(19) DANMARK

(10) DK/EP 2510094 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *C 12 N 9/64 (2006.01)* *C 12 N 9/52 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2017-03-13**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2016-11-30**

(86) Europæisk ansøgning nr.: **10836666.7**

(86) Europæisk indleveringsdag: **2010-12-09**

(87) Den europæiske ansøgnings publiceringsdag: **2012-10-17**

(86) International ansøgning nr.: **US2010059628**

(87) Internationalt publikationsnr.: **WO2011072099**

(30) Prioritet: **2009-12-09 US 285127 P** **2010-10-12 US 392373 P**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

(73) Patenthaver: **Danisco US Inc., 925 Page Mill Road, Palo Alto, California 94304, USA**

(72) Opfinder: **BASLER, Joshua Roy, Danisco US INC., 925 Page Mill Road, Palo Alto, California 94304, USA**
CASCAO-PEREIRA, Luis Gustavo, Danisco Us Inc., 925 Page Mill Road, Palo Alto, California 94304, USA
ESTELL, David A., Danisco Us Inc., 925 Page Mill Road, Palo Alto, California 94304, USA
KELLIS, James T., Danisco US INC., 925 Page Mill Road, Palo Alto, California 94304, USA
PISARCHIK, Alexander, Danisco US INC., 925 Page Mill Road, Palo Alto, California 94304, USA
POULOSE, Ayrookaran J., Danisco US INC., 925 Page Mill Road, Palo Alto, California 94304, USA
TORRES-PAZMINO, Daniel, Danisco US INC., 925 Page Mill Road, Palo Alto, California 94304, USA

(74) Fuldmægtig i Danmark: **Marks & Clerk (Luxembourg) LLP, 44 rue de la Vallée, B.P. 1775, L-1017 Luxembourg, Luxembourg**

(54) Benævnelse: **SAMMENSÆTNINGER OG FREMGANGSMÅDER OMFATTENDE PROTEASEVARIANTER**

(56) Fremdragne publikationer:
US-A1- 2005 239 043
US-B1- 6 586 223
BRODE PHILIP F III ET AL: "Subtilisin BPN' variants: Increased hydrolytic activity on surface-bound substrates via decreased surface activity", BIOCHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 35, no. 10, 1 January 1996 (1996-01-01), pages 3162-3169, XP002380291, ISSN: 0006-2960, DOI: 10.1021/BI951990H
WO-A1-95/29979
WO-A1-95/30010
WO-A2-2009/149144
US-A1- 2002 081 703
US-A1- 2003 118 605
US-A1- 2004 209 343
US-A1- 2005 054 843

Fortsættes ...

US-A1- 2005 148 059

DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention provides protease variants, compositions comprising protease variants, and methods of using such protease variants and compositions thereof.

BACKGROUND OF THE INVENTION

[0002] Although proteases have long been known in the art of industrial enzymes, there remains a need for engineered proteases that are suitable for particular conditions and uses. The present invention fills these and other needs.

[0003] Brode et al. describe the use of site-directed and random mutagenesis to produce variants of subtilisin BPN' (bacillus amyloliquefaciens) protease with variable surface adsorption properties. (biochemistry 1996, 35, 3162-3169.)

[0004] WO 95/29979 describes fabric cleaning compositions containing variants of subtilisin BPN' having modifications at positions 199-220.

[0005] US 2004/0209343 describes methods for contructing variants of parent TY145 and BPN' subtilases having altered properties.

[0006] WO 95/30010 describes BPN' variants having modifications in surface loop regions in order to decrease adsorption to insoluble substrates and increase hydrolysis.

SUMMARY OF THE INVENTION

[0007] The invention provides an isolated protease variant, the variant comprising an amino acid sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO:2 and comprising the amino acid substitutions X024G/R, X053G, X078N, X101N, X128A/S, and X217Q/L, wherein the variant has enhanced proteolytic activity and/or cleaning activity compared to the proteolytic activity and/or cleaning activity of the BPN' protease having the sequence of SEQ ID NO:2, and each amino acid position of the variant is numbered by correspondence to an amino acid position in the amino acid sequence of SEQ ID NO:2 as determined by alignment of the amino acid sequence of the variant with SEQ ID NO:2.

[0008] The variant may further comprise the amino acid substitution X097A.

[0009] For example, the variant amino acid sequence may comprise the amino acid substitutions S024G+S053G+S078N+S101N+G128S+Y217Q or S024G+S053G+S078N+S101N+G128A+Y217Q, and optionally may further comprise:

1. (i) a substitution selected from the group consisting of N109G, N076D, S033T, N243V, S248A, A088T, and S063G; or
2. (ii) a set of amino acid substitutions selected from the group consisting of:

   A088T+N109G+A116T+G131H+N243V+L257G,

   S033T+N076D,

   S009T+N109G+K141R+N243V,

   S162G+K256R,

   N109G+A116T,

   N109G+L257G,

   S162G+L257G,

N061G+N109G+N243V,

N109G+N243V+S248A,

S033T+N076D+N109G+N218S+N243V+S248N+K256R,

N109G+A116T+N243V+K256R,

A088T+N109G+A116T+G131H+N243V,

A088T+N109G,

N109G+N243V,

T158S+L257G,

N061S+N109G+N243V,

P040A+N109G+N243V+S248N+K256R,

S009T+S018T+Y021N+N109G+K141R,

A088T+N109G+A116T+T158S+N243V+K256R,

A088T+N109G+A116T+T158S+N218S+L257G,

N109G+K256R,

N109G+N243V+K256R,

S063G+K256R,

S063G+N109G,

S063G,

S063G+N076D,

S033T+N076D+N218S, and

N076D+N218S

[0010] Such a variant may have enhanced proteolytic activity compared to the proteolytic activity of the protease having the sequence of SEQ ID NO:6.

[0011] The variant may have at least 85% sequence identity to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:6.

[0012] For example, the variant may have the amino acid sequence of SEQ ID NO: 6, or the sequence of:

ai) BPN' S024G-S053G-S078N-S101N-G128S-Y217Q;

a) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+A088T+N109G+A116T+ G131H+N243V+L257G;

b) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+S033T+N076D;

c) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+S009T+N109G+ K141R+N243V;

d) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+S162G+K256R;

e) BPN'-S024G+S053G+S078N+S101N+G 128A+Y217Q+N109G+A116T;

f) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N109G+L257G;

g) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+S162G+L257G;

h) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N061G+N109G+N243V;

i) BPN'-S024G+S053G+S078N+S 101N+G 128S+Y217Q+N109G+N243V+S248A;

j) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+S033T+N076D+N109G+ N218S+N243V+S248N+K256R;

k) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N109G+A116T+N243V+ K256R;

1) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+A088T+N109G+A116T+ G131H+N243V;

m) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+A088T+N109G;

n) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N109G+N243V;

o) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+T158S+L257G;

p) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N061S+N109G+N243V;

q) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+P040A+N109G+N243V+ S248N+K256R;

r) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+S009T+S018T+Y021N+ N109G+K141R;

s) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+A088T+N109G+A116T+ T1585+N243V+K256R;

t) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+A088T+N109G+A116T+ T158S+N218S+L257G;

u) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N109G+K256R;

v) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+N109G+N243V+K256R;

w) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+S063G+K256R;

x) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+S063G+N109G;

y) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+S063G;

z) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+S063G+N076D;

aa) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+S033T+N076D+N218S;

bb) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N076D+N218S; or cc) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q.

[0013] The invention further provides an isolated or recombinant nucleic acid comprising a polynucleotide sequence encoding at least one polypeptide variant (e.g., protease variant) of the invention, or a complementary polynucleotide sequence thereof.

[0014] The invention further provides an isolated or recombinant nucleic acid comprising a polynucleotide sequence having at least 80% sequence identity to the polynucleotide sequence set forth in SEQ ID NO:3 or SEQ ID NO:5, or a complementary polynucleotide sequence thereof.

[0015] The invention further provides an expression vector comprising at least one nucleic acid of the invention. Also provided is a recombinant host cell or cell culture comprising at least one nucleic acid or an expression vector of the invention.

[0016] The invention further provides a method of producing at least one polypeptide (e.g., protease variant) of the invention, the method comprising: (a) introducing a recombinant expression vector of the invention which encodes a polypeptide (e.g., protease variant) of the invention into a population of cells; (b) culturing the cells in a culture medium under conditions conducive to produce the polypeptide (e.g., protease variant) encoded by the expression vector; and optionally (c) isolating or recovering the variant from the cells or from the culture medium.

[0017] The invention further provides a composition comprising at least one protease variant or polypeptide of the invention, optionally in combination with another enzyme. Such composition may comprise an adjunct ingredient, such as a surfactant and/or builder, or a carrier. Such composition may be a cleaning composition or a detergent composition and may be useful in cleaning methods described elsewhere herein. Such composition may be a fabric and home care product or such composition may not be

a fabric and home care product.

[0018] The invention further provides a method for cleaning an item, object, or surface in need of cleaning, the method comprising contacting the item, object, or surface with a polypeptide or protease variant of the invention or a composition of the invention, and optionally rinsing the item, object, or surface with water.

[0019] The invention further provides a method for cleaning an item or surface (e.g., hard surface), the method comprising contacting at least a portion of the item or surface (e.g., hard surface) to be cleaned with a polypeptide or protease variant of the invention or a composition of the invention for a sufficient time and/or under conditions sufficient or effective to clean or wash the item or surface (e.g., hard surface) to a desired degree, and optionally comprising rinsing the item or surface (e.g., hard surface) with water.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020]

Figure 1 provides a plasmid map of pHPLT-BPN'-v3.

Figure 2 provides a plasmid map of pHPLT-BPN'-v3+S78N.

Figure 3 provides a plasmid map of pHPLT-BPN' partial opt.

Figure 4 provides a plasmid map of pHPLT-BPN'-v36.

Figure 5 provides an alignment of the mature reference subtilisin proteases including: BPN' (SEQ ID NO:2) and GG36 (SEQ ID NO:755). Each amino acid position of each protease variant described herein, including each cold water protease variant, is numbered according to the numbering of the corresponding amino acid position in the amino acid sequence of *Bacillus amyloliquefaciens* subtilisin protease BPN' (SEQ ID NO:2), as shown in Figure 5, as determined by alignment of the protease variant amino acid sequence with the *Bacillus amyloliquefaciens* subtilisin protease BPN' amino acid sequence. Thus, unless otherwise specified herein, substitution positions are given in relationship to BPN'.

DESCRIPTION OF THE INVENTION

Definitions

[0021] Unless otherwise indicated, the practice of the present invention involves conventional techniques commonly used in molecular biology, protein engineering, microbiology, and recombinant DNA, which are within the skill of the art. Such techniques are known to those of skill in the art and are described in numerous texts and reference works well known to those of skill in the art.

[0022] Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Many technical dictionaries are known to those of skill in the art. Although any methods and materials similar or equivalent to those described herein find use in the practice of the present invention, some suitable methods and materials are described herein. Accordingly, the terms defined immediately below are more fully described by reference to the specification as a whole. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary, depending upon the context they are used by those of skill in the art.

[0023] The practice of the present invention employs, unless otherwise indicated, conventional techniques of protein purification, molecular biology, microbiology, recombinant DNA techniques and protein sequencing, all of which are within the skill of those in the art.

[0024] Furthermore, the headings provided herein are not limitations of the various aspects of the invention which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification as a whole. Nonetheless, in order to facilitate understanding of the invention, a number of terms are defined below.

[0025] As used herein, the terms "protease" and "proteinase" refer to an enzyme protein that has the ability to break down other proteins. A protease has the ability to conduct "proteolysis," which begins protein catabolism by hydrolysis of peptide bonds that link amino acids together in a peptide or polypeptide chain forming the protein. This activity of a protease as a protein-digesting enzyme is referred to as "proteolytic activity." Many well known procedures exist for measuring proteolytic activity (see, e.g., Kalisz, "Microbial Proteinases," *In:* Fiechter (ed.), Advances in Biochemical Engineering/Biotechnology (1988)). For example, proteolytic activity may be ascertained by comparative assays which analyze the respective protease's ability to hydrolyze a commercial substrate. Exemplary substrates useful in the analysis of protease or proteolytic activity, include, but are not limited to, dimethyl casein (Sigma C-9801), bovine collagen (Sigma C-9879), bovine elastin (Sigma E-1625), and bovine keratin (ICN Biomedical 902111). Colorimetric assays utilizing these substrates are well known in the art (see, e.g., WO 99/34011 and U.S. Pat. No. 6,376,450). The pNA assay (see, e.g., Del Mar et al., Anal. Biochem. 99:316-320 [1979]) also finds use in determining the active enzyme concentration for fractions collected during gradient elution. This assay measures the rate at which *p*-nitroaniline is released as the enzyme hydrolyzes the soluble synthetic substrate, succinyl-alanine-alanine-proline-phenylalanine-*p*-nitroanilide (suc-AAPF-pNA). The rate of production of yellow color from the hydrolysis reaction is measured at 410 nm on a spectrophotometer and is proportional to the active enzyme concentration. In addition, absorbance measurements at 280 nanometers (nm) can be used to determine the total protein concentration. The active enzyme/total protein ratio gives the enzyme purity.

[0026] As used herein, the term "subtilisin" refers any member of the S8 serine protease family as described in MEROPS - The Peptidase Data base (see Rawlings et al., MEROPS: the peptidase database, Nucl. Acids Res., 34 Database issue, D270-272 [2006]). As described therein, the peptidase family S8 contains the serine endopeptidase subtilisin and its homologues (Rawlings and Barrett, Biochem. J.. 290:205-218, [1993]). Family S8, also known as the subtilase family, is the second largest family of serine peptidases. The tertiary structures for several members of family S8 have now been determined. A typical S8 protein structure consists of three layers with a seven-stranded β sheet sandwiched between two layers of helices. Subtilisin (S08.001) is the type structure for clan SB (SB). Despite the different structure, the active sites of subtilisin and chymotrypsin (S01.001) can be superimposed, which suggests the similarity is the result of convergent rather than divergent evolution.

[0027] A "protease variant" (or "variant protease") may refer to a protease that differs in its amino acid sequence from the amino acid sequence of a reference protease or parent protease by at least one amino acid residue. A parent protease or reference protease need not be a wild-type protease, but may itself be a variant of a wild-type protease. It is not intended that the reference or parent protease be limited to any particular amino acid sequence. A protease variant of a reference or parent protease may comprise an amino acid sequence comprising at least 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of the parent protease or reference protease and at least one amino acid substitution, insertion, or deletion relative to the amino acid sequence of the parent protease or reference protease. In one aspect, the invention includes a variant of a serine protease, wherein the variant has at least one mutation relative to the serine protease. In one aspect, the present invention includes a "BPN' variant" (or "BPN' subtilisin variant") comprising an amino acid sequence comprising one or more mutations relative to the mature BPN' sequence of SEQ ID NO:2.

[0028] A parent protease or reference protease can be, but is not limited to, e.g., a known protease (including, but not limited to, e.g., BPN') or a commercially available protease or a variant of the commercially available protease. A parent protease or reference protease may itself be a variant of a known or commercially available protease. A protease variant can be derived from a parent protease that is commercially available or a variant of such commercially available parent protease. Commercially available proteases, include, but are not limited to, e.g., proteases sold under the tradenames SAVINASE®, POLARZYME®, KANNASE®, LIQUANASE®, LIQUANASE ULTRA®, SAVINASE ULTRA®, OVOZYME®, (by Novozymes A/S); MAXACAL®, PROPERASE®, PURAFECT®, FN3®, FN4® and PURAFECT OXP®, PURAFAST™, PURAFECT® PRIME, PURAMAX® (by Danisco US Inc., formerly Genencor International, Inc.); and those available from Henkel/Kemira, namely BLAP (amino acid sequence shown in Figure 29 of US 5,352,604 with the following mutations S99D+S101R+S103A+ V104I+G159S, hereinafter referred to as BLAP) and BLAP X (BLAP with S3T+V4I+V205I).

[0029] As used herein, a "cold water protease" is an enzyme that exhibits one or more of the following four criteria: (a) a performance index of at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from 1.1 to about 10, from 1.1 to about 8, or even from 1.1 to about 5 on BMI at pH 8 and 16°C (60°F) when compared to PURAFECT® Prime (SEQ ID NO:2 with the amino acid substitution Y217L), as defined in the "Test Method" set forth

herein in Example 1; (b) a performance index of at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from 1.3 to about 10, from 1.3 to about 8, or even from 1.3 to about 5 on BMI at pH 8 and 16°C (60°F) when compared to BPN' (SEQ ID NO:2), as defined in the "Test Method" set forth herein in Example 1; (c) a performance index of at least 0.9, at least 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from 0.9 to about 10, from 0.9 to about 8, or even from 0.9 to about 5 on BMI at pH 8 and 16°C (60°F) when compared to BPN'-v3 (SEQ ID NO:4), as defined in the "Test Method" set forth herein in Example 1; and/or (d) a performance index of at least 0.9, at least 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from 0.9 to about 10, from 0.9 to about 8, from 0.9 to about 5, from 1.0 to about 10, from 1.0 to about 8, or even from 1.0 to about 5 on BMI at pH 8 and 16°C (60°F) when compared to BPN'-v36 (SEQ ID NO:6), as defined in the "Test Method" set forth herein in Example 1.

[0030] Some suitable cold water proteases are derived from subtilisins, particularly those derived from subtilisin BPN' (SEQ ID NO:2). A cold water protease can be a variant of BPN' having the amino acid sequence of SEQ ID NO:2 (e.g., "BPN' variant." or "BPN' subtilisin variant"). Some such cold water proteases comprise one or more of the amino acid substitutions set forth herein.

[0031] As used herein, the genus *Bacillus* includes all species within the genus *Bacillus*, as known to those of skill in the art, including but not limited to *B. subtilis, B. licheniformis, B. lentus, B. brevis, B. stearothermophilus, B. alkalophilus, B. amyloliquefaciens, B. clacesii, B. halodurans, B. megaterium, B. coagulans, B. circulans, B. lautus*, and *B. thuringiensis*. It is recognized that the genus *Bacillus* continues to undergo taxonomical reorganization. Thus, it is intended that the genus include species that have been reclassified, including but not limited to such organisms as *B. stearothermophilus*, which is now named *"Geobacillus stearothermophilus."* The production of resistant endospores in the presence of oxygen is considered the defining feature of the genus *Bacillus,* although this characteristic also applies to the recently named *Alicyclobacillus, Amphibacillus, Aneurinibacillus, Anoxybacillus, Brevibacillus,* Filobacillns, *Gracilibacillus, Halobacillus, Paenibacillus, Salibacillus, Thermobacillus, Ureibacillus,* and *Virgibacillus.*

[0032] The terms "polynucleotide" and "nucleic acid," which are used interchangeably herein, refer to a polymer of any length of nucleotide monomers covalently bonded in a chain. DNA (deoxyribonucleic acid), a polynucleotide comprising deoxyribonucleotides, and RNA (ribonucleic acid), a polymer of ribonucleotides, are examples of polynucleotides or nucleic acids having distinct biological function. Polynucleotides or nucleic acids include, but are not limited to, a single-, double- or triple-stranded DNA, genomic DNA, cDNA, RNA, DNA-RNA hybrid, or a polymer comprising purine and pyrimidine bases, or other natural, chemically, biochemically modified, non-natural or derivatized nucleotide bases. The following are non-limiting examples of polynucleotides: genes, gene fragments, chromosomal fragments, expressed sequence tag(s) (EST(s)), exons, introns, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), ribozymes, complementary DNA (cDNA), recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. Some polynucleotides comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, uracyl, other sugars and linking groups such as fluororibose and thioate, and nucleotide branches. A sequence of nucleotides may be interrupted by non-nucleotide components.

[0033] As used herein, the term "vector" refers to a nucleic acid construct or polynucleotide construct used to introduce or transfer nucleic acid(s) or polynucleotide(s) into a target cell or tissue. A vector is typically used to introduce foreign DNA into another cell or tissue. A vector generally comprises a DNA sequence that is a transgene and a larger polynucleotide sequence that serves as the "backbone" of the vector. The vector typically serves to transfers genetic information, such as the inserted transgene, to a target cell or tissue so as to isolate, multiply, or express the insert in the target cell or tissue. Vectors include plasmids, cloning vectors, bacteriophages, viruses (e.g., viral vector), cosmids, expression vectors, shuttle vectors, cassettes, and the like. A vector typically includes an origin of replication, a multicloning site, and a selectable marker. The process of inserting a vector into a target cell is typically referred to as transformation in bacterial and yeast cells and as transfection in mammalian cells. The present invention includes a vector that comprises a DNA sequence encoding a protease variant (e.g., precursor or mature protease variant) that is operably linked to a suitable prosequence (e.g., secretory, signal peptide sequence, etc.) capable of effecting the expression of the DNA sequence in a suitable host.

[0034] As used herein, the term "expression cassette" or "expression vector" refers to a nucleic acid construct or vector generated recombinantly or synthetically for the expression of a nucleic acid of interest (e.g., a foreign nucleic acid or transgene) in a target cell. The nucleic acid of interest typically expresses a protein of interest. An expression vector or expression cassette typically comprises a promoter nucleotide sequence that drives or promotes expression of the foreign nucleic acid. The expression vector or cassette also typically includes any other specified nucleic acid elements that permit transcription of a particular nucleic acid in a target cell. A recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Some expression vectors have the ability to incorporate and express heterologous DNA fragments in a host cell. Many prokaryotic and eukaryotic expression vectors are commercially

available. Selection of appropriate expression vectors is within the knowledge of those of skill in the art. Selection of appropriate expression vectors for expression of a protein from a nucleic acid sequence incorporated into the expression vector is within the knowledge of those of skill in the art.

[0035] A DNA construct is an artificially constructed segment of nucleic acid that may be introduced into a target cell or tissue. A DNA construct typically comprises a DNA insert comprising a nucleotide sequence encoding a protein of interest that has been subcloned into a vector. The vector may contain bacterial resistance genes for growth in bacteria and a promoter for expression of the protein of interest in an organism. The DNA may be generated *in vitro* by PCR or any other suitable technique(s) known to those in the art. The DNA construct may comprise a nucleic acid sequence of interest. In one aspect, the sequence is operably linked to additional elements such as control elements (e.g., promoters, etc.). The DNA construct may further comprise a selectable marker and may further comprise an incoming sequence flanked by homology boxes. The construct may comprise other non-homologous sequences, added to the ends (e.g., stuffer sequences or flanks). The ends of the sequence may be closed such that the DNA construct forms a closed circle. The nucleic acid sequence of interest, which is incorporated into the DNA construct, using techniques well known in the art, may be a wild-type, mutant, or modified nucleic acid. The DNA construct may comprise one or more nucleic acid sequences homologous to the host cell chromosome. The DNA construct may comprise one or more non-homologous nucleotide sequences. Once the DNA construct is assembled *in vitro,* it may be used, e.g., to: 1) insert heterologous sequences into a desired target sequence of a host cell; and/or 2) mutagenize a region of the host cell chromosome (i.e., replace an endogenous sequence with a heterologous sequence); 3) delete target genes; and/or 4) introduce a replicating plasmid into the host. "DNA construct" is used interchangeably herein with "expression cassette."

[0036] As used herein, a "plasmid" refers to an extrachromosomal DNA molecule which is capable of replicating independently from the chromosomal DNA. A plasmid is double stranded (ds) and may be circular and is typically used as a cloning vector.

[0037] As used herein in the context of introducing a nucleic acid sequence into a cell, the term "introduced" refers to any method suitable for transferring the nucleic acid sequence into the cell. Such methods for introduction include but are not limited to protoplast fusion, transfection, transformation, electroporation, conjugation, and transduction (see, e.g., Ferrari et al., "Genetics," in Hardwood et al. (eds.), Bacillus, Plenum Publishing Corp., pp. 57-72 [1989]).

[0038] Transformation refers to the genetic alteration of a cell which results from the uptake, genomic incorporation, and expression of genetic material (e.g., DNA).

[0039] As used herein, a nucleic acid is "operably linked" with another nucleic acid sequence when it is placed into a functional relationship with another nucleic acid sequence. For example, a promoter or enhancer is operably linked to a nucleotide coding sequence if the promoter affects the transcription of the coding sequence. A ribosome binding site may be operably linked to a coding sequence if it is positioned so as to facilitate translation of the coding sequence. Typically, "operably linked" DNA sequences are contiguous. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers may be used in accordance with conventional practice.

[0040] As used herein, "recombinant" when used with reference to a cell typically indicates that the cell has been modified by the introduction of a heterologous nucleic acid sequence or that the cell is derived from a cell so modified. For example, a recombinant cell may comprise a gene not found in identical form within the native (non-recombinant) form of the cell, or a recombinant cell may comprise a native gene (found in the native form of the cell) but which has been modified and re-introduced into the cell. A recombinant cell may comprise a nucleic acid endogenous to the cell that has been modified without removing the nucleic acid from the cell; such modifications include those obtained by gene replacement, site-specific mutation, and related techniques known to those of ordinary skill in the art. Recombinant DNA (rDNA) is a form of artificial DNA that is created by combining two or more nucleotide sequences that would not normally occur together through the process of gene splicing. Recombinant DNA technology includes techniques for the production of recombinant DNA *in vitro* and transfer of the recombinant DNA into cells where it may be expressed or propagated, thereby producing a recombinant polypeptide.

[0041] As used herein, the term nucleic acid or gene "amplification" refers to a process by which specific DNA sequences are disproportionately replicated such that the amplified nucleic acid or gene becomes present in a higher copy number than was initially present in the genome. Selection of cells by growth in the presence of a drug (e.g., an inhibitor of an inhibitable enzyme) may result in the amplification of either the endogenous gene encoding the gene product required for growth in the presence of the drug or by amplification of exogenous (i.e., input) sequences encoding this nucleic acid or gene product or both.

[0042] As used herein, the term "primer" refers to an oligonucleotide (a polymer of nucleotide residues), whether occurring

naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is induced (i.e., in the presence of nucleotides and an inducing agent such as DNA polymerase and at a suitable temperature and pH). A primer is preferably single stranded for maximum efficiency in amplification, but may alternatively be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. The primer may comprise an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent. The exact length of a primer depends on a variety of factors, including temperature, source of primer, and the use of the method.

