wild type GH5 polypeptide from the natural *Opitutaceae* strain**

The *Opitutaceae* strain was cultivated on a rotary shaking table in 500 mL baffled Erlenmeyer flasks each containing 100 ml mineral solution with 0.5% xanthan gum. The strain was cultivated for 20 days at 30°C. A total of 2.0 L supernatant was harvested by centrifugation and was filtered using a 0.2 µm bottle top filter (Nalgene Nunc). The broth was concentrated to 300 mL using ultra-filtration (Sartorius) with 30 kDa cut-off. Equal volume of 3.2 M ammonium sulphate in 40 mM Tris-HCl, pH 7.9 was slowly added with continuous stirring. The sample was filtered using Whatman glass filters (1.7 µm – 0.7 µm) to remove larger particles. The sample was applied on a 20 mL Phenyl-sepharose high performance column (GE Healthcare) pre-equilibrated with 1.6 M ammonium sulphate in 20 mM Tris-HCl, pH 7.9 (equilibration buffer). Unbound protein was eluted by two column volumes of equilibration buffer. Elution was done by a 12 column volume linear gradient from 1.6 M to 0.0 M ammonium sulphate in 20 mM Tris-HCl, pH 7.9. A last elution step of 4 column volume with equilibration buffer was used to elute tightly bound protein. The absorbance at 280 nm was recorded during the entire purification. Protein containing fractions identified by the absorbance at 280 nm in the chromatogram were analyzed by SDS-PAGE (NuPAGE, Invitrogen). Fractions judged as pure were pooled. The sample was concentration from 30 to 4 mL using Macrosep ultra filtration device

with 3 kDa cut-off (Pall). The protein concentration was determined by measuring the absorbance at 280 nm using the calculated extinction coefficient where 1 mg/mL equaled 1.89 absorbance units.

**Example 4: Purification of recombinant GH5 polypeptide produced in *E.coli***

200 mL lysed cells (grown as example 2a) were filtered through Fast PES 0.2 µm bottle-top filters to remove debris and unbroken cells. 200 mL of equilibration buffer (20 mM Tris-HCl, pH 7.5 + 500 mM NaCl) was added to the crude protein solution. A 20 mL HisPrep column loaded with Ni<sup>2+</sup> was equilibrated with equilibration buffer until a stable UV baseline was obtained. The absorbance at 280 nm was continuously monitored throughout the purification. Crude protein was loaded on the column using a flow rate of 4 mL/min. Unbound protein was removed by washing the column with equilibration buffer until a stable UV baseline was obtained. Elution was carried out by a two-step linear gradient using 20 mM Tris-HCl, pH 7.5 + 500 mM NaCl + 500 mM Imidazole (elution buffer). First elution gradient was 10 column volumes 0 to 40 % elution buffer followed by 4 column volumes from 40% to 100 %. Peaks absorbing at 280 nm were analyzed by SDS-PAGE (NuPAGE, Invitrogen). Fractions containing protein with the correct apparent molecular weight were pooled. The pool was desalted and buffer exchanged using a Sephadex G-25 super fine desalting column equilibrated with 20 mM Tris-HCl, pH 8.0. The pool was applied on a 20 mL Source15Q column pre-equilibrated with 20 mM Tris-HCl, pH 8.0. Unbound protein was washed out using 20 mM Tris-HCl, pH 8.0 until a stable UV baseline was obtained. Elution was done by a 10 column volume linear NaCl gradient from 0 to 500 mM NaCl in 20 mM Tris-HCl, pH 8.0. Protein containing fractions were analyzed by SDS-PAGE and fractions judged as pure were pooled. Protein concentration was measured using absorbance at 280 nm using a calculated extinction coefficient where 1 mg/mL corresponded to 1.86 absorbance units.

**Example 5: Purification of recombinant GH5 polypeptide produced in *B. subtilis***

All His-tagged enzymes were purified by immobilized metal chromatography (IMAC) using Ni<sup>2+</sup> as the metal ion on 5 mL HisTrap Excel columns (GE Healthcare Life Sciences). The purification was done at pH 8 and the bound proteins were eluted with imidazole. The purity of the purified enzymes was checked by SDS-PAGE and the concentration of each enzyme determined by Abs 280 nm after a buffer exchange.

**Example 6: Xanthan degrading activity of GH5 polypeptide and xanthan lyase on xanthan gum by measurement of viscosity reduction**

The viscosity reduction measurements were performed using the viscosity pressure assay described in WO2011/107472 and following the method described in WO2013167581. Results presented are the average of three measurements and are shown in table 1 and 2 below.

A sample size of was 400  $\mu$ L was used. The hydrolysis conditions were as follows: 30 °C, either 0.25% or 0.5% xanthan gum (XG) in 50 mM MES buffer + 0.01% triton x-100 pH 7.0 or 100mM CHES buffer + 0.01% triton x-100 pH10. Enzyme was added upon thermal equilibration. Prior to use all enzymes were buffer changed to the MES buffer using NAP 5 columns (GE Healthcare).

The purified enzyme preparations of Example 5 were used for the analysis at a concentration of 31.25 mg/L.

	T= 0 minutes	T= 30 minutes	T= 1 hour	T= 2 hours	T= 3 hours	T= 4 hours
Water (control)	430 $\pm$ 44	504 $\pm$ 50	470 $\pm$ 75	483 $\pm$ 86	466 $\pm$ 60	504 $\pm$ 82
Xanthan gum (control)	1703 $\pm$ 132	1738 $\pm$ 26	1837 $\pm$ 122	1803 $\pm$ 64	1739 $\pm$ 84	1757 $\pm$ 21
Xanthan gum + EXa SEQ ID NO:13	1586 $\pm$ 101	1154 $\pm$ 38	1270 $\pm$ 67	1230 $\pm$ 36	1156 $\pm$ 49	1184 $\pm$ 44
Xanthan gum + XLa SEQ ID NO:21	1963 $\pm$ 93	1884 $\pm$ 67	1890 $\pm$ 84	1840 $\pm$ 131	1886 $\pm$ 50	1950 $\pm$ 25
Xanthan gum + EXa SEQ ID NO:13 + XLa SEQ ID NO:21	1370 $\pm$ 197	861 $\pm$ 23	973 $\pm$ 59	840 $\pm$ 62	916 $\pm$ 47	904 $\pm$ 79

The results presented above show that the GH5 polypeptide alone and in combination with xanthan lyase can degrade the xanthan gum present in the media at pH 7, thus leading to viscosity reduction. A synergistic effect is obtained with combination of GH5 and xanthan lyase.

	T=0	T=0.5 hours	T=1 hours	T=2 hours	T=3,5 hours
Water	370 $\pm$ 10	454 $\pm$ 15	519 $\pm$ 60	411 $\pm$ 29	554 $\pm$ 180
Xanthan gum (XG) control	1740 $\pm$ 151	1734 $\pm$ 21	1819 $\pm$ 67	1795 $\pm$ 29	1898 $\pm$ 75
XG + EXa SEQ ID NO:13	1676 $\pm$ 50	1324 $\pm$ 58	1223 $\pm$ 12	1251 $\pm$ 31	1318 $\pm$ 62
XG + XLa SEQ ID NO:23	2046 $\pm$ 112	1811 $\pm$ 82	1773 $\pm$ 64	1781 $\pm$ 92	1704 $\pm$ 67
XG + EXa SEQ ID NO:13 + XLa SEQ ID NO:23	1573 $\pm$ 227	1057 $\pm$ 21	1153 $\pm$ 12	1161 $\pm$ 40	1188 $\pm$ 89

The results presented above show that the GH5 polypeptide in alone or combination with xanthan lyase can degrade the xanthan gum present in the media at pH 10, thus leading to viscosity reduction.

	T=0	T= 0.5 hours	T= 1 hours	T= 2 hours	T= 3 hours
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Water	441±25	421±40	646±44	535±59	599±74	492±15	494±32
Xanthan gum(XG)	2027±2 3	1707±3 5	1949±5 9	1785±11 6	1746±7 5	1726±1 0	1867±6
XG + EXa SEQ ID NO:13	2054±4 4	1514±1 7	1299±2 1	1112±57	1089±4 5	1046±0	1027±6
XG + EXb SEQ ID NO:16	2067±1 5	1527±8 1	1393±1 2	1229±53	1159±1 2	1136±0	1134±6
XG + EXc SEQ ID NO:17	2061±3 1	1501±5 5	1416±4 4	1175±6	1183±7 8	1169±4 0	1147±1 5
XG + EXa SEQ ID NO:13 + XLb SEQ ID NO:22	2061±6	1274±1 7	1063±4 7	812±59	769±46	729±15	671±26
XG + EXb SEQ ID NO:20 + XLb SEQ ID NO:22	2074±2 6	1411±6 5	1079±1 5	945±92	809±12	796±10	781±10
XG + EXc SEQ ID NO:17 + XLb SEQ ID NO:22	2094±3 0	1491±2 5	1166±0	959±46	889±40	846±0	847±57
XG + XLb SEQ ID NO:22	2097±4 9	1794±6 2	1863±2 3	1685±15	1653±1 0	1679±6	1667±2 9
XG + EXa SEQ ID NO:13 + XLa SEQ ID NO:21	2131±1 5	1227±8 1	1143±8 1	789±62	739±25	716±44	677±55
XG + EXb SEQ ID NO:16 + XLa SEQ ID NO:21	2104±7 9	1324±1 7	1096±4 4	795±31	803±26	792±21	767±12
XG + EXc SEQ ID NO:17 + XLa SEQ ID NO:21	2107±1 2	1241±5 0	1163±3 2	802±15	826±15	846±0	894±15
XG + XLa SEQ ID NO:21	2134±2 0	1741±5 7	1933±2 9	1639±30	1659±2 3	1666±1 7	1637±1 2

The results presented above show that the GH5 polypeptides EXa, EXb and EXc alone and in combination with xanthan lyase can degrade the xanthan gum present in the media at pH 7, thus leading to viscosity reduction. A synergistic effect is obtained with combination of GH5 polypeptide and xanthan lyase.

Table 6: Viscosity measurements (Pa) of EXa, EXb, EXc recombinantly expressed in E.coli (SEQ ID NO:13; SEQ ID NO: 14 or SEQ ID NO: 15 ) and/or Xanthan Lyase (XLc, SEQ ID NO: 23 or SEQ ID NO:24) on 0.5% xanthan gum at pH 10. T=00 is before addition of enzyme and T=0 is right after.

	T=00	T=0	T=30'	T=1hr	T=2hrs	T=3hrs
Water	429±66	502±110	504±50	434±29	478±42	479±26
Xanthan gum (XG)	1932±31	1485±81	1678±12	1641±70	1642±38	1592±92
XG + EXa SEQ ID NO:13	1992±13 8	1332±6	1254±21	1147±51	1192±35	1215±31
XG + EXb SEQ ID NO:14	1989±85	1415±50	1351±66	1321±17	1358±51	1252±21
XG + EXc SEQ ID NO:17	1892±45	1442±10 0	1408±21	1341±50	1332±31	1262±51
XG + EXa SEQ ID NO:13 +XLc SEQ ID NO:23	1899±69	1429±62	1084±76	1131±17	1092±25	1112±40

XG + EXb SEQ ID NO: 14 +XLc SEQ ID NO: 23	2019±62	1465±13 2	1144±23	1121±53	1108±81	1012±59
XG + EXc SEQ ID NO: 15 +XLc SEQ ID NO:23	2085±80	1602±38	1344±15	1321±10	1262±55	1319±10
XG + XLc SEQ ID NO:23	2005±47	1702±75	1588±6	1524±67	1588±60	1569±36
XG + EXa SEQ ID NO:13 +XLd SEQ ID NO:24	1959±72	1462±11 0	1158±38	1144±40	1148±72	1005±45
XG + EXb SEQ ID NO:14 +XLd SEQ ID NO:24	1975±25	1442±35	1211±26	1177±15	1192±72	1182±67
XG + EXc SEQ ID NO: 15 +XLd SEQ ID NO:24	1925±13 3	1422±95	1238±12	1274±58	1208±81	1215±67
XG + XLd SEQ ID NO:24	1839±40	1525±61	1488±21	1447±42	1432±15	1425±76

The results presented above show that the GH5 polypeptides GH5, EXb and EXc in combination with xanthan lyase can degrade the xanthan gum present in the media at pH 10, thus leading to viscosity reduction.

Table 7: Viscosity measurements (Pa) of GH5 polypeptide purified from supernatant of the *Opiritaceae* sp strain and/or Xanthan Lyase (XLa, SEQ ID NO: 21) on 0.25% xanthan gum at pH7

	T=0	T=0.5 hour	T= 1 hour	T= 2 hours	T= 3 hours
Water	471±99	390±46	423±61	433±64	438±36
Xanthan gum (XG)	898±12	880±40	900±17	820±40	908±50
XG + EXa SEQ ID NO:1	856±34	743±46	723±34	672±38	644±55
XG + XLa SEQ ID NO:21	908±29	865±22	860±35	857±32	856±61
XG + EXa SEQ ID NO:1 + XLa SEQ ID NO:21	800±28	597±30	612±31	577±45	648±89

#### **Example 8: Xanthan degrading activity of GH5 polypeptide and xanthan lyase on xanthan gum by measurement of viscosity reduction**

The viscosity measurements were performed using the viscosity pressure assay described in WO2011/107472. 150 µL of each 1 mL hydrolysis or control was the sample size. Results presented are the average of four measurements and are shown in table 8 and 9 below.

Modified xanthan gum was prepared by an adaption of Nankai et al. 1999. "Microbial system for polysaccharide depolymerization: enzymatic route for xanthan depolymerization by *Bacillus* sp strain GL1." Applied and Environmental Microbiology 65(6): 2520-2526.

2.5 g of xanthan gum (CP Kelco) was wetted with 5 mL of 96 % ethanol in a 2 L beaker. 500 mL of 100 mM ACES buffer pH 7.00 was added and the solution stirred at ambient temperature for 2 h. 250 µL of xanthan lyase (*Bacillus* sp., Megazyme) was added and the solution incubated for 20 h at 50 °C. The sample was then cooled by placing the beaker on ice. After hydrolysis was 1400 mL of ice cold 96 % ethanol was added to the 500 mL sample, under stirring. Precipitation occurs, and after approximately 5 min the ethanol was decanted removing the pyruvated mannose residues. The sample was vacuum filtered and transferred to a glass plate. The glasses were dried at 50 °C for 20 h. The sample was collected, weighed, and grinded.

The hydrolysis conditions were as follows: 40 °C, 0.35 % xanthan gum (XG) in 50 mM HEPES buffer + 0.01 % triton X-100 pH 7.0. The modified xanthan gum powder (mXG) was prepared as described above and a 0.7 % solution was prepared using the same procedure as outlined for XG. Enzyme was added upon thermal equilibration. The initial viscosity is measured prior to enzyme addition, after thermal equilibration. Controls are the same with buffer added instead of enzyme. Buffer was monitored to determine the ultimate end point of a total hydrolysis.

Table 8. Viscosity measurements (Pa). EXc SEQ ID NO:17 and XLb (SEQ ID NO:22). Each enzyme dosed in 1.5 ppm. pH 7.0							
Time (Minutes)	0	15	30	45	60	75	90
Buffer 50 mM HEPES Control	645	610	521	502	620	632	600
Xanthan Gum + Buffer Control	2140	2075	1948	2092	2033	2077	2005
Xanthan Gum + EXc	2120	1295	991	957	935	1112	917
Xanthan Gum + EXc + Xanthan Lyase	1977	808	811	837	773	807	777
Xanthan Gum + Xanthan lyase	1972	1853	1838	1802	1750	1737	1677
Modified Xanthan Gum + Buffer Control	2262	2100	2143	2134	2118	2150	2097
Modified Xanthan Gum + EXc	2217	1225	1173	1157	1130	1155	1130

#### Example 9: Wash performance of GH5 polypeptide and xanthan lyase

The wash performance of the GH5 enzyme was assessed in laundry wash experiments using a Mini wash assay, which is a test method where soiled textile is continuously lifted up and down into the test solution and subsequently rinsed. The wash experiment was conducted under the experimental conditions specified in Table 10.

The textiles were subsequently air-dried and the wash performance was measured as the brightness of the color of the textiles. Brightness can be expressed as the Remission (R), which is a measure for the light reflected or emitted from the test material when illuminated with white light. The Remission (R) of the textiles was measured at 460 nm using a Zeiss MCS 521 VIS spectrophotometer. The measurements were done according to the manufacturer's protocol. The performance of the new enzyme (combination) was compared to the performance of detergent alone (blank). An enzyme (combination) is considered to exhibit improved wash performance, if it performs better than the detergent alone (i.e.  $R_{\text{ENZYME}} > R_{\text{BLANK}}$ ) (see Table 13 and 14).



Table 10: Experimental setup of Mini wash assay	
Detergent	Liquid Model detergent A or Model detergent T (see Table 11 and 12)
Detergent dose	3.33 g/l
pH	"as is" in the current detergent solution and was not adjusted
Water hardness	16°dH, adjusted by adding CaCl <sub>2</sub> *2H <sub>2</sub> O, MgCl <sub>2</sub> *6H <sub>2</sub> O and NaHCO <sub>3</sub> (5:1:3) to milli-Q water.
Enzymes	EXc (SEQ ID NO:17), xanthan lyase (XLb, SEQ ID NO:22 or XLc SEQ ID NO:23)
Enzyme dosage	Dosage of GH5: 0.05 mg EP/L (enzyme protein), 0.10 mg EP/L, 0.2 mg EP/L, 0.5 mg EP/L, 1.0 mg EP/L; experiments with combinations of GH5 and XL were conducted with a fixed concentration of 1.0 mg EP/L XL
Volume of test solution	50 ml
Test material	Xanthan Gum with carbon black DN-31D textile swatches (23x3 cm). The test material was obtained from Center for Testmaterials BV, P.O. Box 120, 3133 KT Vlaardingen, the Netherlands, and WFK Testgewebe GmbH, Christenfeld 10, D-41379 Brüggen, Germany
Temperature	40°C
Wash time	30 min
Rinse time	5 min
Test system	Soiled textile continuously lifted up and down into the test solutions, 50 times per minute (up-time 0.4 sec, down-time 0.4 sec, lift time 0.4 sec). The test solutions are kept in 125 ml glass beakers. After wash of the textiles are continuously lifted up and down into tap water, 50 times per minute (up-time 0.4 sec, down-time 0.4 sec, lift time 0.4 sec).

Table 11: Composition of Model Detergent A (Liquid) <sup>1)</sup>	
Detergent ingredients	Wt %
Linear alkylbenzenesulfonic acid (LAS) (Marlon AS3)	13
Sodium alkyl(C12)ether sulfate (AEOS) (STEOL CS-370 E)	10
Coco soap (Radiacid 631)	2.75
Soy soap (Edenor SJ)	2.75
Alcohol ethoxylate (AEO) (Bio-Soft N25-7)	11
Sodium hydroxide	2
Ethanol	3
Propane-1,2-diol (MPG)	6
Glycerol	2
Triethanolamine (TEA)	3
Sodium formate	1
Sodium citrate	2
Diethylenetriaminepentakis(methylenephosphonic acid) (DTMPA)	0.2
Polycarboxylate polymer (PCA) (Sokalan CP-5)	0.2
Water	Up to 100
<sup>1)</sup> The pH of the detergent was adjusted to pH 8 with sodium hydroxide or citric acid.	

Detergent ingredients	Wt %
LAS, sodium salt	11.72
AS, sodium salt	2.0
Soap, sodium salt	2.15
AEO	3.0
Soda ash	14.98
Hydrous sodium silicate	3.12
Zeolite A	18.75
HEDP-Na4	0.15
Sodium citrate	2.0
PCA, copoly(acrylic acid/maleic acid), sodium salt	1.65
SRP	0.5
Sodium sulfate	13.53
Sodium percarbonate	22.20
TAED	3.25
Foam regulator	1.0

Table 13: Remission (R) values obtained in Mini Wash using EXc with and without xanthan lyase (XLb) in liquid model A detergent

Enzyme dosage	No enzyme	EXc	EXc + xanthan lyase
0.05 mg EP/L	29.5	32.8	35.1
0.1 mg EP/L	29.5	33.6	35.4
0.2 mg EP/L	29.5	34.3	35.9
0.5 mg EP/L	29.5	35.1	36.7
1.0 mg EP/L	29.5	35.4	37.3

Table 14 **Remission (R) values obtained in Mini Wash using EXc with and without Xanthan Lyase (XLc) in powder model T detergent**

Enzyme dosage	No enzyme	EXc	EXc + xanthan lyase
0.05 mg EP/L	29.8	29.7	29.7
0.1 mg EP/L	29.8	29.8	29.8
0.2 mg EP/L	29.8	30.0	30.0
0.5 mg EP/L	29.8	30.6	30.9
1.0 mg EP/L	29.8	31.0	31.2

**Example 10: Wash performance of combinations of a GH5 polypeptide and xanthan lyase was tested on specific stains**

The wash performance of variants in liquid and powder detergents was determined by using the following standardized stains, all obtainable from CFT (Center for Testmaterials) B.V., Vlaardingen, Netherlands:

A: Fluid make-up: product no. PCS17

B: Fluid make-up: product no. CS17

For the tests in liquid detergents, a liquid washing agent with the following composition was used as base formulation (all values in weight percent): 0 to 0.5% xanthan gum, 0.2 to 0.4% antifoaming agent, 6 to 7% glycerol, 0.3 to 0.5% ethanol, 0 to 7% FAEOS (fatty alcohol ether sulfate), 10 to 28% nonionic surfactants, 0.5-1% boric acid, 1 to 2% sodium citrate (dihydrate), 2 to 4% soda, 0 to 16% coconut fatty acid, 0.5% HEDP (1-hydroxyethane-(1,1-diphosphonic acid)), 0 to 0.4% PVP (polyvinylpyrrolidone), 0 to 0.05% optical brighteners, 0 to 0.001% dye, remainder deionized water.

Based on this base formulation, detergent was prepared by adding the respective enzyme combination as indicated in table 15. As a reference, the detergent composition without addition of the enzyme combinations was used.

The dosing ratio of the liquid washing agent was 4.7 grams per liter of washing liquor and the washing procedure was performed for 60 minutes at a temperature of 40°C, the water having a water hardness between 15.5 and 16.5° (German degrees of hardness).

For the tests in solid detergents, a European premium detergent was used as base formulation.

The whiteness, i.e. the brightening of the stains, was determined photometrically as an indication of wash performance. A Minolta CM508d spectrometer device was used, which was calibrated beforehand using a white standard provided with the unit.

The results obtained are the difference values between the remission units obtained with the detergents and the remission units obtained with the detergent containing the enzyme combinations. A positive value therefore indicates an improved wash performance due to the enzyme combinations present in the detergent. It is evident from table 15 that enzyme combinations according to the invention show improved wash performance.

Table 15: Wash performance in liquid detergent

Enzyme combination		A	B
XLb SEQ ID NO:22+ EXc SEQ ID NO:17	Diff	3.3	6.4
	HSD	2.4	1.2

Table 16: Wash performance in solid detergent

Enzyme combination		B
XLb SEQ ID NO:22 + EXc SEQ ID NO:17	Diff	1.9
	HSD	1.2

### Example 11: Wash performance of GH5 polypeptides with and without Xanthan Lyase

In this example wash performance of GH5 polypeptides was evaluated in a liquid model detergent A washed in the Automatic Mechanical Stress Assay (AMSA) at 20°C or 40°C. The wash performance of the enzymes was evaluated either alone or in combination with a Xanthan Lyase. The wash conditions used are specified in Table 17 below.

Table 17. Wash conditions used in the example 11:

Detergent	Liquid model detergent A
Detergent conc.	3.3 g/L
pH	"as is" in the current detergent solution and was not adjusted
Temperature	20°C or 40°C
Dosages in AMSA-plate	140µL detergent per slot; 20µL enzyme per slot
Water hardness	16°dH, adjusted by adding CaCl <sub>2</sub> *2H <sub>2</sub> O, MgCl <sub>2</sub> *6H <sub>2</sub> O and NaHCO <sub>3</sub> (5:1:3) to milli-Q water
Enzymes	EXb (SEQ ID NO:16); EXc (SEQ ID NO:17), xanthan lyase (XLb, SEQ ID NO:22)
Enzyme dosage	EXb and EXc concentrations: 0.7, 1.5, 20, 125 ppb XLb concentration: 400 ppb
Test solution volume	160 micro L
Wash time	20 minutes
Stain/ swatch	Mayonnaise with carbon black C-S-05 S from CFT, Center for Testmaterials BV.

The enzyme and wash liquid were dosed into the AMSA plate and washed according to conditions listed in Table 17. After wash the fabric was flushed in tap water and air-dried. The performance of the enzyme was subsequently measured as the brightness of the colour of the textile samples. Brightness was measured as the intensity of the light reflected from the textile sample

when illuminated with white light. Intensity was measured with a professional flatbed scanner EPSON EXPRESSION 10000XL with special designed software that extracted the intensity value from the scanned image through standard vector calculations.

The performance of the enzyme (or combination of enzymes) was compared to the performance of detergent alone (blank) or detergent with the Xanthan lyase (XL). An enzyme (or combination of enzymes) was considered to exhibit improved wash performance if it performed better than the detergent alone (i.e.,  $R_{\text{ENZYME}} > R_{\text{BLANK}}$ ) (see Tables 18, 19, 20 and 21).

Table 18. Intensity and delta intensity of GH5 polypeptides EXb (SEQ ID NO:16) and EXc (SEQ ID NO:17) tested in AMSA at 20°C in model detergent A.

Concentration [ppb]	Intensity				Delta intensity			
	0.7	1.5	20	125	0.7	1.5	20	125
Blank	210.4	210.4	210.4	210.4				
EXb (SEQ ID NO:16)	210.8	212.8	217.2	217.8	0.4	2.4	6.8	7.5
EXc (SEQ ID NO:17)	212.0	214.4	216.5	218.4	1.6	4.1	6.2	8.0

Table 19. Intensity and delta intensity of GH5 polypeptides EXb (SEQ ID NO:16) and EXc (SEQ ID NO:17) tested in AMSA at 40°C in model detergent A.

Concentration [ppb]	Intensity				Delta intensity			
	0.7	1.5	20	125	0.7	1.5	20	125
Blank	220.0	220.0	220.0	220.0				
EXb (SEQ ID NO:16)	221.9	222.9	229.4	230.2	1.9	3.0	9.4	10.2
EXc (SEQ ID NO:17)	223.2	225.4	228.3	229.0	3.3	5.4	8.3	9.0

Table 20. Intensity and delta intensity of GH5 polypeptides EXb (SEQ ID NO:16) and EXc (SEQ ID NO:17) with Xanthan lyase (XLb (SEQ ID NO:22) tested in AMSA at 20°C in model detergent A.

Concentration [ppb]	Intensity				Delta intensity			
	0.7	1.5	20	125	0.7	1.5	20	125
Blank with XLb (SEQ ID NO:22)	214.0	214.0	214.0	214.0				
EXb (SEQ ID NO:16 with XLb (SEQ ID NO:22)	213.0	215.3	220.4	223.7	-1.0	1.3	6.4	9.7
EXc (SEQ ID NO:17) with XLb (SEQ ID NO:22)	212.4	215.1	220.2	221.4	-1.6	1.1	6.2	7.4

Table 21. Intensity and delta intensity of GH5 polypeptides EXb (SEQ ID NO:16) and EXc (SEQ ID NO:17) with Xanthan lyase (XLb (SEQ ID NO:22) tested in AMSA at 40°C in model detergent A.

Concentration [ppb]	Intensity				Delta intensity			
	0.7	1.5	20	125	0.7	1.5	20	125
Blank with XLb (SEQ ID NO:22)	220.6	220.6	220.6	220.6				
EXb (SEQ ID NO:16 with XLb (SEQ ID NO:22)	222.0	225.0	231.0	232.6	1.3	4.4	10.3	12.0
EXc (SEQ ID NO:17) with XLb (SEQ ID NO:22)	222.3	223.9	230.1	231.5	1.7	3.2	9.5	10.9

The results in above tables show that the GH5 polypeptides, e.g., EXb and EXc, have an improved wash performance both when evaluated alone or in combination with the Xanthan Lyase, e.g., XLb.

The invention described and claimed herein is not to be limited in scope by the specific aspects herein disclosed, since these aspects are intended as illustrations of several aspects of the invention. Any equivalent aspects are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. In the case of conflict, the present disclosure including definitions will control.

**Claims**

1. A detergent composition comprising a polypeptide of glycosyl hydrolase family 5 having xanthan degrading activity.

2. The detergent composition of claim 1, wherein the polypeptide is selected from the group consisting of:

(a) a polypeptide having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the mature polypeptide of SEQ ID NO: 6;

(b) a polypeptide encoded by a polynucleotide that hybridizes under medium stringency conditions with (i) the mature polypeptide coding sequence of any of SEQ ID NO: 5, (ii), or the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the mature polypeptide coding sequence of SEQ ID NO: 5;

(d) a variant of the mature polypeptide of any of SEQ ID NO: 6 comprising a substitution, deletion, and/or insertion at one or more positions;

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has xanthan degrading activity; and

(f) a polypeptide comprising the polypeptide of (a), (b), (c), (d), or (e) and a N-terminal and/or C-terminal His-tag.

3. The detergent composition of claim 1, wherein the polypeptide is selected from the group consisting of:

(a) a polypeptide having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the mature polypeptide of any of SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 8;

(b) a polypeptide encoded by a polynucleotide that hybridizes under medium stringency conditions with (i) the mature polypeptide coding sequence of any of SEQ ID NO: 1, SEQ ID NO: 3, or SEQ ID NO: 7 (ii), or the full-length complement of (i);

(c) a polypeptide encoded by a polynucleotide having at least 60%, at least 65%, at

least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the mature polypeptide coding sequence of any of SEQ ID NO: 1, SEQ ID NO: 3, or SEQ ID NO: 7;

(d) a variant of the mature polypeptide of any of SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 8 comprising a substitution, deletion, and/or insertion at one or more positions;

(e) a fragment of the polypeptide of (a), (b), (c), or (d) that has xanthan degrading activity; and

(f) a polypeptide comprising the polypeptide of (a), (b), (c), (d), or (e) and a N-terminal and/or C-terminal His-tag.

4. The detergent composition of any one of claims 1 to 3, the polypeptide having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide of any of SEQ ID NO: 2, 4, 6, or 8.

5. The detergent composition of any of claims 1 to 4, wherein the polypeptide is encoded by a polynucleotide that hybridizes under medium-high stringency conditions with (i) the mature polypeptide coding sequence of any of SEQ ID NO: 1, 3, 5, or 7, or (ii) the full-length complement of (i).

6. The detergent composition of any one of claims 1 to 5, wherein the polypeptide is encoded by a polynucleotide having at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the mature polypeptide coding sequence of any of SEQ ID NO: 1, 3, 5, or 7.

7. The detergent composition of any of claims 1 to 6, wherein the polypeptide is a variant of the mature polypeptide of any of SEQ ID NO: 2, 4, 6, or 8 comprising a substitution, deletion, and/or insertion at one or more positions, such as up to 10, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 positions.

8. The detergent composition of any one of claims 1 to 7, wherein the polypeptide is a fragment of any of SEQ ID NO: 2, 4, 6, or 8, wherein the fragment has xanthan degrading activity.

9. The detergent composition of any one of claims 1 to 8, further comprising a polypeptide having xanthan lyase activity.

10. The detergent composition of claim 9, wherein the polypeptide having xanthan lyase activity



is a polypeptide having the amino acid sequence of any one of SEQ ID NO: 21, 22, 23 or 24.

11. The detergent composition according to any one of claims 1 - 10, wherein the composition is in form of a bar, a homogenous tablet, a tablet having two or more layers, a pouch having one or more compartments, a regular or compact powder, a granule, a paste, a gel, or a regular, compact or concentrated liquid.

12. The detergent composition of any one of claims 1-11, the composition further comprising one or more additional enzymes selected among protease, lipase, cutinase, amylase, carbohydrase, cellulase, pectinase, mannanase, arabinase, galactanase, xylanase, oxidase, xanthanase, laccase, and/or peroxidase.

13. The detergent composition of any one of claims 1-12, wherein the composition is a laundry detergent composition or a dishwashing composition, preferably a machine dishwashing composition.

14. Use of a detergent composition according to any one of claims 1-13 in a cleaning process.

15. The use according to claim 14, wherein the cleaning process is laundry.

16. The use according to claim 15, wherein the cleaning process is hard surface cleaning such as dish wash.

17. A method for removing a stain from a surface which comprises contacting the surface with a detergent composition according to any one of claims 1 - 13.

18. Use of a detergent composition according to any of claims 1 to 13 for degrading xanthan gum.

19. The use of claim 18, wherein the detergent composition has an enzyme detergency benefit.

20. A method for degrading xanthan gum comprising applying a detergent composition according to any of claims 1 to 13 to xanthan gum.

21. The method of claim 20, wherein the xanthan gum is on the surface of a textile or of a hard surface, such as in dish wash.

eol f-seq1  
SEQUENCE LISTING

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<150> 15185640.8

<151> 2015-09-17

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cga	cgc	ttc	tcc	ctc	ccg	ctg	ctc	gcc	gcc	gcg	ctg	ggc	ctc	gcc	gcg		96
Arg	Arg	Phe	Ser	Leu	Pro	Leu	Leu	Ala	Ala	Ala	Leu	Gly	Leu	Ala	Ala		
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Pro	Ala	Arg	Ala	Ala	Asp	Tyr	Tyr	Leu	Lys	Ala	Ser	Gln	Gly	Ala	Ser		
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Ser	Gly	Glu	Thr	Arg	Leu	Hi s	Gly	Gly	Gly	Al a	Val	Arg	Leu	Asp	Val							
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acc	gtc	gcc	ggc	acc	gag	tat	tcg	ccc	ggc	aac	tac	acc	ttc	gcc	gcg	768						
Thr	Val	Al a	Gly	Thr	Glu	Tyr	Ser	Pro	Gly	Asn	Tyr	Thr	Thr	Al a	Al a							
																205	210	215				220
ctc	cag	gcc	gcg	cat	cct	acg	gtg	ttc	acc	tcc	ggc	acc	gcc	ggc	ggc	816						
Leu	Gln	Al a	Al a	Hi s	Pro	Thr	Val	Phe	Thr	Ser	Gly	Thr	Al a	Gly	Gly							
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tcg	atc	acc	gtc	cgc	gcc	ccg	cgc	acc	tgg	tat	ctc	acc	gtg	aat	cag	864						
Ser	I le	Thr	Val	Arg	Al a	Pro	Arg	Thr	Trp	Tyr	Leu	Thr	Val	Asn	Gln							
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ggc	ggc	gtg	cag	aac	tgg	acc	gag	acc	tac	ctt	tcg	aac	tgg	aac	tcc	912						
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gcc	gcc	aat	ggc	tcc	ggc	gtc	gcg	ccg	act	tcg	atc	aac	ggc	tac	gac	960						
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Phe	Tyr	I le	Asp	Gln	Val	Ser	Asn	Arg	Glu	I le	Arg	Thr	Pro	Ser	Thr							
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gcc	tcc	acc	ttc	ggc	ggc	ggc	gcg	ctc	gcc	ctc	gcc	agc	ggc	gcc	aag	1056						
Al a	Ser	Thr	Phe	Gly	Gly	Gly	Al a	Leu	Al a	Leu	Al a	Ser	Gly	Al a	Lys							
																305	310	315				
ctc	acc	ctc	aag	agt	tcg	ccc	ggc	gtc	gtc	agc	acc	atc	ccg	gcg	ttc	1104						
Leu	Thr	Leu	Lys	Ser	Ser	Pro	Gly	Val	Val	Ser	Thr	I le	Pro	Al a	Phe							
																320	325	330				
gtg	aac	acg	aac	tcc	ccg	atc	atc	gtg	aac	ggc	ggc	ggt	agc	ttc	cgc	1152						
Val	Asn	Thr	Asn	Ser	Pro	I le	I le	Val	Asn	Gly	Gly	Gly	Ser	Phe	Arg							
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caa	agt	ctc	gcc	ctc	ggt	gac	tgg	gag	atc	gcc	tcc	ggc	atc	acc	aag	1200						
Gln	Ser	Leu	Al a	Leu	Gly	Asp	Trp	Glu	I le	Al a	Ser	Gly	I le	Thr	Lys							
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ctc	tcc	gcc	ggc	tcc	ggt	cgc	agc	ctc	ggc	ttc	gac	atc	gac	tac	ctc	1248						

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agc Ser	ctc Leu	gac Asp	gac Asp 400	ggc Gly	tcc Ser	ggc Gly	tac Tyr	acc Thr 405	ggc Gly	acg Thr	ctc Leu	aac Asn	cac His 410	gcg Ala	tcc Ser		1344
ggc Gly	gcg Ala	ctc Leu 415	cgc Arg	ttc Phe	gag Glu	tcc Ser	gtc Val 420	ttc Phe	tcc Ser	acc Thr	gag Glu	ggc Gly 425	gcg Ala	ctc Leu	acc Thr		1392
atc Ile	ggc Gly 430	tcc Ser	tcg Ser	gcg Ala	acc Thr	gtc Val 435	cac His	ctc Leu	gac Asp	caa Gln	cag Gln 440	ggt Val	tac Tyr	gtc Val	acg Thr		1440
tcg Ser 445	ttc Phe	tcc Ser	gtc Val	gcc Ala	ggt Gly 450	gtc Val	gcc Ala	aag Lys	gcc Ala	gcc Ala 455	ggc Gly	atc Ile	cac His	acc Thr	tac Tyr 460		1488
gcc Ala	tcg Ser	ctg Leu	aac Asn	gcc Ala 465	gcg Ala	cat His	ccc Pro	gca Ala	cag Gln 470	ttc Phe	acc Thr	gcc Ala	ggc Gly	gcc Ala 475	gcg Ala		1536
ccc Pro	gga Gly	ctc Leu	gtc Val 480	gct Ala	ggt Val	tac Tyr	acg Thr	ccc Pro 485	gac Asp	acc Thr	gcc Ala	ggc Gly	ccc Pro 490	gtc Val	cgc Arg		1584
atg Met	aac Asn	ggc Gly 495	gtc Val	aat Asn	atc Ile	tcc Ser	ggc Gly 500	ccc Pro	gag Glu	agc Ser	aac Asn	acc Thr 505	gcc Ala	aac Asn	ctc Leu		1632
ccc Pro	ggc Gly 510	acc Thr	tac Tyr	ggc Gly	tac Tyr	aac Asn 515	tac Tyr	ggt Val	tac Tyr	ccc Pro	acc Thr 520	gag Glu	gcc Ala	gac Asp	ttc Phe		1680
gac Asp 525	tac Tyr	tac Tyr	gcc Ala	tcc Ser	aag Lys 530	ggc Gly	ctc Leu	aac Asn	ctc Leu	atc Ile 535	cgc Arg	att Ile	ccc Pro	ttc Phe	cgc Arg 540		1728
tgg Trp	gag Glu	cgc Arg	atg Met	cag Gln 545	cac His	ggc Gly	ctg Leu	aac Asn	ggt Val 550	ccg Pro	ctc Leu	aac Asn	acc Thr	gcc Ala 555	cag Gln		1776
ctc Leu	ggc Gly	tac Tyr	atg Met 560	gac Asp	acc Thr	gcc Ala	gtc Val	gcc Ala 565	cgc Arg	gcc Ala	tcc Ser	gcg Ala	cgc Arg 570	ggc Gly	atg Met		1824
aag Lys	gtc Val	atc Ile 575	ctc Leu	gat Asp	atg Met	cac His	aac Asn 580	tac Tyr	gcc Ala	cgc Arg	tgc Cys	aaa Lys 585	gtc Val	ggc Gly	gga Gly		1872
gtc Val 590	acc Thr	tac Tyr	aag Lys	ttc Phe	ggc Gly	gac Asp 595	gcg Ala	cag Gln	ctc Leu	ccc Pro	gcc Ala 600	tcg Ser	gcc Ala	tac Tyr	gcc Ala		1920
gac Asp 605	gtc Val	tgg Trp	cgc Arg	cgt Arg	ctc Leu 610	gcc Ala	gac Asp	cac His	tac Tyr	aaa Lys 615	aac Asn	gag Glu	ccc Pro	gcc Ala	atc Ile 620		1968
tac Tyr	ggc Gly	ttc Phe	gac Asp	atc Ile 625	atg Met	aac Asn	gag Glu	ccc Pro	aac Asn 630	ggc Gly	ctc Leu	tcc Ser	ggc Gly	ggc Gly 635	gtc Val		2016
tgg Trp	ccc Pro	gcc Ala	tac Tyr	gcc Ala	cag Gln	gcc Ala	gcg Gly	gtc Leu	aac Asn	gcc Ala	atc Ile	cgc Arg	gag Glu	gtc Val	aat Asn		2064

eol f-seq1

Trp Pro Ala Tyr Ala Gl n Ala Ala Val Asn Ala Ile Arg Gl u Val Asn  
640 645 650

ctg tcc acc tgg gtc atc gtc gag ggc gag ttt tgg gcc aac gct tgg 2112  
Leu Ser Thr Trp Val Ile Val Gl u Gl y Gl u Phe Trp Ala Asn Ala Trp  
655 660 665

ggc ttc gag acc aag aac ccg tat ctg cac aac gtc cgc gat ccc gtc 2160  
Gly Phe Gl u Thr Lys Asn Pro Tyr Leu His Asn Val Arg Asp Pro Val  
670 675 680

ggc cgc ctc atg ttc tcc gcc cac tcc tac tgg agc gac gcc ggc acc 2208  
Gly Arg Leu Met Phe Ser Ala His Ser Tyr Trp Ser Asp Ala Gly Thr  
685 690 700

gat gtt tac aag acc tac gac gaa gag ggc gcc tat ccc gag atg ggc 2256  
Asp Val Tyr Lys Thr Tyr Asp Gl u Gl u Gl y Ala Tyr Pro Gl u Met Gly  
705 710 715

gtg aac aac gtg aag ccc ttc atc gac tgg ctg aag aag cac gac gcc 2304  
Val Asn Asn Val Lys Pro Phe Ile Asp Trp Leu Lys Lys His Asp Ala  
720 725 730

aag ggc ttc gtc ggc gaa tac ggc gtg ccc aac aac gac ccg cgc tgg 2352  
Lys Gly Phe Val Gly Gl u Tyr Gl y Val Pro Asn Asn Asp Pro Arg Trp  
735 740 745

ctc gtc gtg ctg gac aac ttc ctc gcc tac ctc gcg gcc gag ggc gtg 2400  
Leu Val Val Leu Asp Asn Phe Leu Ala Tyr Leu Ala Ala Gl u Gly Val  
750 755 760

agc ggc acc tac tgg gcc ggc ggc gcc tgg tat tcg ggc agc ccg atc 2448  
Ser Gly Thr Tyr Trp Ala Gly Gl y Ala Trp Tyr Ser Gly Ser Pro Ile  
765 770 775 780

agc tgc cac ccg tcc tcc aac tac acc gtg gat cgc gcc gtc atg agc 2496  
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gtg ctc gaa gac cat cca tga 2517  
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-20 -15 -10 -5