[0043] As used herein, the term "probe" refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, recombinantly or by PCR amplification, which is typically capable of hybridizing to another oligonucleotide of interest. A probe may be single-stranded or double-stranded. Probes are useful in the detection, identification and isolation of particular gene sequences. It is contemplated that any probe used in the present invention will be labeled with any "reporter molecule," so that it is detectable in any detection system, including, but not limited to enzyme (e.g., ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, and luminescent systems. It is not intended that the invention be limited to any particular detection system or label.

[0044] As used herein, the term "polymerase chain reaction" (PCR) refers to the methods of U.S. Patent Nos. 4,683,195 4,683,202, and 4,965,188, which include methods for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target sequence is well known in the art.

[0045] As used herein, the term "amplification reagents" refers to those reagents (e.g., deoxyribonucleotide triphosphates, buffer, etc.) needed for amplification except for primers, nucleic acid template, and the amplification enzyme. Typically, amplification reagents along with other reaction components are placed and contained in a reaction vessel (test tube, microwell, etc.).

[0046] As used herein, the term "restriction endonuclease" or "restriction enzyme" refers to an enzyme (e.g., bacterial enzyme) that is capable of cutting double-stranded or single-stranded DNA at or near a specific sequence of nucleotides known as a restriction site. The nucleotide sequence comprising the restriction site is recognized and cleaved by a given restriction endonuclease or restriction enzyme and is frequently the site for insertion of DNA fragments. A restriction site can be engineered into an expression vector or DNA construct.

[0047] As is known in the art, a DNA sequence can be transcribed by an RNA polymerase to produce an RNA sequence, but an RNA sequence can be reverse transcribed by reverse transcriptase to produce a DNA sequence.

[0048] "Host strain" or "host cell" refers to a suitable host for an expression vector comprising a DNA sequence of interest. A DNA sequence of interest may express a protein of interest in the host strain or host cell.

[0049] A "protein" or "polypeptide" or "peptide" is a polymeric sequence of amino acid residues. A carboxyl group of one amino acid is linked to the amino group of another. The terms "protein" and "polypeptide" and "peptide" may be used interchangeably herein. A peptide comprises two or more amino acids. Peptides typically contain fewer amino acids than do polypeptides or proteins. The single and 3-letter code for amino acids as defined in conformity with the IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN) is used through out this disclosure. The single letter X refers to any of the twenty amino acids. It is also understood that a polypeptide may be coded for by more than one nucleotide sequence due to the degeneracy of the genetic code.

[0050] In describing enzyme variants, the following nomenclature is used typically for ease of reference: Original amino acid(s):position(s):substituted amino acid(s). The accepted IUPAC single letter or triple letter amino acid abbreviation is employed. The single letter "X" refers to any amino acid residue However, when in the context of an amino acid substitution (e.g. "X003C"), it is to be understood that "X" refers to an amino acid residue other than the amino acid residue resulting from the substitution (e.g., X is an amino acid residue other than C). Mutations are typically named by the one letter code for the parent amino acid, followed by a three or two or one digit amino acid position number in an amino acid sequence and then the one letter code for the substituted amino acid. For example, mutating the amino acid glycine (G) at amino acid position 87 in an amino acid sequence by substituting to the amino acid serine (S) for glycine (G) is represented as "G087S" or "G87S". Typically, the substitution of a glycine at position 2 with a threonine is represented as G002T; however, such substitution may also be represented as G02T or G2T. One or two leading zeroes ("0") may be included simply to provide a convenient three number designation for each amino acid position. The amino acid position "001" is the same as "1" and thus "A001C" is the same as "A1C". "X001G" refers to the substitution of glycine (G) at amino acid position 1 in an amino acid sequence, wherein the amino acid that is to be replaced by

glycine is any amino acid. Multiple mutations are indicated by inserting a "-" between the mutations or by using a plus (+) sign between the mutations. For example, amino acid substitutions at amino acid residue positions 87 and 90 in an amino acid sequence are represented as either "G087S-A090Y" or "G87S-A90Y" or "G87S + A90Y" or "G087S + A090Y". For deletions, the one letter code "Z" is used. For an insertion relative to the parent sequence, the one letter code "Z" is on the left side of the position number. For a deletion, the one letter code "Z" is on the right side of the position number. For insertions, the position number is the position number before the inserted amino acid(s), plus 0.01 for each amino acid. For example, an insertion of three amino acids alanine (A), serine (S) and tyrosine (Y) between position 87 and 88 is shown as "Z087.01A-Z087.02S-Z087.03Y." Thus, combining all the mutations above plus a deletion at position 100 is: "G087S- Z087.01A-Z087.02S-Z087.03Y-A090Y-A100Z."

[0051] A "prosequence" or "propetide sequence" refers to an amino acid sequence between the signal peptide sequence and mature protease sequence that is necessary for the secretion of the protease. Cleavage of the prosequence or propeptide sequence results in a mature active protease.

[0052] The term "signal sequence" or "signal peptide" refers to a sequence of amino acid residues that may participate in the secretion or direct transport of the mature or precursor form of a protein. The signal sequence is typically located N-terminal to the precursor or mature protein sequence. The signal sequence may also be referred to as a leader sequence. The signal sequence may be endogenous or exogenous. One exemplary exogenous signal sequence comprises the first seven amino acid residues of the signal sequence from *Bacillus saibtilis* subtilisin fused to the remainder of the signal sequence of the subtilisin from *Bacillus lentus* (ATCC 21536). A signal sequence is normally absent from the mature protein. A signal sequence is typically cleaved from the protein by a signal peptidase after the protein is transported.

[0053] The term "hybrid signal sequence" refers to signal sequences in which part of sequence is obtained from the expression host fused to the signal sequence of the gene to be expressed. Synthetic sequences can be utilized.

[0054] The term "mature" form of a protein, polypeptide, or peptide refers to the functional form of the protein, polypeptide, or peptide without the signal peptide sequence and propeptide sequence.

[0055] The term "precursor" form of a protein or peptide refers to a mature form of the protein having a prosequence operably linked to the amino or carbonyl terminus of the protein. The precursor may also have a "signal" sequence operably linked to the amino terminus of the prosequence. The precursor may also have additional polynucleotides that are involved in post-translational activity (e.g., polynucleotides cleaved therefrom to leave the mature form of a protein, polypeptide, or peptide).

[0056] The term "wild-type" in reference to an amino acid sequence or nucleic acid sequence indicates that the amino acid sequence or nucleic acid sequence is native or naturally occurring sequence. As used herein, the term "naturally-occurring" refers to anything (e.g., proteins, amino acids, or nucleic acid sequences) that is found in nature (e.g., has not been manipulated by means of recombinant or chemical methods). As used herein, the term "non-naturally occurring" refers to anything that is not found in nature (e.g., recombinant or chemically synthesized nucleic acids produced in the laboratory).

[0057] An amino acid residue in a particular amino acid sequence may be numbered by correspondence with an amino acid residue in a position of a reference amino acid sequence. An amino acid residue of an amino acid sequence of interest which is in a position that "corresponds to" or is "corresponding to" or in "correspondence with" the position of an amino acid residue of a reference amino acid sequence indicates that the amino acid residue of the sequence of interest is located at a position that is equivalent or homologous to the position of an amino acid residue in the reference amino acid sequence. One skilled in the art can determine whether a particular residue position in a polypeptide corresponds to a position of a homologous reference sequence. For example, a protease variant may be aligned with that of a reference sequence (e.g., BPN' sequence of SEQ ID NO:2) using known techniques. The positions of the amino acid residues in the reference sequence are used for numbering of the amino acid residues in the sequence of interest. Accordingly, the amino acid residues of the protease variant may be numbered according to the corresponding amino acid residue position numbering of the reference sequence. For example, the amino acid residues in the reference sequence of SEQ ID NO: 2 may be used for determining amino acid residue position numbering of each amino acid residue of a protease variant of interest.

[0058] As used herein, "homology" refers to sequence similarity or identity, with identity being preferred. Homology may be determined using standard techniques known in the art (see, e.g., Smith and Waterman, Adv. Appl. Math. 2:482 [1981]; Needleman and Wunsch, J. Mol. Biol. 48:443 [1970; Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444 [1988]; software programs such as GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package (Genetics Computer Group, Madison, WI); and Devereux et al., Nucl. Acid Res. 12:387-395 [1984]). One example of a useful algorithm is PILEUP.

PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pair-wise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng and Doolittle (see Feng and Doolittle, J. Mol. Evol. 35:351-360 [1987]). The method is similar to that described by Higgins and Sharp (see Higgins and Sharp, CABIOS 5:151-153 [1989]). Useful PILEUP parameters including a default gap weight of 3.00, a default gap length weight of 0.10, and weighted end gaps. Another example of a useful algorithm is the BLAST algorithm, described by Altschul et al., (see Altschul et al., J. Mol. Biol. 215:403-410 [1990]; and Karlin and Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5787 [1993]). A particularly useful BLAST program is the WU-BLAST-2 program (see Altschul et al., Meth. Enzymol. 266:460-480 [1996]). WU-BLAST-2 uses several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span =1, overlap fraction = 0.125, word threshold (T) = 11. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched. However, the values may be adjusted to increase sensitivity.

[0059] The percent sequence identity (% sequence identity or simply % identity) between a subject polypeptide sequence and a reference polypeptide sequence means that the subject amino acid sequence is identical on an amino acid residue-by-amino acid residue basis by a specified percentage to the reference polypeptide sequence over a comparison length when the sequences are optimally aligned, as determined, for example, by an amino acid sequence comparison algorithm or visual inspection. The percent sequence identity between a subject nucleic acid sequence and a reference nucleic acid sequence similarly means the subject nucleotide sequence is identical on a nucleic acid residue-by-nucleic acid residue basis by a specified percentage to the reference nucleotide sequence over a comparison length when the sequences are optimally aligned.

[0060] The percent sequence identity (percent identity or % sequence identity or % identity) between a reference sequence and a subject sequence of interest may be readily determined by one skilled in the art. The percent identity shared by two polypeptide sequences can be determined, for example, by direct comparison of the amino acid residues in each sequence by aligning the residues of the respective sequences for maximum similarity and determining the number of identical amino acid residues between the sequences by using a sequence comparison algorithm known in the art or by visual inspection. The two optimally aligned polypeptide sequences can be compared over the comparison length and the number of positions in the optimal alignment at which identical amino acid residues occur in both polypeptide sequences can be determined, thereby providing the number of matched positions, and the number of matched positions is then divided by the total number of positions over the comparison length. The resulting number is multiplied by 100 to yield the percent identity of the subject polypeptide sequence to the reference (or query) polypeptide sequence. The percent identity shared by two nucleic acid sequences can be similarly determined by direct comparison of the nucleotide residues in each sequence by aligning the residues of the respective sequences for maximum similarity and determining the number of identical nucleic acid residues between the nucleic acid sequences by using a sequence comparison algorithm or by visual inspection. The percent identity between two or more sequences may also be described as the sequences being a particular percent identical.

[0061] An example of an algorithm that is suitable for determining sequence identity is the BLAST algorithm, (see Altschul, et al., J. Mol. Biol., 215:403-410 [1990]). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. These initial neighborhood word hits act as starting points to find longer HSPs containing them. The word hits are expanded in both directions along each of the two sequences being compared for as far as the cumulative alignment score can be increased. Extension of the word hits is stopped when: the cumulative alignment score falls off by the quantity X from a maximum achieved value; the cumulative score goes to zero or below; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 [1992]) alignments (B) of 50, expectation (E) of 10, M'5, N'-4, and a comparison of both strands.

[0062] The BLAST algorithm then performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin and Altschul, *supra)*. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a protease-encoding nucleic acid of this invention if the smallest sum probability in a comparison of the test nucleic acid to a protease-encoding nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001. Where the test nucleic acid encodes a protease polypeptide, it is considered similar to a specified protease-encoding nucleic acid if the comparison results in a smallest sum probability of less than about 0.5, and more preferably less than about 0.2.

[0063] "Optimal alignment" or "optimally aligned" refers to the alignment of two (or more) sequences giving the highest percent

identity score. For example, optimal alignment of two polypeptide sequences can be achieved by manually aligning the sequences such that the maximum number of identical amino acid residues in each sequence are aligned together or by using software programs or procedures described herein or known in the art. Optimal alignment of two nucleic acid sequences can be achieved by manually aligning the sequences such that the maximum number of identical nucleotide residues in each sequence are aligned together or by using software programs or procedures described herein or known in the art.

[0064] Two sequences (e.g., polypeptide sequences) may be deemed "optimally aligned" when they are aligned using defined parameters, such as a defined amino acid substitution matrix, gap existence penalty (also termed gap open penalty), and gap extension penalty, so as to achieve the highest identity score possible for that pair of sequences. The BLOSUM62 scoring matrix (see Henikoff and Henikoff, *supra*) is often used as a default scoring substitution matrix in polypeptide sequence alignment algorithms (e.g., BLASTP). The gap existence penalty is imposed for the introduction of a single amino acid gap in one of the aligned sequences, and the gap extension penalty is imposed for each residue position in the gap. Exemplary alignment parameters employed are: BLOSUM62 scoring matrix, gap existence penalty=11, and gap extension penalty=1. The alignment score is defined by the amino acid positions of each sequence at which the alignment begins and ends (e.g., the alignment window), and optionally by the insertion of a gap or multiple gaps into one or both sequences, so as to achieve the highest possible similarity score.

[0065] Optimal alignment between two or more sequences can be determined manually by visual inspection or by using a computer, such as, but not limited to e.g., the BLASTP program for amino acid sequences and the BLASTN program for nucleic acid sequences (see, e.g., Altschul et al., Nucleic Acids Res. 25(17):3389-3402 (1997); see also the National Center for Biotechnology Information (NCBI) website) or CLUSTALW program.

[0066] A polypeptide of interest may be said to be "substantially identical" to a reference polypeptide if the polypeptide of interest comprises an amino acid sequence having at least about 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% sequence identity to the amino acid sequence of the reference polypeptide. The percent identity between two such polypeptides can be determined manually by inspection of the two optimally aligned polypeptide sequences or by using software programs or algorithms (e.g., BLAST, ALIGN, CLUSTAL) using standard parameters. One indication that two polypeptides are substantially identical is that the first polypeptide is immunologically cross-reactive with the second polypeptide. Typically, polypeptides that differ by conservative amino acid substitutions are immunologically cross-reactive. Thus, a polypeptide is substantially identical to a second polypeptide, e.g., where the two peptides differ only by a conservative amino acid substitution or one or more conservative amino acid substitutions.

[0067] A nucleic acid of interest may be said to be "substantially identical" to a reference nucleic acid if the nucleic acid of interest comprises a nucleotide sequence having at least about 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% sequence identity to the nucleotide sequence of the reference nucleic acid. The percent identity between two such nucleic acids can be determined manually by inspection of the two optimally aligned nucleic acid sequences or by using software programs or algorithms (e.g., BLAST, ALIGN, CLUSTAL) using standard parameters. One indication that two nucleic acid sequences are substantially identical is that the two nucleic acid molecules hybridize to each other under stringent conditions (e.g., within a range of medium to high stringency).

[0068] As used herein, "isolated" in reference to a particular component of interest means that component is essentially or substantially free of other components. For example, an "isolated" polypeptide means the polypeptide is essentially or substantially free of other components, including, but not limited to, e.g., other polypeptides and cellular components. An "isolated" nucleic acid means the nucleic acid is essentially or substantially free of other components, including, but not limited to, e.g., other nucleic acids and cellular components. For purposes of this application, "isolated" refers to nucleic acids or polypeptides that are not part of a library (e.g., screening library).

[0069] Purity and homogeneity are typically determined using analytical chemistry techniques, such as polyacrylamide gel electrophoresis or high-performance liquid chromatography. A protein or nucleic acid that is the predominant species present in a preparation is substantially purified. The term "purified" denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel, chromatographic eluate, and/or a media subjected to density gradient centrifugation. Particularly, "purified" means that when isolated, the isolate contains at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% or more of nucleic acid or protein by weight of the isolate. Purified polypeptides may be obtained by a number of methods including, but not limited to, e.g., laboratory synthesis, chromatography (e.g., high-performance liquid chromatography) preparative electrophoresis, polyacrylamide gel electrophoresis followed by visualization upon staining, centrifugation, precipitation, affinity purification, etc. (see, generally, R Scopes, Protein Purification, Springer-Verlag, N.Y. (1982), Deutscher, Methods in Enzymol., Vol. 182: Guide to Protein Purification, Academic Press, Inc. N.Y. (1990)). The invention includes an isolated or purified

polypeptides (e.g., isolated protease variants or subtilisin variants of the invention) and isolated or purified nucleic acids (e.g., nucleic acids encoding protease variants or subtilisin variants of the invention).

[0070] In a related sense, the invention provides methods of enriching compositions for one or more molecules of the invention, such as one or more polypeptides of the invention (e.g., one or more protease variants of the invention) or one or more nucleic acids of the invention (e.g., one or more nucleic acids encoding one or more protease variants of the invention). A composition is enriched for a molecule when there is a substantial increase in the concentration of the molecule after application of a purification or enrichment technique. A substantially pure polypeptide or nucleic acid will typically comprise at least about 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98, 99%, 99.5% or more by weight (on a molar basis) of all macromolecular species in a particular composition.

[0071] As used herein, the term "combinatorial mutagenesis" refers to methods in which libraries of nucleic acid variants of a reference nucleic acid sequence are generated. In these libraries, the variants contain one or several mutations chosen from a predefined set of mutations. The methods also provide means to introduce random mutations which were not members of the predefined set of mutations. Some such methods include those set forth in U.S. Patent No. 6,582,914. Some such combinatorial mutagenesis methods include and/or encompass methods embodied in commercially available kits (e.g., QUIKCHANGE® Multi Site-Directed Mutagenesis Kit (Stratagene)).

[0072] As used herein, having "improved properties" used in connection with a protease variant refers to a protease variant having improved properties compared to a reference or parent protease. Protease variants of the invention may exhibit one of more of the following properties: enhanced or improved proteolytic activity, enhanced or improved stability, enhanced or improved ability to clean a surface or item, enhanced or improved cleaning performance, enhanced or improved fabric or laundry cleaning performance or wash performance, enhanced or improved hand wash performance, enhanced or improved hand or manual dishwashing performance, enhanced or improved automatic dishwashing performance, enhanced or improved laundry performance compared to a reference protease or parent protease of interest.

[0073] As used herein, the term "functional assay" refers to an assay that provides an indication of a protein's activity. The term typically refers to assay systems in which a protein is analyzed for its ability to function in its usual capacity. For example, in the case of enzymes, a functional assay involves determining the effectiveness of the enzyme in catalyzing a reaction.

[0074] The term "property" or grammatical equivalents thereof in the context of a molecule may refer to any characteristic or attribute of the molecule that can be selected or detected. For example, in the context of a polypeptide, a property may be enzymatic activity (e.g., proteolytic activity), stability, or other property.

[0075] A "mutant" nucleic acid sequence typically refers to a nucleic acid sequence that has an alteration in at least one codon occurring in a host cell's wild-type sequence such that the expression product of the mutant nucleic acid sequence is a protein with an altered amino acid sequence relative to the wild-type protein. The expression product may have an altered functional capacity (e.g., enhanced enzymatic activity).

[0076] As used herein, the term "net charge" is defined as the sum of all charges present in a molecule. "Net charge changes" can be made to a parent protein molecule to provide a protein variant that has a net charge that differs from that of the parent protein molecule (i.e., the variant has a net charge that is not the same as that of the parent molecule). For example, substitution of a neutral amino acid of a protein with a negatively charged amino acid or substitution of a positively charged amino acid of a protein with a neutral amino acid results in net charge of -1 with respect to the unmodified protein. Substitution of a positively charged amino acid of a protein with a negatively charged amino acid results in a net charge of -2 with respect to the unmodified protein. Substitution of a neutral amino acid of a protein with a positively charged amino acid or substitution of a negatively charged amino acid of a protein with a neutral amino acid results in net charge of +1 with respect to the parent. Substitution of a negatively charged amino acid of a protein with a positively charged amino acid results in a net charge of +2 with respect to the unmodified protein. The net charge of a parent protein can also be altered by deletion and/or insertion of one or more charged amino acids.

[0077] The terms "thermally stable" and "thermostable" and "thermostability" in reference to a polypeptide indicates that the polypeptide is resistant to a permanent change in its activity caused solely by heat, such as, e.g., via exposure to higher temperature. For example, a thermally stable enzyme means that the enzyme is resistant to a permanent change in its enzymatic activity caused solely by heat, such as, e.g., via exposure to higher temperature. Typically, a protease that is thermally stable is able to retain at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% of its proteolytic activity after exposure to increased temperatures over a given time period, e.g., at least about 60 minutes, 120 minutes, 180 minutes, 240 minutes, 300 minutes, etc.

[0078] Cleaning activity of a polypeptide or protease may refer to a cleaning performance achieved by a protease. Cleaning activity may be determined by using various assays for cleaning one or more of various enzyme-sensitive stains on an object, item, or surface (e.g., a stain resulting from food, grass, blood, ink, blood/milk/ink, milk/oil/pigment, egg yolk, milk, oil, and/or egg protein). Cleaning performance of a polypeptide (e.g., a polypeptide of the invention (such as a protease variant) or a reference polypeptide (e.g., reference protease) may be determined by subjecting the stain on the object, item, or surface to standard wash condition(s) and assessing the degree to which the stain is removed by using various chromatographic, spectrophotometric, or other quantitative methodologies. Exemplary cleaning assays and methods are known in the art and include, but are not limited to those described in WO 99/34011 and U.S. Pat. 6,605,458, as well as those cleaning assays and methods included in the Examples provided below.

[0079] The term "cleaning effective amount" of a protease variant or reference protease refers to the amount of protease that achieves a desired level of enzymatic activity in a specific cleaning composition. Such effective amounts are readily ascertained by one of ordinary skill in the art and are based on many factors, such as the particular protease used, the cleaning application, the specific composition of the cleaning composition, and whether a liquid or dry (e.g., granular, tablet, bar) composition is required, etc.

[0080] The term "cleaning adjunct material" refers to any liquid, solid, or gaseous material included in cleaning composition other than a protease variant of the invention. The cleaning compositions of the present invention may include one of more cleaning adjunct materials. Each cleaning adjunct material is typically selected depending on the particular type and form of cleaning composition (e.g., liquid, granule, powder, bar, paste, spray, tablet, gel, foam, or other composition). Preferably, each cleaning adjunct material is compatible with the protease enzyme used in the composition.

[0081] The term "enhanced performance" in the context of cleaning activity refers to an increased or greater cleaning activity by an enzyme on certain enzyme sensitive stains such as egg, milk, grass, ink, oil, and/or blood, as determined by usual evaluation after a standard wash cycle and/or multiple wash cycles.

[0082] The term "diminished performance" in the context of cleaning activity refers to a decreased or lesser cleaning activity by an enzyme on certain enzyme sensitive stains such as egg, milk, grass or blood, as determined by usual evaluation after a standard wash cycle.

[0083] As used herein, the term "institutional cleaning composition" refers to products suitable for use in institutions including but not limited to schools, hospitals, factories, stores, corporations, buildings, restaurants, office complexes and buildings, processing and/or manufacturing plants, veterinary hospitals, factory farms, factory ranches, etc.

[0084] As used herein, "fabric and home care product" refers to a product or device generally intended to be used or consumed in the form in which it is sold and that is for treating fabric, hard surface and any other surface in the area of fabric and home care, including: air care including air freshener and scent delivery systems, car care, dishwashing, fabric conditioning (including softening and/or freshening), laundry detergency, laundry and rinse additive and/or care, hard surface cleaning and/or treatment including floor and toilet bowl cleaners, and other cleaning products for consumer and institutional use.

[0085] As used herein, the terms "cleaning composition" and "cleaning formulation" refer to compositions that find use in the removal of undesired compound(s) from an item(s) to be cleaned, such as, but not limited to, e.g., fabric, laundry, dishes, dishware, contact lenses, other solid substrates, hair (including human or animal hair) (shampoos), skin (soaps, cosmetics, and creams), teeth (mouthwashes" toothpastes), non-fabric and home care objects, filters, membranes (e.g., filtration membrane, including, but not limited to, ultrafiltration membranes), hard surfaces and other surfaces, including, but not limited to, e.g., the hard surface of a table (table top or legs), wall, another furniture item or object, floor, ceiling, etc. A cleaning composition or cleaning formulation may be useful in a personal care application and/or in personal care item, including, e.g., but not limited to, shampoo (for cleaning human or animal hair); soap, cream or cosmetics (for skin cleaning and/or skin care); mouthwash (for oral care); toothpaste (for cleaning teeth and/or oral care). The terms encompass any material/compound selected for the particular type of cleaning composition or formulation desired and the form of the product (e.g., liquid, gel, granule, or spray composition), as long as the composition or formulation is compatible with the protease and/or other enzyme(s) used in the composition or formulation. The specific selection of cleaning composition or formulation materials are readily made by considering the surface, object, or item (e.g., fabric) to be cleaned, and the desired form of the composition or formulation for the cleaning conditions during use. In one aspect, a cleaning composition or formulation may be a fabric and home care product (e.g., a cleaning composition for cleaning laundry). In another aspect, a cleaning composition or formulation is not a fabric and home care product (e.g., a cleaning composition for cleaning contact lens, hair, teeth, or skin, or useful in personal care applications and/or personal

care items).

[0086] Cleaning compositions and cleaning formulations include any composition that is suited for cleaning, bleaching, disinfecting, and/or sterilizing any object, item, and/or surface. Such compositions and formulations include, but are not limited to, for example, liquid and/or solid compositions, including cleaning or detergent compositions (e.g., liquid, tablet, gel, bar, granule, and/or solid laundry cleaning or detergent compositions and fine fabric detergent compositions; hard surface cleaning compositions and formulations, such as for glass, wood, ceramic and metal counter tops and windows; carpet cleaners; oven cleaners; fabric fresheners; fabric softeners; and textile, laundry booster cleaning or detergent compositions, laundry additive cleaning compositions, and laundry pre-spotter cleaning compositions; dishwashing compositions, including hand or manual dishwash compositions (e.g., "hand" or "manual" dishwashing detergents) and automatic dishwashing compositions (e.g., "automatic dishwashing detergents").