Pro Ala Arg Ala Ala Asp Tyr Tyr Leu Lys Ala Ser Gl n Gly Ala Ser  
-1 1 5 10

Asn His Trp Ser Ser His Leu Thr Asp Trp Thr Ala Asn Ala Asp Gly  
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Thr Gly Ala Asn Pro Thr Val Ile Gly Leu Ala Asp Thr Phe Asp Thr

eol f-seq1 40

30

35

Asn 45 Asn Arg Thr Leu Arg 50 Thr Pro Ala Val Asn 55 Ala Thr Thr Thr Tyr 60  
 Pro Gly Gly Val 65 Leu Arg Leu Ser Gly 70 Gly Ala Gly Val Ile 75 Gly Met  
 Lys Thr Gly 80 Gly Thr Ala Val Ala 85 Ile Val Pro Lys Leu Val 90 Ser Thr  
 Ala Gly Thr 95 Val Asp Ala Trp His 100 Thr Gly Thr Gln Tyr 105 Phe Arg Ala  
 Asp 110 Asp Trp Glu Asn Leu Ala 115 Ser Gly Thr Gly Phe 120 Thr Ala Leu Lys  
 Ala 125 Val Ala Gly Arg Thr 130 Leu Lys Val Ser Val 135 Gly Lys Leu Thr Gly  
 Ser Gly Glu Thr Arg 145 Leu His Gly Gly Gly Ala Val Arg Leu Asp 155 Val  
 Thr Asp Gly Glu 160 Arg Tyr Leu Gly Val 165 Val Arg Val Ser Ser Gly Ala  
 Ala Asp Phe 175 Asp Asn Asn Val Phe 180 Val Ser Gly Pro Leu Val 185 Ile Glu  
 Thr Gly 190 Ala Thr Val Val Leu Asp Gln Ala Val Ser 200 Phe Ala Gly Leu  
 Thr 205 Val Ala Gly Thr Glu 210 Tyr Ser Pro Gly Asn 215 Tyr Thr Phe Ala Ala  
 Leu Gln Ala Ala His 225 Pro Thr Val Phe Thr 230 Ser Gly Thr Ala Gly 235 Gly  
 Ser Ile Thr Val 240 Arg Ala Pro Arg Thr 245 Trp Tyr Leu Thr Val 250 Asn Gln  
 Gly Gly Val 255 Gln Asn Trp Thr Glu 260 Thr Tyr Leu Ser Asn 265 Trp Asn Ser  
 Ala 270 Ala Asn Gly Ser Gly Val 275 Ala Pro Thr Ser Ile 280 Asn Gly Tyr Asp  
 Phe 285 Tyr Ile Asp Gln Val 290 Ser Asn Arg Glu Ile 295 Arg Thr Pro Ser Thr 300  
 Ala Ser Thr Phe Gly Gly Gly Ala Leu Ala Leu Ala Ser Gly Ala Lys

eol f-seq1  
310

305

315

Leu Thr Leu Lys Ser Ser Pro Gly Val Val Ser Thr Ile Pro Ala Phe  
320 325 330

Val Asn Thr Asn Ser Pro Ile Ile Val Asn Gly Gly Gly Ser Phe Arg  
335 340 345

Gln Ser Leu Ala Leu Gly Asp Trp Glu Ile Ala Ser Gly Ile Thr Lys  
350 355 360

Leu Ser Ala Gly Ser Gly Arg Ser Leu Gly Phe Asp Ile Asp Tyr Leu  
365 370 375 380

Gly Gly Ala Gly Gly Leu Val Thr Gln Asn Gly Gly Ser Tyr Phe Leu  
385 390 395

Ser Leu Asp Asp Gly Ser Gly Tyr Thr Gly Thr Leu Asn His Ala Ser  
400 405 410

Gly Ala Leu Arg Phe Glu Ser Val Phe Ser Thr Glu Gly Ala Leu Thr  
415 420 425

Ile Gly Ser Ser Ala Thr Val His Leu Asp Gln Gln Val Tyr Val Thr  
430 435 440

Ser Phe Ser Val Ala Gly Val Ala Lys Ala Ala Gly Ile His Thr Tyr  
445 450 455 460

Ala Ser Leu Asn Ala Ala His Pro Ala Gln Phe Thr Ala Gly Ala Ala  
465 470 475

Pro Gly Leu Val Ala Val Tyr Thr Pro Asp Thr Ala Gly Pro Val Arg  
480 485 490

Met Asn Gly Val Asn Ile Ser Gly Pro Glu Ser Asn Thr Ala Asn Leu  
495 500 505

Pro Gly Thr Tyr Gly Tyr Asn Tyr Val Tyr Pro Thr Glu Ala Asp Phe  
510 515 520

Asp Tyr Tyr Ala Ser Lys Gly Leu Asn Leu Ile Arg Ile Pro Phe Arg  
525 530 535 540

Trp Glu Arg Met Gln His Gly Leu Asn Val Pro Leu Asn Thr Ala Gln  
545 550 555

Leu Gly Tyr Met Asp Thr Ala Val Ala Arg Ala Ser Ala Arg Gly Met  
560 565 570

Lys Val Ile Leu Asp Met His Asn Tyr Ala Arg Cys Lys Val Gly Gly

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575

580

585

Val Thr Tyr Lys Phe Gly Asp Ala Gl n Leu Pro Ala Ser Ala Tyr Ala  
 590 595 600  
 Asp Val Trp Arg Arg Leu Ala Asp Hi s Tyr Lys Asn Gl u Pro Ala Ile  
 605 610 615 620  
 Tyr Gly Phe Asp Ile Met Asn Gl u Pro Asn Gly Leu Ser Gly Gly Val  
 625 630 635  
 Trp Pro Ala Tyr Ala Gl n Ala Ala Val Asn Ala Ile Arg Gl u Val Asn  
 640 645 650  
 Leu Ser Thr Trp Val Ile Val Gl u Gly Gl u Phe Trp Ala Asn Ala Trp  
 655 660  
 Gly Phe Gl u Thr Lys Asn Pro Tyr Leu Hi s Asn Val Arg Asp Pro Val  
 670 675 680  
 Gly Arg Leu Met Phe Ser Ala Hi s Ser Tyr Trp Ser Asp Ala Gly Thr  
 685 690 695 700  
 Asp Val Tyr Lys Thr Tyr Asp Gl u Gl u Gly Ala Tyr Pro Gl u Met Gly  
 705 710 715  
 Val Asn Asn Val Lys Pro Phe Ile Asp Trp Leu Lys Lys Hi s Asp Ala  
 720 725 730  
 Lys Gly Phe Val Gly Gl u Tyr Gly Val Pro Asn Asn Asp Pro Arg Trp  
 735 740 745  
 Leu Val Val Leu Asp Asn Phe Leu Ala Tyr Leu Ala Ala Gl u Gly Val  
 750 755 760  
 Ser Gly Thr Tyr Trp Ala Gly Gly Ala Trp Tyr Ser Gly Ser Pro Ile  
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eol f-seql

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Pro Phe Leu Ala Thr Leu Ala Thr Ile Leu Gly Leu Ala Ala Ser Val	
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tcc tcc gtc tcc gcc gcc gac tgg tat ctc gat aaa aac cag gcc cgc	144
Ser Ser Val Ser Ala Ala Asp Trp Tyr Leu Asp Lys Asn Gln Ala Arg	
-5 -1 1 5 10	
tac gcc agc tgg gac acc ctc gcc gac tgg aaa ccc aac ccc gac ggc	192
Tyr Ala Ser Trp Asp Thr Leu Ala Asp Trp Lys Pro Asn Pro Asp Gly	
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agc ggc tcc aac ccc tcc gcc ctc tcc ccc tcc gcc acc tac cac ctc	240
Ser Gly Ser Asn Pro Ser Ala Leu Ser Pro Ser Asp Thr Tyr His Leu	
30 35 40	
aac ggc ttc atg ctc cgc acc ccc gag ggc ggc tcc acc tac acc ttc	288
Asn Gly Phe Met Leu Arg Thr Pro Glu Gly Gly Ser Thr Tyr Thr Phe	
45 50 55	
acc ggc ggc ctc ctc agc ctc gcc aac aac gcc gac aac ttc gcc ctc	336
Thr Gly Gly Leu Leu Ser Leu Ala Asn Asn Ala Asp Asn Phe Ala Leu	
60 65 70 75	
aag acc acc ggc tcc ggc gtc tcc atc atc ccc gcc ctg cgc acc acc	384
Lys Thr Thr Gly Ser Gly Val Ser Ile Ile Pro Ala Leu Arg Thr Thr	
80 85 90	
gcc ggc ctc gtc caa aac gtc ggc tcc ggc acg caa aac ctc cag gtt	432
Ala Gly Leu Val Gln Asn Val Gly Ser Thr Gly Thr Gln Asn Leu Gln Val	
95 100 105	
ggc cac tac caa aac ctc tcc ggc acg acc tcc tac tac gcc cag acc	480
Gly His Tyr Gln Asn Leu Ser Gly Thr Thr Ser Tyr Tyr Ala Gln Thr	
110 115 120	
ggg cgc ggc ctc aac ctc gcc atc acc acc ctc gtg ggc tcc ggc cag	528
Gly Arg Gly Leu Asn Leu Ala Ile Thr Thr Leu Val Gly Ser Gly Gln	
125 130 135	
ttc cgc ttc tac ggc ggc ggc acc tac tac ctc tcc ctc gcc aac tcc	576
Phe Arg Phe Tyr Gly Gly Gly Thr Tyr Tyr Leu Ser Leu Ala Asn Ser	
140 145 150 155	
ccg acc tac gac ggc gac atc tac gtc caa tcc ggc acc atc gat ttc	624
Pro Thr Tyr Asp Gly Asp Ile Tyr Val Gln Ser Gly Thr Ile Asp Phe	
160 165 170	
aac aac gac ctc gcc acc gcc ggc act ctc acc gtc aac acc ggt gcc	672
Asn Asn Asp Leu Ala Thr Ala Gly Thr Leu Thr Val Asn Thr Gly Ala	
175 180 185	

## eol f-seql

aag Lys	gtc Val	gcc Ala 190	ctc Leu	gac Asp	cag Gln	gcc Ala	gtc Val 195	acc Thr	ttc Phe	acc Thr	ggc Gly	ctc Leu 200	acc Thr	ata Ile	gcc Ala	720
ggc Gly	aca Thr 205	gcg Ala	tat Tyr	cca Pro	gtt Val	gga Gly 210	aac Asn	tac Tyr	agc Ser	tac Tyr	gcc Ala 215	gcg Ala	ctt Leu	cag Gln	gcc Ala	768
gcc Ala 220	cac His	ccc Pro	gcc Ala	gtt Val	ttc Phe 225	gtc Val	tcc Ser	ggc Gly	acc Thr	tcc Ser 230	ggc Gly	gga Gly	gcc Ala	atc Ile	aac Asn 235	816
gtc Val	cgc Arg	gcc Ala	ccg Pro	cgc Arg 240	aac Asn	tgg Trp	tat Tyr	ctc Leu	tcc Ser 245	acc Thr	cac His	caa Gln	ccc Pro	gtc Val 250	ggc Gly	864
gcc Ala	agc Ser	tgg Trp	aac Asn 255	acc Thr	ctc Leu	gcc Ala	cat His	tgg Trp 260	cgc Arg	gcc Ala	aac Asn	ccc Pro	gac Asp 265	ggc Gly	acc Thr	912
ggc Gly	gcc Ala 270	acc Thr	gcc Ala	gac Asp	tcc Ser	atc Ile	aac Asn 275	tcc Ser	ttc Phe	gac Asp	aac Asn	tac Tyr 280	atc Ile	aac Asn	caa Gln	960
gtc Val	tcc Ser 285	ggc Gly	cgc Arg	acc Thr	ctg Leu	cgc Arg 290	acc Thr	ccc Pro	gaa Glu	acc Thr	acc Thr 295	gcc Ala	acc Thr	ttc Phe	gcc Ala	1008
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gcc Ala	tcc Ser	cag Gln	atc Ile 415	ggc Gly	acc Thr	ggc Gly	ggc Gly	acc Thr 420	ctc Leu	gtc Val	gtc Val	gaa Glu 425	tcc Ser 425	acc Thr	ggc Gly	1392
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ggc Gly	acg Thr 445	ccc Pro	ctc Leu	gcc Ala	ccc Pro	ggc Gly 450	tac Tyr	cac His	acc Thr	tac Tyr	gcc Ala 455	gcg Ala	ctc Leu	aaa Lys	gcc Ala	1488

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ctc Leu	gcc Ala	ggc Gly	ggc Gly 495	gaa Glu	ttc Phe	ggc Gly	acc Thr	ccg Pro 500	atg Met	ccc Pro	ggc Gly	ggt Val	tac Tyr 505	ggc Gly	acc Thr	1632
gac Asp	tac Tyr	atc Ile 510	tac Tyr	ccg Pro	agc Ser	gcc Ala	gcc Ala 515	gcc Ala	ttc Phe	gat Asp	tac Tyr	tac Tyr 520	cac His	ggc Gly	aaa Lys	1680
ggc Gly	ctc Leu 525	aaa Lys	ctc Leu	atc Ile	cgc Arg	ctc Leu 530	ccc Pro	ttt Phe	aag Lys	tgg Trp	gaa Glu 535	cgc Arg	ctc Leu	cag Gln	cac His	1728
acc Thr 540	ctc Leu	aac Asn	gcc Ala	ccc Pro	ctc Leu 545	aac Asn	gcc Ala	gcc Ala	gag Glu	ctc Leu 550	gcc Ala	cgc Arg	atc Ile	gac Asp	acc Thr 555	1776
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cac His	aac Asn	tac Tyr	gcc Ala 575	cgc Arg	cgc Arg	aaa Lys	gaa Glu	agc Ser 580	ggc Gly	acc Thr	acc Thr	tac Tyr	ctc Leu 585	atc Ile	ggc Gly	1872
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gcc Ala	gat Asp 605	cac His	tac Tyr	aag Lys	ggc Gly	aac Asn 610	ccc Pro	gcc Ala	atc Ile	tac Tyr	ggc Gly 615	tac Tyr	ggc Gly	atc Ile	atg Met	1968
aac Asn 620	gag Glu	ccc Pro	tac Tyr	tcc Ser	acc Thr 625	aac Asn	acc Thr	acc Thr	tgg Trp	ccc Pro 630	cag Gln	atg Met	gcc Ala	cag Gln	acc Thr 635	2016
gcc Ala	gtc Val	aac Asn	gcc Ala	atc Ile 640	cgc Arg	acc Thr	ggt Val	gac Asp	ctc Leu 645	acc Thr	acc Thr	cac His	gtc Val	atc Ile 650	gtc Val	2064
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aac Asn	ctc Leu	gac Asp 670	acc Thr	cag Gln	gac Asp	ccc Pro	gtc Val 675	ggc Gly	cgc Arg	ctc Leu	atc Ile	tac Tyr 680	gaa Glu	gcc Ala	cac His	2160
tgc Cys	tac Tyr 685	ttc Phe	gat Asp	tcc Ser	aac Asn	ctc Leu 690	tcc Ser	ggc Gly	acc Thr	tac Tyr	acc Thr 695	caa Gln	agc Ser	tac Tyr	gat Asp	2208
gcc Ala 700	gcc Ala	ggc Gly	gcc Ala	cac His	ccc Pro 705	atg Met	atc Ile	ggc Gly	gtg Val	gac Asp 710	cgc Arg	gtg Val	cgc Arg	gaa Glu	ttc Phe 715	2256
gtc Val	gag Glu	tgg Trp	ctt Leu	cag Gln 720	gaa Glu	acc Thr	ggc Gly	aac Asn	aaa Lys 725	ggc Gly	ttc Phe	atc Ile	ggc Gly	gaa Glu 730	tac Tyr	2304

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ggc gtc ccc ggc aac gac ccc cgc tgg ctc gtc gtg ctc gac aac ttc 2352  
 Gly Val Pro Gly Asn Asp Pro Arg Trp Leu Val Val Leu Asp Asn Phe  
 735 740 745

ctc gcc tac ctc gac gcc aac ggc gtc tcc ggc acc tac tgg gcc ggc 2400  
 Leu Ala Tyr Leu Asp Ala Asn Gly Val Ser Gly Thr Tyr Trp Ala Gly  
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ggt cct tgg tgg ggc aac tac ccg ctc agc tgc gaa ccc acc tcc aac 2448  
 Gly Pro Trp Trp Gly Asn Tyr Pro Leu Ser Cys Glu Pro Thr Ser Asn  
 765 770 775

tac acc gtg gac aaa ccc cag atg agc gtc ctc gaa aac tac aac tga 2496  
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Ser Ser Val Ser Ala Ala Asp Trp Tyr Leu Asp Lys Asn Gl n Ala Arg  
 -5 -1 1 5 10

Tyr Ala Ser Trp Asp Thr Leu Ala Asp Trp Lys Pro Asn Pro Asp Gly  
 15 20 25

Ser Gly Ser Asn Pro Ser Ala Leu Ser Pro Ser Asp Thr Tyr His Leu  
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Asn Gly Phe Met Leu Arg Thr Pro Gl u Gly Gly Ser Thr Tyr Thr Phe  
 45 50 55

Thr Gly Gly Leu Leu Ser Leu Ala Asn Asn Ala Asp Asn Phe Ala Leu  
 60 65 70 75

Lys Thr Thr Gly Ser Gly Val Ser Ile Ile Pro Ala Leu Arg Thr Thr  
 80 85 90

Ala Gly Leu Val Gl n Asn Val Gly Ser Gly Thr Gl n Asn Leu Gl n Val  
 95 100 105

Gly His Tyr Gl n Asn Leu Ser Gly Thr Thr Ser Tyr Tyr Ala Gl n Thr  
 110 115 120

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Gly Arg Gly Leu Asn Leu Ala Ile Thr Thr Leu Val Gly Ser Gly Gln  
125 130 135

Phe Arg Phe Tyr Gly Gly Gly Thr Tyr Tyr Leu Ser Leu Ala Asn Ser  
140 145 150 155

Pro Thr Tyr Asp Gly Asp Ile Tyr Val Gln Ser Gly Thr Ile Asp Phe  
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Asn Asn Asp Leu Ala Thr Ala Gly Thr Leu Thr Val Asn Thr Gly Ala  
175 180 185

Lys Val Ala Leu Asp Gln Ala Val Thr Phe Thr Gly Leu Thr Ile Ala  
190 195 200

Gly Thr Ala Tyr Pro Val Gly Asn Tyr Ser Tyr Ala Ala Leu Gln Ala  
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Ala His Pro Ala Val Phe Val Ser Gly Thr Ser Gly Gly Ala Ile Asn  
220 225 230 235

Val Arg Ala Pro Arg Asn Trp Tyr Leu Ser Thr His Gln Pro Val Gly  
240 245 250

Ala Ser Trp Asn Thr Leu Ala His Trp Arg Ala Asn Pro Asp Gly Thr  
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Gly Ala Thr Ala Asp Ser Ile Asn Ser Phe Asp Asn Tyr Ile Asn Gln  
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Val Ser Gly Arg Thr Leu Arg Thr Pro Glu Thr Thr Ala Thr Phe Ala  
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Gly Gly Ser Leu Val Leu Ala Asp Gly Gly Asn Leu Ser Leu Lys Ala  
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Pro Ala Gly His Ser Ser Thr Ile Pro Ala Phe Ala Thr Ser Gly Ser  
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Ile Ser Ile Thr Asn Gly Phe Ser Ser Ile Thr Gln Pro Leu Val Ile  
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Gly Asp Trp His Leu Gly Ala Gly Thr Ala Gln Val Ser Val Pro Ser  
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Thr Ser Thr Val Gln Leu Thr Val Asp Lys Leu Ser Gly Asp Gly Thr  
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Leu Gln Phe Gln Asn Gly Gly Lys Tyr Thr Leu Asn Ile Arg Gly Ala  
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Ser Ala Phe Thr Gly Thr Leu Arg His Leu Ser Gly Thr Leu Thr Val  
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Ala Ser Gln Ile Gly Thr Gly Gly Thr Leu Val Val Glu Ser Thr Gly  
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Ala Val Lys Leu Asp His Pro Gly Phe Phe Thr Gly Val Thr Val Ala  
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Gly Thr Pro Leu Ala Pro Gly Tyr His Thr Tyr Ala Ala Leu Lys Ala  
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Ala His Pro Ala Arg Phe Pro Thr Gly Ser Thr Asn Ala Phe Leu Ala  
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Val Tyr Pro Pro Asp Thr Thr Gly Pro Ala His Met Phe Gly Val Asn  
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Leu Ala Gly Gly Glu Phe Gly Thr Pro Met Pro Gly Val Tyr Gly Thr  
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Asp Tyr Ile Tyr Pro Ser Ala Ala Ala Phe Asp Tyr Tyr His Gly Lys  
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Gly Leu Lys Leu Ile Arg Leu Pro Phe Lys Trp Glu Arg Leu Gln His  
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Thr Leu Asn Ala Pro Leu Asn Ala Ala Glu Leu Ala Arg Ile Asp Thr  
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Val Val Gly Tyr Ala Ser Ala Arg Gly Met Lys Val Val Leu Asp Met  
560 565 570

His Asn Tyr Ala Arg Arg Lys Glu Ser Gly Thr Thr Tyr Leu Ile Gly  
575 580 585

Thr Gly Pro Val Thr Met Asp Ala Phe Gly Asp Val Trp Arg Arg Ile  
590 595 600

Ala Asp His Tyr Lys Gly Asn Pro Ala Ile Tyr Gly Tyr Gly Ile Met  
605 610 615

Asn Glu Pro Tyr Ser Thr Asn Thr Thr Trp Pro Gln Met Ala Gln Thr  
620 625 630 635

Ala Val Asn Ala Ile Arg Thr Val Asp Leu Thr Thr His Val Ile Val  
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Ala Gly Asp Gly Trp Ser Asn Ala Thr Gly Trp Arg Ser Lys Asn Pro  
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Asn Leu Asp Thr Gln Asp Pro Val Gly Arg Leu Ile Tyr Glu Ala His  
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Cys Tyr Phe Asp Ser Asn Leu Ser Gly Thr Tyr Thr Gln Ser Tyr Asp  
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Ala Ala Gly Ala His Pro Met Ile Gly Val Asp Arg Val Arg Glu Phe  
 700 705 710 715

Val Glu Trp Leu Gln Glu Thr Gly Asn Lys Gly Phe Ile Gly Glu Tyr  
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Gly Val Pro Gly Asn Asp Pro Arg Trp Leu Val Val Leu Asp Asn Phe  
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Leu Ala Tyr Leu Asp Ala Asn Gly Val Ser Gly Thr Tyr Trp Ala Gly  
 750 755 760

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aac Asn	cgc Arg	acg Thr	ctc Leu	cgc Arg 50	acc Thr	ccc Pro	gcc Ala	gtc Val	ggc Gly 55	gtc Val	aac Asn	gcc Ala	acc Thr	ttc Phe 60	ccc Pro	288		
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ccc Pro	tcc Ser	gcc Ala 80	ttc Phe	tcc Ser	atc Ile	gcc Ala	ccc Pro 85	aag Lys	ctc Leu	gtc Val	tcc Ser	acc Thr 90	gcc Ala	ggc Gly	gcc Ala	384		
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gtc Val 190	gtc Val	ctc Leu	gac Asp	cag Gln	gcc Ala 195	gtc Val	tcc Ser	ttc Phe	gcc Ala	ggc Gly 200	ctc Leu	gcc Ala	gtc Val	gga Gly	gcc Ala 205	720		
acc Thr	gag Glu	tat Tyr	cca Pro	ccc Pro 210	ggc Gly	aac Asn	tac Tyr	acc Thr	ctc Leu 215	gcc Ala	gcc Ala	ctg Leu	caa Gln	gcc Ala 220	gcc Ala	768		
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acc Thr	gag Glu 255	gcc Ala	ttc Phe	ctc Leu	tcc Ser	aac Asn 260	tgg Trp	aac Asn	tcc Ser	gcc Ala	gcc Ala 265	aac Asn	ggc Gly	tcc Ser	ggc Gly	912		
gtc Val 270	gcc Ala	ccg Pro	aac Asn	tac Tyr	atc Ile 275	aac Asn	ggc Gly	cac His	gac Asp	atc Ile 280	tac Tyr	ctc Leu	aac Asn	cag Gln	gtg Val 285	960		
aac Asn	aac Asn	cgc Arg	gag Glu	ctc Leu	cgc Arg	acg Thr	ccc Pro	tac Tyr	acc Thr	gcc Ala	agc Ser	acc Thr	ttc Phe	acc Thr	ggc Gly	1008		



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Gly	Thr	Leu	Ala	Leu	Thr	Phe	Gly	Ser	Lys	Leu	Val	Val	Lys	Thr	Ser				
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ccc	aac	ctc	gtc	agc	acc	atc	ccc	gcc	ctc	gtc	acc	tcc	ggc	acc	ccg	1104			
Pro	Asn	Leu	Val	Ser	Thr	Ile	Pro	Ala	Leu	Val	Thr	Ser	Gly	Thr	Pro				
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cag	ttc	gcc	aac	ggc	agc	ggc	agc	cgc	caa	aac	ctc	gcc	atc	ggc	gac	1152			
Gln	Phe	Ala	Asn	Gly	Ser	Gly	Ser	Arg	Gln	Asn	Leu	Ala	Ile	Gly	Asp				
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tgg	gac	atc	atc	tcc	ggc	acc	agc	cgc	ctc	gtc	gcc	ggc	tcc	acc	cgg	1200			
Trp	Asp	Ile	Ile	Ser	Gly	Thr	Ser	Arg	Leu	Val	Ala	Gly	Ser	Thr	Arg				
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tcc	ctc	ggc	ttc	gac	atc	ggc	tgg	ctc	acc	ggc	gcg	ggc	aac	ctc	cag	1248			
Ser	Leu	Gly	Phe	Asp	Ile	Gly	Trp	Leu	Thr	Gly	Ala	Gly	Asn	Leu	Gln				
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acc	gaa	ggc	ggc	ggc	tcg	ttc	ttc	ctc	cgc	ctc	atc	gac	ggc	tcc	ggc	1296			
Thr	Glu	Gly	Gly	Gly	Ser	Phe	Phe	Leu	Arg	Leu	Ile	Asp	Gly	Ser	Gly				
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tac	acc	ggc	gcc	atc	aac	cac	aac	tcc	ggc	gcc	ctc	cgc	ttc	gag	tcc	1344			
Tyr	Thr	Gly	Ala	Ile	Asn	His	Asn	Ser	Gly	Ala	Leu	Arg	Phe	Glu	Ser				
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gtc	ttc	tcc	acc	gcc	ggt	gcc	ctc	aac	atc	ggc	gcc	tcc	gcg	acc	gtc	1392			
Val	Phe	Ser	Thr	Ala	Gly	Ala	Leu	Asn	Ile	Gly	Ala	Ser	Ala	Thr	Val				
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cac	ctc	gac	aag	ccc	gtc	tat	gtc	agc	ggc	ctc	tcc	gtc	gcc	ggc	gtc	1440			
His	Leu	Asp	Lys	Pro	Val	Tyr	Val	Ser	Gly	Leu	Ser	Val	Ala	Gly	Val				
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gcc	aaa	ccc	gcc	ggc	atc	cac	acc	tac	gcc	tcg	ctg	aac	gcc	gcg	cat	1488			
Ala	Lys	Pro	Ala	Gly	Ile	His	Thr	Tyr	Ala	Ser	Leu	Asn	Ala	Ala	His				
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ccc	gcg	cag	ttc	aac	gcc	ggc	gcc	gcg	ccc	gga	ctc	gtc	gcc	ggt	tac	1536			
Pro	Ala	Gln	Phe	Asn	Ala	Gly	Ala	Ala	Pro	Gly	Leu	Val	Ala	Val	Tyr				
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aca	ccc	aac	act	gcc	gcc	ccc	gtc	cgc	atg	aac	ggc	gtc	aac	ctc	tcc	1584			
Thr	Pro	Asn	Thr	Ala	Ala	Pro	Val	Arg	Met	Asn	Gly	Val	Asn	Leu	Ser				
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ggc	ccc	gaa	tcc	gtc	ggc	ggc	gcc	ggc	acg	ccc	ttt	ccc	ggc	acc	tac	1632			
Gly	Pro	Glu	Ser	Val	Gly	Gly	Ala	Gly	Thr	Pro	Phe	Pro	Gly	Thr	Tyr				
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Gly	Phe	Gln	Trp	Ile	Tyr	Pro	Thr	Val	Ala	Asp	Tyr	Asp	Tyr	Tyr	Ala				
			510							515				520					
gcc	aag	ggc	ctt	aac	ctc	atc	cgc	atc	cca	ttc	cgc	tgg	gaa	cgc	atg	1728			
Ala	Lys	Gly	Leu	Asn	Leu	Ile	Arg	Ile	Pro	Phe	Arg	Trp	Glu	Arg	Met				
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caa	ggc	acc	ctt	aac	ggt	ccc	ctc	atc	gcc	gcc	gaa	ctc	gct	cgc	atg	1776			
Gln	Gly	Thr	Leu	Asn	Gly	Pro	Leu	Ile	Ala	Ala	Glu	Leu	Ala	Arg	Met				
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gac	aac	gcc	atc	gcc	ctc	gcc	tcc	gcg	cgc	ggc	atg	aag	gtc	atc	ctc	1824			
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Asp	Met	His	Asn	Tyr	Ala	Arg	Tyr	Arg	Thr	Pro	Thr	Ala	Ser	Tyr	Val	
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ttt	ggt	gac	gcc	cag	ctc	ccc	gcc	tcc	gcc	ttc	gcc	gac	gtc	tgg	cgc	1920
Phe	Gly	Asp	Ala	Gln	Leu	Pro	Ala	Ser	Ala	Phe	Ala	Asp	Val	Trp	Arg	
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aag	ctc	gcc	gat	cac	tac	aaa	aac	gaa	ccc	gcc	atc	tac	ggt	ttc	gac	1968
Lys	Leu	Ala	Asp	His	Tyr	Lys	Asn	Glu	Pro	Ala	Ile	Tyr	Gly	Phe	Asp	
				610					615					620		
atc	atg	aac	gag	ccg	cac	agc	atg	ccc	acc	ccc	acc	acc	tgg	ccc	acc	2016
Ile	Met	Asn	Glu	Pro	His	Ser	Met	Pro	Thr	Pro	Thr	Thr	Trp	Pro	Thr	
			625					630					635			
tac	gcc	caa	gcc	gcc	gtc	cac	gcc	atc	cgc	gag	gtc	aac	ctc	gac	acc	2064
Tyr	Ala	Gln	Ala	Ala	Val	His	Ala	Ile	Arg	Glu	Val	Asn	Leu	Asp	Thr	
		640					645					650				
tgg	atc	atc	gta	gag	ggc	gag	acc	tat	gcc	aac	tcc	tgg	aaa	ttc	ggg	2112
Trp	Ile	Ile	Val	Glu	Gly	Glu	Thr	Tyr	Ala	Asn	Ser	Trp	Lys	Phe	Gly	
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gaa	aaa	aat	ccc	cac	ctc	cac	aac	gtg	cgc	gac	ccc	gtc	ggc	cgc	ctc	2160
Glu	Lys	Asn	Pro	His	Leu	His	Asn	Val	Arg	Asp	Pro	Val	Gly	Arg	Leu	
670					675					680					685	
atg	ttc	tcc	gcc	cac	tcc	tac	tgg	tgc	aaa	aac	ggc	gac	gac	aga	tac	2208
Met	Phe	Ser	Ala	His	Ser	Tyr	Trp	Cys	Lys	Asn	Gly	Asp	Asp	Arg	Tyr	
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ggc	acc	tac	gac	gcg	gaa	aac	ggc	cac	ccc	cag	atg	ggc	gtg	gac	agc	2256
Gly	Thr	Tyr	Asp	Ala	Glu	Asn	Gly	His	Pro	Gln	Met	Gly	Val	Asp	Ser	
			705					710					715			
ctc	aag	cac	ttc	gtt	gac	tgg	ctc	cgc	aaa	cac	aac	gcc	cac	ggc	ttc	2304
Leu	Lys	His	Phe	Val	Asp	Trp	Leu	Arg	Lys	His	Asn	Ala	His	Gly	Phe	
		720					725					730				
gtc	ggc	gaa	tac	ggc	gtc	ccc	aac	aac	gac	ccc	cgc	tgg	ctc	gaa	gtc	2352
Val	Gly	Glu	Tyr	Gly	Val	Pro	Asn	Asn	Asp	Pro	Arg	Trp	Leu	Glu	Val	
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ctt	gaa	aac	gcg	ctc	atc	tac	ctg	gcg	aat	gaa	aac	atc	agc	ggc	acc	2400
Leu	Glu	Asn	Ala	Leu	Ile	Tyr	Leu	Ala	Asn	Glu	Asn	Ile	Ser	Gly	Thr	
	750				755					760					765	
tac	tgg	gcc	ggc	ggc	gcc	tgg	ctc	gcc	ggc	agc	cac	atc	agc	tgc	cac	2448
Tyr	Trp	Ala	Gly	Gly	Ala	Trp	Leu	Ala	Gly	Ser	His	Ile	Ser	Cys	His	
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ccg	tcc	tcc	aac	tac	acc	gtg	gac	cgc	ccc	gtc	atg	agc	gtc	ctc	caa	2496
Pro	Ser	Ser	Asn	Tyr	Thr	Val	Asp	Arg	Pro	Val	Met	Ser	Val	Leu	Gln	
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Asn	Tyr	Pro														
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Gly Gly Val Leu Gly Leu Asn Gly Gly Val Ile Gly Ile Lys Thr Gly  
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Pro Ser Ala Phe Ser Ile Ala Pro Lys Leu Val Ser Thr Ala Gly Ala  
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Ile Glu Ser Trp Gly Thr Pro Gln Asn Phe Arg Ala Asp Asp Trp Glu  
 95 100 105

Ser Asn Ala Pro Phe Pro Thr Phe Thr Gly Leu Arg Thr Ala Ser Asn  
 110 115 120 125

His Thr Leu Lys Val Ser Val Gly Lys Leu Ser Gly Thr Gly Glu Ile  
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Arg Val His Gly Gly Gly Thr Val Leu Leu Asp Val Thr Asp Ala Glu  
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Asn Tyr Leu Gly Thr Leu Cys Val Ala Ser Gly Ala Leu Asn Phe Asp  
 160 165 170

Asn Ala Val Phe Ser Ser Gly Pro Leu Asp Ile Lys Thr Gly Ala Thr  
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Val Val Leu Asp Gln Ala Val Ser Phe Ala Gly Leu Ala Val Gly Ala  
 190 195 200 205

Thr Glu Tyr Pro Pro Gly Asn Tyr Thr Leu Ala Ala Leu Gln Ala Ala  
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 Ala Pro Arg Thr Trp Tyr Leu Thr Val Ser Gl n Gly Ser Gl n Asn Trp  
 240 245 250  
 Thr Gl u Ala Phe Leu Ser Asn Trp Asn Ser Ala Ala Asn Gly Ser Gly  
 255 260 265  
 Val Ala Pro Asn Tyr Ile Asn Gly Hi s Asp Ile Tyr Leu Asn Gl n Val  
 270 275 280 285  
 Asn Asn Arg Gl u Leu Arg Thr Pro Tyr Thr Ala Ser Thr Phe Thr Gly  
 290 295 300  
 Gly Thr Leu Ala Leu Thr Phe Gly Ser Lys Leu Val Val Lys Thr Ser  
 305 310 315  
 Pro Asn Leu Val Ser Thr Ile Pro Ala Leu Val Thr Ser Gly Thr Pro  
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 Gl n Phe Ala Asn Gly Ser Gly Ser Arg Gl n Asn Leu Ala Ile Gly Asp  
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 Trp Asp Ile Ile Ser Gly Thr Ser Arg Leu Val Ala Gly Ser Thr Arg  
 350 355 360 365  
 Ser Leu Gly Phe Asp Ile Gly Trp Leu Thr Gly Ala Gly Asn Leu Gl n  
 370 375 380  
 Thr Gl u Gly Gly Gly Ser Phe Phe Leu Arg Leu Ile Asp Gly Ser Gly  
 385 390 395  
 Tyr Thr Gly Ala Ile Asn Hi s Asn Ser Gly Ala Leu Arg Phe Gl u Ser  
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 Val Phe Ser Thr Ala Gly Ala Leu Asn Ile Gly Ala Ser Ala Thr Val  
 415 420 425  
 Hi s Leu Asp Lys Pro Val Tyr Val Ser Gly Leu Ser Val Ala Gly Val  
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 Ala Lys Pro Ala Gly Ile Hi s Thr Tyr Ala Ser Leu Asn Ala Ala Hi s  
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 Pro Ala Gl n Phe Asn Ala Gly Ala Ala Pro Gly Leu Val Ala Val Tyr  
 465 470 475  
 Thr Pro Asn Thr Ala Ala Pro Val Arg Met Asn Gly Val Asn Leu Ser  
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eol f-seql

Gly 495 Pro Glu Ser Val Gly 500 Gly Ala Gly Thr Pro Phe 505 Pro Gly Thr Tyr  
 Gly 510 Phe Gln Trp Ile Tyr 515 Pro Thr Val Ala Asp 520 Tyr Asp Tyr Tyr Ala 525  
 Ala Lys Gly Leu Asn 530 Leu Ile Arg Ile Pro Phe 535 Arg Trp Glu Arg Met 540  
 Gln Gly Thr Leu 545 Asn Gly Pro Leu Ile Ala Ala Glu Leu Ala Arg Met 555  
 Asp Asn Ala 560 Ile Ala Leu Ala Ser 565 Ala Arg Gly Met Lys 570 Val Ile Leu  
 Asp Met 575 His Asn Tyr Ala Arg 580 Tyr Arg Thr Pro Thr Ala Ser Tyr Val  
 Phe 590 Gly Asp Ala Gln Leu 595 Pro Ala Ser Ala Phe 600 Ala Asp Val Trp Arg 605  
 Lys Leu Ala Asp His 610 Tyr Lys Asn Glu Pro Ala 615 Ile Tyr Gly Phe Asp 620  
 Ile Met Asn Glu 625 Pro His Ser Met Pro Thr Pro Thr Thr Trp Pro Thr 635  
 Tyr Ala Gln Ala Ala Val His Ala 645 Ile Arg Glu Val Asn 650 Leu Asp Thr  
 Trp Ile Ile Val Glu Gly Glu 660 Thr Tyr Ala Asn Ser 665 Trp Lys Phe Gly  
 Glu 670 Lys Asn Pro His Leu 675 His Asn Val Arg Asp 680 Pro Val Gly Arg Leu 685  
 Met Phe Ser Ala His 690 Ser Tyr Trp Cys Lys 695 Asn Gly Asp Asp Arg Tyr 700  
 Gly Thr Tyr Asp 705 Ala Glu Asn Gly His 710 Pro Gln Met Gly Val 715 Asp Ser  
 Leu Lys His 720 Phe Val Asp Trp Leu 725 Arg Lys His Asn Ala 730 His Gly Phe  
 Val Gly Glu Tyr Gly Val Pro 740 Asn Asn Asp Pro Arg 745 Trp Leu Glu Val  
 Leu 750 Glu Asn Ala Leu Ile 755 Tyr Leu Ala Asn Glu 760 Asn Ile Ser Gly Thr 765

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Tyr Trp Ala Gly Gly Ala Trp Leu Ala Gly Ser His Ile Ser Cys His  
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Asn Tyr Pro  
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gcc ggc ttc tcg atc ccc gcc acc tcc gca aac cgc gcc gcg ttc gtg 240  
 Ala Gly Phe Ser Ile Pro Ala Thr Ser Ala Asn Arg Ala Ala Phe Val  
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gcc ggc gct tcg gta cga ctg gca gac ggt cag gta cgc aag atc agc 288  
 Ala Gly Ala Ser Val Arg Leu Ala Asp Gly Gln Val Arg Lys Ile Ser  
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cgc gcg caa atc gtc ggc agc aac atg agc atc ttc ctg gaa ggt gca 336  
 Arg Ala Gln Ile Val Gly Ser Asn Met Ser Ile Phe Leu Glu Gly Ala  
 65 70 75

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 Lys Leu Asp Gly Asn Lys Val Gly Ala Pro Gln Val Val Thr Ile Gly  
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 Ser Thr Ala Val Thr Ala Pro Asp Thr Ser Ala Pro Ile Thr Thr Pro  
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## eol f-seq1

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Asn	Ser	Pro	Leu	Asn	Ala	Glu	Glu	Phe	Ala	Arg	Leu	Lys	Gln	Ser	Leu	
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Asp	Phe	Ala	Gln	Lys	His	Asn	Val	Lys	Val	Ile	Leu	Asp	Leu	His	Asn	
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Tyr	Tyr	Arg	Tyr	Tyr	Gly	Lys	Leu	Ile	Gly	Ser	Lys	Glu	Val	Pro	Ile	
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Val	Asp	Lys	Asp	Phe	Ser	Gly	Asn	Tyr	Phe	Asp	Lys	Ala	Glu	Lys	Phe	
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Lys	Gln	His	Lys	Leu	Arg	Gly	Tyr	Ile	Gly	Glu	His	Gly	Val	Pro	Asp	
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Phe	Ser	Pro	Ser	Ala	Ile	Val	Ala	Thr	Asp	Asn	Leu	Leu	Ala	Tyr	Leu	
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Gly	Glu	Tyr	Ala	Met	Ser	Leu	Asp	Val	Ser	Ser	Gly	Lys	His	Arg	Pro	
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cag	ctg	ccg	gtt	ctg	cag	aag	cac	gcc	aaa	acc	gca	aac	agc	tgc	acc	2064
Gln	Leu	Pro	Val	Leu	Gln	Lys	His	Ala	Lys	Thr	Ala	Asn	Ser	Cys	Thr	
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Ala Gly Ala Ser Val Arg Leu Ala Asp Gly Gln Val Arg Lys Ile Ser  
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Arg Ala Gln Ile Val Gly Ser Asn Met Ser Ile Phe Leu Glu Gly Ala  
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Lys Leu Asp Gly Asn Lys Val Gly Ala Pro Gln Val Val Thr Ile Gly  
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Ser Thr Ala Val Thr Ala Pro Asp Thr Ser Ala Pro Ile Thr Thr Pro  
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Pro Thr Val Thr Ala His Ser Thr Ser Ile Asn Ala Phe Thr Asn Asn  
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Asp Trp Leu Asn Gly Val Trp Arg Lys Ser Pro Gly Phe Ser Ile Pro  
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Ala Ser Ala Ala Asn Lys Ala Ala Phe Lys Val Gly Ala Thr Ala Lys  
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Leu Ala Asp Gly Gln Val Arg Lys Ile Thr Gln Val Gln Val Val Gly  
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Ala Asn Met Ser Val Tyr Leu Glu Gly Ala Ala Val Asn Gly Ser Val  
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Val Gly Ala Pro Asn Lys Leu Ala Leu Ala Thr Thr Ser Thr Thr Ser  
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Pro Ala Pro Thr Pro Ala Pro Ser Ala Pro Thr Pro Ser Val Ile Ala  
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Thr Ser Asn Leu Asn Asn Tyr Thr Asn Ala Gln Trp Leu Asn Gly Met  
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Tyr Arg Thr Ala Ala Gly Phe Ser Ile Gln Ala Ser Ser Ala Asn Val  
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Ala Ala Phe Lys Ala Gly Ala Leu Val Arg Leu Ala Asp Gly Gln Thr  
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Arg Lys Val Leu Arg Ala Gln Leu Val Gly Ser Asn Met Ser Val Phe  
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Leu Asp Gly Ala Val Ile Asn Gly Thr Thr Leu Gly Tyr Pro Lys Thr  
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Ile Ser Val Val Ser Thr Ser Thr Gly Thr Pro Ser Ser Pro Ala Leu  
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335 340 345