[0087] Cleaning compositions or cleaning formulations, as used herein, include, unless otherwise indicated, granular or powder-form all-purpose or heavy-duty washing agents, especially cleaning detergents; liquid, granular, gel, solid, tablet, or paste-form all-purpose washing agents, especially the so-called heavy-duty liquid (HDL) detergent or heavy-duty powder detergent (HDD) types; liquid fine-fabric detergents; hand or manual dishwashing agents, including those of the high-foaming type; hand or manual dishwashing, automatic dishwashing, or dishware or tableware washing agents, including the various tablet, powder, solid, granular, liquid, gel, and rinse-aid types for household and institutional use; liquid cleaning and disinfecting agents, including antibacterial hand-wash types, cleaning bars, mouthwashes, denture cleaners, car shampoos, carpet shampoos, bathroom cleaners; personal care items, such as, but not limited to, e.g., hair shampoos and/or hair-rinses for humans (and other animals), shower gels and foam baths, skin care items, cosmetics, creams, bath and personal human soaps; metal cleaners; as well as cleaning auxiliaries, such as bleach additives and "stain-stick" or pre-treat types. Some granular compositions are in "compact" form; some liquid compositions are in a "concentrated" form.

[0088] In one aspect, the invention provides a cleaning composition or detergent composition comprising at least one protease variant or polypeptide of the invention, wherein the cleaning composition or detergent composition is useful for cleaning contact lens(es). In another aspect, the invention provides a cleaning composition or detergent composition comprising at least one protease variant or polypeptide of the invention, wherein the cleaning composition or detergent composition is useful in a personal care application. In another aspect, the invention provides a cleaning composition or detergent composition comprising at least one protease variant or polypeptide of the invention, wherein the cleaning composition or detergent composition is useful for cleaning or rinsing hair, including human hair and/or animal hair (e.g., hair shampoo and/or hair-rinse). In another aspect, the invention provides a cleaning composition or detergent composition comprising at least one protease variants or polypeptide of the invention, wherein the cleaning composition or detergent composition is useful for cleaning or treating skin (e.g., human and/or animal skin) (e.g., shower gel, foam bath, skin care cleaner, cosmetic, cream, and/or bath soap). In another aspect, the invention provides a cleaning composition or detergent composition comprising at least one protease variant or polypeptide of the invention, wherein the cleaning composition or detergent composition is useful for cleaning teeth and/or dentures and/or for oral care. Such cleaning compositions or detergent compositions may comprise at least one adjunct ingredient or carrier, at least one additional enzyme, at least one builder, and/or at least one surfactant and may be formulated or in a form appropriate to their use. Such cleaning or detergent compositions may contain phosphate or may be phosphate-free. Additional details regarding compositions of the invention are provided elsewhere herein.

[0089] As used herein, "fabric cleaning compositions" include hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the soaking and/or pretreatment of stained fabrics (e.g., clothes, linens, and other textile materials).

[0090] As used herein, "non-fabric cleaning compositions" include non-textile (i.e., non-fabric) surface cleaning compositions, including, but not limited to, for example, hand or manual or automatic dishwashing detergent compositions, oral cleaning compositions, denture cleaning compositions, and personal cleansing compositions.

[0091] As used herein, the term "detergent composition" or "detergent formulation" is used in reference to a composition intended for use in a wash medium for the cleaning of soiled or dirty objects, including particular fabric and/or non-fabric objects or items. Such compositions of the present invention are not limited to any particular detergent composition or formulation. Indeed, the detergents of the invention may comprise at least one protease variant of the invention and, in addition, one or more surfactants, transferase(s), hydrolytic enzymes, perhydrolases, oxido reductases, builders (e.g., a builder salt), bleaching agents, bleach activators, bluing agents, fluorescent dyes, caking inhibitors, masking agents, enzyme activators, antioxidants, and/or solubilizers. In some instances, a builder salt is a mixture of a silicate salt and a phosphate salt, preferably with more silicate (e.g., sodium metasilicate) than phosphate (e.g., sodium tripolyphosphate). Some compositions of the invention, such as, but not limited to, cleaning compositions or detergent compositions, do not contain any phosphate (e.g., phosphate salt or phosphate builder).

[0092] As used herein, "dishwashing composition" refers to all forms of compositions for cleaning dishware, including cutlery, including, but not limited to, granular and liquid forms. In some aspects, the dishwashing composition is an "automatic dishwashing" composition that finds use in automatic dishwashing machines. It is not intended that the present invention be limited to any particular type of dishware composition. Indeed, the present invention finds use in cleaning dishware (e.g., dishes, including, but not limited to plates, cups, glasses, bowls, etc.) and cutlery (e.g., utensils, including, but not limited to ,spoons, knives, forks, serving utensils, etc.) of any material, including, but not limited to, ceramics, plastics, metals, china, glass, acrylics, etc. The term "dishware" is used herein in reference to both dishes and cutlery.

[0093] As used herein, the term "bleaching" refers to the treatment of a material (e.g., fabric, laundry, pulp, etc.) or surface for a sufficient length of time and/or under appropriate pH and/or temperature conditions to effect a brightening *(i.e.,* whitening) and/or cleaning of the material. Examples of chemicals suitable for bleaching include, but are not limited to, for example, ClO_2, H_2O_2, peracids, NO_2, etc.

[0094] As used herein, "wash performance" of a protease (e.g., a protease variant of the invention) refers to the contribution of a protease variant to washing that provides additional cleaning performance to the detergent as compared to the detergent without the addition of the protease variant to the composition. Wash performance is compared under relevant washing conditions. In some test systems, other relevant factors, such as detergent composition, sud concentration, water hardness, washing mechanics, time, pH, and/or temperature, can be controlled in such a way that condition(s) typical for household application in a certain market segment (e.g., hand or manual dishwashing, automatic dishwashing, dishware cleaning, tableware cleaning, fabric cleaning, etc.) are imitated.

[0095] The term "relevant washing conditions" is used herein to indicate the conditions, particularly washing temperature, time, washing mechanics, sud concentration, type of detergent and water hardness, actually used in households in a hand dishwashing, automatic dishwashing, or laundry detergent market segment.

[0096] The term "improved wash performance" is used to indicate that a better end result is obtained in stain removal under relevant washing conditions, or that less protease variant, on weight basis, is needed to obtain the same end result relative to the corresponding wild-type or starting parent protease.

[0097] As used herein, the term "disinfecting" refers to the removal of contaminants from the surfaces, as well as the inhibition or killing of microbes on the surfaces of items. It is not intended that the present invention be limited to any particular surface, item, or contaminant(s) or microbes to be removed.

[0098] The "compact" form of the cleaning compositions herein is best reflected by density and, in terms of composition, by the amount of inorganic filler salt. Inorganic filler salts are conventional ingredients of detergent compositions in powder form. In conventional detergent compositions, the filler salts are present in substantial amounts, typically about 17 to about 35% by weight of the total composition. In contrast, in compact compositions, the filler salt is present in amounts not exceeding about 15% of the total composition. The filler salt can be present in amounts that do not exceed about 10%, or more preferably, about 5%, by weight of the composition. The inorganic filler salts can be selected from the alkali and alkaline-earth-metal salts of sulfates and chlorides. The filler salt may be sodium sulfate.

[0099] The position of an amino acid residue in a given amino acid sequence is typically numbered herein using the numbering of the position of the corresponding amino acid residue of the *B. anyloliquefaciens* subtilisin BPN' amino acid sequence shown in SEQ ID NO:2. The *B. amyloliquefaciens* subtilisin BPN' amino acid sequence of SEQ ID NO:2 thus serves as a reference sequence. A given amino acid sequence, such as a protease variant amino acid sequence described herein, can be aligned with the BPN' sequence (SEQ ID NO:2) using an alignment algorithm as described herein, and an amino acid residue in the given amino acid sequence that aligns (preferably optimally aligns) with an amino acid residue in the BPN' sequence can be conveniently numbered by reference to the corresponding amino acid residue in the subtilisin BPN' sequence.

[0100] Generally, the nomenclature used herein and many of the laboratory procedures in cell culture, molecular genetics, molecular biology, nucleic acid chemistry, and protein chemistry described below are well known and commonly employed by those of ordinary skill in the art. Methods for production and manipulation of recombinant nucleic acid methods, nucleic acid synthesis, cell culture methods, and transgene incorporation (e.g., transfection, electroporation) are known to those skilled in the art and are described in numerous standard texts. Oligonucleotide synthesis and purification steps are typically performed according to specifications. Techniques and procedures are generally performed according to conventional methods well known in the art and various general references that are provided throughout this document. Procedures therein are believed to be well

known to those of ordinary skill in the art and are provided for the convenience of the reader.

[0101] A fabric and home care product may comprise a protease (including a protease variant), including one or more protease variants of the invention and a material selected from the group consisting of an encapsulate comprising a perfume, a hueing agent, an Amphiphilic cleaning polymer and mixtures thereof, with the balance of any aspects of the aforementioned composition is made up of one or more adjunct materials, is disclosed. In one aspect of the aforementioned fabric and home care product, said fabric and home care product may comprise, based on total fabric and home care product weight, from about 0.005 weight percent (0.0005 wt%) to about 0.1 wt%, from about 0.001 wt% to about 0.05 wt%, or even from about 0.002 wt% to about 0.03 wt% of said protease. In one aspect of the aforementioned fabric and home care product, said fabric and home care product may comprise, based on total fabric and home care product weight, about 0.00003 wt% to about 0.1 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. In one aspect of the aforementioned fabric and home care product, said fabric and home care product may comprise, based on total fabric and home care product weight, from about 0.001 wt% to about 5 wt%, from about 0.01 wt% to about 2 wt%, or even from about 0.03 wt% to about 0.5 wt%, perfume capsules. In one aspect of the aforementioned fabric and home care product, said fabric and home care product may comprise, based on total fabric and home care product weight, from about 0.1 wt% to about 5 wt%, from about 0.25 wt% to about 2.5 wt%, or even from about 0.3 wt% to about 1.5 wt% amphiphilic cleaning polymer.

Polypeptides of the Invention

[0102] The present invention provides novel polypeptides, which may be collectively referred to as "polypeptides of the invention". Polypeptides of the invention include isolated, recombinant, substantially pure, or non-naturally occurring protease variants, including, for example, subtilisin protease variant polypeptides, which have enzymatic activity (e.g., proteolytic activity) and/or additional properties discussed in greater detail elsewhere herein (e.g., cleaning activity, stability, etc.).

[0103] As noted above, the protease variant polypeptides of the invention have enzymatic activities (e.g., proteolytic activities) and thus are useful in cleaning applications, including but not limited to, methods for cleaning dishware items, tableware items, fabrics, and items having hard surfaces (e.g., the hard surface of a table, table top, wall, furniture item, floor, ceiling, etc.). Exemplary cleaning compositions comprising one or more protease variant polypeptides of the invention are described *infra*. The enzymatic activity (e.g., protease activity) of a protease variant polypeptide of the invention can be determined readily using procedures well known to those of ordinary skill in the art. The Examples presented *infra* describe methods for evaluating the enzymatic activity, cleaning performance, and/or washing performance. The performance of protease variants of the invention in removing stains (e.g., a proteinaceous stain), cleaning hard surfaces, or cleaning laundry, dishware or tableware item(s) can be readily determined using procedures well known in the art and/or by using procedures set forth in the Examples.

[0104] A polypeptide of the invention can be subject to various changes, such as one or more amino acid insertions, deletions, and/or substitutions, either conservative or non-conservative, including where such changes do not substantially alter the enzymatic activity of the polypeptide. Similarly, a nucleic acid of the invention can also be subject to various changes, such as one or more substitutions of one or more nucleic acids in one or more codons such that a particular codon encodes the same or a different amino acid, resulting in either a silent variation (e.g., mutation in a nucleotide sequence results in a silent mutation in the amino acid sequence, for example when the encoded amino acid is not altered by the nucleic acid mutation) or non-silent variation, one or more deletions of one or more nucleic acids (or codons) in the sequence, one or more additions or insertions of one or more nucleic acids (or codons) in the sequence, and/or cleavage of or one or more truncations of one or more nucleic acids (or codons) in the sequence. Many such changes in the nucleic acid sequence may not substantially alter the enzymatic activity of the resulting encoded protease variant compared to the protease variant encoded by the original nucleic acid sequence. A nucleic acid of the invention can also be modified to include one or more codons that provide for optimum expression in an expression system (e.g., bacterial expression system), while, if desired, said one or more codons still encode the same amino acid(s).

[0105] The present invention includes a genus of polypeptides comprising protease variant polypeptides having the desired enzymatic activity (e.g., protease activity or cleaning performance activity) which comprise sequences having the amino acid substitutions described herein and also which comprise one or more additional amino acid substitutions, such as conservative and non-conservative substitutions, wherein the polypeptide exhibits, maintains, or approximately maintains the desired enzymatic activity (e.g., protease activity or subtilisin activity, as reflected in the cleaning activity or performance of the protease variant). Amino acid substitutions in accordance with the invention may include, but are not limited to, one or more non-conservative substitutions and/or one or more conservative amino acid substitutions. A conservative amino acid residue substitution typically involves exchanging a member within one functional class of amino acid residues for a residue that belongs to the same

functional class (identical amino acid residues are considered functionally homologous or conserved in calculating percent functional homology). A conservative amino acid substitution typically involves the substitution of an amino acid in an amino acid sequence with a functionally similar amino acid. For example, alanine, glycine, serine, and threonine are functionally similar and thus may serve as conservative amino acid substitutions for one another. Aspartic acid and glutamic acid may serve as conservative substitutions for one another. Asparagine and glutamine may serve as conservative substitutions for one another. Arginine, lysine, and histidine may serve as conservative substitutions for one another. Isoleucine, leucine, methionine, and valine may serve as conservative substitutions for one another. Phenylalanine, tyrosine, and tryptophan may serve as conservative substitutions for one another.

[0106] Other conservative amino acid substitution groups can be envisioned. For example, amino acids can be grouped by similar function or chemical structure or composition (e.g., acidic, basic, aliphatic, aromatic, sulfur-containing). For instance, an aliphatic grouping may comprise: Glycine (G), Alanine (A), Valine (V), Leucine (L), Isoleucine (I). Other groups containing amino acids that are considered conservative substitutions for one another include: aromatic: Phenylalanine (F), Tyrosine (Y), Tryptophan (W); sulfur-containing: Methionine (M), Cysteine (C); Basic: Arginine (R), Lysine (K), Histidine (H); Acidic: Aspartic acid (D), Glutamic acid (E); non-polar uncharged residues, Cysteine (C), Methionine (M), and Proline (P); hydrophilic uncharged residues: Serine (S), Threonine (T), Asparagine (N), and Glutamine (Q). Additional groupings of amino acids are well-known to those of skill in the art and described in various standard textbooks. Listing of a polypeptide sequence herein, in conjunction with the above substitution groups, provides an express listing of all conservatively substituted polypeptide sequences.

[0107] More conservative substitutions exist within the amino acid residue classes described above, which also or alternatively can be suitable. Conservation groups for substitutions that are more conservative include: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. Thus, for example, the invention includes an isolated or recombinant protease variant polypeptide (e.g., subtilisin variant) having proteolytic activity, said protease variant polypeptide comprising an amino acid sequence having at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 99.5% sequence identity to the amino acid sequence of SEQ ID NO:2. A conservative substitution of one amino acid for another in a protease variant of the invention is not expected to alter significantly the enzymatic activity or cleaning performance activity of the protease variant. Enzymatic activity or cleaning performance activity of the resultant protease can be readily determined using the standard assays and the assays described herein.

[0108] The present invention provides protease variants, including serine protease variants, having one or more substitutions as compared to a reference serine protease. In one aspect, the present invention provides cold water proteases. In addition, the present invention provides compositions comprising one or more such protease variants, e.g., serine protease variants. A composition may comprise at least one such protease variant of the invention and an adjunct ingredient, as described elsewhere herein. In one aspect, the present invention provides cleaning compositions comprising at least one of these protease variants, e.g., serine protease variants. A cleaning composition may be a detergent composition. The trend in cleaning is to use lower wash temperatures to save energy. Enzymes have lower activity at lower temperatures resulting in reduced cleaning performance. There is a need in the art for enzymes with enhanced performance at coldwater washing conditions over those currently known in the art. The present invention addresses this need.

[0109] The present invention provides cleaning compositions comprising at least one serine protease variant described herein, such as a subtilisin protease variant. The subtilisin protease variant may be a BPN' protease variant. As discussed in further detail *supra,* the invention includes a composition comprising a BPN' protease variant and a GG36 protease variant. Some protease variants, including, but not limited to, e.g., subtilisin variants of the invention, are cold water proteases. The cleaning composition may be a laundry detergent. The laundry detergent may be a detergent having a pH between 3 and 11 (e.g., between pH 4, pH 5, pH 6, pH 7, pH 7.5, pH 8, pH 9, pH 10, pH 10.5, etc.) cold water detergent, a low pH detergent (e.g., pH 3-6), neutral pH detergent (e.g., pH 6.5-7.5), alkaline pH detergent (e.g., pH 9-11) or a compact detergent. A detergent may include phosphate or be without phosphate. The cleaning composition may be a dishwashing detergent. In one aspect, the dishwashing detergent is a phosphate-free detergent, while in another aspect, the dishwashing detergent is a phosphate-containing detergent. In one aspect, the cleaning composition of the invention further comprises at least one additional enzyme, which may optionally be selected from the group of a neutral metalloprotease, lipase, cutinase, amylase, carbohydrase, cellulase, pectinase, mannanase, arabinase, galactanase, xylanase, perhydrolase, oxidase, and peroxidase. Also provided are isolated nucleic acids encoding a serine protease variant of the invention, expression vectors comprising one or more such nucleic acids of the invention, and host cells comprising at least one such expression vector of the invention.

[0110] Throughout the specification, for ease of reference, a "set of amino acid substitutions" or "set of substitutions" may refer to a set of multiple amino acid substitutions (i.e., G097A+G128A+Y217Q) or set of a single amino acid substitution (i.e., Y217Q). Thus, a BPN' variant comprising the BPN' sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128A-Y217Q and Y217Q indicates the BPN' variant comprises an amino acid

sequence comprising BPN'-G097A-G128A-Y217Q or BPN'-Y217Q.

[0111] The amino acid sequence of the mature BPN'-v3 subtilisin protease variant, which is set forth in SEQ ID NO:4, can be represented as BPN'-G097A-G128A-Y217Q, which means the BPN' amino acid sequence of SEQ ID NO:2 with the three substitutions G097A,G128A, and Y217Q. In this format, each dash (-) is equivalent to using a plus sign (+). Thus, BPN'-G097A-G128A-Y217Q can be written alternatively as BPN'+G097A+G128A+Y217Q. The amino acid sequence of the mature BPN'-v36 subtilisin protease variant, which is set forth in SEQ ID NO:6, can be written as BPN'-S24G-S53G-S78N-S101N-G128A-Y217Q or BPN'+S24G+S53G+S78N+S101N+G128A+Y217Q. BPN' variants of the invention may be depicted using these formats.

[0112] The present invention also provides dishwashing compositions comprising the subtilisin variant(s), and fabric cleaning compositions comprising the subtilisin variant(s). In some preferred aspects, the dishwashing and fabric cleaning compositions further comprise at least one additional enzyme. In some preferred aspects, the additional enzyme is selected from: a protease (e.g., a neutral metalloprotease, a wild type serine protease, or a second variant serine protease) a lipase, a cutinase, an amylase, a carbohydrase, a cellulase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, a perhydrolase, an oxidase, and a peroxidase. Moreover, the present invention provides dishwashing methods, comprising the steps of: providing i) the dishwashing composition comprising the subtilisin variant, and ii) dishware in need of cleaning; and contacting the dishware with the dishwashing composition under conditions effective to provide cleaning of the dishware. Similarly, the present invention provides fabric cleaning methods, comprising the steps of: providing i) the fabric cleaning composition comprising the subtilisin variant, and ii) laundry in need of cleaning; and contacting the laundry with the fabric cleaning composition under conditions effective to provide cleaning of the laundry. In still further aspects, the present invention provides an isolated nucleic acid encoding the variant, an expression vector comprising the isolated nucleic acid in operable combination with a promoter, and/or host cells comprising the expression vector are provided.

[0113] The present invention also provides cleaning compositions comprising the subtilisin variants provided herein. In one aspect, the cleaning compositions comprise a liquid, gel, tablet, powder and/or granule detergent. In some further aspects, the cleaning compositions are selected from laundry detergents and dish detergents. In some preferred aspects, the cleaning compositions comprise laundry detergents. In some particularly preferred aspects, the cleaning compositions are heavy duty detergents. In some additional aspects, the cleaning compositions comprise dish detergents selected from hand dishwashing and automatic dishwashing detergents. In some further preferred aspects, the cleaning compositions provided herein further comprise at least one bleaching agent. In some additional aspects, the cleaning compositions provided herein are phosphate-free, while in some alternative aspects, the cleaning compositions provided herein are phosphate-containing detergents. In some still further aspects, the cleaning compositions provided herein are cold water detergents. In yet some additional aspects, the cleaning compositions provided herein further comprise at least one additional enzyme. In one aspect, the cleaning compositions comprise at least one additional enzyme selected from the group consisting of a hemicellulase, cellulase, peroxidase, protease, metalloprotease, xylanase, lipase, phospholipase, esterase, perhydrolase, cutinase, pectinase, pectate lyase, mannanase, keratinase, reductase, oxidase, phenoloxidase, lipoxygenase, ligninase, pullulanase, tannase, pentosanase, malanase, ß-glucanase arabinosidase, hyaluronidase, chondroitinase, laccase, and amylase; or mixtures of any thereof.

[0114] The present invention also provides methods for cleaning, comprising providing an item to be cleaned and a composition comprising at least one cleaning composition provided herein, and contacting them with the composition, under conditions effective to provide cleaning of the item. In one aspect, the methods further comprise the step of rinsing the item after contacting the item with the cleaning composition. In some preferred aspects, the item to be cleaned comprises dishware. In some alternative aspects, the item to be cleaned comprises fabric.

Nucleic Acids of the Invention

[0115] The invention provides isolated, non-naturally occurring, or recombinant nucleic acids (also referred to herein as "polynucleotides"), which may be collectively referred to as "nucleic acids of the invention" or "polynucleotides of the invention", which encode polypeptides (e.g., protease variants) of the invention. Nucleic acids of the invention, including all described below, are useful in recombinant production (e.g., expression) of polypeptides of the invention, typically through expression of a plasmid expression vector comprising a sequence encoding the polypeptide of interest or fragment thereof.

[0116] The nucleic acid may comprise a polynucleotide sequence having at least 80%, 85%, 90%, 9.1%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the polynucleotide sequence set forth in SEQ ID NO:3 or SEQ ID NO:5, or a complementary polynucleotide sequence thereof.

[0117] Nucleic acids of the invention can be generated by using any suitable synthesis, manipulation, and/or isolation techniques, or combinations thereof. For example, a polynucleotide of the invention may be produced using standard nucleic acid synthesis techniques, such as solid-phase synthesis techniques that are well-known to those skilled in the art. In such techniques, fragments of up to 50 or more nucleotide bases are typically synthesized, then joined (e.g., by enzymatic or chemical ligation methods, or polymerase mediated recombination methods) to form essentially any desired continuous nucleic acid sequence. The synthesis of the nucleic acids of the invention can be also facilitated (or alternatively accomplished) by any suitable method known in the art, including but not limited to chemical synthesis using the classical phosphoramidite method (see, e.g., Beaucage et al. Tetrahedron Letters 22:1859-69 (1981)); or the method described by Matthes et al., EMBO J. 3:801-805 (1984), as is typically practiced in automated synthetic methods. Nucleic acids of the invention also can be produced by using an automatic DNA synthesizer. Customized nucleic acids can be ordered from a variety of commercial sources (e.g., The Midland Certified Reagent Company, the Great American Gene Company, Operon Technologies Inc., and DNA2.0). Other techniques for synthesizing nucleic acids and related principles are known in the art (see, e.g., Itakura et al., Ann. Rev. Biochem. 53:323 (1984); and Itakura et al., Science 198:1056 (1984)).

[0118] As indicated above, recombinant DNA techniques useful in modification of nucleic acids are well known in the art. For example, techniques such as restriction endonuclease digestion, ligation, reverse transcription and cDNA production, and polymerase chain reaction (e.g., PCR) are known and readily employed by those of skill in the art. Nucleotides of the invention may also be obtained by screening cDNA libraries (e.g., cDNA libraries generated using mutagenesis techniques commonly used in the art, including those described herein) using one or more oligonucleotide probes that can hybridize to or PCR-amplify polynucleotides which encode a protease variant polypeptide(s) of the invention. Procedures for screening and isolating cDNA clones and PCR amplification procedures are well known to those of skill in the art and described in standard references known to those skilled in the art. Some nucleic acids of the invention can be obtained by altering a naturally occurring polynucleotide backbone (e.g., that encodes an enzyme or parent protease) by, for example, a known mutagenesis procedure (e.g., site-directed mutagenesis, site saturation mutagenesis, *and in vitro* recombination).

[0119] Nucleic acids hybridize due to a variety of well-characterized physico-chemical forces, such as hydrogen bonding, solvent exclusion, base stacking and the like. An extensive guide to the hybridization of nucleic acids is found in P. Tijssen (1993) Laboratory Techniques in Biochemistry and Molecular Biology - Hybridization with Nucleic Acid Probes, vol. 24, part I, chapter 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays," (Elsevier, N.Y.) (hereinafter "Tijssen"). See also Hames and Higgins (1995) Gene Probes 1 and 2. An indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under at least stringent conditions. Stringent hybridization conditions in the context of nucleic acid hybridization experiments, such as Southern and northern hybridizations, are sequence dependent, and are different under different environmental parameters. High stringency conditions are typically selected such that hybridization occurs at about 5°C or less than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the test sequence hybridizes to a perfectly matched probe. The Tm indicates the temperature at which the nucleic acid duplex is 50% denatured under the given conditions and its represents a direct measure of the stability of the nucleic acid hybrid. Thus, the Tm corresponds to the temperature corresponding to the midpoint in transition from helix to random coil; it depends on length, nucleotide composition, and ionic strength for long stretches of nucleotides. Under stringent conditions, a probe will typically hybridize to its target subsequence, but to no other sequences. Very stringent condition" are selected to be equal to the Tm for a particular probe.