Ala Gly Phe Gly Pro Ser Val Val Pro Gly Lys His Gly Thr Asn Tyr  
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Thr Tyr Pro Ala Glu Ser Tyr Tyr Lys Lys Tyr Ser Asp Leu Gly Met  
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Pro Leu Val Arg Leu Pro Phe Leu Trp Glu Arg Ile Gln Pro Lys Leu  
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Asn Ser Pro Leu Asn Ala Glu Glu Phe Ala Arg Leu Lys Gln Ser Leu  
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Asp Phe Ala Gln Lys His Asn Val Lys Val Ile Leu Asp Leu His Asn  
415 420 425

Tyr Tyr Arg Tyr Tyr Gly Lys Leu Ile Gly Ser Lys Glu Val Pro Ile  
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Ser Ser Phe Ala Ala Val Trp Lys Gln Ile Val Gln Gln Val Val Asn  
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Asn Gly Leu Trp Pro Gln Ala Ala Leu Ala Ala Ala Gln Ala Ile Arg  
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Ser Ala Phe His Trp Pro His Tyr Asn Thr Gln Leu Val Thr Asn Pro  
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Trp Met Arg Asp Pro Lys Asn Asn Leu Val Tyr Glu Ala His Met Tyr  
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Val Asp Lys Asp Phe Ser Gly Asn Tyr Phe Asp Lys Ala Glu Lys Phe  
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Asp Pro Met Ile Gly Val Asn Arg Val Lys Pro Phe Val Asp Trp Leu  
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Lys Gln His Lys Leu Arg Gly Tyr Ile Gly Glu His Gly Val Pro Asp  
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Phe Ser Pro Ser Ala Ile Val Ala Thr Asp Asn Leu Leu Ala Tyr Leu  
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Gly Glu Tyr Ala Met Ser Leu Asp Val Ser Ser Gly Lys His Arg Pro  
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eol f-seq1

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gtagcaciaa ttccggcact ggttacatct ggaacaccgc aatttgcgaa tggcagcggc	1020
tcaagacaaa atctggcaat tggcgattgg gatattatct caggcacatc aagactggtt	1080
gcaggctcaa caagatcact gggctttgat attggctggc tgacaggcgc tggcaatctg	1140
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gctattaacc ataattctgg cgctctgaga tttgaaagcg tttttagcac agctggcgca	1260
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gcaggtacac cttttccggg aacatatggc tttcaatgga tttatccgac agtcgcggat	1560
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gacattatga atgaaccgca ttcaatgccg acaccgacaa cgtggccgac atatgcacaa	1920
gcagcagttc atgcaattag agaagtcaat ctggatacat ggattatcgt tgaaggcgaa	1980
acatatgcca actcatggaa atttggcgaa aaaaatccgc atctgcataa tgtttagagat	2040
ccggttggca gactgatgtt ttcagcacat tcatattggt gcaaaaatgg cgacgatcgc	2100
tatggcacgt atgatcgga aatggccat ccgcaaatgg gcgttgattc actgaaacat	2160
ttgttgatt ggctgcgcaa acataatgca catggctttg ttggcgaata tggcgttccg	2220
aataatgatc cgagatggct ggaagtctg gaaaatgcac tgatttatct ggccaacgaa	2280
aacattagcg gcacatattg ggcaggcgga gcatggctgg caggctcaca tatttcatgc	2340
catccgtcat ctaactatac agttgatcgt ccggttatga gcgtcctgca aaattatccg	2400
taa	2403

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eol f-seq1

gcagcatttg ttgcaggcgc atcagttaga ctggcagatg gccaagttag aaaaattagc 180  
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aataaagttg gcgcaccgca agttgttaca attggctcaa cagcagttac agcaccggat 300  
acatcagcac cgattacaac accgcctaca gtcacagcac attcaacatc aattaacgcc 360  
tttacaata atgactggct taacggcggt tggcgcaaat caccgggatt tagcattccg 420  
gcatctgcag cgaataaagc ggcttttaa gttggagcaa cagcaaaact tgcggatgga 480  
caggttcgca aaattacaca agttcaagtt gttggcgcta acatgagcgt ttatcttgaa 540  
ggcgagcag tcaatggctc agttgttga gcaccgaata aactggcact ggcaacaaca 600  
agcacaacat caccggcacc gacaccggct ccgtcagctc cgacaccgtc agttattgca 660  
acatcaaatc tgaacaacta tacaatgcg cagtggctga acggaatgta tagaacagca 720  
gcgggatttt ctattcaagc atcaagcga aatgtcgcag cttttaaagc aggcgactg 780  
gtcagacttg ctgatggcca gacaagaaa gttctgagag cacaactggg ttgctcaaat 840  
atgtcagtct ttcttgatgg cgctgtcatt aatggcacia cactgggcta tccgaaaaca 900  
atttcagttg ttagcacatc aacaggcaca ccgtcatctc cggcactgac aacacctccg 960  
gttgaaccgg ctctgcacc ggttccgaca gcgcctgata caacaaatgg caaaccgctg 1020  
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ggcaciaaatt atacatatcc ggcaaaaagc tactacaaaa aatactcaga tctgggcatg 1140  
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aatgcagaag aatttgaag actgaaacag agcctggatt ttgcgcagaa acataacggt 1260  
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catccggcag ttgaaggcta tggcctgatg aatgaaccgc atagcaciaa tggcctgtgg 1440  
cctcaagcag cactggcagc agcacaagca attagaacag ttgatagcaa acgctggatt 1500  
tatgtcgcag gcgatagatg gtcatcagca tttcattggc ctattataa cacacagctg 1560  
gttaciaaatc cgtggatgag agatccgaaa aataacctgg tttatgaagc gcatatgtat 1620  
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ggagaatatg caatgtcact ggatgtttca agcggcaaac atagaccgca acttccggtt 1920  
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eol f-seql

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<400> 13

Met Hi s Hi s Hi s Hi s Hi s Hi s Pro Arg Ala Asp Tyr Tyr Leu Lys Ala  
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Ser Gl n Gly Ala Ser Asn Hi s Trp Ser Ser Hi s Leu Thr Asp Trp Thr  
 20 25 30

Ala Asn Ala Asp Gly Thr Gly Ala Asn Pro Thr Val Ile Gly Leu Ala  
 35 40 45

Asp Thr Phe Asp Thr Asn Asn Arg Thr Leu Arg Thr Pro Ala Val Asn  
 50 55 60

Ala Thr Thr Thr Tyr Pro Gly Gly Val Leu Arg Leu Ser Gly Gly Ala  
 65 70 75 80

Gly Val Ile Gly Met Lys Thr Gly Gly Thr Ala Val Ala Ile Val Pro  
 85 90 95

Lys Leu Val Ser Thr Ala Gly Thr Val Asp Ala Trp Hi s Thr Gly Thr  
 100 105 110

Gl n Tyr Phe Arg Ala Asp Asp Trp Gl u Asn Leu Ala Ser Gly Thr Gly  
 115 120 125

Phe Thr Ala Leu Lys Ala Val Ala Gly Arg Thr Leu Lys Val Ser Val  
 130 135 140

Gly Lys Leu Thr Gly Ser Gly Gl u Thr Arg Leu Hi s Gly Gly Gly Ala  
 145 150 155 160

Val Arg Leu Asp Val Thr Asp Gly Gl u Arg Tyr Leu Gly Val Val Arg  
 165 170 175

Val Ser Ser Gly Ala Ala Asp Phe Asp Asn Asn Val Phe Val Ser Gly  
 180 185 190

Pro Leu Val Ile Gl u Thr Gly Ala Thr Val Val Leu Asp Gl n Ala Val  
 195 200 205

Ser Phe Ala Gly Leu Thr Val Ala Gly Thr Gl u Tyr Ser Pro Gly Asn  
 210 215 220

eol f-seq1

Tyr Thr Phe Ala Ala Leu Gln Ala Ala His Pro Thr Val Phe Thr Ser  
 225 230 235 240

Gly Thr Ala Gly Gly Ser Ile Thr Val Arg Ala Pro Arg Thr Trp Tyr  
 245 250 255

Leu Thr Val Asn Gln Gly Gly Val Gln Asn Trp Thr Glu Thr Tyr Leu  
 260 265 270

Ser Asn Trp Asn Ser Ala Ala Asn Gly Ser Gly Val Ala Pro Thr Ser  
 275 280 285

Ile Asn Gly Tyr Asp Phe Tyr Ile Asp Gln Val Ser Asn Arg Glu Ile  
 290 295 300

Arg Thr Pro Ser Thr Ala Ser Thr Phe Gly Gly Gly Ala Leu Ala Leu  
 305 310 315 320

Ala Ser Gly Ala Lys Leu Thr Leu Lys Ser Ser Pro Gly Val Val Ser  
 325 330 335

Thr Ile Pro Ala Phe Val Asn Thr Asn Ser Pro Ile Ile Val Asn Gly  
 340 345 350

Gly Gly Ser Phe Arg Gln Ser Leu Ala Leu Gly Asp Trp Glu Ile Ala  
 355 360 365

Ser Gly Ile Thr Lys Leu Ser Ala Gly Ser Gly Arg Ser Leu Gly Phe  
 370 375 380

Asp Ile Asp Tyr Leu Gly Gly Ala Gly Gly Leu Val Thr Gln Asn Gly  
 385 390 395 400

Gly Ser Tyr Phe Leu Ser Leu Asp Asp Gly Ser Gly Tyr Thr Gly Thr  
 405 410 415

Leu Asn His Ala Ser Gly Ala Leu Arg Phe Glu Ser Val Phe Ser Thr  
 420 425 430

Glu Gly Ala Leu Thr Ile Gly Ser Ser Ala Thr Val His Leu Asp Gln  
 435 440 445

Gln Val Tyr Val Thr Ser Phe Ser Val Ala Gly Val Ala Lys Ala Ala  
 450 455 460

Gly Ile His Thr Tyr Ala Ser Leu Asn Ala Ala His Pro Ala Gln Phe  
 465 470 475 480

Thr Ala Gly Ala Ala Pro Gly Leu Val Ala Val Tyr Thr Pro Asp Thr  
 485 490 495

eol f-seq1

Al a Gly Pro Val Arg Met Asn Gly Val Asn Ile Ser Gly Pro Glu Ser  
500 505 510

Asn Thr Ala Asn Leu Pro Gly Thr Tyr Gly Tyr Asn Tyr Val Tyr Pro  
515 520 525

Thr Glu Ala Asp Phe Asp Tyr Tyr Ala Ser Lys Gly Leu Asn Leu Ile  
530 535 540

Arg Ile Pro Phe Arg Trp Glu Arg Met Gl n His Gly Leu Asn Val Pro  
545 550 555 560

Leu Asn Thr Ala Gl n Leu Gly Tyr Met Asp Thr Ala Val Ala Arg Ala  
565 570 575

Ser Ala Arg Gly Met Lys Val Ile Leu Asp Met His Asn Tyr Ala Arg  
580 585 590

Cys Lys Val Gly Gly Val Thr Tyr Lys Phe Gly Asp Ala Gl n Leu Pro  
595 600 605

Al a Ser Ala Tyr Ala Asp Val Trp Arg Arg Leu Ala Asp His Tyr Lys  
610 615 620

Asn Glu Pro Ala Ile Tyr Gly Phe Asp Ile Met Asn Glu Pro Asn Gly  
625 630 635 640

Leu Ser Gly Gly Val Trp Pro Ala Tyr Ala Gl n Ala Ala Val Asn Ala  
645 650 655

Ile Arg Glu Val Asn Leu Ser Thr Trp Val Ile Val Glu Gly Glu Phe  
660 665 670

Trp Ala Asn Ala Trp Gly Phe Glu Thr Lys Asn Pro Tyr Leu His Asn  
675 680 685

Val Arg Asp Pro Val Gly Arg Leu Met Phe Ser Ala His Ser Tyr Trp  
690 695 700

Ser Asp Ala Gly Thr Asp Val Tyr Lys Thr Tyr Asp Glu Glu Gly Ala  
705 710 715 720

Tyr Pro Glu Met Gly Val Asn Asn Val Lys Pro Phe Ile Asp Trp Leu  
725 730 735

Lys Lys His Asp Ala Lys Gly Phe Val Gly Glu Tyr Gly Val Pro Asn  
740 745 750

Asn Asp Pro Arg Trp Leu Val Val Leu Asp Asn Phe Leu Ala Tyr Leu  
755 760 765

Ala Ala Glu Gly Val Ser Gly Thr Tyr Trp Ala Gly Gly Ala Trp Tyr  
770 775 780 eol f-seq1

Ser Gly Ser Pro Ile Ser Cys His Pro Ser Ser Asn Tyr Thr Val Asp  
785 790 795 800

Arg Ala Val Met Ser Val Leu Glu Asp His Pro  
805 810

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Met His His His His His His Pro Arg Ala Asp Trp Tyr Leu Asp Lys  
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Asn Gln Ala Arg Tyr Ala Ser Trp Asp Thr Leu Ala Asp Trp Lys Pro  
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Asn Pro Asp Gly Ser Gly Ser Asn Pro Ser Ala Leu Ser Pro Ser Asp  
35 40 45

Thr Tyr His Leu Asn Gly Phe Met Leu Arg Thr Pro Glu Gly Gly Ser  
50 55 60

Thr Tyr Thr Phe Thr Gly Gly Leu Leu Ser Leu Ala Asn Asn Ala Asp  
65 70 75 80

Asn Phe Ala Leu Lys Thr Thr Gly Ser Gly Val Ser Ile Ile Pro Ala  
85 90 95

Leu Arg Thr Thr Ala Gly Leu Val Gln Asn Val Gly Ser Gly Thr Gln  
100 105 110

Asn Leu Gln Val Gly His Tyr Gln Asn Leu Ser Gly Thr Thr Ser Tyr  
115 120 125

Tyr Ala Gln Thr Gly Arg Gly Leu Asn Leu Ala Ile Thr Thr Leu Val  
130 135 140

Gly Ser Gly Gln Phe Arg Phe Tyr Gly Gly Gly Thr Tyr Tyr Leu Ser  
145 150 155 160

eof-seq1

Leu Ala Asn Ser Pro Thr Tyr Asp Gly Asp Ile Tyr Val Gln Ser Gly  
 165 170 175

Thr Ile Asp Phe Asn Asn Asp Leu Ala Thr Ala Gly Thr Leu Thr Val  
 180 185 190

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

Leu Thr Ile Ala Gly Thr Ala Tyr Pro Val Gly Asn Tyr Ser Tyr Ala  
 210 215 220

Ala Leu Gln Ala Ala His Pro Ala Val Phe Val Ser Gly Thr Ser Gly  
 225 230 235 240

Gly Ala Ile Asn Val Arg Ala Pro Arg Asn Trp Tyr Leu Ser Thr His  
 245 250 255

Gln Pro Val Gly Ala Ser Trp Asn Thr Leu Ala His Trp Arg Ala Asn  
 260 265 270

Pro Asp Gly Thr Gly Ala Thr Ala Asp Ser Ile Asn Ser Phe Asp Asn  
 275 280 285

Tyr Ile Asn Gln Val Ser Gly Arg Thr Leu Arg Thr Pro Glu Thr Thr  
 290 295 300

Ala Thr Phe Ala Gly Gly Ser Leu Val Leu Ala Asp Gly Gly Asn Leu  
 305 310 315 320

Ser Leu Lys Ala Pro Ala Gly His Ser Ser Thr Ile Pro Ala Phe Ala  
 325 330 335

Thr Ser Gly Ser Ile Ser Ile Thr Asn Gly Phe Ser Ser Ile Thr Gln  
 340 345 350

Pro Leu Val Ile Gly Asp Trp His Leu Gly Ala Gly Thr Ala Gln Val  
 355 360 365

Ser Val Pro Ser Thr Ser Thr Val Gln Leu Thr Val Asp Lys Leu Ser  
 370 375 380

Gly Asp Gly Thr Leu Gln Phe Gln Asn Gly Gly Lys Tyr Thr Leu Asn  
 385 390 395 400

Ile Arg Gly Ala Ser Ala Phe Thr Gly Thr Leu Arg His Leu Ser Gly  
 405 410 415

Thr Leu Thr Val Ala Ser Gln Ile Gly Thr Gly Gly Thr Leu Val Val  
 420 425 430

eol f-seq1

Gl u Ser Thr Gly Ala Val Lys Leu Asp His Pro Gly Phe Phe Thr Gly  
 435 440 445

Val Thr Val Ala Gly Thr Pro Leu Ala Pro Gly Tyr His Thr Tyr Ala  
 450 455 460

Ala Leu Lys Ala Ala His Pro Ala Arg Phe Pro Thr Gly Ser Thr Asn  
 465 470 475 480

Ala Phe Leu Ala Val Tyr Pro Pro Asp Thr Thr Gly Pro Ala His Met  
 485 490 495

Phe Gly Val Asn Leu Ala Gly Gly Gl u Phe Gly Thr Pro Met Pro Gly  
 500 505

Val Tyr Gly Thr Asp Tyr Ile Tyr Pro Ser Ala Ala Ala Phe Asp Tyr  
 515 520 525

Tyr His Gly Lys Gly Leu Lys Leu Ile Arg Leu Pro Phe Lys Trp Gl u  
 530 535 540

Arg Leu Gl n His Thr Leu Asn Ala Pro Leu Asn Ala Ala Gl u Leu Ala  
 545 550 555 560

Arg Ile Asp Thr Val Val Gly Tyr Ala Ser Ala Arg Gly Met Lys Val  
 565 570 575

Val Leu Asp Met His Asn Tyr Ala Arg Arg Lys Gl u Ser Gly Thr Thr  
 580 585 590

Tyr Leu Ile Gly Thr Gly Pro Val Thr Met Asp Ala Phe Gly Asp Val  
 595 600 605

Trp Arg Arg Ile Ala Asp His Tyr Lys Gly Asn Pro Ala Ile Tyr Gly  
 610 615 620

Tyr Gly Ile Met Asn Gl u Pro Tyr Ser Thr Asn Thr Thr Trp Pro Gl n  
 625 630 635 640

Met Ala Gl n Thr Ala Val Asn Ala Ile Arg Thr Val Asp Leu Thr Thr  
 645 650 655

His Val Ile Val Ala Gly Asp Gly Trp Ser Asn Ala Thr Gly Trp Arg  
 660 665 670

Ser Lys Asn Pro Asn Leu Asp Thr Gl n Asp Pro Val Gly Arg Leu Ile  
 675 680 685

Tyr Gl u Ala His Cys Tyr Phe Asp Ser Asn Leu Ser Gly Thr Tyr Thr  
 690 695 700

eof-seq1

Gln Ser Tyr Asp Ala Ala Gly Ala His Pro Met Ile Gly Val Asp Arg  
705 710 715 720

Val Arg Glu Phe Val Glu Trp Leu Gln Glu Thr Gly Asn Lys Gly Phe  
725 730 735

Ile Gly Glu Tyr Gly Val Pro Gly Asn Asp Pro Arg Trp Leu Val Val  
740 745 750

Leu Asp Asn Phe Leu Ala Tyr Leu Asp Ala Asn Gly Val Ser Gly Thr  
755 760 765

Tyr Trp Ala Gly Gly Pro Trp Trp Gly Asn Tyr Pro Leu Ser Cys Glu  
770 775 780

Pro Thr Ser Asn Tyr Thr Val Asp Lys Pro Gln Met Ser Val Leu Glu  
785 790 800

Asn Tyr Asn

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<400> 15

Met His His His His His His Pro Arg Ala Asp Tyr Tyr Leu Lys Val  
1 5 10 15

Asn Gln Pro His Pro Asn Ser Trp Ala Ser Pro Val Thr Asp Trp Ala  
20 25 30

Ala Asn Pro Asp Gly Thr Gly Ala Ala Pro Ala Ala Ile Ala Ala Pro  
35 40 45

Asp Thr Phe Tyr Thr Asn Asn Arg Thr Leu Arg Thr Pro Ala Val Gly  
50 55 60

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

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

eol f-seq1

Val Ser Thr Ala Gly Ala Ile Glu Ser Trp Gly Thr Pro Gln Asn Phe  
100 105 110

Arg Ala Asp Asp Trp Glu Ser Asn Ala Pro Phe Pro Thr Phe Thr Gly  
115 120 125

Leu Arg Thr Ala Ser Asn His Thr Leu Lys Val Ser Val Gly Lys Leu  
130 135 140

Ser Gly Thr Gly Glu Ile Arg Val His Gly Gly Gly Thr Val Leu Leu  
145 150 155 160

Asp Val Thr Asp Ala Glu Asn Tyr Leu Gly Thr Leu Cys Val Ala Ser  
165 170 175

Gly Ala Leu Asn Phe Asp Asn Ala Val Phe Ser Ser Gly Pro Leu Asp  
180 185 190

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

Gly Leu Ala Val Gly Ala Thr Glu Tyr Pro Pro Gly Asn Tyr Thr Leu  
210 215 220

Ala Ala Leu Gln Ala Ala His Pro Gly Val Phe Thr Gly Thr Ala Ala  
225 230 235 240