[0120] The Tm of a DNA-DNA duplex can be estimated using equation (1): Tm (°C) = 81.5°C+16.6 (logioM)+0.41 (% G+C)-0.72 (% f)-500/n, where M is the molarity of the monovalent cations (usually Na+), (% G+C) is the percentage of guanosine (G) and cytosine (C) nucleotides, (% f) is the percentage of formalize and n is the number of nucleotide bases (i.e., length) of the hybrid. The Tm of an RNA-DNA duplex can be estimated using equation (2): Tm (°C) = 79.8°C+18.5 ($\log_{10}$M)+0.58 (% G+C)-11.8(% G+C)^2-0.56 (% f)-820/n, where M is the molarity of the monovalent cations (usually Na+), (% G+C) is the percentage of guanosine (G) and cytosine (C) nucleotides, (% f) is the percentage of formamide and n is the number of nucleotide bases (i.e., length) of the hybrid. Equations 1 and 2 above are typically accurate only for hybrid duplexes longer than about 100-200 nucleotides. The Tm of nucleic acid sequences shorter than 50 nucleotides can be calculated as follows: Tm (°C) = 4G+C)+2(A+T), where A (adenine), C, T (thymine), and G are the numbers of the corresponding nucleotides.

[0121] Non-hybridized nucleic acid material is typically removed by a series of washes, the stringency of which can be adjusted depending upon the desired results, in conducting hybridization analysis. Low stringency washing conditions (e.g., using higher salt and lower temperature) increase sensitivity, but can product nonspecific hybridization signals and high background signals. Higher stringency conditions (e.g., using lower salt and higher temperature that is closer to the hybridization temperature) lower the background signal, typically with only the specific signal remaining. For additional guidance, see Hames and Higgins, *supra*.

[0122] Exemplary stringent conditions for analysis of at least two nucleic acids comprising at least 100 nucleotides include incubation in a solution or on a filter in a Southern or northern blot comprises 50% formalin (or formamide) with 1 milligram (mg) of heparin at 42°C, with the hybridization being carried out overnight. A regular stringency wash can be carried out using a solution comprising 0.2x SSC buffer wash at about 65°C for about 15 minutes (see Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, and the third edition thereof (2001) for a description of SSC buffer). The regular stringency wash can be preceded by a low stringency wash to remove background probe signal. A low stringency wash can be carried out in, for example, a solution comprising 2x SSC buffer at about 40°C for about 15 minutes. A highly stringent wash can be carried out using a solution comprising 0.15 M NaCl at about 72°C for about 15 minutes. Exemplary moderate stringency conditions include overnight incubation at 37°C in a solution comprising 20% formalin (or formamide), 0.5x SSC, 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1x SSC at about 37-50°C. High stringency conditions are conditions that (a) use low ionic strength and high temperature for washing, such as 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate (SDS) at 50°C, (b) employ a denaturing agent during hybridization, such as formamide, e.g., 50% (v/v) formamide with 0.1% bovine serum albumin (BSA)/0.1% FicoII/0.1% polyvinylpyrrolidone (PVP)/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C, or (c) employ 50% formamide, 5x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5x Denhardt's solution, sonicated salmon sperm DNA (50µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C with washes at (1) 42°C in 0.2x SSC, (2) 55°C in 50% formamide, and (3) 55°C in 0.1x SSC (preferably with EDTA). A signal to noise ratio of 2x or 2.5x-5x that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Detection of at least stringent hybridization between two sequences in the context of the present invention indicates relatively strong structural similarity or homology to a nucleic acid of the invention.

Vectors, Cells, and Methods for Making Protease Variant Polypeptides of the Invention

[0123] A variety of methods are known in the art that are suitable for generating modified polynucleotides of the invention that encode protease variants of the invention (such as cold water proteases of the invention), including, but not limited to, e.g., site-saturation mutagenesis, scanning mutagenesis, insertional mutagenesis, deletion mutagenesis, random mutagenesis, site-directed mutagenesis, and directed-evolution, as well as various other recombinatorial approaches. Methods for making modified polynucleotides and proteins (e.g., protease variants) include DNA shuffling methodologies (see, e.g., Stemmer WP, Proc. Natl. Acad. Sci. USA 91(22):10747-51 (1994)); methods based on non-homologous recombination of genes, e.g., ITCHY (Ostermeier et al., Bioorg. Med. Chem.7:2139-44 [1999]); SCRATCHY (Lutz et al., Proc. Natl. Acad. Sci. USA 98:11248-53 [2001]); SHIPREC (Sieber et al., Nat. Biotechnol. 19:456-60 [2001]); NRR (Bittker et al., Nat. Biotechnol. 20:1024-9 [2001]; Bittker et al., Proc Natl. Acad. Sci. USA 101:7011-6 [2004]); methods that rely on the use of oligonucleotides to insert random and targeted mutations, deletions and/or insertions (Ness et al., Nat. Biotechnol. 20:1251-5 [2002]; Coco et al., Nat. Biotechnol. 20:1246-50 [2002]; Zha et al., Chembiochem. 4:34-9 [2003]; Glaser et al., J. Immunol. 149:3903-13 [1992]); see also Arkin and Youvan, Biotechnology 10:297-300 (1992); Reidhaar-Olson et al., Methods Enzymol. 208:564-86 (1991).

[0124] In one aspect, a full-length parent polynucleotide is ligated into an appropriate expression plasmid, and the following mutagenesis method is used to facilitate the construction of the modified protease of the present invention, although other methods may be used. The method is based on that described by Pisarchik et al. (Pisarchik et al., Prot. Eng. Des. Select. 20:257-265 [2007]). In one aspect, an added advantage is provided in that the restriction enzyme cuts outside its recognition sequence, which allows digestion of practically any nucleotide sequence and precludes formation of a restriction site scar.

[0125] In one approach, a naturally-occurring gene encoding a full-length protease is obtained and sequenced and scanned for one or more points at which it is desired to make a mutation (e.g., deletion, insertion, substitution) at one or more amino acids. Mutation of the gene in order to change its sequence to conform to the desired sequence is accomplished by primer extension in accord with generally known methods. Fragments to the left and to the right of the desired point(s) of mutation are amplified by PCR and to include the *Eam*1104I restriction site. The left and right fragments are digested with Eam1104I to generate a plurality of fragments having complementary three base overhangs, which are then pooled and ligated to generate a library of modified sequences containing one or more mutations. This method avoids the occurrence of frame-shift mutations. This method also simplifies the mutagenesis process because all of the oligonucleotides can be synthesized so as to have the same restriction site, and no synthetic linkers are necessary to create the restriction sites as is required by some other methods.

[0126] In another aspect, the invention provides a vector comprising a nucleic acid or polynucleotide of the invention. The vector may be an expression vector or expression cassette in which a polynucleotide sequence of the invention which encodes a protease variant of the invention is operably linked to one or additional nucleic acid segments required for efficient gene

expression (e.g., a promoter operably linked to the polynucleotide of the invention which encodes a protease variant of the invention). A vector may include a transcription terminator and/or a selection gene, such as an antibiotic resistance gene that enables continuous cultural maintenance of plasmid-infected host cells by growth in antimicrobial-containing media.

[0127] An expression vector may be derived from plasmid or viral DNA, or in alternative aspects, contains elements of both. Exemplary vectors include, but are not limited to pXX, pC194, pJH101, pE194, pH13 (Harwood and Cutting (eds.)), Molecular Biological Methods for Bacillus, John Wiley & Sons (1990), see, e.g., chapter 3; suitable replicating plasmids for *B. subtilis* include those listed on p. 92; Perego, M. (1993) Integrational Vectors for Genetic Manipulations in Bacillus subtilis, pp. 615-624; A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), Bacillus subtilis and other Gram-positive bacteria: biochemistry, physiology and molecular genetics, American Society for Microbiology, Washington, D.C.

[0128] For expression and production of a protein of interest (e.g., protease variant) in a cell, at least one expression vector comprising at least one copy of a polynucleotide encoding the modified protease, and preferably comprising multiple copies, is transformed into the cell under conditions suitable for expression of the protease. In one aspect, a polynucleotide sequence encoding the protease variant (as well as other sequences included in the vector) is integrated into the genome of the host cell, while in another aspect, a plasmid vector comprising a polynucleotide sequence encoding the protease variant remains as autonomous extra-chromosomal element within the cell. The invention provides both extrachromosomal nucleic acid elements as well as incoming nucleotide sequences that are integrated into the host cell genome. The vectors described herein are useful for production of the protease variants of the invention. In one aspect, a polynucleotide construct encoding the protease variant is present on an integrating vector that enables the integration and optionally the amplification of the polynucleotide encoding the protease variant into the bacterial chromosome. Examples of sites for integration include are well known to those skilled in the art. In one aspect, transcription of a polynucleotide encoding a protease variant of the invention is effectuated by a promoter that is the wild-type promoter for the selected precursor protease. In some other aspects, the promoter is heterologous to the precursor protease, but is functional in the host cell. Specifically, examples of suitable promoters for use in bacterial host cells include, but are not limited to, e.g., the amyE, amyQ, amyL, pstS, sacB, pSPAC, pAprE, pVeg, pHpall promoters, the promoter of the *B. stearothermophilus* maltogenic amylase gene, the *B. amyloliquefaciens* (BAN) amylase gene, the *B. subtilis* alkaline protease gene, the *B. clausii* alkaline protease gene the B. *pumilis* xylosidase gene, the *B. thuringiensis* cryIIIA, and the *B. licheniformis* alpha-amylase gene. Additional promoters include, but are not limited to the A4 promoter, as well as phage Lambda P_R or P_L promoters, and the *E. coli* lac, trp or tac promoters.

[0129] Protease variants of the invention can be produced in host cells of any suitable Gram-positive microorganism, including bacteria and fungi. For example, in one aspect, the protease variant is produced in host cells of fungal and/or bacterial origin. In one aspect, the host cells are *Bacillus sp., Streptomyces sp., Escherichia sp. or Aspergillus sp.* In one aspect, the protease variants are produced by *Bacillus sp.* host cells. Examples of *Bacillus* sp. host cells that find use in the production of the protease variants of the invention include, but are not limited to *B. licheniformis*, *B. lentus*, *B. subtilis*, *B. amyloliquefaciens*, *B. lentus*, *B. brevis*, *B. stearothermophilus*, *B. alkalophilus*, *B. coagulans*, *B. circulans*, *B. pumilis*, *B. thuringiensis*, *B. clausii*, and *B. megaterium*, as well as other organisms within the genus *Bacillus*. In one aspect, *B. subtilis* host cells are used for production of protease variants. U.S. Patents 5,264,366 and 4,760,025 (RE 34,606) describe various *Bacillus* host strains that can be used for producing protease variants of the invention, although other suitable strains can be used.

[0130] Several industrial bacterial strains that can be used to produce protease variants of the invention include non-recombinant (i.e., wild-type) *Bacillus sp.* strains, as well as variants of naturally-occurring strains and/or recombinant strains. In one aspect, the host strain is a recombinant strain, wherein a polynucleotide encoding a polypeptide of interest has been introduced into the host. In one aspect, the host strain is a *B. subtilis* host strain and particularly a recombinant *Bacillus subtilis* host strain. Numerous *B. subtilis* strains are known, including, but not limited to, e.g., 1A6 (ATCC 39085), 168 (1A01), SB19, W23, Ts85, B637, PB1753 through PB1758, PB3360, JH642, 1A243 (ATCC 39,087), ATCC 21332, ATCC 6051, MI113, DE100 (ATCC 39,094), GX4931, PBT 110, and PEP 211strain (see, e.g., Hoch et al., Genetics 73:215-228 (1973)) (see also U.S. Patent Nos. 4,450,235 and 4,302,544, and EP 0134048). The use of *B. subtilis* as an expression host cells is well known in the art (see, e.g., Palva et al., Gene 19:81-87 (1982); Fahnestock and Fischer, J. Bacteriol. 165:796-804 (1986); and Wang et al., Gene 69:39-47 (1988)).

[0131] In one aspect, the *Bacillus* host cell is a *Bacillus* sp. that includes a mutation or deletion in at least one of the following genes, *degU, degS, degR* and *degQ*. The mutation may be in a *degU* gene, and in some instances the mutation may be *degU*(*Hy*)*32*. See, e.g., Msadek et al., J. Bacteriol. 172:824-834 (1990) and Olmos et al., Mol. Gen. Genet. 253:562-567 (1997)). A typical host strain is a *Bacillus subtilis* carrying a *degU32*(*Hy*) mutation. The *Bacillus* host may comprise an amino acid mutation (e.g., substitution) or deletion in *scoC4* (see, e.g., Caldwell et al., J. Bacteriol. 183:7329-7340 (2001)); *spoIIE* (see, e.g., Arigoni et al., Mol. Microbiol. 31:1407-1415 (1999)); and/or *oppA* or other genes of the *opp* operon (see, e.g., Perego et al., Mol. Microbiol.

5:173-185 (1991)). Indeed, it is contemplated that any mutation in the *opp* operon that causes the same phenotype as a mutation in the *oppA* gene will find use in one aspect of the altered *Bacillus* strain of the invention. Such mutations may occur alone or combinations of mutations may be present. In one aspect, an altered *Bacillus* host cell strain that can be used to produce a protease variant of the invention is a *Bacillus* host strain that already includes a mutation in one or more of the above-mentioned genes. In addition, *Bacillus* sp. host cells that comprise mutation(s) and/or deletions of endogenous protease genes find use. The *Bacillus* host cell may comprise a deletion of the *aprE* and the *nprE* genes. The *Bacillus* sp. host cell may comprise a deletion of 5 protease genes, or the *Bacillus sp.* host cell may comprise a deletion of 9 protease genes (see, e.g., U.S. Pat. Appn. Pub. No. 2005/0202535).

[0132] Host cells are transformed with at least one nucleic acid encoding at least one protease variant of the invention using any suitable method known in the art. Whether the nucleic acid is incorporated into a vector or is used without the presence of plasmid DNA, it is typically introduced into a microorganism, in one aspect, preferably an *E. coli* cell or a competent *Bacillus* cell. Methods for introducing a nucleic acid (e.g., DNA) into *Bacillus* cells or *E. coli* cells utilizing plasmid DNA constructs or vectors and transforming such plasmid DNA constructs or vectors into such cells are well known. In one aspect, the plasmids are subsequently isolated from *E. coli* cells and transformed into *Bacillus* cells. However, it is not essential to use intervening microorganisms such as *E. coli,* and in one aspect, a DNA construct or vector is directly introduced into a *Bacillus* host.

[0133] Those of skill in the art are well aware of suitable methods for introducing nucleic acid or polynucleotide sequences of the invention into *Bacillus* cells (see, e.g., Ferrari et al., "Genetics," in Harwood et al. (ed.), Bacillus, Plenum Publishing Corp. (1989), pp. 57-72; Saunders et al., J. Bacteriol. 157:718-726 (1984); Hoch et al., J. Bacteriol. 93:1925 -1937 (1967); Mann et al., Current Microbiol. 13:131-135 (1986); and Holubova, Folia Microbiol. 30:97 (1985); Chang et al., Mol. Gen. Genet. 168:11-115 (1979); Vorobjeva et al., FEMS Microbiol. Lett. 7:261-263 (1980); Smith et al., Appl. Env. Microbiol. 51:634 (1986); Fisher et al., Arch. Microbiol. 139:213-217 (1981); and McDonald, J. Gen. Microbiol. 130:203 (1984)). Indeed, such methods as transformation, including protoplast transformation and congression, transduction, and protoplast fusion are well known and suited for use in the present invention. Methods of transformation are used to introduce a DNA construct or vector comprising a nucleic acid encoding a protease variant of the present invention into a host cell. Methods known in the art to transform *Bacillus* cells include such methods as plasmid marker rescue transformation, which involves the uptake of a donor plasmid by competent cells carrying a partially homologous resident plasmid (Contente et al., Plasmid 2:555-571 (1979); Haima et al., Mol. Gen. Genet. 223:185-191 (1990); Weinrauch et al., J. Bacteriol. 154:1077-1087 (1983); and Weinrauch et al., J. Bacteriol. 169:1205-1211 (1987)). In this method, the incoming donor plasmid recombines with the homologous region of the resident "helper" plasmid in a process that mimics chromosomal transformation.

[0134] In addition to commonly used methods, in one aspect, host cells are directly transformed with a DNA construct or vector comprising a nucleic acid encoding a protease variant of the invention (i.e., an intermediate cell is not used to amplify, or otherwise process, the DNA construct or vector prior to introduction into the host cell). Introduction of the DNA construct or vector of the invention into the host cell includes those physical and chemical methods known in the art to introduce a nucleic acid sequence (e.g., DNA sequence) into a host cell without insertion into a plasmid or vector. Such methods include, but are not limited to calcium chloride precipitation, electroporation, naked DNA, liposomes and the like. In additional aspects, DNA constructs or vector are co-transformed with a plasmid, without being inserted into the plasmid. In further aspects, a selective marker is deleted from the altered *Bacillus* strain by methods known in the art (see Stahl et al., J. Bacteriol. 158:411-418 (1984); and Palmeros et al., Gene 247:255 -264 (2000)).

[0135] In one aspect, the transformed cells of the present invention are cultured in conventional nutrient media. The suitable specific culture conditions, such as temperature, pH and the like are known to those skilled in the art. In addition, some culture conditions may be found in the scientific literature such as Hopwood (2000) Practical Streptomyces Genetics, John Innes Foundation, Norwich UK; Hardwood et al., (1990) Molecular Biological Methods for Bacillus, John Wiley and from the American Type Culture Collection (ATCC). In one aspect, the invention provides a culture (e.g., cell culture) comprising at least one protease variant or at least one nucleic acid of the invention. Also provided is a composition comprising at least one nucleic acid, vector, or DNA construct of the invention.

[0136] Host cells transformed with at least one polynucleotide sequence encoding at least one protease variant of the invention may be cultured in a suitable nutrient medium under conditions permitting the expression of the present protease, after which the resulting protease is recovered from the culture. The medium used to culture the cells comprises any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g., in catalogues of the American Type Culture Collection). The protease produced by the cells may be recovered from the culture medium by conventional procedures, including, but not limited to, e.g., separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt (e.g., ammonium sulfate), chromatographic

purification (e.g., ion exchange, gel filtration, affinity, etc.). Any method suitable for recovering or purifying a protease variant of the invention can be used.

[0137] In one aspect, a protease variant produced by a recombinant host cell is secreted into the culture medium. A nucleic acid sequence that encodes a purification facilitating domain may be used to facilitate purification of soluble proteins. A vector or DNA construct comprising a polynucleotide sequence encoding a protease variant may further comprise a nucleic acid sequence encoding a purification facilitating domain to facilitate purification of the protease variant (see, e.g., Kroll, D.J. et al., DNA Cell Biol. 12:441-53 (1993)). Such purification facilitating domains include, but are not limited to, e.g., metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals (Porath J., Protein Expr. Purif. 3:263-281 (1992)), protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the heterologous protein also find use to facilitate purification.

[0138] Assays for detecting and measuring the enzymatic activity of an enzyme, such as a protease variant of the invention, are well known. Various assays for detecting and measuring activity of proteases, such as, e.g., protease variants of the invention, are also known to those of ordinary skill in the art. In particular, assays are available for measuring protease activity that are based on the release of acid-soluble peptides from casein or hemoglobin, measured as absorbance at 280 nm or colorimetrically using the Folin method (see, e.g., Bergmeyer et al., "Methods of Enzymatic Analysis" vol. 5, Peptidases, Proteinases and their Inhibitors, Verlag Chemie, Weinheim (1984)). Other exemplary assays involve the solubilization of chromogenic substrates (see, e.g., Ward, "Proteinases," in Fogarty (ed.). Microbial Enzymes and Biotechnology, Applied Science, London, (1983), pp. 251-317). Other exemplary assays include, but are not limited to succinyl-Ala-Ala-Pro-Phe-pam nitroanilide assay (SAAPFpNA) and the 2,4,6-trinitrobenzene sulfonate sodium salt assay (TNBS assay). Numerous additional references known to those in the art provide suitable methods (see, e.g., Wells et al., Nucleic Acids Res. 11:7911-7925 (1983); Christianson et al., Anal. Biochem. 223:119 -129 (1994); and Hsia et al., Anal Biochem. 242:221-227 (1999)).

[0139] A variety of methods can be used to determine the level of production of a mature protease (e.g., mature protease variant of the invention) in a host cell. Such methods include, but are not limited to, e.g., methods that utilize either polyclonal or monoclonal antibodies specific for the protease. Exemplary methods include, but are not limited, e.g., to enzyme-linked immunosorbent assays (ELISA), radioimmunoassays (RIA), fluorescent immunoassays (FIA), and fluorescent activated cell sorting (FACS). These and other assays are well known in the art (see, e.g., Maddox et al., J. Exp. Med. 158:1211 (1983)).

[0140] In another aspect, the invention provides methods for making or producing a mature protease variant of the invention. A mature protease variant does not include a signal peptide or a propeptide sequence. Some such methods comprising making or producing a protease variant of the invention in a recombinant bacterial host cell, such as, e.g., a *Bacillus sp.* cell, including, e.g., *Bacillus subtilis* cell. In one aspect, the invention provides a method of producing a protease variant of the invention, the method comprising cultivating a recombinant host cell comprising a recombinant expression vector comprising a nucleic acid encoding a protease variant of the invention under conditions conducive to the production of the protease variant. Some such methods further comprise recovering the protease variant from the culture.

[0141] In one aspect, the invention provides a method of producing a protease variant of the invention, the method comprising: (a) introducing a recombinant expression vector comprising a nucleic acid encoding a protease variant of the invention into a population of cells (e.g., bacterial cells, such as *Bacillus subtilis* cells); and (b) culturing the cells in a culture medium under conditions conducive to produce the protease variant encoded by the expression vector. Some such methods further comprise: (c) isolating the protease variant from the cells or from the culture medium.

[0142] In addition to recombinant production, the protease variant polypeptides of the invention may be produced by direct peptide synthesis using solid-phase techniques (see, e.g., Stewart et al. (1969) Solid-Phase Peptide Synthesis, W.H. Freeman Co, San Francisco; Merrifield (1963) J. Am. Chem. Soc 85:2149-2154). Peptide synthesis may be performed using manual or automated techniques. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City, Calif.) in accordance with the instructions provided by the manufacturer. For example, subsequences may be chemically synthesized separately and combined using chemical methods to provide protease variants or functional fragments thereof. Alternatively, such variant polypeptide sequences may be ordered from any number of companies that specialize in production of polypeptides. Most commonly, polypeptides of the invention are produced by expressing coding nucleic acids and recovering polypeptides, e.g., as described above and in the Examples.

[0143] In another aspect, the invention provides a method of producing a protease variant, the method comprising cultivating a

recombinant host cell of the invention, said host cell comprising an expression vector which comprising at least one nucleic acid of the invention, under conditions conducive to produce the variant. The method may further comprise recovering the variant from the culture.

[0144] In another aspect, the invention includes a method of producing a protease variant, the method comprising: (a) introducing the recombinant expression vector of the invention into a population of cells; and (b) culturing the cells in a culture medium under conditions conducive to produce the subtilisin variant encoded by the expression vector. The method may further comprise (c) isolating the variant from the cells or from the culture medium.

[0145] In another aspect, the invention provides methods for producing a serine protease variant of a *Bacillus* serine protease, comprising: transforming a host cell with an expression vector comprising a nucleic acid encoding the serine protease variant; and cultivating the transformed host cell under conditions suitable for the production of the serine protease variant. In one aspect, the methods further comprise the step of harvesting the produced serine protease variant. In one aspect, the host cell is a *Bacillus* cell, and in a subset of these aspects, the *Bacillus* cell is a *B. subtilis* cell. Furthermore, the present invention provides methods of cleaning, comprising the step of contacting a surface and/or an article comprising a fabric with a cleaning composition comprising a serine protease variant. In some alternative methods, the present invention provides methods of cleaning, comprising the step of contacting a surface and/or an article comprising dishware with a cleaning composition comprising a serine protease variant.

Compositions of the Invention

[0146] The invention includes a composition comprising at least one polypeptide (e.g., at least one protease variant) of the invention. Such compositions may comprise at least one excipient, carrier, adjunct ingredient, or other substituent, component, or material.

[0147] For example, in one aspect, the invention includes a composition comprising at least one protease variant and at least one excipient, carrier, adjunct ingredient, or other substituent, component, or material. Such at least one protease variant may be a BPN' protease variant. For example, a composition of the invention may be a BPN' protease variant, such as BPN'-v36 (SEQ ID NO:6). Such composition may be a fabric and home care product or fabric and home care composition.

[0148] A composition according to the invention may comprise at least one additional enzyme selected from the group consisting of hemicellulase, cellulase, amylase, peroxidase, protease, xylanase, lipase, phospholipase, esterase, cutinase, pectinase, pectate lyase, mannanase, keratinase, reductase, oxidase, phenoloxidase, lipoxygenase, ligninase, pullulanase, tannase, pentosanase, malanase, ß-g1ucanase, arabinosidase, hyaluronidase, chondroitinase, and laccase. A composition according to the seventeenth aspect of the invention may comprise two or more additional enzymes selected from the group consisting of hemicellulase, cellulase, amylase, peroxidase, protease, xylanase, lipase, phospholipase, esterase, cutinase, pectinase, pectate lyase, mannanase, keratinase, reductase, oxidase, phenoloxidase, lipoxygenase, ligninase, pullulanase, tannase, pentosanase, malanase, ß-glucanase, arabinosidase, hyaluronidase, chondroitinase, and laccase.

[0149] A composition according to the invention may comprise phosphate or may not contain phosphate. A composition according to the invention may comprise at least one builder and/or at least one surfactant.