Gly Ser Ile Thr Val Arg Ala Pro Arg Thr Trp Tyr Leu Thr Val Ser  
245 250 255

Gln Gly Ser Gln Asn Trp Thr Glu Ala Phe Leu Ser Asn Trp Asn Ser  
260 265 270

Ala Ala Asn Gly Ser Gly Val Ala Pro Asn Tyr Ile Asn Gly His Asp  
275 280 285

Ile Tyr Leu Asn Gln Val Asn Asn Arg Glu Leu Arg Thr Pro Tyr Thr  
290 295 300

Ala Ser Thr Phe Thr Gly Gly Thr Leu Ala Leu Thr Phe Gly Ser Lys  
305 310 315 320

Leu Val Val Lys Thr Ser Pro Asn Leu Val Ser Thr Ile Pro Ala Leu  
325 330 335

Val Thr Ser Gly Thr Pro Gln Phe Ala Asn Gly Ser Gly Ser Arg Gln  
340 345 350

Asn Leu Ala Ile Gly Asp Trp Asp Ile Ile Ser Gly Thr Ser Arg Leu  
355 360 365



eol f-seq1

Val Ala Gly Ser Thr Arg Ser Leu Gly Phe Asp Ile Gly Trp Leu Thr  
370 375 380

Gly Ala Gly Asn Leu Gln Thr Glu Gly Gly Gly Ser Phe Phe Leu Arg  
385 390 395 400

Leu Ile Asp Gly Ser Gly Tyr Thr Gly Ala Ile Asn His Asn Ser Gly  
405 410 415

Ala Leu Arg Phe Glu Ser Val Phe Ser Thr Ala Gly Ala Leu Asn Ile  
420 425 430

Gly Ala Ser Ala Thr Val His Leu Asp Lys Pro Val Tyr Val Ser Gly  
435 440 445

Leu Ser Val Ala Gly Val Ala Lys Pro Ala Gly Ile His Thr Tyr Ala  
450 455 460

Ser Leu Asn Ala Ala His Pro Ala Gln Phe Asn Ala Gly Ala Ala Pro  
465 470 475 480

Gly Leu Val Ala Val Tyr Thr Pro Asn Thr Ala Ala Pro Val Arg Met  
485 490 495

Asn Gly Val Asn Leu Ser Gly Pro Glu Ser Val Gly Gly Ala Gly Thr  
500 505 510

Pro Phe Pro Gly Thr Tyr Gly Phe Gln Trp Ile Tyr Pro Thr Val Ala  
515 520 525

Asp Tyr Asp Tyr Tyr Ala Ala Lys Gly Leu Asn Leu Ile Arg Ile Pro  
530 535 540

Phe Arg Trp Glu Arg Met Gln Gly Thr Leu Asn Gly Pro Leu Ile Ala  
545 550 555 560

Ala Glu Leu Ala Arg Met Asp Asn Ala Ile Ala Leu Ala Ser Ala Arg  
565 570 575

Gly Met Lys Val Ile Leu Asp Met His Asn Tyr Ala Arg Tyr Arg Thr  
580 585 590

Pro Thr Ala Ser Tyr Val Phe Gly Asp Ala Gln Leu Pro Ala Ser Ala  
595 600 605

Phe Ala Asp Val Trp Arg Lys Leu Ala Asp His Tyr Lys Asn Glu Pro  
610 615 620

Ala Ile Tyr Gly Phe Asp Ile Met Asn Glu Pro His Ser Met Pro Thr  
625 630 635 640

eol f-seq1

Pro Thr Thr Trp Pro Thr Tyr Ala Gln Ala Ala Val His Ala Ile Arg  
645 650 655

Glu Val Asn Leu Asp Thr Trp Ile Ile Val Glu Gly Glu Thr Tyr Ala  
660 665 670

Asn Ser Trp Lys Phe Gly Glu Lys Asn Pro His Leu His Asn Val Arg  
675 680 685

Asp Pro Val Gly Arg Leu Met Phe Ser Ala His Ser Tyr Trp Cys Lys  
690 695 700

Asn Gly Asp Asp Arg Tyr Gly Thr Tyr Asp Ala Glu Asn Gly His Pro  
705 710 715 720

Gln Met Gly Val Asp Ser Leu Lys His Phe Val Asp Trp Leu Arg Lys  
725 730 735

His Asn Ala His Gly Phe Val Gly Glu Tyr Gly Val Pro Asn Asn Asp  
740 745 750

Pro Arg Trp Leu Glu Val Leu Glu Asn Ala Leu Ile Tyr Leu Ala Asn  
755 760 765

Glu Asn Ile Ser Gly Thr Tyr Trp Ala Gly Gly Ala Trp Leu Ala Gly  
770 775 780

Ser His Ile Ser Cys His Pro Ser Ser Asn Tyr Thr Val Asp Arg Pro  
785 790 795 800

Val Met Ser Val Leu Gln Asn Tyr Pro  
805

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<400> 16

His His His His His His Pro Arg Ala Asp Trp Tyr Leu Asp Lys Asn  
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eol f-seq1

Gln Ala Arg Tyr Ala Ser Trp Asp Thr Leu Ala Asp Trp Lys Pro Asn  
20 25 30

Pro Asp Gly Ser Gly Ser Asn Pro Ser Ala Leu Ser Pro Ser Asp Thr  
35 40 45

Tyr His Leu Asn Gly Phe Met Leu Arg Thr Pro Glu Gly Gly Ser Thr  
50 55 60

Tyr Thr Phe Thr Gly Gly Leu Leu Ser Leu Ala Asn Asn Ala Asp Asn  
65 70 75 80

Phe Ala Leu Lys Thr Thr Gly Ser Gly Val Ser Ile Ile Pro Ala Leu  
85 90 95

Arg Thr Thr Ala Gly Leu Val Gln Asn Val Gly Ser Gly Thr Gln Asn  
100 105 110

Leu Gln Val Gly His Tyr Gln Asn Leu Ser Gly Thr Thr Ser Tyr Tyr  
115 120 125

Ala Gln Thr Gly Arg Gly Leu Asn Leu Ala Ile Thr Thr Leu Val Gly  
130 135 140

Ser Gly Gln Phe Arg Phe Tyr Gly Gly Gly Thr Tyr Tyr Leu Ser Leu  
145 150 155 160 165

Ala Asn Ser Pro Thr Tyr Asp Gly Asp Ile Tyr Val Gln Ser Gly Thr  
165 170 175

Ile Asp Phe Asn Asn Asp Leu Ala Thr Ala Gly Thr Leu Thr Val Asn  
180 185 190

Thr Gly Ala Lys Val Ala Leu Asp Gln Ala Val Thr Phe Thr Gly Leu  
195 200 205

Thr Ile Ala Gly Thr Ala Tyr Pro Val Gly Asn Tyr Ser Tyr Ala Ala  
210 215 220

Leu Gln Ala Ala His Pro Ala Val Phe Val Ser Gly Thr Ser Gly Gly  
225 230 235 240

Ala Ile Asn Val Arg Ala Pro Arg Asn Trp Tyr Leu Ser Thr His Gln  
245 250 255

Pro Val Gly Ala Ser Trp Asn Thr Leu Ala His Trp Arg Ala Asn Pro  
260 265 270

Asp Gly Thr Gly Ala Thr Ala Asp Ser Ile Asn Ser Phe Asp Asn Tyr  
275 280 285

eof-seq1

Ile Asn Gln Val Ser Gly Arg Thr Leu Arg Thr Pro Glu Thr Thr Ala  
 290 295 300  
 Thr Phe Ala Gly Gly Ser Leu Val Leu Ala Asp Gly Gly Asn Leu Ser  
 305 310 315 320  
 Leu Lys Ala Pro Ala Gly His Ser Ser Thr Ile Pro Ala Phe Ala Thr  
 325 330 335  
 Ser Gly Ser Ile Ser Ile Thr Asn Gly Phe Ser Ser Ile Thr Gln Pro  
 340 345 350  
 Leu Val Ile Gly Asp Trp His Leu Gly Ala Gly Thr Ala Gln Val Ser  
 355 360 365  
 Val Pro Ser Thr Ser Thr Val Gln Leu Thr Val Asp Lys Leu Ser Gly  
 370 375 380  
 Asp Gly Thr Leu Gln Phe Gln Asn Gly Gly Lys Tyr Thr Leu Asn Ile  
 385 390 395 400  
 Arg Gly Ala Ser Ala Phe Thr Gly Thr Leu Arg His Leu Ser Gly Thr  
 405 410 415  
 Leu Thr Val Ala Ser Gln Ile Gly Thr Gly Gly Thr Leu Val Val Glu  
 420 425 430  
 Ser Thr Gly Ala Val Lys Leu Asp His Pro Gly Phe Phe Thr Gly Val  
 435 440 445  
 Thr Val Ala Gly Thr Pro Leu Ala Pro Gly Tyr His Thr Tyr Ala Ala  
 450 455 460  
 Leu Lys Ala Ala His Pro Ala Arg Phe Pro Thr Gly Ser Thr Asn Ala  
 465 470 475 480  
 Phe Leu Ala Val Tyr Pro Pro Asp Thr Thr Gly Pro Ala His Met Phe  
 485 490 495  
 Gly Val Asn Leu Ala Gly Gly Glu Phe Gly Thr Pro Met Pro Gly Val  
 500 505 510  
 Tyr Gly Thr Asp Tyr Ile Tyr Pro Ser Ala Ala Ala Phe Asp Tyr Tyr  
 515 520 525  
 His Gly Lys Gly Leu Lys Leu Ile Arg Leu Pro Phe Lys Trp Glu Arg  
 530 535 540  
 Leu Gln His Thr Leu Asn Ala Pro Leu Asn Ala Ala Glu Leu Ala Arg  
 545 550 555 560

Ile Asp Thr Val Val Gly Tyr Ala Ser Ala Arg Gly Met Lys Val Val  
 565 570 575  
 Leu Asp Met His Asn Tyr Ala Arg Arg Lys Glu Ser Gly Thr Thr Tyr  
 580 585 590  
 Leu Ile Gly Thr Gly Pro Val Thr Met Asp Ala Phe Gly Asp Val Trp  
 595 600 605  
 Arg Arg Ile Ala Asp His Tyr Lys Gly Asn Pro Ala Ile Tyr Gly Tyr  
 610 615 620  
 Gly Ile Met Asn Glu Pro Tyr Ser Thr Asn Thr Thr Trp Pro Glu Met  
 625 630 635 640  
 Ala Glu Thr Ala Val Asn Ala Ile Arg Thr Val Asp Leu Thr Thr His  
 645 650 655  
 Val Ile Val Ala Gly Asp Gly Trp Ser Asn Ala Thr Gly Trp Arg Ser  
 660 665 670  
 Lys Asn Pro Asn Leu Asp Thr Glu Asp Pro Val Gly Arg Leu Ile Tyr  
 675 680 685  
 Glu Ala His Cys Tyr Phe Asp Ser Asn Leu Ser Gly Thr Tyr Thr Glu  
 690 695 700  
 Ser Tyr Asp Ala Ala Gly Ala His Pro Met Ile Gly Val Asp Arg Val  
 705 710 715 720  
 Arg Glu Phe Val Glu Trp Leu Glu Glu Thr Gly Asn Lys Gly Phe Ile  
 725 730 735  
 Gly Glu Tyr Gly Val Pro Gly Asn Asp Pro Arg Trp Leu Val Val Leu  
 740 745 750  
 Asp Asn Phe Leu Ala Tyr Leu Asp Ala Asn Gly Val Ser Gly Thr Tyr  
 755 760 765  
 Trp Ala Gly Gly Pro Trp Trp Gly Asn Tyr Pro Leu Ser Cys Glu Pro  
 770 775 780  
 Thr Ser Asn Tyr Thr Val Asp Lys Pro Glu Met Ser Val Leu Glu Asn  
 785 790 795 800  
 Tyr Asn

<210> 17  
 <211> 808  
 <212> PRT  
 <213> Artificial

eol f-seql

<220>

<223> HASTAG' ed

<220>

<221> MI SC\_FEATURE

<222> (1) . (8)

<223> Has tag

<400> 17

Hi s Hi s Hi s Hi s Hi s Hi s Pro Arg Ala Asp Tyr Tyr Leu Lys Val Asn  
1 5 10 15

Gl n Pro Hi s Pro Asn Ser Trp Ala Ser Pro Val Thr Asp Trp Ala Ala  
20 25 30

Asn Pro Asp Gly Thr Gly Ala Ala Pro Ala Ala Ile Ala Ala Pro Asp  
35 40 45

Thr Phe Tyr Thr Asn Asn Arg Thr Leu Arg Thr Pro Ala Val Gly Val  
50 55 60

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

Gly Ile Lys Thr Gly Pro Ser Ala Phe Ser Ile Ala Pro Lys Leu Val  
85 90 95

Ser Thr Ala Gly Ala Ile Glu Ser Trp Gly Thr Pro Gl n Asn Phe Arg  
100 105 110

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

Arg Thr Ala Ser Asn Hi s Thr Leu Lys Val Ser Val Gly Lys Leu Ser  
130 135 140

Gly Thr Gly Glu Ile Arg Val Hi s Gly Gly Gly Thr Val Leu Leu Asp  
145 150 155 160

Val Thr Asp Ala Glu Asn Tyr Leu Gly Thr Leu Cys Val Ala Ser Gly  
165 170 175

Ala Leu Asn Phe Asp Asn Ala Val Phe Ser Ser Gly Pro Leu Asp Ile  
180 185 190

Lys Thr Gly Ala Thr Val Val Leu Asp Gl n Ala Val Ser Phe Ala Gly  
195 200 205

Leu Ala Val Gly Ala Thr Glu Tyr Pro Pro Gly Asn Tyr Thr Leu Ala  
210 215 220

eol f-seq1

Ala 225 Leu Gln Ala Ala His 230 Pro Gly Val Phe Thr Gly Thr Ala Ala Gly 240

Ser Ile Thr Val Arg 245 Ala Pro Arg Thr Trp 250 Tyr Leu Thr Val Ser 255 Gln

Gly Ser Gln Asn 260 Trp Thr Glu Ala Phe 265 Leu Ser Asn Trp Asn 270 Ser Ala

Ala Asn Gly 275 Ser Gly Val Ala Pro 280 Asn Tyr Ile Asn Gly 285 His Asp Ile

Tyr Leu 290 Asn Gln Val Asn Asn 295 Arg Glu Leu Arg Thr 300 Pro Tyr Thr Ala

Ser Thr Phe Thr Gly 310 Gly Thr Leu Ala Leu Thr 315 Phe Gly Ser Lys Leu 320

Val Val Lys Thr Ser 325 Pro Asn Leu Val Ser 330 Thr Ile Pro Ala Leu Val 335

Thr Ser Gly Thr 340 Pro Gln Phe Ala Asn 345 Gly Ser Gly Ser Arg 350 Gln Asn

Leu Ala Ile 355 Gly Asp Trp Asp Ile 360 Ile Ser Gly Thr Ser 365 Arg Leu Val

Ala Gly 370 Ser Thr Arg Ser Leu 375 Gly Phe Asp Ile Gly 380 Trp Leu Thr Gly

Ala Gly 385 Asn Leu Gln Thr 390 Glu Gly Gly Gly Ser 395 Phe Phe Leu Arg Leu 400

Ile Asp Gly Ser Gly 405 Tyr Thr Gly Ala Ile 410 Asn His Asn Ser Gly 415 Ala

Leu Arg Phe Glu 420 Ser Val Phe Ser Thr 425 Ala Gly Ala Leu Asn 430 Ile Gly

Ala Ser Ala 435 Thr Val His Leu Asp 440 Lys Pro Val Tyr 445 Val Ser Gly Leu

Ser Val 450 Ala Gly Val Ala Lys 455 Pro Ala Gly Ile His 460 Thr Tyr Ala Ser

Leu Asn Ala Ala His 470 Pro Ala Gln Phe Asn Ala 475 Gly Ala Ala Pro Gly 480

Leu Val Ala Val Tyr 485 Thr Pro Asn Thr Ala 490 Ala Pro Val Arg Met 495 Asn

eol f-seq1

Gly Val Asn Leu Ser Gly Pro Glu Ser Val Gly Gly Ala Gly Thr Pro  
500 505 510

Phe Pro Gly Thr Tyr Gly Phe Gl n Trp Ile Tyr Pro Thr Val Ala Asp  
515 520 525

Tyr Asp Tyr Tyr Ala Ala Lys Gly Leu Asn Leu Ile Arg Ile Pro Phe  
530 535 540

Arg Trp Glu Arg Met Gl n Gly Thr Leu Asn Gly Pro Leu Ile Ala Ala  
545 550 555 560

Glu Leu Ala Arg Met Asp Asn Ala Ile Ala Leu Ala Ser Ala Arg Gly  
565 570 575

Met Lys Val Ile Leu Asp Met His Asn Tyr Ala Arg Tyr Arg Thr Pro  
580 585 590

Thr Ala Ser Tyr Val Phe Gly Asp Ala Gl n Leu Pro Ala Ser Ala Phe  
595 600 605

Ala Asp Val Trp Arg Lys Leu Ala Asp His Tyr Lys Asn Glu Pro Ala  
610 615 620

Ile Tyr Gly Phe Asp Ile Met Asn Glu Pro His Ser Met Pro Thr Pro  
625 630 635 640

Thr Thr Trp Pro Thr Tyr Ala Gl n Ala Ala Val His Ala Ile Arg Glu  
645 650 655

Val Asn Leu Asp Thr Trp Ile Ile Val Glu Gly Glu Thr Tyr Ala Asn  
660 665 670

Ser Trp Lys Phe Gly Glu Lys Asn Pro His Leu His Asn Val Arg Asp  
675 680 685

Pro Val Gly Arg Leu Met Phe Ser Ala His Ser Tyr Trp Cys Lys Asn  
690 695 700

Gly Asp Asp Arg Tyr Gly Thr Tyr Asp Ala Glu Asn Gly His Pro Gl n  
705 710 715 720

Met Gly Val Asp Ser Leu Lys His Phe Val Asp Trp Leu Arg Lys His  
725 730 735

Asn Ala His Gly Phe Val Gly Glu Tyr Gly Val Pro Asn Asn Asp Pro  
740 745 750

Arg Trp Leu Glu Val Leu Glu Asn Ala Leu Ile Tyr Leu Ala Asn Glu  
755 760 765



eof-seq1

Asn Ile Ser Gly Thr Tyr Trp Ala Gly Gly Ala Trp Leu Ala Gly Ser  
 770 775 780

His Ile Ser Cys His Pro Ser Ser Asn Tyr Thr Val Asp Arg Pro Val  
 785 790 795 800

Met Ser Val Leu Gln Asn Tyr Pro  
 805

<210> 18  
 <211> 665  
 <212> PRT  
 <213> Artificial

<220>  
 <223> HASTAG'ed

<220>  
 <221> MISC\_FEATURE  
 <222> (1)..(8)  
 <223> Has tag

<400> 18

His His His His His His Pro Arg Ser Ser Val Ala Ala Val Ser Val  
 1 5 10 15

Ser Ala Lys Ile Asn Ala Phe Thr Asn Ser Asp Trp Leu Asn Gly Ile  
 20 25 30

Trp Arg Thr Gly Ala Gly Phe Ser Ile Pro Ala Thr Ser Ala Asn Arg  
 35 40 45

Ala Ala Phe Val Ala Gly Ala Ser Val Arg Leu Ala Asp Gly Gln Val  
 50 55 60

Arg Lys Ile Ser Arg Ala Gln Ile Val Gly Ser Asn Met Ser Ile Phe  
 65 70 75 80

Leu Glu Gly Ala Lys Leu Asp Gly Asn Lys Val Gly Ala Pro Gln Val  
 85 90 95

Val Thr Ile Gly Ser Thr Ala Val Thr Ala Pro Asp Thr Ser Ala Pro  
 100 105 110

Ile Thr Thr Pro Pro Thr Val Thr Ala His Ser Thr Ser Ile Asn Ala  
 115 120 125

Phe Thr Asn Asn Asp Trp Leu Asn Gly Val Trp Arg Lys Ser Pro Gly  
 130 135 140

Phe Ser Ile Pro Ala Ser Ala Ala Asn Lys Ala Ala Phe Lys Val Gly  
 145 150 155 160

eol f-seq1

Al a Thr Al a Lys Leu Al a Asp Gly Gl n Val Arg Lys Ile Thr Gl n Val  
165 170 175

Gl n Val Val Gly Al a Asn Met Ser Val Tyr Leu Gl u Gly Al a Al a Val  
180 185 190

Asn Gly Ser Val Val Gly Al a Pro Asn Lys Leu Al a Leu Al a Thr Thr  
195 200 205

Ser Thr Thr Ser Pro Al a Pro Thr Pro Al a Pro Ser Al a Pro Thr Pro  
210 215 220

Ser Val Ile Al a Thr Ser Asn Leu Asn Asn Tyr Thr Asn Al a Gl n Trp  
225 230 235 240

Leu Asn Gly Met Tyr Arg Thr Al a Al a Gly Phe Ser Ile Gl n Al a Ser  
245 250 255

Ser Al a Asn Val Al a Al a Phe Lys Al a Gly Al a Leu Val Arg Leu Al a  
260 265 270

Asp Gly Gl n Thr Arg Lys Val Leu Arg Al a Gl n Leu Val Gly Ser Asn  
275 280 285

Met Ser Val Phe Leu Asp Gly Al a Val Ile Asn Gly Thr Thr Leu Gly  
290 295 300

Tyr Pro Lys Thr Ile Ser Val Val Ser Thr Ser Thr Gly Thr Pro Ser  
305 310 315 320

Ser Pro Al a Leu Thr Thr Pro Pro Val Gl u Pro Al a Pro Al a Pro Val  
325 330 335

Pro Thr Al a Pro Asp Thr Thr Asn Gly Lys Pro Leu Leu Val Gly Val  
340 345 350

Asn Leu Ser Gly Al a Gly Phe Gly Pro Ser Val Val Pro Gly Lys His  
355 360 365

Gly Thr Asn Tyr Thr Tyr Pro Al a Gl u Ser Tyr Tyr Lys Lys Tyr Ser  
370 375 380

Asp Leu Gly Met Pro Leu Val Arg Leu Pro Phe Leu Trp Gl u Arg Ile  
385 390 395 400

Gl n Pro Lys Leu Asn Ser Pro Leu Asn Al a Gl u Gl u Phe Al a Arg Leu  
405 410 415

Lys Gl n Ser Leu Asp Phe Al a Gl n Lys His Asn Val Lys Val Ile Leu  
420 425 430

eol f-seq1

Asp Leu His Asn Tyr Tyr Arg Tyr Tyr Gly Lys Leu Ile Gly Ser Lys  
435 440 445

Glu Val Pro Ile Ser Ser Phe Ala Ala Val Trp Lys Gl n Ile Val Gl n  
450 455 460

Gl n Val Val Asn His Pro Ala Val Gl u Gly Tyr Gly Leu Met Asn Gl u  
465 470 475 480

Pro His Ser Thr Asn Gly Leu Trp Pro Gl n Ala Ala Leu Ala Ala Ala  
485 490 495

Gl n Ala Ile Arg Thr Val Asp Ser Lys Arg Trp Ile Tyr Val Ala Gly  
500 505 510

Asp Arg Trp Ser Ser Ala Phe His Trp Pro His Tyr Asn Thr Gl n Leu  
515 520 525

Val Thr Asn Pro Trp Met Arg Asp Pro Lys Asn Asn Leu Val Tyr Gl u  
530 535 540

Ala His Met Tyr Val Asp Lys Asp Phe Ser Gly Asn Tyr Phe Asp Lys  
545 550 555 560 565

Ala Gl u Lys Phe Asp Pro Met Ile Gly Val Asn Arg Val Lys Pro Phe  
565 570 575

Val Asp Trp Leu Lys Gl n His Lys Leu Arg Gly Tyr Ile Gly Gl u His  
580 585 590

Gly Val Pro Asp Phe Ser Pro Ser Ala Ile Val Ala Thr Asp Asn Leu  
595 600 605

Leu Ala Tyr Leu Arg Gl n Asn Cys Ile Pro Ser Thr Tyr Trp Ala Ala  
610 615 620

Gly Pro Trp Trp Gly Gl u Tyr Ala Met Ser Leu Asp Val Ser Ser Gly  
625 630 635 640

Lys His Arg Pro Gl n Leu Pro Val Leu Gl n Lys His Ala Lys Thr Ala  
645 650 655

Asn Ser Cys Thr Ser Ile Gly Pro Leu  
660 665

<210> 19  
<211> 8  
<212> PRT  
<213> Arti fici al

<220>  
<223> Has tag

eol f-seq1

<400> 19

His His His His His His Pro Arg  
1 5

<210> 20

<211> 27

<212> PRT

<213> Bacillus clausii

<400> 20

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

Ser Val Ala Phe Ser Ser Ser Ile Ala Ser Ala  
20 25

<210> 21

<211> 795

<212> PRT

<213> Paenibacillus sp

<220>

<221> mat\_peptide

<222> (28)..(795)

<400> 21

Met Lys Lys Pro Leu Gly Lys Ile Val Ala Ser Thr Ala Leu Leu Ile  
-25 -20 -15

Ser Val Ala Phe Ser Ser Ser Ile Ala Ser Ala His His His His His  
-10 -5 -1 1 5

His Pro Arg Ala Glu Ala Ser Asp Met Phe Asp Glu Leu Arg Glu Lys  
10 15 20

Tyr Ala Thr Met Leu Thr Gly Gly Thr Ala Tyr Ser Leu Ser Asp Pro  
25 30 35

Asp Ile Ala Ala Arg Val Ala Ser Ile Thr Thr Asn Ala Gln Thr Leu  
40 45 50

Trp Thr Ser Met Lys Lys Asp Ala Asn Arg Val Arg Leu Trp Asp Asn  
55 60 65

Ala Pro Leu Gly Asn Asp Ser Ala Ser Ile Thr Thr Ser Tyr Arg Gln  
70 75 80 85

Leu Ala Ala Met Ala Leu Ala Tyr Arg Thr Tyr Gly Ser Ser Leu Met  
90 95 100

Gly Asp Pro Asp Leu Arg Asp Asp Ile Ile Asp Gly Leu Asp Trp Ile  
105 110 115

eol f-seql

Asn Thr Phe Gln His Gly Phe Cys Glu Gly Cys Ser Met Tyr Gln Asn  
120 125 130

Trp Trp His Trp Gln Ile Gly Gly Pro Ile Ala Leu Asn Glu Val Ile  
135 140 145

Ala Leu Met Tyr Asp Glu Leu Thr Gln Thr Gln Ile Asp Ser Tyr Ile  
150 155 160 165

Ala Ala Ile Asn Tyr Ala Gln Pro Ser Val Asn Met Thr Gly Ala Asn  
170 175 180

Arg Leu Trp Glu Ser Gln Val Ile Ala Leu Ala Gly Ile Asn Gly Lys  
185 190 195

Asn Gly Asp Lys Ile Ala His Ala Arg Asp Gly Leu Ser Ala Leu Leu  
200 205 210

Thr Tyr Val Val Gln Gly Asp Gly Phe Tyr Glu Asp Gly Ser Phe Val  
215 220 225

Gln His Ser Tyr Tyr Ser Tyr Asn Gly Gly Tyr Gly Leu Asp Leu Leu  
230 235 240 245

Lys Gly Ile Ala Asp Leu Thr Tyr Leu Leu His Asp Ser Asn Trp Glu  
250 255 260

Val Val Asp Pro Asn Lys Gln Asn Ile Phe Asn Trp Val Tyr Asp Ser  
265 270 275

Phe Glu Pro Phe Ile Tyr Asn Gly Asn Leu Met Asp Met Val Arg Gly  
280 285 290

Arg Glu Ile Ser Arg His Ala Arg Gln Ser Asn Val Val Gly Val Glu  
295 300 305

Ala Val Ala Ala Ile Leu Arg Leu Ser His Val Ala Pro Pro Ala Asp  
310 315 320 325

Ala Ala Ala Phe Lys Ser Met Val Lys His Trp Leu Gln Glu Gly Gly  
330 335 340

Gly Ser Gln Phe Leu Gln Gln Ala Ser Ile Thr His Ile Leu Ser Ala  
345 350 355

Gln Asp Val Leu Asn Asp Ser Gly Ile Val Pro Arg Gly Glu Leu Glu  
360 365 370

Ala Tyr Arg Gln Phe Ala Gly Met Asp Arg Ala Leu Gln Leu Arg Gln  
375 380 385

eol F-seqI

Gly Tyr Gly Phe Gly Ile Ser Met Phe Ser Ser Arg Ile Gly Gly His  
 390 395 400 405  
 Glu Ala Ile Asn Ala Glu Asn Asn Lys Gly Trp His Thr Gly Ala Gly  
 410 415 420  
 Met Thr Tyr Leu Tyr Asn Asn Asp Leu Ser Gln Phe Asn Asp His Phe  
 425 430 435  
 Trp Pro Thr Val Asn Ser Tyr Arg Leu Pro Gly Thr Thr Val Leu Arg  
 440 445 450  
 Asp Thr Pro Gln Ala Ala Asn Thr Arg Gly Asp Arg Ser Trp Ala Gly  
 455 460 465  
 Gly Thr Asp Met Leu Gly Leu Tyr Gly Ile Thr Gly Met Glu Tyr His  
 470 475 480 485  
 Ala Ile Gly Lys Ser Leu Thr Ala Lys Lys Ser Trp Phe Met Phe Asp  
 490 495 500  
 Asp Glu Ile Val Ala Leu Gly Ala Asp Ile Thr Ser Gly Asp Gly Val  
 505 510 515  
 Ala Val Glu Thr Ile Val Glu Asn Arg Lys Leu Asn Gly Ala Gly Asp  
 520 525 530  
 Asn Ser Leu Thr Val Asn Gly Thr Ala Lys Pro Ala Thr Leu Gly Trp  
 535 540 545  
 Ser Glu Thr Met Gly Thr Thr Ser Tyr Ala His Leu Gly Gly Ser Val  
 550 555 560 565  
 Ala Asp Ser Asp Ile Gly Tyr Tyr Phe Pro Asp Gly Gly Ala Thr Leu  
 570 575 580  
 His Ala Leu Arg Glu Ala Arg Thr Gly Asn Trp Arg Gln Ile Asn Ser  
 585 590 595  
 Ala Gln Gly Ser Pro Asn Ala Pro His Thr Arg Asn Tyr Leu Thr Met  
 600 605 610  
 Trp Leu Glu His Gly Val Asn Pro Ser Asn Gly Ala Tyr Ser Tyr Val  
 615 620 625  
 Leu Leu Pro Asn Lys Thr Ser Ala Ala Thr Ala Ser Tyr Ala Ala Ser  
 630 635 640 645  
 Pro Asp Ile Thr Ile Ile Glu Asn Ser Ser Ser Ala Gln Ala Val Lys  
 650 655 660

eol f-seq1

Glu Asn Gly Leu Asn Met Ile Gly Val Asn Phe Trp Asn Asn Glu Arg  
665 670 675

Lys Thr Ala Gly Gly Ile Thr Ser Asn Ala Lys Ala Ser Val Met Thr  
680 685 690

Arg Glu Thr Ala Ser Glu Leu Asn Val Ser Val Ser Asp Pro Thr Glu  
695 700 705

Ser Asn Val Gly Met Ile Tyr Ile Glu Ile Asp Lys Ser Ala Thr Gly  
710 715 720 725

Leu Ile Ala Lys Asp Asp Ala Val Thr Val Leu Glu Tyr Ser Pro Thr  
730 735 740

Ile Lys Phe Lys Val Asp Val Asn Lys Ala Arg Gly Lys Ser Phe Lys  
745 750 755

Ala Ala Phe Ser Leu Thr Gly Ala Glu Glu Pro  
760 765

<210> 22  
<211> 1073  
<212> PRT  
<213> Paenibacillus sp

<220>  
<221> mat\_peptide  
<222> (28)..(1973)

<400> 22

Met Lys Lys Pro Leu Gly Lys Ile Val Ala Ser Thr Ala Leu Leu Ile  
-25 -20 -15

Ser Val Ala Phe Ser Ser Ser Ile Ala Ser Ala His His His His His  
-10 -5 -1 1 5

His Pro Arg Ala Asp Glu Phe Asp Thr Leu Arg Glu Lys Tyr Lys Ala  
10 15 20

Met Leu Asn Gly Gly Thr Thr Tyr Asn Leu Ser Asp Pro Asp Ile Ala  
25 30 35

Ala Arg Val Asn Ala Ile Thr Val Thr Ala Glu Gly Tyr Trp Asp Ser  
40 45 50

Met Leu Lys Asp Pro Asn Arg Asn Arg Leu Trp Asn Asp Ala Pro Phe  
55 60 65

Gly Ser Asp Ser Thr Ser Ile Thr Thr Thr Tyr Arg His Leu Tyr Asp  
70 75 80 85

eol F-seqI

Met Ala Leu Ala Tyr Thr Thr Tyr Gly Ser Ser Leu Gln Gly Asn Ala  
90 95 100

Ala Leu Lys Ala Asp Ile Ile Ser Gly Leu Asp Trp Met Asn Ala Asn  
105 110 115

Gln Phe Tyr Asn Gly Cys Ser Gln Tyr Gln Asn Trp Trp His Trp Gln  
120 125 130

Ile Gly Gly Pro Met Ala Leu Asn Asp Ile Val Ala Leu Met Tyr Thr  
135 140 145

Glu Leu Thr Ala Thr Gln Ile Ser Asn Tyr Met Ala Ala Ile Tyr Tyr  
150 155 160 165

Thr Gln Ala Ser Val Thr Met Thr Gly Ala Asn Arg Leu Trp Glu Ser  
170 175 180

Gln Val Ile Ala Ile Ser Gly Ile Leu Asn Lys Asp Ser Ala Arg Val  
185 190 195

Ala Ala Gly Arg Asp Gly Ile Ser Ala Leu Leu Pro Tyr Val Ala Lys  
200 205 210

Gly Asp Gly Phe Tyr Asn Asp Gly Ser Phe Val Gln His Thr Tyr Tyr  
215 220 225

Ala Tyr Asn Gly Gly Tyr Gly Ser Glu Leu Leu Ser Gly Ile Ala Asp  
230 235 240 245

Leu Ile Phe Ile Leu Asn Gly Ser Ser Trp Gln Val Thr Asp Pro Asn  
250 255 260

Lys Asn Asn Val Tyr Arg Trp Ile Tyr Asp Ser Tyr Glu Pro Phe Ile  
265 270 275

Tyr Lys Gly Asn Leu Met Asp Met Val Arg Gly Arg Glu Ile Ser Arg  
280 285 290

His Gly Leu Gln Asp Asp Lys Ala Ala Val Thr Val Met Ala Ser Ile  
295 300 305

Ile Arg Leu Ser Gln Thr Ala Ala Ser Ala Asp Ala Thr Ala Phe Lys  
310 315 320 325

Arg Met Val Lys Tyr Trp Leu Leu Leu Asp Thr Asp Lys Thr Phe Leu  
330 335 340

Lys Ala Val Ser Ile Asp Leu Ile Ile Ala Ala Asn Gln Leu Val Asn  
345 350 355



eol f-seql

Asp Ser Thr Val Thr Ser Arg Gly Glu Leu Val Lys Tyr Lys Gl n Phe  
360 365 370

Ser Gly Met Asp Arg Ala Val Gl n Leu Arg Pro Gly Phe Gly Phe Gly  
375 380 385

Leu Ser Met Phe Ser Ser Arg Ile Gly Asn Tyr Glu Ser Ile Asn Ala  
390 395 400 405

Glu Asn Asn Lys Gly Trp His Thr Gly Asp Gly Met Thr Tyr Leu Tyr  
410 415

Asn Thr Asp Leu Ser Gl n Phe Asn Asp His Phe Trp Ala Thr Val Asp  
425 430 435

Asn Tyr Arg Leu Pro Gly Thr Thr Val Leu Gl n Asn Thr Thr Gl n Thr  
440 445 450

Ala Asn Ser Arg Ser Asp Lys Ser Trp Ala Gly Gly Thr Asp Ile Leu  
455 460 465

Gly Gl n Tyr Gly Val Ser Gly Met Glu Leu His Thr Val Gly Lys Ser  
470 475 480 485

Leu Thr Ala Lys Lys Ser Trp Phe Met Phe Asp Asp Glu Ile Val Ala  
490 495 500

Leu Gly Ser Gly Ile Ala Ser Thr Asp Gly Ile Ala Thr Glu Thr Ile  
505 510 515

Val Glu Asn Arg Lys Leu Asn Ser Ser Gly Asn Asn Ala Leu Ile Val  
520 525 530

Asn Gly Thr Ala Lys Pro Gly Ser Leu Gly Trp Ser Glu Thr Met Thr  
535 540 545

Gly Thr Asn Tyr Ile His Leu Ala Gly Ser Val Pro Gly Ser Asp Ile  
550 555 560 565

Gly Tyr Tyr Phe Pro Gly Gly Ala Ala Val Lys Gly Leu Arg Glu Ala  
570 575 580

Arg Ser Gly Ser Trp Ser Ser Leu Asn Ser Ser Ala Ser Trp Lys Asp  
585 590 595

Ser Thr Leu His Thr Arg Asn Phe Met Thr Leu Trp Phe Asp His Gly  
600 605 610

Met Asn Pro Thr Asn Gly Ser Tyr Ser Tyr Val Leu Leu Pro Asn Lys  
615 620 625

eol f-seql

Thr Ser Ser Ala Val Ala Ser Tyr Ala Ala Thr Pro Gln Ile Ser Ile  
630 635 640 645

Leu Glu Asn Ser Ser Ser Ala Gln Ala Val Lys Glu Thr Gln Leu Asn  
650 655 660

Val Thr Gly Ile Asn Phe Trp Asn Asp Glu Pro Thr Thr Val Gly Leu  
665 670

Val Thr Ser Asn Arg Lys Ala Ser Val Met Thr Lys Glu Thr Ala Ser  
680 685 690

Asp Phe Glu Ile Ser Val Ser Asp Pro Thr Gln Ser Asn Val Gly Thr  
695 700 705

Ile Tyr Ile Asp Val Asn Lys Ser Ala Thr Gly Leu Ile Ser Lys Asp  
710 715 720 725

Asn Glu Ile Thr Val Ile Gln Tyr Tyr Pro Thr Met Lys Phe Lys Val  
730 735 740

Asn Val Asn Asn Ser Gly Gly Lys Ser Tyr Lys Val Lys Phe Ser Leu  
745 750 755

Thr Gly Thr Pro Gly Ser Asn Pro Ser Pro Ile Pro Ile Pro Asn Pro  
760 765 770

Tyr Glu Ala Glu Ala Leu Pro Ile Asn Ala Leu Thr Asp Thr Pro Val  
775 780 785

Val Tyr Asn Asp Ala Asn Ala Ser Gly Gly Lys Lys Leu Gly Phe Asn  
790 795 800 805

Asn Asn Ala Val Asp Asp Tyr Val Glu Phe Ser Leu Asp Val Thr Gln  
810 815 820

Pro Gly Thr Tyr Asp Val Lys Ser Arg Ile Met Lys Ser Thr Asn Ser  
825 830 835

Gly Ile Tyr Gln Leu Ser Ile Asn Gly Thr Asn Val Gly Ser Ala Gln  
840 845 850

Asp Met Phe Trp Thr Thr Ser Glu Leu Ser Lys Glu Phe Thr Met Gly  
855 860 865

Ser Tyr Ser Phe Ser Thr Pro Gly Ser Tyr Leu Phe Arg Leu Lys Thr  
870 875 880 885

Thr Gly Lys Asn Val Ser Ser Ser Gly Tyr Lys Leu Met Leu Asp Asn  
890 895 900

eol f-seql

Phe Ser Leu Val Ser Thr Gly Ile Asp Thr Thr Val Ile Val Asp Asn  
 905 910 915

Ala Asp Ala Ala Gly Val Thr Lys Val Gly Thr Trp Thr Gly Thr Asn  
 920 925 930

Thr Gln Thr Asp Arg Tyr Gly Ala Asp Tyr Ile His Asp Gly Asn Thr  
 935 940 945

Gly Lys Gly Thr Lys Ser Val Thr Phe Thr Pro Asn Val Pro Ile Ser  
 950 955 960

Gly Thr Tyr Gln Val Tyr Met Met Trp Ala Ala His Thr Asn Arg Ala  
 970 975 980

Thr Asn Val Pro Val Asp Val Thr His Ser Gly Gly Thr Ala Thr Leu  
 985 990 995

Asn Val Asn Gln Gln Gly Asn Gly Gly Val Trp Asn Leu Leu Gly  
 1000 1005 1010

Thr Tyr Ser Phe Asn Ala Gly Ser Thr Gly Ala Ile Lys Ile Arg  
 1015 1020 1025

Thr Asp Ala Thr Asn Gly Tyr Val Val Ala Asp Ala Val Lys Leu  
 1030 1035 1040

Val Lys Val Pro  
 1045

<210> 23  
 <211> 1078  
 <212> PRT  
 <213> Paenibacillus sp

<220>  
 <221> mat\_peptide  
 <222> (28)..(1078)

<400> 23

Met Lys Lys Pro Leu Gly Lys Ile Val Ala Ser Thr Ala Leu Leu Ile  
 -25 -20 -15

Ser Val Ala Phe Ser Ser Ser Ile Ala Ser Ala His His His His His  
 -10 -5 -1 1 5

His Pro Arg Ala Glu Ala Ser Asp Met Phe Asp Glu Leu Arg Glu Lys  
 10 15 20

Tyr Ala Thr Met Leu Thr Gly Gly Thr Ala Tyr Ser Leu Ser Asp Pro  
 25 30 35

eol f-seql

Asp Ile Ala Ala Arg Val Ala Ser Ile Thr Thr Asn Ala Gln Thr Leu  
40 45 50

Trp Thr Ser Met Lys Lys Asp Ala Asn Arg Val Arg Leu Trp Asp Asn  
55 60 65

Ala Pro Leu Gly Asn Asp Ser Ala Ser Ile Thr Thr Ser Tyr Arg Gln  
70 75 80 85

Leu Ala Ala Met Ala Leu Ala Tyr Arg Thr Tyr Gly Ser Ser Leu Met  
90 95 100

Gly Asp Pro Asp Leu Arg Asp Asp Ile Ile Asp Gly Leu Asp Trp Ile  
105 110 115

Asn Thr Phe Gln His Gly Phe Cys Glu Gly Cys Ser Met Tyr Gln Asn  
120 125 130

Trp Trp His Trp Gln Ile Gly Gly Pro Ile Ala Leu Asn Glu Val Ile  
135 140 145

Ala Leu Met Tyr Asp Glu Leu Thr Gln Thr Gln Ile Asp Ser Tyr Ile  
150 155 160 165

Ala Ala Ile Asn Tyr Ala Gln Pro Ser Val Asn Met Thr Gly Ala Asn  
170 175 180

Arg Leu Trp Glu Ser Gln Val Ile Ala Leu Ala Gly Ile Asn Gly Lys  
185 190 195

Asn Gly Asp Lys Ile Ala His Ala Arg Asp Gly Leu Ser Ala Leu Leu  
200 205 210

Thr Tyr Val Val Gln Gly Asp Gly Phe Tyr Glu Asp Gly Ser Phe Val  
215 220 225

Gln His Ser Tyr Tyr Ser Tyr Asn Gly Gly Tyr Gly Leu Asp Leu Leu  
230 235 240 245