[0150] As further elsewhere herein, the polypeptides of the invention, including the protease variants of the invention, are useful in a variety of cleaning applications, including laundry cleaning applications, automatic dishwashing applications, hand dishwashing applications, hard surface cleaning applications, personal care applications, and other applications described herein. Thus, for example, in one aspect, the invention provides cleaning compositions comprising at least one polypeptide (e.g., protease variant) of the invention. As noted above, such cleaning compositions include, but are not limited to, e.g., automatic and hand dishwashing detergent compositions, laundry detergent compositions (including, e.g., liquid and powder laundry detergent compositions), fabric cleaning compositions, hard surface cleaning compositions (including, but not limited to, e.g., hard surface of a non-dishware item, non-tableware item, table, table top, furniture item, wall, floor, ceiling, etc.). Such cleaning compositions, which are useful in methods of cleaning an item or a surface in need of cleaning, may comprise, e.g., but not limited to, at least one excipient, carrier, and/or other substituent, component, or material.

[0151] In another aspect, the invention provides a composition comprising any polypeptide of the invention (e.g., any protease variant or subtilisin variant of the invention) described herein, wherein said composition is a fabric and home care composition or a fabric and home care product.

[0152] In another aspect, the invention provides a composition comprising any polypeptide of the invention (e.g., any protease variant or subtilisin variant of the invention) described herein, wherein said composition is not a fabric and home care composition or not a fabric and home care product. A composition of the invention comprising a protease variant of the invention may further comprise at least one adjunct material selected from perfume encapsulate; fabric hueing agent; cold-water soluble brightener; a bleach catalyst that may comprise a material selected from an iminium cation, iminium polyion, iminium zwitterion; modified amine; modified amine oxide; N-sulphonyl imine; N-phosphonyl imine; N-acyl imine; thiadiazole dioxide; perfluoroimine; cyclic sugar ketone; first wash lipase; bacterial cleaning cellulase; Guerbet nonionic surfactant; and mixture of any thereof. Compositions of the invention may further comprise at least one additional non-immunoequivalent protease selected from subtilisins (EC 3.4.21.62); trypsin-like or chymotrypsin-like proteases; metalloproteases; and mixtures thereof.

[0153] Compositions of the invention may further comprise at least one additional non-immunoequivalent protease selected from: subtilisins (EC 3.4.21.62) derived from *B. subtilis, B. amyloliquefaciens, B. pumilus* and *B. gibsonii;* trypsin proteases and/or chymotrypsin proteases derived from *Cellulomonas;* metalloproteases derived from *B. amyloliquefaciens;* and mixtures thereof.

[0154] Compositions of the invention further comprise at least one additional enzyme selected from first-wash lipases; alpha-amylases; bacterial cleaning cellulases; and mixtures thereof.

[0155] A composition of the invention may further comprise at least one of the following: an encapsulate comprising a perfume comprises a perfume micro capsule; a hueing agent comprising a material selected from basic, acid, hydrophobic, direct and polymeric dyes, and dye-conjugates having a peak absorption wavelength of from 550 nm to 650 nm and mixtures thereof; a detersive surfactant comprising a material selected from anionic detersive surfactants, non-ionic detersive surfactant, cationic detersive surfactants, zwitterionic detersive surfactants and amphoteric detersive surfactants and mixtures thereof; a builder comprising a material selected from zeolites, phosphates and mixtures thereof; a silicate salt comprising a material selected from sodium silicate, potassium silicate and mixtures thereof; a brightener comprising a material selected from cold-water soluble brightener and mixtures thereof; a carboxylate polymer comprising a material selected from maleate/acrylate random copolymer or polyacrylate homopolymer and mixtures thereof; a soil release polymer comprising a material selected from terephthalate co-polymer and mixtures thereof; a cellulosic polymer comprising a material selected from alkyl cellulose, alkyl alkoxyalkyl cellulose, carboxyalkyl cellulose, alkyl carboxyalkyl cellulose and mixtures thereof; a bleach catalyst comprising a material selected from an iminium cation; iminium polyion; iminium zwitterion; modified amine; modified amine oxide; N-sulphonyl imine; N-phosphonyl imine; N-acyl imine; thiadiazole dioxide; perfluoroimine; cyclic sugar ketone and any mixture thereof; a bleach activator comprising a material selected from dodecanoyl oxybenzene sulphonate, decanoyl oxybenzene sulphonate, decanoyl oxybenzoic acid or salt thereof, 3,5,5-trimethyl hexanoyloxybenzene sulphonate, tetraacetyl ethylene diamine (TAED), nonanoyloxybenzene sulphonate (NOBS) and mixtures thereof; a source of hydrogen peroxide comprising a material selected from an inorganic perhydrate salt, including an alkali metal salt, such as sodium salts of perborate (usually mono- or tetra-hydrate), percarbonate, persulphate, perphosphate, persilicate salt, and any mixture thereof; a chelant comprising a material selected from DTPA (diethylene triamine pentaacetic acid), HEDP (hydroxyethane diphosphonic acid), DTPMP (diethylene triamine penta(methylene phosphonic acid)), ethylenediaminedisuccinic acid (EDDS), 1,2-dihydroxybenzene-3,5-disulfonic acid disodium salt hydrate, derivative of said chelant; and any mixture thereof.

[0156] A composition of the invention may comprise a fabric hueing agent selected from the group consisting of a dye; dye-clay conjugate comprising at least one cationic-basic dye and a smectite clay; and any mixture thereof.

[0157] A composition of the invention may comprise at least one fabric hueing agent selected from small molecule dye, polymeric dye, and any mixture thereof; dye-clay conjugate comprising at least one cationic-basic dye and a smectite clay; and any mixture thereof.

[0158] A composition comprising a protease of the invention may be provided in single or multiple-compartment unit doses. The composition may be a multi-compartment unit dose, wherein the protease variant is in a different compartment than any source of hydrogen peroxide and/or chelant and/or additional enzyme. A composition comprising at least one protease variant or polypeptide of the invention may comprise a wash liquor.

[0159] A composition comprising at least one protease variant of the invention may comprise one or more of the following ingredients (based on total composition weight): from about 0.0005 wt% to about 0.1 wt%, from about 0.001 wt% to about 0.05 wt%, or even from about 0.002 wt% to about 0.03 wt% of said protease variant; and one or more of the following: from about 0.00003 wt% to about 0.1 wt% fabric hueing agent; from about 0.001 wt% to about 5 wt %, perfume capsules; from about 0.001 wt% to about 1 wt%, cold-water soluble brighteners; from about 0.00003 wt% to about 0.1 wt% bleach catalysts; from about 0.00003 wt% to about 0.1 wt% first wash lipases; from about 0.00003 wt% to about 0.1 wt% bacterial cleaning cellulases; and/or from about 0.05 wt% to about 20 wt% Guerbet nonionic surfactants.

[0160] A composition may be a granular or powder laundry detergent comprising a cold water protease or comprising a protease variant that is not a cold water protease.

[0161] A composition of the invention may be provided in any suitable form, including a fluid or solid. The composition may be in the form of a unit dose pouch, especially when in the form of a liquid, and the composition may be at least partially, or even completely, enclosed by a water-soluble pouch. In addition, the composition may have any combination of parameters and/or characteristics detailed above.

[0162] Unless otherwise noted, all component or composition levels provided herein are made in reference to the active level of that component or composition, and are exclusive of impurities, for example, residual solvents or by-products, which may be present in commercially available sources. Enzyme components weights are based on total active protein. All percentages and ratios are calculated by weight unless otherwise indicated. All percentages and ratios are calculated based on the total composition unless otherwise indicated. In the exemplified detergent compositions, the enzymes levels are expressed by pure enzyme by weight of the total composition and, unless otherwise specified, the detergent ingredients are expressed by weight of the total compositions.

[0163] As indicated herein, in one aspect, the cleaning compositions of the present invention may further comprise one or more adjunct materials or ingredients including, but not limited to, e.g., one or more surfactants, builders, bleaches, bleach activators, bleach catalysts, other enzymes, enzyme stabilizing systems, chelants, optical brighteners, soil release polymers, dye transfer agents, dispersants, suds suppressors, dyes, perfumes, perfume capsules, colorants, filler salts, hydrotropes, photoactivators, fluorescers, fabric conditioners, fabric softeners, hydrolyzable surfactants, preservatives, anti-oxidants, anti-shrinkage agents, anti-wrinkle agents, germicides, fungicides, color speckles, silvercare, anti-tarnish and/or anti-corrosion agents, alkalinity sources, solubilizing agents, carriers, excipients, processing aids, pigments, rinse aid (e.g., a rinse aid containing at least one surfactant to prevent water droplet formation by making water drain from the surface of the item being cleaned in a thin sheet, rather than forming droplets), solvents, and/or pH control agents (see, e.g., U.S. Pat. Nos. 6,610,642, 6,605,458, 5,705,464, 5,710,115, 5,698,504, 5,695,679, 5,686,014 and 5,646,101, all of which are incorporated herein). Aspects of specific cleaning composition materials are exemplified in detail below. If a cleaning adjunct material(s) is not compatible with a protease variant of the present invention in a desired cleaning composition, then a suitable method of keeping the cleaning adjunct material(s) and the protease variant(s) separated (i.e., not in contact with one anther) until combination of the two components is appropriate is used. Such separation methods include any suitable method known in the art (e.g., gelcaps, encapsulation, tablets, physical separation, etc.).

[0164] The cleaning compositions of the present invention are advantageously employed, for example, in laundry applications, hard surface cleaning applications, hand or manual dishwashing applications, automatic dishwashing applications, eyeglass cleaning applications, as well as cosmetic applications, such as for cleaning dentures, teeth, hair, and skin. Due to the unique advantages of increased effectiveness in lower temperature solutions, the protease variant enzymes of the present invention are suited for laundry applications and dishwashing applications, including hand and automatic dishwashing applications. Furthermore, the protease variant enzymes of the present invention find use in solid, gel, granular, and/or liquid compositions, including solid, gel, granular, and/or liquid detergent compositions and/or formulations.

[0165] The protease variants of the present invention also find use cleaning additive product compositions. In one aspect, a protease variant of the invention is useful in low temperature solution cleaning applications and methods. In one aspect, the invention provides cleaning additive product compositions which include at least one protease variant enzyme of the present invention and which are ideally suited for inclusion in a wash process when additional bleaching effectiveness is desired. Such instances include, but are not limited to, e.g., low temperature solution cleaning applications. In one aspect, the additive product composition is in its simplest form - i.e., one or more protease variants of the invention. In one aspect, the additive product composition is packaged in dosage form for addition to a cleaning process. In one aspect, the additive product composition is packaged in dosage form for addition to a cleaning process where a source of peroxygen is employed and increased bleaching effectiveness is desired. Any suitable single dosage unit form may be used, including but not limited to, e.g., pills, tablets, gelcaps, or other single dosage units, such as pre-measured powders or liquids. Thus, in one aspect, the invention provides a cleaning product composition comprising at least one protease variant of the invention, wherein the product is formulated in suitable form (e.g., as a liquid, powder solid, pill, tablet, gelcap or other suitable form) in a suitable single dosage unit such that a single dose of the protease variant is provided. Such cleaning products are useful in a variety of cleaning methods and applications, including but not limited to, e.g., machine or hand laundry methods and applications, automatic dishwashing or hand dishwashing methods and applications, etc. Such cleaning methods and applications may be conducted at low temperature or low pH conditions. In one aspect, at least one filler and/or at least one carrier material is included to increase the volume of such compositions. Suitable filler or carrier materials include, but are not limited to, e.g., various salts of sulfate, carbonate and silicate as well as talc, clay and

the like. Suitable filler or carrier materials for liquid compositions include, but are not limited to, e.g., water or low molecular weight primary and secondary alcohols including polyols and diols. Examples of such alcohols include, but are not limited to, e.g., methanol, ethanol, propanol and isopropanol. In one aspect, the compositions contain from about 5% to about 90% of such filler or carrier materials. Acidic fillers may be included in such compositions to reduce the pH of the resulting solution in the cleaning method or application. Alternatively, in one aspect, the cleaning additive includes one or more adjunct ingredients, as more fully described below.

[0166] The present cleaning compositions and cleaning additives require an effective amount of at least one protease variant of the invention, alone or in combination with other proteases and/or additional enzymes. The required level of enzyme is achieved by the addition of one or more protease variants of the invention. Typically, a cleaning composition comprises at least about 0.0001 weight percent to about 20 weight percent, from about 0.0001 to about 10 weight percent, from about 0.0001 to about 1 weight percent, from about 0.001 to about 1 weight percent, or from about 0.01 to about 0.1 weight percent of at least one protease variant of the invention. In one aspect, a composition of the invention (e.g., cleaning composition of the invention) comprises from about 0.01 milligram (mg) to about 10 mg, about 0.01 to about 5 mg, about 0.01 mg to about 2 mg, about 0.01 to about 1 mg, about 0.5 mg to about 10 mg, about 0.5 to about 5 mg, about 0.5 to about 4 mg, about 0.5 to about 4 mg, about 0.5 to about 3 mg, about 0.5 to about 2 mg, about 0.5 to about 1 mg, about 0.1 to about 10 mg, about 0.1 to about 5 mg, about 0.1 to about 4 mg, about 0.1 to about 3 mg, about 0.1 to about 2 mg, about 0.1 to about 2 mg, about 0.1 to about 1 mg, about 0.1 to about 0.5 mg of at least one active protease variant of the invention per gram of the composition.

[0167] The invention includes a cleaning composition comprising an amount of a protease variant of the invention (said composition optionally comprising one or more adjunct ingredients) such that when the cleaning composition is added to wash water the resultant protease concentration in the resultant wash liquor is 0.01 ppm to 10 ppm, including, e.g., 0.1 ppm to 1 ppm, 0.1 to 5 ppm, 1 to 5 ppm, 1 ppm to 10 ppm, 5 ppm to 10 ppm, 5 ppm to 7 ppm.

[0168] The cleaning compositions of the invention are typically formulated such that, during use in aqueous cleaning operations, the wash water will have a pH of from about 5.0 to about 11.5 or even from about 7.5 to about 10.5. Liquid product compositions or formulations are typically formulated to have a neat pH from about 3.0 to about 9.0 or from about 3.0 to about 5.0. Granular laundry product compositions are typically formulated to have a pH from about 9 to about 11. Hand dishwashing and automatic dishwashing detergent compositions are typically formulated to have a pH from about 8 to about 11.5, including, but not limited to, e.g., pH ranges of about 8 to about 10, from about 9 to about 11.5, and from about 9.5 to about 11.5 depending on the method and specific application. Techniques for controlling pH at recommended usage levels include the use of buffers, alkalis, acids, etc., and are well known to those skilled in the art.

[0169] Suitable low pH cleaning compositions typically have a neat pH of from about 3 to about 5, and are typically free of surfactants that hydrolyze in such a pH environment. Such surfactants include sodium alkyl sulfate surfactants that comprise at least one ethylene oxide moiety or even from about 1 to about 16 moles of ethylene oxide. Such cleaning compositions typically comprise a sufficient amount of a pH modifier, such as sodium hydroxide, monoethanolamine, or hydrochloric acid, to provide such cleaning composition with a neat pH of from about 3 to about 5. Such compositions typically comprise at least one acid stable enzyme. In one aspect, the compositions are liquids, while in other aspects, they are solids. The pH of such liquid compositions is typically measured as a neat pH. The pH of such solid compositions is measured as a 10% solids solution of said composition wherein the solvent is distilled water. In these aspects, all pH measurements are taken at 20°C, unless otherwise indicated.

[0170] In one aspect, when the protease variant(s) of the invention is/are employed in a granular composition or liquid, it is desirable for the protease variant to be in the form of an encapsulated particle to protect the protease variant from other components of the granular composition during storage. In addition, encapsulation is also a means of controlling the availability of the protease variant during the cleaning process. In one aspect, encapsulation enhances the performance of the protease variant(s) and/or additional enzymes. In this regard, the protease variants of the present invention are encapsulated with any suitable encapsulating material known in the art. In one aspect, the encapsulating material typically encapsulates at least part of the catalyst for the protease variant(s) of the invention. Typically, the encapsulating material is water-soluble and/or water-dispersible. In one aspect, the encapsulating material has a glass transition temperature (Tg) of 0°C or higher. Glass transition temperature is described in more detail in WO 97/11151. The encapsulating material is typically selected from consisting of carbohydrates, natural or synthetic gums, chitin, chitosan, cellulose and cellulose derivatives, silicates, phosphates, borates, polyvinyl alcohol, polyethylene glycol, paraffin waxes, and combinations thereof. When the encapsulating material is a carbohydrate, it is typically selected from monosaccharides, oligosaccharides, polysaccharides, and combinations thereof. In some typical aspects, the encapsulating material is a starch (see, e.g., EP 0 922 499; U.S. Patent Nos. 4,977,252, 5,354,559, and 5,935,826). In one aspect, the encapsulating material is a microsphere made from plastic such as thermoplastics, acrylonitrile, methacrylonitrile, polyacrylonitrile, polymethacrylonitrile and mixtures thereof; commercially available microspheres that find use

include, but are not limited to those supplied by EXPANCEL® (Stockviksverken, Sweden), and PM 6545, PM 6550, PM 7220, PM 7228, EXTENDOSPHERES®, LUXSIL®, Q-CEL®, and SPHERICEL® (PQ Corp., Valley Forge, PA).

[0171] As described herein, the protease variants of the invention find particular use in the cleaning methods and applications, including, but not limited to, e.g., cleaning, laundry, hand dishwashing, and automatic dishwashing detergent compositions. These applications place enzymes under various environmental stresses. The protease variants of the invention provide advantages over many currently used enzymes in such cleaning applications due to their proteolytic activity and stability under various conditions.

[0172] There are a variety of wash conditions including varying detergent formulations, wash water volumes, wash water temperatures, and lengths of wash time, to which proteases involved in washing are exposed. In addition, detergent formulations used in different geographical areas have different concentrations of their relevant components present in the wash water. For example, European detergents typically have about 4500-5000 parts per million (ppm) of detergent components in the wash water, while Japanese detergents typically have approximately 667 ppm of detergent components in the wash water. In North America, particularly the United States, detergents typically have about 975 ppm of detergent components present in the wash water.

[0173] A low detergent concentration system includes detergents where less than about 800 ppm of the detergent components are present in the wash water. Japanese detergents are typically considered low detergent concentration system as they have approximately 667 ppm of detergent components present in the wash water.

[0174] A medium detergent concentration includes detergents where between about 800 ppm and about 2000 ppm of the detergent components are present in the wash water. North American detergents are generally considered to be medium detergent concentration systems as they have approximately 975 ppm of detergent components present in the wash water. Brazil typically has approximately 1500 ppm of detergent components present in the wash water.

[0175] A high detergent concentration system includes detergents where greater than about 2000 ppm of the detergent components are present in the wash water. European detergents are generally considered to be high detergent concentration systems as they have approximately 4500-5000 ppm of detergent components in the wash water.

[0176] Latin American detergents are generally high suds phosphate builder detergents and the range of detergents used in Latin America can fall in both the medium and high detergent concentrations as they range from 1500 ppm to 6000 ppm of detergent components in the wash water. As mentioned above, Brazil typically has approximately 1500 ppm of detergent components present in the wash water. However, other high suds phosphate builder detergent geographies, not limited to other Latin American countries, may have high detergent concentration systems up to about 6000 ppm of detergent components present in the wash water.

[0177] In light of the foregoing, it is evident that concentrations of detergent compositions in typical wash solutions throughout the world varies from less than about 800 ppm of detergent composition ("low detergent concentration geographies"), e.g., about 667 ppm in Japan, to between about 800 ppm to about 2000 ppm ("medium detergent concentration geographies"), e.g., about 975 ppm in U.S. and about 1500 ppm in Brazil, to greater than about 2000 ppm ("high detergent concentration geographies"), e.g., about 4500 ppm to about 5000 ppm in Europe and about 6000 ppm in high suds phosphate builder geographies.

[0178] The concentrations of the typical wash solutions are determined empirically. For example, in the U.S., a typical washing machine holds a volume of about 64.4 L of wash solution. Accordingly, in order to obtain a concentration of about 975 ppm of detergent within the wash solution about 62.79 g of detergent composition must be added to the 64.4 L of wash solution. This amount is the typical amount measured into the wash water by the consumer using the measuring cup provided with the detergent.

[0179] As a further example, different geographies use different wash temperatures. The temperature of the wash water in Japan is typically less than that used in Europe. For example, the temperature of the wash water in North America and Japan is typically between about 10 and about 30°C (e.g., about 20°C), whereas the temperature of wash water in Europe is typically between about 30 and about 60°C (e.g., about 40°C). However, in the interest of saving energy, many consumers are switching to using cold water washing. In addition, in some further regions, cold water is typically used for laundry, as well as in dishwashing applications. In one aspect, the "cold water washing" of the present invention utilizes washing at temperatures from about 4°C to about 10°C, from about 10°C to about 40°C, or from about 20°C to about 30°C, from about 15°C to about 25°C, from about 10°C to about 20°C, from about 14°C to about 18°C, as well as all other combinations within the range of about 15°C to about 35°C,

and all ranges within 10°C to 40°C, and about 16°C.

[0180] As a further example, different geographies typically have different water hardness. Water hardness is usually described in terms of the grains per gallon mixed Ca^{2+}/Mg^{2+}. Hardness is a measure of the amount of calcium (Ca^{2+}) and magnesium (Mg^{2+}) in the water. Most water in the United States is hard, but the degree of hardness varies. Moderately hard (60-120 ppm) to hard (121-181 ppm) water has 60 to 181 parts per million (parts per million converted to grains per U.S. gallon is ppm # divided by 17.1 equals grains per gallon) of hardness minerals.

Water	Grains per gallon	Parts per million
Soft	less than 1.0	less than 17
Slightly hard	1.0 to 3.5	17 to 60
Moderately hard	3.5 to 7.0	60 to 120
Hard	7.0 to 10.5	120 to 180
Very hard	greater than 10.5	greater than 180

[0181] European water hardness is typically greater than about 10.5 (e.g., about 10.5 to about 20.0) grains per gallon mixed Ca^{2+}/Mg^{2+} (e.g., about 15 grains per gallon mixed Ca^{2+}/Mg^{2+}). North American water hardness is typically greater than Japanese water hardness, but less than European water hardness. For example, North American water hardness can be between about 3 to about 10 grains, about 3 to about 8 grains or about 6 grains. Japanese water hardness is typically lower than North American water hardness, usually less than about 4, for example about 3 grains per gallon mixed Ca^{2+}/Mg^{2+}.

[0182] Accordingly, in one aspect, the invention provides protease variants that show surprising wash performance in at least one set of wash conditions (e.g., water temperature, water hardness, and/or detergent concentration). In one aspect, the protease variants of the invention are comparable in wash performance to other subtilisin proteases. In one aspect, the protease variants of the present invention exhibit enhanced wash performance as compared to subtilisin proteases currently commercially available. Thus, in one aspect of the invention, the protease variants provided herein exhibit enhanced oxidative stability, enhanced thermal stability, enhanced cleaning capabilities under various conditions, and/or enhanced chelator stability. In addition, the protease variants of the invention find use in cleaning compositions that do not include detergents, again either alone or in combination with builders and stabilizers.

[0183] In one aspect, the invention provides a cleaning composition comprising at least one protease variant of the present invention that is present at a level from about 0.00001 % to about 10% by weight of the composition with the balance (e.g., about 99.999% to about 90.0%) comprising one or more cleaning adjunct materials by weight of composition. In another aspect, the invention provides a cleaning composition comprising at least one protease variant of the invention that is present at a level of about 0.0001 % to about 10%, about 0.001% to about 5%, about 0.001% to about 2%, or about 0.005% to about 0.5% by weight of the composition with the balance of the cleaning composition (e.g., about 99.9999% to about 90.0%, about 99.999 % to about 98%, about 99.995% to about 99.5% by weight) comprising one or more cleaning adjunct materials.

[0184] In one aspect, a cleaning composition of the invention comprises, in addition to at least one protease variant of the invention, one or more additional enzymes, which provide cleaning performance and/or fabric care and/or hand or manual dishwashing and/or automatic dishwashing benefits. Examples of suitable enzymes include, but are not limited to, e.g., hemicellulases, cellulases, peroxidases, proteases, xylanases, lipases, phospholipases, esterases, perhydrolases, cutinases, pectinases, pectate lyases, mannanases, keratinases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, ß-glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, and/or amylases, neutral metalloprotease enzymes (abbreviated as "nprE"), or mixtures of any thereof. In one aspect, the cleaning composition comprises, in addition to at least one protease variant of the invention, a combination of additional enzymes (i.e., a "cocktail") comprising conventional applicable enzymes such as, e.g., at least one additional protease, lipase, cutinase, cellulose, and/or amylase.

[0185] In addition to the protease variants provided herein, any other suitable protease may find use and be included in a composition of the invention. In one aspect, the invention provides a composition (e.g., cleaning composition) comprising at least one protease variant of the invention and at least one additional protease. Suitable proteases include those of animal, vegetable, or microbial origin. In one aspect, a microbial protease may be included. A chemically or genetically modified mutant of a protease may be included. In one aspect, the at least one additional protease is a serine protease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases include subtilisins, especially those derived from *Bacillus* (e.g.,

subtilisin, *B. lentus* subtilisin (i.e., GG36), *B. amyloliquefaciens* subtilisin (i.e., BPN'), subtilisin Carlsberg, subtilisin 309, subtilisin 147, PB92, and subtilisin 168). Additional examples include those mutant proteases (i.e., protease variants) described in U.S. Pat. Nos. RE 34,606, 5,955,340, 5,700,676, 6,312,936, and 6,482,628. Additional proteases include, but are not limited to trypsin (e.g., of porcine or bovine origin), and the *Fusarium* protease described in WO 89/06270. A composition of the invention comprising at least one protease variant of the invention may also comprise at least one commercially available protease enzyme. Commercially available protease enzymes that find use in compositions of the invention include, but are not limited to, e.g., MAXATASE®, MAXACAL™, MAXAPEM™, OPTICLEAN®, OPTIMASE®, PROPERASE®, PURAFECT®, PURAFECT® OXP, PURAMAX™, EXCELLASE™, and PURAFAST™ (Genencor); ALCALASE®, SAVINASE®, PRIMASE®, DURAZYM™, POLARZYME®, OVOZYME®, KANNASE®, LIQUANASE®, NEUTRASE®, RELASE® and ESPERASE® (Novozymes); KAP *Bacillus* alkalophilus subtilisin with A230V+S256G+S259N (Kao); and BLAP™ *B. lentus* protease, BLAP X, and BLAP S (Henkel Kommanditgesellschaft auf Aktien, Duesseldorf, Germany). Additional proteases that may be included in compositions of the invention include those described in WO95/23221, WO 92/21760, U.S. Pat. Pub. No. 2008/0090747, and U.S. Pat. Nos. 5,801,039, 5,340,735, 5,500,364, 5,855,625, RE 34,606, 5,955,340, 5,700,676, 6,312,936, and 6,482,628, and various other patents. Metalloproteases may be included in compositions of the invention. Such metalloproteases, include, but are not limited to, e.g., the neutral metalloprotease enzyme (nprE) described in WO 07/044993.

[0186] In one aspect, the invention provides a composition (e.g., cleaning composition) comprising at least one protease variant of the invention and at least one lipase. Suitable lipases include, but are not limited to, e.g., those of bacterial or fungal origin. A chemically or genetically modified mutant of a lipase may be included in the composition. Examples of useful lipases include *Humicola lanuginosa* lipase (see, e.g., EP 258 068, and EP 305 216), *Rhizomucor miehei* lipase (see, e.g., EP 238 023), *Candida* lipase, such as *C. antartica* lipase (e.g., *C. antartica* lipase A or B; see, e.g., EP 214 761), *Pseudomonas* lipases such as *P. alcaligenes* lipase and *P. pseudoalcaligenes* lipase (see, e.g., EP 218 272), *P. cepacia* lipase (see, e.g., EP 331 376), *P. stutzeri* lipase (see, e.g., GB 1,372,034), *P.fluorescens* lipase, *Bacillus* lipase (e.g., *B. subtilis* lipase (Dartois et al., Biochem. Biophys. Acta 1131:253-260 (1993)); *B. stearothermophilus* lipase (see, e.g., JP 64/744992); and *B. pumilus* lipase (see, e.g., WO 91/16422)).

[0187] Furthermore, a number of cloned lipases find use in compositions (e.g., cleaning compositions) of the present invention, including, but not limited to, e.g., *Penicillium camembertii* lipase (Yamaguchi et al., Gene 103:61-67 (1991)), *Geotricum candidum* lipase (Schimada et al., J. Biochem. 106:383-388 (1989)), and various *Rhizopus* lipases such as *R. delemar* lipase (Hass et al., Gene 109:117-113 (1991)), a *R. niveus* lipase (Kugimiya et al., Biosci. Biotech. Biochem. 56:716-719 (1992)) and *R. oryzae* lipase.

[0188] Other types of lipolytic enzymes, such as cutinases, also find use in one aspect of the present invention, including, but not limited to, e.g., the cutinase derived from *Pseudomonas mendocina* (see WO 88/09367), and the cutinase derived from *Fusarium solani pisi* (see WO 90/09446).

[0189] Additional suitable lipases include commercially available lipases such as M1 LIPASE™, LUMA FAST™, and LIPOMAX™ (Genencor); LIPOLASE® and LIPOLASE® ULTRA (Novozymes); and LIPASE P™ "Amano" (Amano Pharmaceutical Co. Ltd., Japan).

[0190] In one aspect, the invention provides compositions (e.g., cleaning compositions) comprising at least one protease variant of the invention and at least one lipase that is present at a level from about 0.00001 % to about 10% of additional lipase by weight of the composition and the balance of one or more cleaning adjunct materials by weight of composition. In one aspect, a cleaning composition of the present invention comprises, in addition to at least one protease variant of the invention, at least one lipase at a level of about 0.0001 % to about 10%, about 0.001% to about 5%, about 0.001% to about 2%, or about 0.005% to about 0.5% lipase by weight of the composition.

[0191] Also included is a composition (e.g., cleaning composition) comprising at least one variant of the invention and at least one amylase. Any amylase (e.g., alpha and/or beta) suitable for use in alkaline solutions may be useful to include in such a composition. Suitable amylases include, but are not limited to, e.g., those of bacterial or fungal origin. A chemically or genetically modified mutant of an amylase may be included. Amylases that find use in compositions of the invention, include, but are not limited to, e.g., α-amylases obtained from *B. licheniformis* (see, e.g., GB 1,296,839). Commercially available amylases that find use in compositions of the invention include, but are not limited to, e.g., DURAMYL®, TERMAMYL®, FUNGAMYL®, STAINZYME®, STAINZYME PLUS®, STAINZYME ULTRA®, and BAN™ (Novozymes), as well as POWERASE™, RAPIDASE® and MAXAMYL® P (Genencor).

[0192] In one aspect, the invention provides a cleaning composition comprising at least one protease variant or at least one amylase, wherein the amylase is present at a level from about 0.00001 % to about 10% of additional amylase by weight of the

composition and the balance of one or more cleaning adjunct materials by weight of composition. In another aspect, the invention includes a cleaning composition comprising at least one protease variant and at least one amylase that is present at a level of about 0.0001 % to about 10%, about 0.001% to about 5%, about 0.001% to about 2%, about 0.005% to about 0.5% amylase by weight of the composition.

[0193] Any suitable cellulase may find used in a cleaning composition of the present invention. In one aspect, the invention provides a cleaning composition comprising at least one protease variant of the invention and one or at least one cellulase. Suitable cellulases include, but are not limited to, e.g., those of bacterial or fungal origin. A chemically or genetically modified mutant of a cellulase may be included in a composition of the invention. Suitable cellulases include, but are not limited to, e.g., *Humicola insolens* cellulases (see, e.g., U.S. Pat. No. 4,435,307) and cellulases having color care benefits (see, e.g., EP 0 495 257). Additional suitable cellulases are known in the art. Commercially available cellulases that find use and may be included in a composition of the invention include, but are not limited to, e.g., CELLUZYME®, CAREZYME® (Novozymes), and KAC-500(B)™ (Kao Corporation), Puradax 7000L, Puradax HA 4000G (Genencor). In one aspect, cellulases are incorporated as portions or fragments of mature wild-type or variant cellulases, wherein a portion of the N-terminus is deleted (see, e.g., U.S. Pat. No. 5,874,276). In one aspect, a cleaning composition of the invention comprises at least one protease variant of the invention and at least one cellulase at a level from about 0.00001 % to about 10% of additional cellulase by weight of the composition and the balance of one or more cleaning adjunct materials by weight of composition. In another aspect, the invention provides a cleaning composition comprising at least one protease variant of the invention and at least one cellulase at a level of about 0.0001 % to about 10%, about 0.001% to about 5%, about 0.001% to about 2%, about 0.005% to about 0.5% cellulase by weight of the composition.

[0194] Any mannanase suitable for use in detergent compositions also finds use in and thus may be included in a cleaning composition of the invention. In one aspect, the invention provides a cleaning composition comprising at least one protease variant of the invention and least one mannanase. Suitable mannanases include, but are not limited to, e.g., those of bacterial or fungal origin. A chemically or genetically modified mutant of a mannanase may be included in a composition of the invention. Various mannanases are known which are useful and may be included in a composition of invention (see, e.g., mannanases described in U.S. Pat. Nos. 6,566,114, 6,602,842, and 6,440,991). In one aspect, the invention provides a cleaning composition comprising at least one protease variant of the invention and at least one mannanase at a level from about 0.00001 % to about 10% of additional mannanase by weight of the composition and the balance of one or more cleaning adjunct materials by weight of composition. In some such cleaning compositions, each mannanase is present at a level of about 0.0001 % to about 10%, about 0.001% to about 5%, about 0.001% to about 2%, or about 0.005% to about 0.5% mannanase by weight of the composition.

[0195] A peroxidase may be used in combination with hydrogen peroxide or a source thereof (e.g., a percarbonate, perborate or persulfate) in a composition of the invention. An oxidase may be used in combination with oxygen in a composition of the invention. Both such types of enzymes are used for "solution bleaching" (i.e., to prevent transfer of a textile dye from a dyed fabric to another fabric when the fabrics are washed together in a wash liquor), preferably together with an enhancing agent (see, e.g., WO 94/12621 and WO 95/01426). Suitable peroxidases/oxidases that may be included in compositions of the invention include, but are not limited to, e.g., those of plant, bacterial, or fungal origin. A chemically or genetically modified mutant of a peroxidase or oxidase may be included in a composition of the invention. In one aspect, the invention provides a cleaning composition comprising at least one protease variant of the invention and at least one peroxidase and/or at least one oxidase enzyme. Each such peroxidase or oxidase may be present in the composition at a level from about 0.00001 % to about 10% of peroxidase or oxidase by weight of the composition and the balance of one or more cleaning adjunct materials by weight of composition. In another aspect, the invention provides a cleaning composition comprising at least one protease variant of the invention and at least one peroxidase and/or at least oxidase enzyme, wherein each such peroxidase or oxidase is present at a level of about 0.0001 % to about 10%, about 0.001% to about 5%, about 0.001% to about 2%, or about 0.005% to about 0.5% peroxidase or oxidase enzyme, respectively, by weight of the composition.

[0196] In one aspect, the invention provides a composition (e.g., cleaning composition) comprising at least one protease variant of the invention and one or more additional enzymes find use, including but not limited to, e.g., one or more perhydrolases (see, e.g., WO 05/056782).

[0197] In another aspect, the invention provides a composition (e.g., cleaning composition) comprising at least one protease variant of the invention and one or more mixtures of the above-mentioned enzymes are encompassed, such as, e.g., one or more additional proteases, amylases, lipases, mannanases, and/or cellulases. Indeed, it is contemplated that various mixtures of these enzymes will find use in compositions of the present invention. It is also contemplated that the varying levels of the protease variant(s) and one or more additional enzymes may both independently range to about 10%, the balance of the cleaning composition being one or more cleaning adjunct materials. The specific selection of a cleaning adjunct material is readily made by

considering the surface or item (e.g., dishware item, tableware item, or fabric item) or fabric to be cleaned, and the desired form of the composition for the cleaning conditions during use (e.g., through the hand or automatic dishwashing detergent use).

[0198] In one aspect, the invention provides a composition (e.g., cleaning composition) comprising at least one protease variant of the invention (and optionally at least one additional enzyme, if desired) and one or more cleaning adjunct materials. Examples of suitable cleaning adjunct materials include, but are not limited to, e.g., surfactants, builders, bleaches, bleach activators, bleach catalysts, other enzymes, enzyme stabilizing systems, chelants, optical brighteners, soil release polymers, dye transfer agents, dye transfer inhibiting agents, catalytic materials, hydrogen peroxide, sources of hydrogen peroxide, preformed peracids, polymeric dispersing agents, clay soil removal agents, structure elasticizing agents, dispersants, suds suppressors, dyes, perfumes, colorants, filler salts, hydrotropes, perfume, perfume capsule, photoactivators, fluorescers, fabric conditioners, fabric softeners, carriers, hydrotropes, processing aids, solvents, pigments, hydrolyzable surfactants, preservatives, anti-oxidants, anti-shrinkage agents, anti-wrinkle agents, germicides, fungicides, color speckles, silvercare, anti-tarnish and/or anticorrosion agents, alkalinity sources, solubilizing agents, carriers, processing aids, pigments, and pH control agents (see, e.g., U.S. Pat. Nos. 6,610,642, 6,605,458, 5,705,464, 5,710,115, 5,698,504, 5,695,679, 5,686,014 and 5,646,101). Aspects of specific cleaning composition materials are exemplified in detail below. As noted above, if a cleaning adjunct material is not compatible with a protease variant of the present invention included in a desired cleaning composition, then a suitable method of keeping the cleaning adjunct material(s) and the protease(s) separated (i.e., not in contact with each other) until combination of the components is appropriate is used. Such separation methods include any suitable method known in the art (e.g., gelcap, encapsulation, tablets, physical separation, etc.).

[0199] In one aspect, a composition (e.g., cleaning composition) of the invention comprises an effective amount of at least one protease variant of the invention that is useful or effective for cleaning a surface in need of proteinaceous stain removal. Such cleaning compositions include cleaning compositions for such applications as cleaning hard surfaces, laundry, fabrics, dishes, tableware, or dishware (e.g., by hand or manual dishwashing or automatic dishwashing). Indeed, in one aspect, the present invention provides fabric cleaning compositions, while in another aspect, the invention provides non-fabric cleaning compositions. Notably, the invention also provides cleaning compositions comprising at least one protease variant of the invention, wherein such cleaning compositions are suitable for personal care, including oral care (including dentifrices, toothpastes, mouthwashes, etc., as well as denture cleaning compositions), suitable for cleaning skin and hair, and suitable for cleaning eyeglasses. It is intended that the present invention encompass detergent compositions in any form (i.e., liquid, granular, bar, solid, semi-solid, gels, emulsions, tablets, capsules, etc.).

[0200] By way of example, several cleaning compositions wherein the protease variants of the invention find use are described in greater detail below. In one aspect in which the cleaning compositions of the invention are formulated as compositions suitable for use in laundry machine washing method(s), the compositions of the invention preferably contain at least one surfactant and at least one builder compound, as well as one or more cleaning adjunct materials, including, e.g., one or more cleaning adjunct materials selected from the group of organic polymeric compounds, bleaching agents, additional enzymes, suds suppressors, dispersants, lime-soap dispersants, soil suspension and anti-redeposition agents, and corrosion inhibitors. In one aspect, laundry compositions also contain one or more softening agents (i.e., as additional cleaning adjunct materials). Additional exemplary laundry or fabric cleaning compositions and formulations to which one or more protease variants of the invention can be added are presented in the Examples below.

[0201] The compositions of the invention also find use as detergent additive products in solid or liquid form. Such additive products are intended to supplement and/or boost the performance of conventional detergent compositions and can be added at any stage of the cleaning process. In one aspect, the density of the laundry detergent composition ranges from about 400 to about 1200 g/liter, while in other aspects, it ranges from about 500 to about 950 g/liter of composition measured at 20°C.

[0202] In one aspect, the invention provides cleaning compositions, such as those described in U.S. Pat. No. 6,605,458, comprising at least protease variant of the invention. In one aspect, the composition comprising at least one protease variant of the invention is a compact granular fabric cleaning composition, while in other aspects, the composition is a granular fabric cleaning composition useful in the laundering of colored fabrics; in another aspect, the composition is a granular fabric cleaning composition which provides softening through the wash capacity. In another aspect, the composition is a heavy duty liquid fabric cleaning composition. In another aspect, the invention provides a composition comprising at least one protease variant of the invention, wherein the composition is a fabric cleaning composition, such as one described in U.S. Pat. Nos. 6,610,642 and 6,376,450. Also provided are granular laundry detergent compositions of particular utility under European or Japanese washing conditions (see, e.g., U.S. Pat. No. 6,610,642) which comprise at least one protease variant of the invention.

[0203] In one aspect, the invention provides hard surface cleaning compositions comprising at least one protease variant provided herein. Some such compositions comprise hard surface cleaning compositions such as those described in U.S. Pat. Nos.

6,610,642, 6,376,450, and 6,376,450 that include at least one such protease variant.

[0204] The invention includes hand dishwashing or automatic dishwashing detergent compositions comprising at least one protease variant provided herein. Some such compositions comprise hard surface cleaning compositions, such as those described in U.S. Pat. Nos. 6,610,642 and 6,376,450.

[0205] In one aspect, the invention provides cleaning compositions for use in manual or hand dishwashing or automatic dishwashing methods comprising at least one protease variant of the invention and/or at least one surfactant and/or at least one additional cleaning adjunct material selected from the group of organic polymeric compounds, suds enhancing agents, group II metal ions, solvents, hydrotropes, and additional enzymes.

[0206] In one aspect in which the cleaning compositions of the invention are formulated as compositions suitable for use in automatic dishwashing machine method(s), the compositions of the invention typically contain at least one surfactant and/or at least one builder compound and may contain one or more cleaning adjunct materials preferably selected from organic polymeric compounds, bleaching agents, additional enzymes, suds suppressors, dispersants, lime-soap dispersants, soil suspension and anti-redeposition agents and corrosion inhibitors. Additional exemplary dishwashing compositions and formulations to which one or more protease variants of the invention can be added are presented in the Examples below.

[0207] In another aspect, the invention provides oral care compositions comprising at least one protease variant of the present invention that are useful for oral care (e.g., cleaning teeth and dentures); components of oral care compositions that may be useful and included in such compositions include those described in U.S. Pat. No. 6,376,450. Compositions of the invention may further comprise cleaning adjunct materials and compounds described in the U.S. Pat. Nos. 6,376,450, 6,605,458, and 6,610,642.

[0208] The cleaning compositions of the invention are formulated into any suitable form and prepared by any process chosen by the formulator, non-limiting examples of which are described in U.S. Pat. Nos. 5,879,584, 5,691,297, 5,574,005, 5,569,645, 5,565,422, 5,516,448, 5,489,392, and 5,486,303. When a low pH cleaning composition is desired, the pH of such composition is adjusted via the addition of a material such as monoethanolamine or an acidic material such as hydrogen chloride (HCl).

[0209] While not essential for the purposes of the present invention, the non-limiting list of adjuncts illustrated hereinafter are suitable for use in the instant cleaning compositions. In one aspect, these adjuncts are incorporated, e.g., to assist or enhance cleaning performance for treatment of the substrate to be cleaned or to modify the aesthetics of the cleaning composition as is the case with perfumes, colorants, dyes or the like. It is understood that such adjuncts are in addition to the protease variants of the present invention. The precise nature of these additional components, and levels of incorporation thereof, will depend on the physical form of the composition and the nature of the cleaning operation for which it is to be used. Suitable adjunct materials include, but are not limited to, e.g., surfactants, builders, chelating agents, dye transfer inhibiting agents, deposition aids, dispersants, additional enzymes, and enzyme stabilizers, catalytic materials, bleach activators, bleach boosters, hydrogen peroxide, sources of hydrogen peroxide, preformed peracids, polymeric dispersing agents, clay soil removal/anti-redeposition agents, brighteners, suds suppressors, dyes, perfumes, structure elasticizing agents, fabric softeners, carriers, hydrotropes, processing aids and/or pigments. In addition to the disclosure below, suitable examples of such other adjuncts and levels of use are found in U.S. Patent Nos. 5,576,282, 6,306,812, and 6,326,348. The aforementioned adjunct ingredients may constitute the balance of the cleaning compositions of the present invention.

[0210] In one aspect, cleaning compositions of the invention comprise at least one surfactant and/or a surfactant system, wherein the surfactant is selected from nonionic surfactants, anionic surfactants, cationic surfactants, ampholytic surfactants, zwitterionic surfactants, semi-polar nonionic surfactants and mixtures thereof. In some low pH cleaning composition aspects (e.g., compositions having a neat pH of from about 3 to about 5), the composition typically does not contain alkyl ethoxylated sulfate, as it is believed that such surfactant may be hydrolyzed by such compositions the acidic contents. In one aspect, the surfactant is present at a level of from about 0.1% to about 60%, while in alternative aspects the level is from about 1% to about 50%, while in still further aspects the level is from about 5% to about 40%, by weight of the cleaning composition.

[0211] In one aspect, cleaning compositions of the invention comprise one or more detergent builders or builder systems. In some such compositions incorporating at least one builder, the cleaning compositions comprise at least about 1%, from about 3% to about 60% or even from about 5% to about 40% builder by weight of the cleaning composition. Builders include, but are not limited to, e.g., alkali metal, ammonium and alkanolammonium salts of polyphosphates, alkali metal silicates, alkaline earth and alkali metal carbonates, aluminosilicates, polycarboxylate compounds, ether hydroxypolycarboxylates, copolymers of maleic anhydride with ethylene or vinyl methyl ether, 1, 3, 5-trihydroxy benzene-2, 4, 6-trisulphonic acid, and carboxymethyloxysuccinic acid, the various alkali metal, ammonium and substituted ammonium salts of polyacetic acids such as ethylenediamine tetraacetic

acid and nitrilotriacetic acid, as well as polycarboxylates, such as mellitic acid, succinic acid, citric acid, oxydisuccinic acid, polymaleic acid, benzene 1,3,5-tricarboxylic acid, carboxymethyloxysuccinic acid, and soluble salts thereof. It is contemplated that any suitable builder will find use in various compositions of the invention.

[0212] In some such compositions, the builders form water-soluble hardness ion complexes (e.g., sequestering builders), such as citrates and polyphosphates (e.g., sodium tripolyphosphate and sodium tripolyphospate hexahydrate, potassium tripolyphosphate, and mixed sodium and potassium tripolyphosphate, etc.). It is contemplated that any suitable builder will find use in the present invention, including those known in the art (see, e.g., EP 2 100 949).

[0213] Some cleaning compositions of the invention comprise at least one chelating agent in addition to at least one protease variant. Suitable chelating agents include, but are not limited to, e.g., copper, iron, and/or manganese chelating agents and mixtures thereof. In aspects in which at least one chelating agent is used, the cleaning compositions of the present invention comprise from about 0.1% to about 15% or even from about 3% to about 10% chelating agent by weight of the subject cleaning composition.

[0214] Some cleaning compositions provided herein comprise at least one deposition aid in addition to at least one protease variant. Suitable deposition aids include, but are not limited to, e.g., polyethylene glycol, polypropylene glycol, polycarboxylate, soil release polymers such as polytelephthalic acid, clays such as kaolinite, montmorillonite, atapulgite, illite, bentonite, halloysite, and mixtures thereof.

[0215] As indicated herein, in one aspect, anti-redeposition agents find use in one aspect of the present invention. In one aspect, non-ionic surfactants find use. These non-ionic surfactants also find use in preventing the re-deposition of soils. In one aspect, the anti-redeposition agent is a non-ionic surfactant as known in the art (see, e.g., EP 2 100 949).

[0216] In one aspect, the cleaning compositions of the present invention 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, polyvinyloxazolidones and polyvinylimidazoles or mixtures thereof. In aspects in which at least one dye transfer inhibiting agent is used, the cleaning compositions of the present invention comprise 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 cleaning composition.

[0217] In one aspect, silicates are included within the compositions of the present invention. In some such aspects, sodium silicates (e.g., sodium disilicate, sodium metasilicate, and crystalline phyllosilicates) find use. In one aspect, silicates are present at a level of from about 1% to about 20%. In one aspect, silicates are present at a level of from about 5% to about 15% by weight of the composition.

[0218] In one aspect, the cleaning compositions of the invention also comprise dispersants. Suitable water-soluble organic materials include, but are not limited to, e.g., 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.

[0219] In one aspect, the enzymes (e.g., protease variants of the invention or other additional enzymes) used in the cleaning compositions are stabilized any suitable technique. In one aspect, the enzymes employed herein are stabilized by the presence of water-soluble sources of calcium and/or magnesium ions in the finished compositions that provide such ions to the enzymes. In one aspect, the enzyme stabilizers include oligosaccharides, polysaccharides, and inorganic divalent metal salts, including alkaline earth metals, such as calcium salts. It is contemplated that various techniques for enzyme stabilization will find use in the present invention. For example, in one aspect, the enzymes employed herein are stabilized by the presence of water-soluble sources of zinc (II), calcium (II) and/or magnesium (II) ions in the finished compositions that provide such ions to the enzymes, as well as other metal ions (e.g., barium (II), scandium (II), iron (II), manganese (II), aluminum (III), Tin (II), cobalt (II), copper (II), nickel (II), and oxovanadium (IV). Chlorides and sulfates also find use in one aspect of the present invention. Examples of suitable oligosaccharides and polysaccharides (e.g., dextrins) are known in the art (see, e.g., WO 07/145964). In one aspect, reversible protease inhibitors also find use, such as boron-containing compounds (e.g., borate, phenyl boronic acid, 4-formyl phenyl boronic acid, other phenyl boronic acid derivatives, peptide inhibitors, and the like) and/or a tripeptide aldehyde find use in compositions of the invention to further improve stability, as desired.

[0220] In one aspect, one or more bleaches, bleach activators, and/or bleach catalysts are included in the compositions of the invention. In one aspect, the cleaning compositions of the invention comprise inorganic and/or organic bleaching compound(s). Inorganic bleaches include, but are not limited to perhydrate salts (e.g., perborate, percarbonate, perphosphate, persulfate, and persilicate salts). In one aspect, inorganic perhydrate salts are alkali metal salts. In one aspect, inorganic perhydrate salts are

included as the crystalline solid, without additional protection, although in some other aspects, the salt is coated. Any suitable salt known in the art finds use in the compositions of the invention (see, e.g., EP 2 100 949).

[0221] In one aspect, bleach activators are used in the compositions of the invention. Bleach activators are typically organic peracid precursors that enhance the bleaching action in the course of cleaning at temperatures of 60°C and below. Bleach activators suitable for use herein include compounds which, under perhydrolysis conditions, give aliphatic peroxycarboxylic acids having preferably from about 1 to about 10 carbon atoms, in particular from about 2 to about 4 carbon atoms, and/or optionally substituted perbenzoic acid. Additional bleach activators are known in the art and find use in the composition of the invention (see, e.g., EP 2 100 949).

[0222] In one aspect and as further described herein, the cleaning compositions of the invention further comprise at least one bleach catalyst. In one aspect, the manganese triazacyclononane and related complexes find use, as well as cobalt, copper, manganese, and iron complexes. Additional bleach catalysts find use in the present invention (see, e.g., U.S. Pat Nos. 4,246,612, 5,227,084, and 4,810410; WO 99/06521; and EP 2 100 949).