Lys Gly Ile Ala Asp Leu Thr Tyr Leu Leu His Asp Ser Asn Trp Glu  
250 255 260

Val Val Asp Pro Asn Lys Gln Asn Ile Phe Asn Trp Val Tyr Asp Ser  
265 270 275

Phe Glu Pro Phe Ile Tyr Asn Gly Asn Leu Met Asp Met Val Arg Gly  
280 285 290

Arg Glu Ile Ser Arg His Ala Arg Gln Ser Asn Val Val Gly Val Glu  
295 300 305

eol F-seqI

Ala Val Ala Ala Ile Leu Arg Leu Ser His Val Ala Pro Pro Ala Asp  
 310 315 320 325

Ala Ala Ala Phe Lys Ser Met Val Lys His Trp Leu Gl n Gl u Gly Gly  
 330 335 340

Gly Ser Gl n Phe Leu Gl n Gl n Ala Ser Ile Thr His Ile Leu Ser Ala  
 345 350 355

Gl n Asp Val Leu Asn Asp Ser Gly Ile Val Pro Arg Gly Gl u Leu Gl u  
 360 365 370

Ala Tyr Arg Gl n Phe Ala Gly Met Asp Arg Ala Leu Gl n Leu Arg Gl n  
 375 380 385

Gly Tyr Gly Phe Gly Ile Ser Met Phe Ser Ser Arg Ile Gly Gly His  
 390 395 400 405

Gl u Ala Ile Asn Ala Gl u Asn Asn Lys Gly Trp His Thr Gly Ala Gly  
 410 415 420

Met Thr Tyr Leu Tyr Asn Asn Asp Leu Ser Gl n Phe Asn Asp His Phe  
 425 430 435

Trp Pro Thr Val Asn Ser Tyr Arg Leu Pro Gly Thr Thr Val Leu Arg  
 440 445 450

Asp Thr Pro Gl n Ala Ala Asn Thr Arg Gly Asp Arg Ser Trp Ala Gly  
 455 460 465

Gly Thr Asp Met Leu Gly Leu Tyr Gly Ile Thr Gly Met Gl u Tyr His  
 470 475 480 485

Ala Ile Gly Lys Ser Leu Thr Ala Lys Lys Ser Trp Phe Met Phe Asp  
 490 495 500

Asp Gl u Ile Val Ala Leu Gly Ala Asp Ile Thr Ser Gly Asp Gly Val  
 505 510 515

Ala Val Gl u Thr Ile Val Gl u Asn Arg Lys Leu Asn Gly Ala Gly Asp  
 520 525 530

Asn Ser Leu Thr Val Asn Gly Thr Ala Lys Pro Ala Thr Leu Gly Trp  
 535 540 545

Ser Gl u Thr Met Gly Thr Thr Ser Tyr Ala His Leu Gly Gly Ser Val  
 550 555 560 565

Ala Asp Ser Asp Ile Gly Tyr Tyr Phe Pro Asp Gly Gly Ala Thr Leu  
 570 575 580

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Hi s Ala Leu Arg Glu Ala Arg Thr Gly Asn Trp Arg Gln Ile Asn Ser  
 585 590 595

Ala Gln Gly Ser Pro Asn Ala Pro His Thr Arg Asn Tyr Leu Thr Met  
 600 605 610

Trp Leu Glu His Gly Val Asn Pro Ser Asn Gly Ala Tyr Ser Tyr Val  
 615 620 625

Leu Leu Pro Asn Lys Thr Ser Ala Ala Thr Ala Ser Tyr Ala Ala Ser  
 630 635 640 645

Pro Asp Ile Thr Ile Ile Glu Asn Ser Ser Ser Ala Gln Ala Val Lys  
 650 655 660

Glu Asn Gly Leu Asn Met Ile Gly Val Asn Phe Trp Asn Asn Glu Arg  
 665 670 675

Lys Thr Ala Gly Gly Ile Thr Ser Asn Ala Lys Ala Ser Val Met Thr  
 680 685 690

Arg Glu Thr Ala Ser Glu Leu Asn Val Ser Val Ser Asp Pro Thr Gln  
 695 700 705

Ser Asn Val Gly Met Ile Tyr Ile Glu Ile Asp Lys Ser Ala Thr Gly  
 710 715 720 725

Leu Ile Ala Lys Asp Asp Ala Val Thr Val Leu Gln Tyr Ser Pro Thr  
 730 735 740

Ile Lys Phe Lys Val Asp Val Asn Lys Ala Arg Gly Lys Ser Phe Lys  
 745 750 755

Ala Ala Phe Ser Leu Thr Gly Ala Gln Gln Pro Asn Pro Ala Pro Ile  
 760 765 770

Pro Ile Pro Asn Pro Tyr Glu Ala Glu Leu Leu Pro Ile Ser Ala Thr  
 775 780 785

Thr Lys Thr Pro Thr Leu Ser Asn Asp Ser Asn Ala Ser Gly Gly Lys  
 790 795 800 805

Lys Leu Gly Leu Asn Ser Ser Val Val Gly Asp Tyr Thr Glu Phe Ser  
 810 815 820

Leu Asp Val Thr Gln Pro Gly Thr Tyr Asp Ile Ala Ala Lys Ile Met  
 825 830 835

Lys Val Ser Asn Asn Gly Ile Tyr Gln Phe Ser Ile Asn Gly Glu Pro  
 840 845 850

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Val Gly Asp Pro Val Asp Met Tyr Trp Asn Thr Ser Glu Ser Thr Lys  
855 860 865

Ser Phe Ser Pro Gly Ser Tyr Thr Phe Ser Glu Pro Gly Ser Tyr Leu  
870 875 880 885

Leu Arg Val Thr Val Thr Gly Lys His Pro Ser Ser Ser Gly Tyr Lys  
890 895 900

Leu Met Leu Asp His Phe Thr Leu Glu Glu Ile Pro Val Ser Leu Pro  
905 910 915

Asn Pro Tyr Glu Ala Glu Thr Leu Pro Ile His His Arg Thr Gl n Thr  
920 925 930

Val Thr Ile Tyr Asn Asp Ser Asn Thr Ser Gly Gly Gl n Arg Leu Gly  
935 940 945

Leu Asn His Lys Val Val Gly Asp Tyr Thr Glu Phe Ile Leu Asp Val  
950 955 960 965

Pro Gl n Ala Gly Thr Tyr Asp Ile Thr Ala Arg Val Leu Lys Phe Ser  
970 975 980

Asp Asn Gly Ile Tyr Gl n Phe Ser Ile Asp Gly Asn Pro Val Gly Ala  
985 990 995

Pro Ile Asp Thr Tyr Trp Asn Thr Ala Gly Tyr Ile Arg Asp Phe  
1000 1005 1010

Thr Pro Gly Ser Tyr Thr Phe Ser Glu Pro Gly Ser Tyr Leu Leu  
1015 1020 1025

Arg Leu Thr Ala Thr Gly Lys Asn Pro Ser Ala Ser Gly Leu Lys  
1030 1035 1040

Ile Met Leu Asp Tyr Ile Trp Leu Asp  
1045 1050

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-25 -20 -15

eol f-seql

Ser Val Ala Phe Ser Ser Ser Ile Ala Ser Ala His His His His His  
 -10 -5 -1 1 5

His Pro Arg Gly Gly Glu Ala Ser Gly Ser Ala Asp Asp Ala Ala Glu  
 10 15 20

Thr Ala Glu Ala Ala Glu Gly Glu Asn Ile Glu Asp Lys Met Val Ser  
 25 30 35

Ala Tyr Asn Met Asp Ala Phe Asp Ile Met Arg Glu Val Arg Arg Thr  
 40 45 50

Met Leu Thr Gly Gly Ala Ala Leu Asn Pro Ala Asp Pro Asp Ala Ala  
 55 60 65

Ala Ala Val Ala Ala Leu Ala Ser Glu Ala Asn Gln Tyr Trp Gln Thr  
 70 75 80 85

Met Asp Asp Ser Pro Gly Arg Thr Ser Leu Trp Ser Asp Asn Pro Gly  
 90 95 100

Thr Gly Asn Ser Ile His Ile Arg Ile Thr Tyr Glu Arg Leu Lys Thr  
 105 110 115

Met Ala Leu Ala Tyr Ala Ala Ala Gly Ser Pro Leu His Ser Asn Ala  
 120 125 130

Ser Leu Glu Ala Asp Ile Val Asp Ala Leu Asp Tyr Met Tyr Ala Thr  
 135 140 145

Arg Tyr His Glu Asn Val Thr Thr Thr Pro Ser Gly Thr Ser Asn Trp  
 150 155 160 165

Trp Asp Trp Gln Ile Gly Ile Pro Met Gln Leu Asn Asp Thr Val Val  
 170 175 180

Leu Met Tyr Asp Ser Leu Thr Pro Ala Gln Ile Ala Asn Tyr Met Asn  
 185 190 195

Ala Val Glu Arg Phe Thr Pro Thr Val Asn Leu Thr Gly Ala Asn Arg  
 200 205 210

Ser Trp Lys Ala Ile Val Val Ala Val Arg Gly Ile Leu Val Lys Asp  
 215 220 225

Gly Ala Lys Ile Ala Ala Ala Arg Asp Gly Leu Ser Gln Ile Phe Asn  
 230 235 240 245

Tyr Ala Val Ser Gly Asp Gly Phe Tyr Arg Asp Gly Ser Phe Ile Gln  
 250 255 260



eol f-seql

His Gly Asn Ile Pro Tyr Asn Gly Gly Tyr Gly Leu Asp Leu Leu Leu  
 265 270 275  
 Ala Val Ser Asp Leu Met Thr Leu Leu His Gly Ser Ala Trp Gln Val  
 280 285 290  
 Thr Asp Pro Asn Gln Ala Asn Val Trp Glu Trp Val Tyr Arg Ala Tyr  
 295 300 305  
 Gln Pro Leu Ile Tyr Lys Gly Ala Met Met Asp Met Val Arg Gly Arg  
 310 315 320 325  
 Glu Ile Ser Arg Val Tyr Arg Gln Asp His Ala Ala Gly His Ile Ala  
 330 335 340  
 Met Gln Gly Ile Leu Arg Leu Ser Ala Val Ala Pro Pro Ala Gln Ala  
 345 350 355  
 Glu Asp Phe Lys Arg Met Val Lys Gly Trp Met Val Val Asp Gly Phe  
 360 365 370  
 Met Arg Phe Tyr Glu Gln Ala Pro Leu Gly Leu Ile Pro Leu Ala Lys  
 375 380 385  
 Ala Val Glu Gly Asp Ala Ser Ile Ala Pro Ala Ser Glu Leu Ile Gln  
 390 395 400 405  
 Tyr Arg Gln Tyr Ala Ala Met Asp Arg Ala Val Gln Leu Arg Pro Gly  
 410 415 420  
 Tyr Gly Phe Gly Leu Ala Met Tyr Ser Ser Arg Ile Gly Ser Phe Glu  
 425 430 435  
 Ala Ile Asn Ser Glu Asn Leu Arg Gly Trp Tyr Thr Ser Ala Gly Met  
 440 445 450  
 Thr Ser Leu Tyr Asn Gly Asp Leu Gly His Tyr Ser Glu Asp Tyr Trp  
 455 460 465  
 Pro Thr Val Asn Ala Tyr Arg Leu Pro Gly Thr Thr Val Leu Ser Gly  
 470 475 480 485  
 Thr Ala Ala Ala Ser His Thr Ser Pro Asn Asn Trp Thr Gly Gly Thr  
 490 495 500 505  
 Asp Met Gln Gly Leu Tyr Gly Val Ser Gly Met Asp Leu Lys Tyr Ala  
 505 510 515  
 Ser Asn Ser Leu Ala Ala Arg Lys Ser Trp Phe Met Phe Asp Asp Glu  
 520 525 530

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Ile Val Ala Leu Gly Ala Gly Ile Ser Ser Ala Asp Gly Ile Pro Val  
535 540 545

Glu Thr Ile Ile Glu Asn Arg Arg Ile Gly Gly Ala Gly Asp Asn Ala  
550 555 560 565

Phe Leu Ala Asp Gly Ala Ala Met Pro Ala Glu Leu Gly Trp Ser Gly  
570 575 580

Thr Leu Glu Gly Val Arg Trp Ala His Leu Thr Gly Thr Ala Ala Gly  
585 590 595

Ala Asp Ile Gly Tyr Tyr Phe Pro Glu Pro Ala Ala Val His Ala Val  
600 605 610

Arg Glu Ala Arg Thr Gly Asn Trp Arg Gln Ile Asn Asn Arg Pro Val  
615 620 625

Thr Pro Ala Ala Ser Val Thr Arg Asn Tyr Leu Thr Phe Trp Phe Asp  
630 635 640 645

His Gly Ala Asn Pro Thr Asn Ala Asp Tyr Gln Tyr Val Leu Leu Pro  
650 655 660

Asn Lys Ser Gly Ala Gln Val Ala Gly Tyr Ala Ala Asn Pro Asp Val  
665 670 675

Glu Val Leu Ala Asn Ser Pro Glu Val Gln Ala Val Lys Glu Ser Ser  
680 685 690

Leu Gly Ile Ile Gly Ala Asn Phe Trp Ser Asp Gly Val Arg Thr Val  
695 700 705

Asp Leu Ile Thr Val Asn Lys Lys Ala Ser Val Met Thr Arg Glu Thr  
710 715 720 725

Pro Gly Ala Ile Leu Asp Leu Ser Val Ser Asp Pro Thr Gln Val Asn  
730 735 740

Ala Gly Thr Ile Glu Ile Glu Leu Asn Arg Ala Ala Ser Gly Phe Thr  
745 750 755

Ala Asp Pro Gly Val Thr Val Thr Arg Leu Ser Pro Thr Ile Lys Leu  
760 765 770

Thr Val Gln Val Ala Gly Ala Lys Gly Arg Ser Phe Lys Ala Ser Phe  
775 780 785

Glu Leu Gly Glu Ala Ser Gly Pro Gly Pro Asp Pro Gly Pro Gly Pro  
790 795 800 805

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Ser Glu Ile Ile Val Asp Asn Gly Asp Ala Ala Gly Val Thr Lys Ile  
810 815 820

Gly Ser Trp Lys Thr Gly Thr Val Gln Thr Asp Arg Tyr Gly Pro Asp  
825 830 835

Tyr Leu His Asp Asp Asn Thr Gly Lys Gly Gly Lys Ser Val Arg Phe  
840 845 850

Thr Pro Asp Leu Pro Thr Ala Gly Thr Tyr Asp Val Tyr Met Met Trp  
855 860 865

Pro Gln His Phe Asn Arg Ala Thr Asn Ile Pro Val Thr Ile Ala His  
870 875 880 885

Ala Gly Gly Thr Ala Thr Val Thr Ile Asp Gln Thr Val Ser Gly Gly  
890 895 900

Val Trp Asn Tyr Leu Gly Ser Tyr Ser Phe Asp Thr Gly Ser Gly Gly  
905 910 915

Ser Val Thr Ile Ser Asn Ala Gly Thr Asn Gly Tyr Val Val Ala Asp  
920 925 930

Ala Val Lys Phe Glu Tyr Val Pro  
935 940

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<220>  
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<400> 25

Met Leu Lys Gln Gly Met Lys Arg Trp Thr Ser Val Cys Leu Ala Ile  
1 5 10 15

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

Glu Ala Ser Asp Met Phe Asp Glu Leu Arg Glu Lys Tyr Ala Thr Met  
35 40 45

Leu Thr Gly Gly Thr Ala Tyr Ser Leu Ser Asp Pro Asp Ile Ala Ala  
50 55 60

Arg Val Ala Ser Ile Thr Thr Asn Ala Gln Thr Leu Trp Thr Ser Met  
65 70 75 80

Lys Lys Asp Ala Asn Arg Val Arg Leu Trp Asp Asn Ala Pro Leu Gly

eol f-seq1  
90

85

95

Asn Asp Ser Ala Ser Ile Thr Thr Ser Tyr Arg Gl n Leu Ala Ala Met  
100 105 110

Ala Leu Ala Tyr Arg Thr Tyr Gly Ser Ser Leu Met Gly Asp Pro Asp  
115 120 125

Leu Arg Asp Asp Ile Ile Asp Gly Leu Asp Trp Ile Asn Thr Phe Gl n  
130 135 140

His Gly Phe Cys Glu Gly Cys Ser Met Tyr Gl n Asn Trp Trp His Trp  
145 150 155 160

Gl n Ile Gly Gly Pro Ile Ala Leu Asn Gl u Val Ile Ala Leu Met Tyr  
165 170 175

Asp Gl u Leu Thr Gl n Thr Gl n Ile Asp Ser Tyr Ile Ala Ala Ile Asn  
180 185 190

Tyr Ala Gl n Pro Ser Val Asn Met Thr Gly Ala Asn Arg Leu Trp Gl u  
195 200 205

Ser Gl n Val Ile Ala Leu Ala Gly Ile Asn Gly Lys Asn Gly Asp Lys  
210 215 220

Ile Ala His Ala Arg Asp Gly Leu Ser Ala Leu Leu Thr Tyr Val Val  
225 230 235 240

Gl n Gly Asp Gly Phe Tyr Gl u Asp Gly Ser Phe Val Gl n His Ser Tyr  
245 250 255

Tyr Ser Tyr Asn Gly Gly Tyr Gly Leu Asp Leu Leu Lys Gly Ile Ala  
260 265 270

Asp Leu Thr Tyr Leu Leu His Asp Ser Asn Trp Gl u Val Val Asp Pro  
275 280 285

Asn Lys Gl n Asn Ile Phe Asn Trp Val Tyr Asp Ser Phe Gl u Pro Phe  
290 295 300

Ile Tyr Asn Gly Asn Leu Met Asp Met Val Arg Gly Arg Gl u Ile Ser  
305 310 315 320

Arg His Ala Arg Gl n Ser Asn Val Val Gly Val Gl u Ala Val Ala Ala  
325 330 335

Ile Leu Arg Leu Ser His Val Ala Pro Pro Ala Asp Ala Ala Ala Phe  
340 345 350

Lys Ser Met Val Lys His Trp Leu Gl n Gl u Gly Gly Gly Ser Gl n Phe

## eol f-seql

355

360

365

Leu Gln Gln Ala Ser Ile Thr His Ile Leu Ser Ala Gln Asp Val Leu  
 370 375 380

Asn Asp Ser Gly Ile Val Pro Arg Gly Glu Leu Glu Ala Tyr Arg Gln  
 385 390 395 400

Phe Ala Gly Met Asp Arg Ala Leu Gln Leu Arg Gln Gly Tyr Gly Phe  
 405 410 415

Gly Ile Ser Met Phe Ser Ser Arg Ile Gly Gly His Glu Ala Ile Asn  
 420 425 430

Ala Glu Asn Asn Lys Gly Trp His Thr Gly Ala Gly Met Thr Tyr Leu  
 435 440 445

Tyr Asn Asn Asp Leu Ser Gln Phe Asn Asp His Phe Trp Pro Thr Val  
 450 455 460

Asn Ser Tyr Arg Leu Pro Gly Thr Thr Val Leu Arg Asp Thr Pro Gln  
 465 470 475 480

Ala Ala Asn Thr Arg Gly Asp Arg Ser Trp Ala Gly Gly Thr Asp Met  
 485 490 495

Leu Gly Leu Tyr Gly Ile Thr Gly Met Glu Tyr His Ala Ile Gly Lys  
 500 505 510

Ser Leu Thr Ala Lys Lys Ser Trp Phe Met Phe Asp Asp Glu Ile Val  
 515 520 525

Ala Leu Gly Ala Asp Ile Thr Ser Gly Asp Gly Val Ala Val Glu Thr  
 530 535 540

Ile Val Glu Asn Arg Lys Leu Asn Gly Ala Gly Asp Asn Ser Leu Thr  
 545 550 555 560

Val Asn Gly Thr Ala Lys Pro Ala Thr Leu Gly Trp Ser Glu Thr Met  
 565 570 575

Gly Thr Thr Ser Tyr Ala His Leu Gly Gly Ser Val Ala Asp Ser Asp  
 580 585 590

Ile Gly Tyr Tyr Phe Pro Asp Gly Gly Ala Thr Leu His Ala Leu Arg  
 595 600 605

Glu Ala Arg Thr Gly Asn Trp Arg Gln Ile Asn Ser Ala Gln Gly Ser  
 610 615 620

Pro Asn Ala Pro His Thr Arg Asn Tyr Leu Thr Met Trp Leu Glu His



## eol f-seq1

900

905

910

Val Thr Gly Lys His Pro Ser Ser Ser Gly Tyr Lys Leu Met Leu Asp  
 915 920 925

His Phe Thr Leu Glu Glu Ile Pro Val Ser Leu Pro Asn Pro Tyr Glu  
 930 935 940

Ala Glu Thr Leu Pro Ile His His Arg Thr Gln Thr Val Thr Ile Tyr  
 945 950 955 960 965 970

Asn Asp Ser Asn Thr Ser Gly Gly Gln Arg Leu Gly Leu Asn His Lys  
 965 970 975

Val Val Gly Asp Tyr Thr Glu Phe Ile Leu Asp Val Pro Gln Ala Gly  
 980 985 990

Thr Tyr Asp Ile Thr Ala Arg Val Leu Lys Phe Ser Asp Asn Gly Ile  
 995 1000 1005

Tyr Gln Phe Ser Ile Asp Gly Asn Pro Val Gly Ala Pro Ile Asp  
 1010 1015 1020

Thr Tyr Trp Asn Thr Ala Gly Tyr Ile Arg Asp Phe Thr Pro Gly  
 1025 1030 1035

Ser Tyr Thr Phe Ser Glu Pro Gly Ser Tyr Leu Leu Arg Leu Thr  
 1040 1045 1050

Ala Thr Gly Lys Asn Pro Ser Ala Ser Gly Leu Lys Ile Met Leu  
 1055 1060 1065

Asp Tyr Ile Trp Leu Asp  
 1070