[0223] In one aspect, the cleaning compositions of the invention comprise one or more catalytic metal complexes. In one aspect, a metal-containing bleach catalyst finds use. In some aspects, the metal bleach catalyst comprises a catalyst system comprising a transition metal cation of defined bleach catalytic activity (e.g., copper, iron, titanium, ruthenium, tungsten, molybdenum, or manganese cations), an auxiliary metal cation having little or no bleach catalytic activity (e.g., zinc or aluminum cations), and a sequestrate having defined stability constants for the catalytic and auxiliary metal cations, particularly ethylenediaminetetraacetic acid, ethylenediaminetetra (methylenephosphonic acid) and water-soluble salts thereof are used (see, e.g., U.S. Pat. No. 4,430,243). In one aspect, the cleaning compositions of the invention are catalyzed by means of a manganese compound. Such compounds and levels of use are well known in the art (see, e.g., U.S. Patent No. 5,576,282). In additional aspects, cobalt bleach catalysts find use and are included in the cleaning compositions of the invention. Various cobalt bleach catalysts are known in the art (see, e.g., U.S. Patent Nos. 5,597,936 and 5,595,967) and are readily prepared by known procedures.

[0224] In one aspect, the cleaning compositions of the invention include a transition metal complex of a macropolycyclic rigid ligand (MRL). As a practical matter, and not by way of limitation, in one aspect, the compositions and cleaning processes provided by the invention are adjusted to provide on the order of at least one part per hundred million of the active MRL species in the aqueous washing medium, and in one aspect, provide from about 0.005 ppm to about 25 ppm, from about 0.05 ppm to about 10 ppm, and from about 0.1 ppm to about 5 ppm, of the MRL in the wash liquor.

[0225] In one aspect, transition-metals in the instant transition-metal bleach catalyst include, but are not limited to, e.g., manganese, iron and chromium. Preferred MRLs also include, but are not limited to, e.g., special ultra-rigid ligands that are cross-bridged (e.g., 5,12-diethyl-1,5,8,12-tetraazabicyclo(6.6.2)hexadecane). Suitable transition metal MRLs are readily prepared by known procedures (see, e.g., WO 2000/32601 and U.S. Pat. No. 6,225,464).

[0226] In one aspect, the invention provides an automatic dishwashing detergent composition formulated as a detergent tablet. Such tablet comprises at least one protease variant of the invention and a builder, such as, e.g., a builder salt. Some such tablets have an alkalinity of at least equivalent to 3 grams (g) of sodium hydroxide per 100 grams of the tablet composition and a density of at least 1.4 grams/cubic centimeter. The builder salt can comprise a mixture of a silicate salt and a phosphate salt, preferably with more silicate (e.g., sodium metasilicate) than phosphate (e.g., sodium tripolyphosphate). Some such tablets are free of surfactant materials and are especially adapted for use in automatic dishwashing machines.

[0227] In one aspect, the cleaning compositions of the present invention comprise metal care agents. Metal care agents are useful in preventing and/or reducing the tarnishing, corrosion, and/or oxidation of metals, including aluminum, stainless steel, and non-ferrous metals (e.g., silver and copper). Suitable metal care agents include those described in EP 2 100 949, WO 9426860, and WO 94/26859). In some such cleaning compositions, the metal care agent is a zinc salt. Some such cleaning compositions comprise from about 0.1% to about 5% by weight of one or more metal care agent.

[0228] As indicated above, the cleaning compositions of the present invention are formulated into any suitable form and prepared by any process chosen by the formulator, non-limiting examples of which are described in U.S. Pat. Nos. 5,879,584, 5,691,297, 5,574,005, 5,569,645, 5,516,448, 5,489,392, and 5,486,303. In one aspect in which a low pH cleaning composition is desired, the pH of such composition is adjusted via the addition of an acidic material such as hydrogen chloride (HCl).

[0229] The cleaning compositions disclosed herein find use in cleaning a situs (e.g., a surface, dishware, tableware, or fabric). Typically, at least a portion of the situs is contacted with a present cleaning composition of the invention in neat form or diluted in

a wash liquor, and then the situs is optionally washed and/or rinsed. For purposes of the present invention, "washing" includes, but is not limited to, e.g., scrubbing and mechanical agitation. In one aspect, the cleaning compositions are employed at concentrations of from about 500 ppm to about 15,000 ppm in solution. When the wash solvent is water, the water temperature typically ranges from about 5°C to about 90°C and when the situs comprises a fabric, the water to fabric mass ratio is typically from about 1:1 to about 30:1.

Processes of Making and Using Compositions

[0230] The compositions of the invention described herein and throughout, including, e.g., cleaning compositions, can be formulated into any suitable form and prepared by any suitable process chosen by the formulator (see, e.g., US Patent Nos. 5,879,584, 5,691,297, 5,574,005, 5,569,645, 5,565,422, 5,516,448, 5,489,392, 5,486,303, 4,515,705, 4,537,706, 4,515,707, 4,550,862, 4,561,998, 4,597,898, 4,968,451, 5,565,145, 5,929,022, 6,294,514 and 6,376,445).

[0231] In one aspect, the cleaning compositions of the invention are provided in unit dose form, including tablets, capsules, sachets, pouches, and multi-compartment pouches. In one aspect, the unit dose format is designed to provide controlled release of the ingredients within a multi-compartment pouch (or other unit dose format). Suitable unit dose and controlled release formats are known in the art (see, e.g., EP 2 100 949, WO 02/102955, U.S. Pat. Nos. 4,765,916 and 4,972,017, and WO 04/111178 for materials suitable for use in unit dose and controlled release formats). In one aspect, the unit dose form is provided by tablets wrapped with a water-soluble film or water-soluble pouches. Various formats for unit doses are provided in EP 2 100 947, and are known in the art.

[0232] Additional aspects of the invention relating to processes of making and using compositions of the invention are described elsewhere herein.

Methods of the Invention

[0233] The invention provides methods for cleaning or washing an item or surface (e.g., hard surface) in need of cleaning, including, but not limited to, e.g., methods for cleaning or washing a dishware item, a tableware item, a fabric item, a laundry item, personal care item, eye glass, etc., or the like, and methods for cleaning or washing a hard or soft surface, such as, e.g., a hard surface of an item.

[0234] In one aspect, the invention provides a method for cleaning an item, object, or surface in need of cleaning, the method comprising contacting the item or surface (or a portion of the item or surface desired to be cleaned) with a protease variant of any of the invention or a composition of the invention for a sufficient time and/or under conditions suitable or effective to clean the item, object, or surface to a desired degree. Some such methods further comprise rinsing the item, object, or surface with water. For some such methods, the cleaning composition is a dishwashing detergent composition and the item or object to be cleaned is a dishware item or tableware item. A dishware item is a dish item generally used in serving or eating food. A dishware item can be, but is not limited to, e.g., a dish, plate, cup, bowl, etc., and the like. Tableware is a broader term that includes, but is not limited, to, e.g., dishes, cutlery, knives, forks, spoons, chopsticks, glassware, pitchers, sauce boats, drinking vessels, etc., and the like; a tableware item includes any of these or similar items for serving or eating food. For some such methods, the cleaning composition is an automatic dishwashing detergent composition or a hand dishwashing detergent composition and the item or object to be cleaned is a dishware or tableware item. For some such methods, the cleaning composition is a laundry detergent composition, such as, e.g., a power laundry detergent composition or a liquid laundry detergent composition, and the item to be cleaned is a fabric item.

EXPERIMENTAL

[0235] In the experimental disclosure, the following abbreviations apply: PI (Performance Index), ppm (parts per million); M (molar); mM (millimolar); µM (micromolar); nM (nanomolar); mol (moles); mmol (millimoles); µmol (micromoles); nmol (nanomoles); gm (grams); mg (milligrams); µg (micrograms); pg (picograms); L (liters); ml and mL (milliliters); µl and µL (microliters); cm (centimeters); mm (millimeters); µm (micrometers); nm (nanometers); U (units); V (volts); MW (molecular weight); sec (seconds); min(s) (minute/minutes); h(s) and hr(s) (hour/hours); °C (degrees Centigrade); QS (quantity sufficient); ND (not done); rpm (revolutions per minute); GH (degrees German hardness); H_2O (water); dH_2O (deionized water); HCl (hydrochloric acid); aa

(amino acid); bp (base pair); kb (kilobase pair); kD (kilodaltons); cDNA (copy or complementary DNA); DNA (deoxyribonucleic acid); ssDNA (single stranded DNA); dsDNA (double stranded DNA); dNTP (deoxyribonucleotide triphosphate); RNA (ribonucleic acid); $MgCl_2$ (magnesium chloride); NaCl (sodium chloride); w/v (weight to volume); v/v (volume to volume); w/w (weight to weight); g (gravity); OD (optical density); ppm (parts per million); Dulbecco's phosphate buffered solution (DPBS); SOC (2% Bacto-Tryptone, 0.5% Bacto Yeast Extract, 10 mM NaCl, 2.5 mM KCl); Terrific Broth (TB; 12 g/l Bacto-Tryptone, 24 g/l glycerol, 2.31 g/l KH_2PO_4, and 12.54 g/l K_2HPO_4) OD_{280} (optical density at 280 nm); OD_{600} (optical density at 600 nm); A_{405} (absorbance at 405 nm); Vmax (the maximum initial velocity of an enzyme catalyzed reaction); PAGE (polyacrylamide gel electrophoresis); PBS (phosphate buffered saline [150 mM NaCl, 10 mM sodium phosphate buffer, pH 7.2]); PBST (PBS+0.25% TWEEN®-20); PEG (polyethylene glycol); PCR (polymerase chain reaction); RT-PCR (reverse transcription PCR); SDS (sodium dodecyl sulfate); Tris (tris(hydroxymethyl)aminomethane); HEPES (N-[2-Hydroxyethyl]piperazine-N-[2-ethanesulfonic acid]); HBS (HEPES buffered saline); Tris-HCl (tris[Hydroxymethyl]aminomethane-hydrochloride); Tricine (N-[tris-(hydroxymethyl)-methyl]-glycine); CHES (2-(N-cyclo-hexylamino) ethane-sulfonic acid); TAPS (3-{[tris-(hydroxymethyl)-methyl]-amino{-propanesulfonic acid); CAPS (3-(cyclo-hexylamino)-propane-sulfonic acid; DMSO (dimethyl sulfoxide); DTT (1,4-dithio-DL-threitol); SA (sinapinic acid (s,5-dimethoxy-4-hydroxy cinnamic acid); TCA (trichloroacetic acid); Glut and GSH (reduced glutathione); GSSG (oxidized glutathione); TCEP (Tris[2-carboxyethyl] phosphine); Ci (Curies); mCi (milliCuries); µCi (microCuries); HPLC (high-performance liquid chromatography); RP-HPLC (reverse phase high pressure liquid chromatography); TLC (thin layer chromatography); MALDI-TOF (matrix-assisted laser desorption/ionization--time of flight); Ts (tosyl); Bn (benzyl); Ph (phenyl); Ms (mesyl); Et (ethyl), Me (methyl); *Taq* (*thermus aquaticus* DNA polymerase); Klenow (DNA polymerase I large (Klenow) fragment); EGTA (ethylene glycol-bis(ß-aminoethyl ether) N, N, N', N'-tetraacetic acid); EDTA (ethylenediaminetetracetic acid); bla (β-lactamase or ampicillin-resistance gene); HDL (heavy duty liquid); HDD (heavy duty powder detergent); HSG (high suds granular detergent); CEE (Central and Eastern Europe); WE (Western Europe); NA, when used in reference to detergents (North America); Japan and JPN, when used in reference to detergents (Japan); MTP (microtiter plate); MJ Research (MJ Research, Reno, NV); Baseclear (Baseclear BV, Inc., Leiden, The Netherlands); PerSeptive (PerSeptive Biosystems, Framingham, MA); ThermoFinnigan (ThermoFinnigan, San Jose, CA); Argo (Argo BioAnalytica, Morris Plains, NJ);Seitz EKS (SeitzSchenk Filtersystems GmbH, Bad Kreuznach, Germany); Pall (Pall Corp., East Hills, NY and Bad Kreuznach, Germany); Spectrum (Spectrum Laboratories, Dominguez Rancho, CA); Molecular Structure (Molecular Structure Corp., Woodlands, TX); Accelrys (Accelrys, Inc., San Diego, CA); Chemical Computing (Chemical Computing Corp., Montreal, Canada); New Brunswick (New Brunswick Scientific, Co., Edison, NJ); CFT (Center for Test Materials, Vlaardingen, The Netherlands); P&G and Procter & Gamble (Procter & Gamble, Inc., Cincinnati, OH); GE Healthcare (GE Healthcare, Chalfont St. Giles, United Kingdom); DNA2.0 (DNA2.0, Menlo Park, CA); OXOID (Oxoid, Basingstoke, Hampshire, UK); Megazyme (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co., Wicklow, Ireland); Finnzymes (Finnzymes Oy, Espoo, Finland); Kelco (CP Kelco, Wilmington, DE); Corning (Corning Life Sciences, Corning, NY); (NEN (NEN Life Science Products, Boston, MA); Pharma AS (Pharma AS, Oslo, Norway); Dynal (Dynal, Oslo, Norway); Bio-Synthesis (Bio-Synthesis, Lewisville, TX); ATCC (American Type Culture Collection, Rockville, MD); Gibco/BRL (Gibco/BRL, Grand Island, NY); Sigma (Sigma Chemical Co., St. Louis, MO); Pharmacia (Pharmacia Biotech, Piscataway, NJ); NCBI (National Center for Biotechnology Information); Applied Biosystems (Applied Biosystems, Foster City, CA); BD Biosciences and/or Clontech (BD Biosciences CLONTECH Laboratories, Palo Alto, CA); Operon Technologies (Operon Technologies, Inc., Alameda, CA); MWG Biotech (MWG Biotech, High Point, NC); Oligos Etc (Oligos Etc. Inc, Wilsonville, OR); Bachem (Bachem Bioscience, Inc., King of Prussia, PA); Difco (Difco Laboratories, Detroit, MI); Mediatech (Mediatech, Herndon, VA; Santa Cruz (Santa Cruz Biotechnology, Inc., Santa Cruz, CA); Oxoid (Oxoid Inc., Ogdensburg, NY); Worthington (Worthington Biochemical Corp., Freehold, NJ); GIBCO BRL or Gibco BRL (Life Technologies, Inc., Gaithersburg, MD); Millipore (Millipore, Billerica, MA); Bio-Rad (Bio-Rad, Hercules, CA); Invitrogen (Invitrogen Corp., San Diego, CA); NEB (New England Biolabs, Beverly, MA); Sigma (Sigma Chemical Co., St. Louis, MO); Pierce (Pierce Biotechnology, Rockford, IL); Takara (Takara Bio Inc. Otsu, Japan); Roche (Hoffinann-La Roche, Basel, Switzerland); Gene Oracle (Gene Oracle, Inc., Mountain View, CA); EM Science (EM Science, Gibbstown, NJ); Qiagen (Qiagen, Inc., Valencia, CA); Biodesign (Biodesign Intl., Saco, Maine); Aptagen (Aptagen, Inc., Herndon, VA); Sorvall (Sorvall brand, from Kendro Laboratory Products, Asheville, NC); Molecular Devices (Molecular Devices, Corp., Sunnyvale, CA); R&D Systems (R&D Systems, Minneapolis, MN); Siegfried Handel (Siegfried Handel AG, Zofingen, Switzerland); Stratagene (Stratagene Cloning Systems, La Jolla, CA); Marsh (Marsh Biosciences, Rochester, NY); Geneart (Geneart GmbH, Regensburg, Germany); Bio-Tek (Bio-Tek Instruments, Winooski, VT); (Biacore (Biacore, Inc., Piscataway, NJ); PeproTech (PeproTech, Rocky Hill, NJ); SynPep (SynPep, Dublin, CA); New Objective (New Objective brand; Scientific Instrument Services, Inc., Ringoes, NJ); Waters (Waters, Inc., Milford, MA); Matrix Science (Matrix Science, Boston, MA); Dionex (Dionex, Corp., Sunnyvale, CA); Monsanto (Monsanto Co., St. Louis, MO); Wintershall (Wintershall AG, Kassel, Germany); BASF (BASF Co., Florham Park, NJ); Huntsman (Huntsman Petrochemical Corp., Salt Lake City, UT); Shell Chemicals (Shell Chemicals, Inc., London, UK); Stepan (Stepan, Northfield, IL); Clariant (Clariant, Sulzbach, Germany); Industrial Zeolite (Industrial Zeolite Ltd., Grays, Essex, UK); Jungbunzlauer (Jungbunzlauer, Basel, Switzerland); Solvay (Solvay, Brussels, Belgium); 3V Sigma (3V Sigma, Bergamo, Italy); Innospec (Innospec, Ellesmere Port, UK); Thermphos (Thermphos, Vlissiggen-Ost, The Netherlands); Ciba Specialty (Ciba Specialty Chemicals, Basel, Switzerland); Dow Corning (Dow Corning, Barry, UK); Enichem (Enichem Iberica, Barcelona, Spain); Fluka Chemie AG (Fluka Chemie AG, Buchs, Switzerland); Gist-Brocades (Gist-Brocades, NV, Delft, The Netherlands); Dow Coming

(Dow Coming Corp., Midland, MI); Mettler-Toledo (Mettler-Toledo Inc, Columbus, OH); RB (Reckitt-Benckiser, Slough, UK); and Microsoft (Microsoft, Inc., Redmond, WA).

[0236] In the exemplified detergent compositions provided herein, the enzymes levels are expressed by pure enzyme by weight of the total composition and unless otherwise specified, the detergent ingredients are expressed by weight of the total compositions. The abbreviated component identifications therein have the following meanings:

Abbreviation	**Ingredient**
LAS	: Sodium linear C_{11-13} alkyl benzene sulfonate
NaC16-17HSAS	: Sodium C_{16-17} highly soluble alkyl sulfate
TAS	: Sodium tallow alkyl sulphate
CxyAS	: Sodium C_{1x} - C_{1y} alkyl sulfate
CxyEz	: C_{1x} - C_{1y} predominantly linear primary alcohol condensed with an average of z moles of ethylene oxide
CxyAEzS	: C_{1x} - C_{1y} sodium alkyl sulfate condensed with an average of z moles of ethylene oxide. Added molecule name in the examples.
Nonionic	: Mixed ethoxylated/propoxylated fatty alcohol e.g. Plurafac LF404 being an alcohol with an average degree of ethoxylation of 3.8 and an average degree of propoxylation of 4.5.
QAS	: $R_2.N^+(CH_3)_2(C_2H_4OH)$ with $R_2 = C_{12}$-C_{14}
Silicate	: Amorphous Sodium Silicate (SiO_2:Na_2O ratio = 1.6-3.2:1)
Metasilicate	: Sodium metasilicate (SiO_2:Na_2O ratio = 1.0)
Zeolite A	: Hydrated aluminosilicate of formula $Na_{12}(AlO_2SiO_2)_{12}\cdot 27H_2O$
SKS-6	: Crystalline layered silicate of formula δ-$Na_2Si_2O_5$
Sulfate	: Anhydrous sodium sulphate
STPP	: Sodium Tripolyphosphate
MA/AA	: Random copolymer of 4:1 acrylate/maleate, average molecular weight about 70,000-80,000
AA	: Sodium polyacrylate polymer of average molecular weight (MW) 4,500
Polycarboxylate	: Copolymer comprising mixture of carboxylated monomers such as acrylate, maleate and methyacrylate with a MW ranging between 2,000-80,000 such as Sokolan commercially available from BASF, being a copolymer of acrylic acid, MW 4,500
BB1	: 3-(3,4-Dihydroisoquinolinium)propane sulfonate
BB2	1-(3,4-dihydroisoquinolinium)-decane-2-sulfate
PB 1	: Sodium perborate monohydrate
PB4	: Sodium perborate tetrahydrate of nominal formula $NaBO_3\cdot 4H_2O$
Percarbonate	: Sodium percarbonate of nominal formula $2Na_2CO_3\cdot 3H_2O_2$
TAED	: Tetraacetyl ethylene diamine
NOBS	: Nonanoyloxybenzene sulfonate in the form of the sodium salt
DTPA	: Diethylene triamine pentaacetic acid
HEDP	: 1,1-hydroxyethane diphosphonic acid
DETPMP	: Diethyltriamine penta (methylene) phosphonate, marketed by Monsanto under the Trade name Dequest 2060
EDDS	: Ethylenediamine-N,N'-disuccinic acid, (S,S) isomer in the form of its sodium salt
Diamine	: Dimethyl aminopropyl amine; 1,6-hezane diamine; 1,3-propane diamine; 2-methyl-1,5-pentane diamine; 1,3-pentanediamine; 1-methyl-diaminopropane
DETBCHD	5, 12- diethyl-1,5,8,12-tetraazabicyclo [6,6,2] hexadecane, dichloride, Mn(II) SALT
PAAC	: Pentaamine acetate cobalt(III) salt
Paraffin	: Paraffin oil sold under the tradename Winog 70 by Wintershall

Abbreviation	Ingredient
Paraffin Sulfonate	: A Paraffin oil or wax in which some of the hydrogen atoms have been replaced by sulfonate groups
Aldose oxidase	: Oxidase enzyme sold under the tradename Aldose Oxidase by Novozymes A/S
Galactose oxidase	: Galactose oxidase from Sigma
nprE	: The recombinant form of neutral metalloprotease expressed in *Bacillus subtilis* (see, e.g., WO 07/044993)
PMN	: Purified neutral metalloprotease from *Bacillus amyloliquefaciens*
Amylase	: A suitable amylolytic enzyme, such as those sold under the tradenames PURAFECT® Ox described in WO 94/18314, WO96/05295 sold by Genencor; NATALASE®, TERMAMYL®, FUNGAMYL® and DURAMYL™, all available from Novozymes A/S.
Lipase	: A suitable lipolytic enzyme such as those sold under the tradenames LIPEX®, LIPOLASE®, LIPOLASE® Ultra by Novozymes A/S and Lipomax™ by Gist-Brocades.
Cellulase	: A suitable cellulytic enzyme such as those sold under the tradenames CAREZYME®, CELLUZYME®, and/or ENDOLASE® by Novozymes A/S.
Pectin Lyase	: A suitable pectin lyase, such as those sold under the tradenames XPECT®, PECTAWAY® and PECTAWASH® available from Novozymes A/S.
PVP	: Polyvinylpyrrolidone with an average molecular weight of 60,000
PVNO	: Polyvinylpyridine-N-Oxide, with an average molecular weight of 50,000
PVPVI	: Copolymer of vinylimidazole and vinylpyrrolidone, with an average molecular weight of 20,000
Brightener 1	: Disodium 4,4'-bis(2-sulphostyryl)biphenyl
Silicone antifoam	: Polydimethylsiloxane foam controller with siloxane-oxyalkylene copolymer as dispersing agent with a ratio of said foam controller to said dispersing agent of 10:1 to 100:1
Suds Suppressor	: 12% Silicone/silica, 18% stearyl alcohol, 70% starch in granular form
SRP 1	: Anionically end capped poly esters
PEG X	: Polyethylene glycol of a molecular weight of x
PVP K60®	: Vinylpyrrolidone homopolymer (average MW 160,000)
Jeffamine® ED-2001	: Capped polyethylene glycol from Huntsman
Isachem® AS	: A branched alcohol alkyl sulphate from Enichem
MME PEG (2000)	: Monomethyl ether polyethylene glycol (MW 2000) from Fluka Chemie AG
DC3225C	: Silicone suds suppresser, mixture of Silicone oil and Silica from Dow Corning
TEPAE	: Tetreaethylenepentaamine ethoxylate
BTA	: Benzotriazole
Betaine	: $(CH_3)_3N^+CH_2COO^-$
Sugar	: Industry grade D-glucose or food grade sugar
CFAA	: C_{12}-C_{14} alkyl N-methyl glucamide
TPKFA	: C_{12}-C_{14} topped whole cut fatty acids
Clay	: A hydrated aluminumu silicate in a general formula $Al_2O_3SiO_2 \cdot xH_2O$. Types: Kaolinite, montmorillonite, atapulgite, illite, bentonite, halloysite
pH	: Measured as a 1% solution in distilled water at 20°C

[0237] For North American (NA) and Western European (WE) heavy duty liquid laundry (HDL) detergents, heat inactivation of the enzymes present in commercially-available detergents is performed by placing pre-weighed liquid detergent (in a glass bottle) in a water bath at 95°C for 2 hours. The incubation time for heat inactivation ofNA and WE auto dish washing (ADW) detergents is 8 hours. Both un-heated and heated detergents are assayed within 5 minutes of dissolving the detergent to accurately determine percentage deactivated. Enzyme activity is tested by the AAPF assay.

[0238] For testing of enzyme activity in heat-inactivated detergents, working solutions of detergents are made from the heat inactivated stocks. Appropriate amounts of water hardness (e.g., 6 gpg or 12 gpg) and buffer are added to the detergent solutions to match the desired conditions. The solutions are mixed by vortexing or inverting the bottles. The following Table provides information regarding some of the commercially-available detergents and test conditions used herein. In some experiments, additional and/or other commercially available detergents find use in the following Examples.

Table 1.1. Laundry and Dish Washing Conditions

Region	Form	Dose	Detergent*	Buffer	gpg	pH	T (°C)
Laundry (Heavy Duty Liquid and Granular)							
NA	HDL	0.78 g/l	P&G TIDE® 2X	5 mM HEPES	6	8.0	20
WE	HDL	5.0 g/L	Henkel PERSIL™	5 mM HEPES	12	8.2	40
WE	HDG	8.0 g/L	P&G ARIEL®	2 mM Na_2CO_3	12	10.5	40
JPN	HDG	0.7 g/L	P&G TIDE®	2 mM Na_2CO_3	6	10.0	20
NA	HDG	1.0 g/L	P&G TIDE®	2 mM Na_2CO_3	6	10.0	20
Automatic Dish Washing							
WE	ADW	3.0 g/L	RB CALGONIT™	2 mM Na_2CO_3	21	10.0	40
NA	ADW	3.0 g/L	P&G CASCADE®	2 mM Na_2CO_3	9	10.0	40

[0239] In some additional aspects, the following solutions find use:

Table 1-2. Working Detergent Solutions

Detergent	Temp (C)	Detergent g/L	pH	Buffer	gpg
TIDE® 2X Cold	16	0.98	8	5mM HEPES	6
TIDE® 2X Cold	32	0.98	8	5mM HEPES	6
TIDE® 2X Cold	16	0.98	7	5mM MOPS	6

[0240] The examples, which are intended to be purely exemplary of the invention and should therefore not be considered to limit the invention in any way, also describe and detail aspects and embodiments of the invention discussed above. The foregoing examples and detailed description are offered by way of illustration and not by way of limitation. Not all of the variants described are embodiments of the invention.

Table of Detergents

[0241] The compositions of the detergents used in the assays in Examples are shown in Table 1-3. BPN' variant protein samples were added to the detergent compositions as described in Example 1 to assay for the various properties tested.

[0242] The following are liquid laundry detergent compositions suitable for top-loading automatic washing machines (1, 2 & 4) and front loading washing machines (3).

Table 1-3. Composition of Detergents Used in the Assays to Test BPN' Variants

Ingredient	Composition (wt% of composition)			
	1	2	3	4
C_{12-15} Alkylethoxy(1.8)sulfate	14.7	11.6		16.31
$C_{11.8}$Alkylbenzene sulfonate	4.3	11.6	8.3	7.73
C_{16-17} Branched alkyl sulfate	1.7	1.29		3.09
C_{12-14}Alkyl -9-ethoxylate	0.9	1.07		1.31
C_{12} dimethylamine oxide	0.6	0.64		1.03

Table 1-3. Composition of Detergents Used in the Assays to Test BPN' Variants				
Ingredient	**Composition (wt% of composition)**			
	1	**2**	**3**	**4**
Citric acid	3.5	0.65	3	0.66
C_{12-18} fatty acid	1.5	2.32	3.6	1.52
Sodium Borate (Borax)	2.5	2.46	1.2	2.53
Sodium C_{12-14} alkyl ethoxy 3 sulfate			2.9	
C_{14-15} alkyl 7-ethoxylate			4.2	
C_{12-14}Alkyl -7-ethoxylate			1.7	
Ca formate	0.09	0.09		0.09
A compound having the following general structure: bis((C_2H_5O)(C_2H_4O)n)(CH_3)-N^+-C_xH_{2x}-N^+-(CH_3)- bis((C_2H_5O)(C_2H_4O)n), wherein n = from 20 to 30, and x = from 3 to 8, or sulphated or sulphonated variants thereof			1.2	
Random graft co-polymer[1]		1.46	0.5	
Ethoxylated Polyethylenimine [2]	1.5	1.29		1.44
Diethylene triamine pentaacetic acid	0.34	0.64		0.34
Diethylene triamine penta(methylene phosphonic acid)			0.3	
Tinopal AMS-GX		0.06		
Tinopal CBS-X	0.2	0.17		0.29
Amphiphilic alkoxylated grease cleaning polymer [3]	1.28	1	0.4	1.93
Ethanol	2	1.58	1.6	5.4
Propylene Glycol	3.9	3.59	1.3	4.3
Diethylene glycol	1.05	1.54		1.15
Polyethylene glycol	0.06	0.04		0.1
Monoethanolamine	3.05	2.41	0.4	1.26
NaOH	2.44	1.8		3.01
Sodium Cumene Sulphonate			1	
Sodium Formate		0.11		0.09
Water, Aesthetics (Dyes, perfumes) and Minors (Enzymes, solvents, structurants)	balance	balance	balance	

[1] Random graft copolymer is a polyvinyl acetate grafted polyethylene oxide copolymer having a polyethylene oxide backbone and multiple polyvinyl acetate side chains. The molecular weight of the polyethylene oxide backbone is about 6000 and the weight ratio of the polyethylene oxide to polyvinyl acetate is about 40 to 60 and no more than 1 grafting point per 50 ethylene oxide units.
[2] Polyethylenimine (MW = 600) with 20 ethoxylate groups per -NH.
[3] Amphiphilic alkoxylated grease cleaning polymer is a polyethylenimine (MW = 600) with 24 ethoxylate groups per -NH and 16 propoxylate groups per -NH

EXAMPLE 1

Assays

[0243] Various assays were used as set forth below. Any deviations from the protocols provided below are indicated in the

subsequent Examples.

A. TCA Assay for Protein Content Detemination in 96-well Microtiter Plates

[0244] For BPN' and BPN' variants, this assay was started using filtered *B. subtilis* bacterial culture supernatant from microtiter plates grown 3-4 days at 33-37°C with shaking at 230-250 rpm and humidified aeration. A fresh 96-well flat bottom microtiter plate (MTP) was used for the assay. First, 100 µL/well of 0.25 N HCl was placed in each well. Then, 25 µL of filtered culture broth was added. The light scattering/absorbance at 405 nm (use 5 sec mixing mode in the plate reader) was then determined in order to provide the "blank" reading. For the test, 100 µL/well of 30% (w/v) trichloroacetic acid (TCA) was placed in the plates and incubated for 10 minutes at room temperature. The light scattering/absorbance at 405 nm (use 5 sec mixing mode in the plate reader) was then determined. The equipment used was a Biomek FX Robot (Beckman Coulter) and a SpectraMAX (type 340; Molecular Devices) MTP Reader; the MTPs were from Costar (type 9017).

[0245] The calculations were performed by subtracting the blank (no TCA) from the test reading with TCA to provide a relative measure of the protein content in the samples. If desired, a standard curve can be created by calibrating the TCA readings with AAPF assays of clones with known conversion factors. However, the TCA results are linear with respect to protein concentration from 250 to 2500 micrograms protein per ml (ppm) and can thus be plotted directly against enzyme performance for the purpose of choosing good-performing variants. The turbidity/light scatter increase in the samples correlates to the total amount of precipitable protein in the culture supernatant.

B. AAPF Protease Assay in 96-well Microtiter Plates

[0246] In order to determine the protease activity of the proteases and variants thereof of the present invention, the hydrolysis of N-succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenyl-*p*-nitroanilide (suc-AAPF-pNA) was measured. The reagent solutions used were: 100 mM Tris/HCl, pH 8.6, containing 0.005% TWEEN®-80 (Tris dilution buffer); 100 mM Tris buffer, pH 8.6, containing 10 mM $CaCl_2$ and 0.005% TWEEN®-80 (Tris/Ca buffer); and 160 mM suc-AAPF-pNA in DMSO (suc-AAPF-pNA stock solution) (Sigma: S-7388). To prepare a suc-AAPF-pNA working solution, 1 ml suc-AAPF-pNA stock solution was added to 100 ml Tris/Ca buffer and mixed well for at least 10 seconds. The assay was performed by adding 10 µl of diluted protease solution to each well, immediately followed by the addition of 190 µl 1 mg/ml suc-AAPF-pNA working solution. The solutions were mixed for 5 sec., and the absorbance change in kinetic mode (25 readings in 5 minutes) was read at 405 nm in an MTP reader, at 25°C. The protease activity was expressed as AU (activity = $\Delta OD \cdot min^{-1}\ ml^{-1}$).

C. BMI Microswatch Assay

[0247] Blood, milk and ink (BMI) stained microswatches of 5.5 millimeter circular diameter were obtained from CFT. Before cutting the swatches, the fabric (EMPA 116) was washed with water. One microswatch was placed in each well of a 96-well non-binding microtiter plate (Corning 3641). The detergents used for the assays included Detergent Composition 1, Detergent Composition 2, and Detergent Composition 4. The detergents were diluted in Milli-Q (deionized) water to a working strength concentration of 0.788 g/L. These detergents were buffered with 5 mM HEPES pH 8.2 or pH 7.2, which upon addition to detergent, buffers at pH 8 or pH 7, respectively. Additionally, 6 grains per gallon (gpg) water hardness (3:1 Ca:Mg - $CaCl_2$: $MgCl_2 \cdot 6H_2O$) was added. The detergent solution was pre-equilibrated in an ice-water bath for 16°C assays (room temperature for 32°C assays) and pumped into a circulating reservoir (Beckman FX). Then, 190 µl of the desired detergent solution was added to each well of the MTP that contained microswatches. To this mixture, 10 µl of the diluted enzyme master dilution solution was added, providing an approximate enzyme concentration of 0.4 -0.5 µg/mL. The master dilution was prepared from the culture supernatants at 8 µg/mL, where the approximate enzyme concentrations of the culture supernatants and BPN'-v3 or BPN'-v36 parent controls were determined using the AAPF protease activity assay, basing the concentration on a purified BPN'-v3 or BPN'-v36 standard of known concentration. The MTP was sealed with tape and placed in the iEMS incubator/shaker (Thermo/Labsystems) pre-set at 16°C in a refrigerated dairy case or at 32°C on the benchtop for 20 minutes, with agitation at 1400 rpm. Following incubation under the appropriate conditions, the sealing tape was removed from each plate and 125 µl (150 µl if pipetting by hand for smaller screens) of the solution from each well was transferred into a fresh MTP (Corning 9017). The new MTP containing 125 µl - 150 µl of solution/well was read at 600 nm (with 5 sec mixing mode in the plate reader) using a MTP SpectraMax reader (type 340; Molecular Devices). Blank controls containing a microswatch and detergent but no enzyme were also included. The absorbance value obtained was corrected for the blank value (substrate without enzyme), providing a measure of hydrolytic activity. For each

sample (variant), the performance index was calculated as described below. This BMI Microswatch Assay, run at 60°F (16°C) and pH 8, is referred to herein as the "Test Method."

D. Egg Microswatch Assay

[0248] CS38 aged egg yolk with pigment stained cotton microswatches of 5.5 millimeter circular diameter were obtained from CFT. These swatches were not pre-rinsed in water. One microswatch was placed in each well of a 96-well non-binding microtiter plate (Corning 3641). Detergent Composition 4 was diluted in Milli-Q (deionized) water to a working strength concentration of 0.788 g/L. The detergents were buffered with 5 mM HEPES pH 8.2 which upon addition to detergent, buffers at pH 8. Additionally 6 grains per gallon (gpg) water hardness (3:1 Ca:Mg - $CaCl_2$: $MgCl_2 \cdot 6H_2O$); was added. The detergent solution was pre-equilibrated in an ice-water bath for 16°C assays (room temperature for 32°C assays) and pumped into a circulating reservoir (Beckman FX). Then, 185 µl of the desired detergent solution was added to each well of the MTP, containing microswatches. To this mixture, 15 µl of the diluted enzyme master dilution solution was added, providing an approximate enzyme concentration of 0.6 µg/mL in the reaction. The master dilution was prepared from the culture supernatants at 8 µg/mL, where the approximate enzyme concentration of the culture supernatants and BPN'-v3 or BPN'-v36 parent control was determined using the AAPF protease activity assay, basing the concentration on a purified BPN'-v3 or BPN'-v36 standard of known concentration. The MTP was sealed with tape and placed in the iEMS incubator/shaker (Thermo/Labsystems) pre-set at 16°C in a refrigerated dairy case or at 32°C on the benchtop for 30 minutes, with agitation at 1400 rpm. Following incubation under the appropriate conditions, the sealing tape was removed from each plate and 125 µl (150 µl if pipetting by hand for smaller screens) of the solution from each well was transferred into a fresh MTP (Corning 9017). The new MTP containing 125 µl - 150 µl of solution/well was read at 405 nm (with 5 sec mixing mode in the plate reader) using a MTP SpectraMax reader (type 340; Molecular Devices). Blank controls containing a microswatch and detergent but no enzyme were also included. The absorbance value obtained was corrected for the blank value (substrate without enzyme), providing a measure of hydrolytic activity. For each sample (variant), the performance index was calculated as described below.

E. Grass Microswatch Assay

[0249] Warwick Equest scrubbed grass on woven cotton swatches were obtained from Warwick. These swatches were cut into 5.5 millimeter circular diameter microswatches using a custom made 96-well punch machine that places one microswatch in each well of a 96-well non-binding microtiter plate (Corning 3641). After cutting of the swatches, the fabric was washed in the wells (pre-rinsed) with 100 µL per well of 50% working strength Detergent Composition 4 diluted in water. After 20 minutes of prerinsing the 100 µl of 50% detergent rinse was removed carefully pipetting by hand. Detergent Composition 4 was diluted in Milli-Q (deionized) water to a working strength concentration of 0.788 g/L. These detergents were buffered with 5 mM HEPES pH 8.2, which upon addition to detergent, buffers at pH 8. Additionally 6 grains per gallon (gpg) water hardness (3:1 Ca:Mg - CaCh: $MgCl_2 \cdot 6H_2O$); was added. The detergent solution was pre-equilibrated in an ice-water bath for 16°C assays (room temperature for 32°C assays). Then, 180 µl of the desired detergent solution was added to each well of the MTP containing the microswatches, immediately after the pre-rinsing was complete. To this mixture, 20 µl of the diluted enzyme master dilution solution was added making the approximate enzyme in the reaction at 0.8 µg/mL. The master dilution was prepared from the culture supernatants at 8 µg/mL where the approximate enzyme concentration of the culture supernatants and BPN'-v36 parent control was determined using the AAPF protease assay basing the concentration on a purified BPN'-v36 standard of known concentration. The MTP was sealed with tape and placed in the iEMS incubator/shaker (Thermo/Labsystems) pre-set at 16°C in a refrigerated dairy case or at 32°C on the benchtop for 30 minutes, with agitation at 1400 rpm. Following incubation under the appropriate conditions, the sealing tape was removed from each plate and 125 µl (150 µl if pipetting by hand for smaller screens) of the solution from each well was transferred into a fresh MTP (Corning 9017). The new MTP containing 125 µl - 150 µl of solution/well was read at both 430 nm and 670 nm (with 5 sec mixing mode in the plate reader) using a MTP SpectraMax reader (type 340; Molecular Devices). Blank controls containing a microswatch and detergent but no enzyme were also included. The absorbance value obtained was corrected for the blank value (substrate without enzyme), providing a measure of hydrolytic activity. For each sample (variant), the performance index was calculated as described below.

F. Stability Assay

[0250] The stability of protease variants was determined in the presence of 40% concentrated Detergent Composition 3 diluted in water. The reagents used were Detergent Composition 3 diluted to 50% in Milli-Q water, 10 mM MES 0.01 % TWEEN®-80 pH

5.8 master dilution buffer, AAPF reagents: see protocol AAPF assay. The equipment used was F-bottom MTP (Corning 9017) for dilution of diluted enzyme into detergent as well as for suc-AAPF-pNA plates, Biomek FX (Beckman Coulter), Spectramax Plus 384 MTP Reader (Molecular Devices), iEMS Incubator/Shaker (1 mm amplitude) (Thermo Electron Corporation), sealing tape: Nunc (236366), circulating reservoir (Beckman Fx).

[0251] Detergent Composition 3 was initially diluted to 50% in water. This detergent was kept at room temperature and cycled through the circulating reservoir. The iEMS incubators/shakers (Thermo/Labsystems) were pre-set at 43°C. Culture supernatants were diluted into plates containing master dilution buffer to a concentration of ~ 20 ppm (master dilution plate). Then, 40 µl of sample from the master dilution plate was added to plates containing 160 µl 50% Detergent Composition 3 to give a final incubation concentration of 4 ppm. The contents were mixed and kept at room temperature and triplicate AAPF assays were performed immediately on these plates and recorded as unstressed reads. The AAPF assay was modified such that 20 µL of sample from the step above was added to 190 µL of suc-AAPF-pNA working solution. The plates were immediately covered with sealing tape and placed in 43°C iEMS shakers for 30 min at 650 rpm. Following 30 minutes of incubation, triplicate AAPF assays were performed on these stress plates and recorded as stressed reads. The stability of the samples was determined by calculating the ratio of the residual and initial AAPF activity as follows: Residual Activity (%) = [mOD.min^{-1} stressed]*100 / [mOD. min^{-1} unstressed]. For each sample (variant), the performance index was calculated as described below.

G. LAS/EDTA Stability Assay

[0252] The stability of protease variants in the presence of a representative anionic surfactant (LAS=linear alkylbenzene sulfonate, specifically, sodium dodecylbenzenesulfonate-DOBS) and disodium EDTA was measured after incubation under defined conditions and the residual activity was determined using the AAPF assay. The reagents used were dodecyllbenzene sulfonate, sodium salt (DOBS, Sigma No. D-2525), TWEEN®-80 (Sigma No. P-8074), di-sodium EDTA (Siegfried Handel No. 164599-02), HEPES (Sigma No. H-7523), unstressed buffer: 50 mM HEPES (11.9 g/l) + 0.005% TWEEN®-80, pH 8.0, Stress buffer: 50 mM HEPES (11.9 g/l), 0.1% (w/v) DOBS (1 g/l), 10 mM EDTA (3.36 g/l), pH 8.0, reference protease and protease variant culture supernatants, containing 200 - 400 µg/ml protein. The equipment used was V- or U-bottom MTPs as dilution plates (Greiner 651101 and 650161, respectively), F-bottom MTPs (Corning 9017) for unstressed and LAS/EDTA buffer as well as for suc-AAPF-pNA plates, Biomek FX (Beckman Coulter), Spectramax Plus 384 MTP Reader (Molecular Devices), iEMS Incubator/Shaker (1 mm amplitude) (Thermo Electron Corporation), and Nunc sealing tape (236366).

[0253] The iEMS incubator/shaker (Thermo/Labsystems) was set at 29°C. Culture supernatants were diluted into plates containing unstressed buffer to a concentration of ~ 25 ppm (master dilution plate). Then, 20 µl of sample from the master dilution plate was added to plates containing 180 µl unstressed buffer to give a final incubation concentration of 2.5 ppm. The contents were mixed and kept at room temperature and an AAPF assay was performed on this plate. Then, 20 µl of sample from the master dilution plate was also added to plates containing 180 µl stress buffer (50 mM HEPES (11.9 g/l), 0.1% (w/v) DOBS (1 g/l), 10 mM EDTA (3.36 g/l), pH 8.0). The solutions were mixed and immediately placed in 29°C iEMS shaker for 30 min at 400 rpm. Following 30 minutes of incubation, an AAPF assay was performed on the stress plate. The stability of the samples was determined by calculating the ratio of the residual and initial AAPF activity as follows: Residual Activity (%) = [mOD.min-1 stressed]* 100 / [mOD. min-1 unstressed]. For each sample (variant), the performance index was calculated as described below.

Performance Index

[0254] The performance index provides a comparison of the performance of a variant (actual value) and a standard or reference protease enzyme (theoretical value) at the same protein concentration. The theoretical values can be calculated using the parameters of a performance dose response curve (i.e. using a Langmuir equation to generate the performance curve) of the standard/reference protease. A performance index (PI) that is greater than 1 (PI>1) identifies a better variant as compared to the standard or reference protease (which may be, e.g., wild-type protease or another protease variant), while a PI of 1 (PI=1) identifies a variant that performs the same as the standard or reference protease, and a PI that is less than 1 (PI<1) identifies a variant that performs worse than the standard or reference protease. Thus, the PI identifies winners (e.g., variants having enhanced proteolytic activity compared to that of the standard/reference protease) as well as variants that may be less desirable for use under certain circumstances (e.g., variants having proteolytic activity lower than the proteolytic activity of the standard/reference protease).

[0255] It is important to note that protease variants having performance index values lower than that of a reference or standard

protease are nevertheless useful in the applications and methods described herein. For example, protease variants having performance index values lower than that of a reference or standard protease have proteolytic activity and thus are useful in the compositions of the invention, such as, but not limited to, e.g., cleaning compositions (including, but not limited, to, e.g., detergent cleaning compositions) for cleaning a variety of surfaces and items, including, but not limited to, e.g., laundry, fabrics, and dishware, and in personal care applications and compositions as described elsewhere herein; such protease variants are also useful in fabric and home care products and compositions and in non-fabric and home care products and compositions described elsewhere herein and in methods of the invention, including, but not limited, to, e.g., cleaning methods, methods for personal care, etc., described elsewhere herein.

[0256] Various terms set forth below are used to describe the variant: non-deleterious variants have a PI >0.05; deleterious variants have a PI less than or equal to 0.05; combinable variants are those for which the variant has performance index values greater than or equal to 0.2 for at least one property, and >0.05 for all properties. Combinable variants are those that can be combined to deliver proteins with appropriate performance indices for one or more desired properties. These data find use in engineering any subtilisin/subtilase or protease. Even if the subtilase or protease to be engineered has an amino acid different from that of subtilisin BPN' at one or more particular positions, these data find use in identifying amino acid substitutions that alter the desired properties by identifying the best choices for substitutions, including substitutions of the BPN' wild type amino acid.

EXAMPLE 2

Construction of BPN' Library and Cleaning Performance of BPN' Variants

a) Description of the BPN'-v3 expression cassette used for library construction

[0257] The BPN'-v3 (BPN' protease containing G097A-G128A-Y217Q substitutions) expression cassette used for combinatorial library construction was generated using the BPN' expression cassette, which comprises the aprE-BPN' hybrid leader sequence (i.e., signal sequence), BPN' pro and BPN' mature sequence from *B. amyloliquefaciens*. The DNA sequence is shown below as SEQ ID NO: 1 and encodes the BPN' precursor protein shown below as SEQ ID NO:168.

GTGAGAAGCAAAAAATTGTGGATCAGTTTGCTGTTTGCTTTAGCGTTAATCTTTACGATGGC
GTTCGGCAGCACATCCTCTGCCCAGGCGGCAGGGAAATCAAACGGGGAAAAGAAATATATT
GTCGGGTTTAAACAGACAATGAGCACGATGAGCGCCGCTAAGAAGAAAGATGTCATTTCTG
AAAAAGGCGGGAAAGTGCAAAAGCAATTCAAATATGTAGACGCAGCTTCAGCTACATTAAA

CGAAAAAGCTGTAAAAGAATTGAAAAAAGACCCGAGCGTCGCTTACGTTGAAGAAGATCAC
GTAGCACATGCGTACGCGCAGTCCGTGCCTTACGGCGTATCACAAATTAAAGCCCCTGC
TCTGCACTCTCAAGGCTACACTGGATCAAATGTTAAAGTAGCGGTTATCGACAGCGGT
ATCGACTCGAGCCATCCAGATCTTAAAGTCGCTGGAGGGGCTTCTATGGTGCCGTCCG
AAACAAACCCGTTTCAAGATAACAATTCTCATGGCACACACGTCGCAGGAACGGTTGC
GGCGTTAAACAATTCTATTGGCGTGCTTGGTGTAGCCCCGTCTGCTTCGCTCTACGCC
GTTAAAGTTCTTGGCGCAGACGGATCAGGCCAATACTCATGGATTATCAACGGCATCG
AATGGGCCATCGCGAATAACATGGATGTAATCAACATGAGCCTGGGAGGACCAAGCG
GCAGTGCGGCACTTAAAGCAGCAGTTGATAAAGCTGTTGCATCTGGTGTCGTCGTAGT
AGCGGCAGCTGGGAATGAGGGAACATCCGGATCATCGAGTACCGTCGGTTATCCAGG
CAAGTACCCTTCAGTGATTGCAGTGGGCGCTGTAGACTCTTCAAATCAACGTGCCTCT
TTTTCCTCCGTGGGACCGGAGCTGGATGTCATGGCCCCTGGCGTTTCTATTCAATCGA
CGCTTCCAGGGAACAAGTATGGTGCGTATAACGGGACTTCCATGGCCTCGCCGCATGT
AGCTGGGGCGGCCGCATTGATTCTTTCTAAGCACCCGAACTGGACAAACACTCAAGTC
CGCAGCAGTTTAGAAAACACCACTACAAAACTTGGTGATTCTTTCTACTATGGAAAAG
GGCTGATCAACGTACAGGCGGCAGCTCAG (SEQ ID NO:1)

[0258] In the nucleotide sequence of SEQ ID NO:1, the DNA sequence encoding the mature protease is shown in bold, the nucleotide sequence encoding leader sequence (aprE-BPN' hybrid leader sequence) is shown in standard (non-underlined) text, and the nucleotide sequence encoding the pro sequence (BPN') is underlined. In the amino acid sequence (aprE-BPN' hybrid leader sequence, BPN' pro sequence, and BPN' mature protein sequence) of the BPN' precursor protein set forth in SEQ ID NO:168, the bolded portion indicates the mature BPN' subtilisin protease.

VRSKKLWISLLFALALIFTMAFGSTSSAQAAGKSNGEKKYIVGFKQTMSTMSAAKKKDVISEKG
GKVQKQFKYVDAASATLNEKAVKELKKDPSVAYVEEDHVAHAY**AQSVPYGVSQIKAPALHS**
QGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETNPFQDNNSHGTHVAGTVAALNNSI
GVLGVAPSASLYAVKVLGADGSGQYSWIINGIEWAIANNMDVINMSLGGPSGSAALKAAVD
KAVASGVVVVAAAGNEGTSGSSSTVGYPGKYPSVIAVGAVDSSNQRASFSSVGPELDVMAP
GVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTKLGDSFY
YGKGLINVQAAAQ (SEQ ID NO:168)

[0259] Thus, the amino acid sequence of the mature BPN' subtilisin protease is:
AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETNPFQDNNSH
GTHVAGTVAALNNSIGVLGVAPSASLYAVKVLGADGSGQYSWIINGIEWAIANNMDVINMSLG
GPSGSAALKAAVDKAVASGVVVVAAAGNEGTSGSSSTVGYPGKYPSVIAVGAVDSSNQRASFS
SVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTT
TKLGDSFYYGKGLINVQAAAQ (SEQ ID NO:2)

[0260] The nucleotide sequence of the mature BPN'-v3 gene is shown below (the signal sequence and propeptide sequence used in the BPN'-v3 expression cassette is the same as that for BPN' shown in SEQ ID NO:1):
GCGCAGTCCGTGCCTTACGGCGTATCACAAATTAAAGCCCCTGCTCTGCACTCTCAAGGCTA
CACTGGATCAAATGTTAAAGTAGCGGTTATCGACAGCGGTATCGACTCGAGCCATCCAGAT
CTTAAAGTCGCTGGAGGGGCTTCTATGGTGCCGTCCGAAACAAACCCGTTTCAAGATAACA
ATTCTCATGGCACACACGTCGCAGGAACGGTTGCGGCGTTAAACAATTCTATTGGCGTGCTT
GGTGTAGCCCCGTCTGCTTCGCTCTACGCCGTTAAAGTTCTTGCAGCAGACGGATCAGGCCA
ATACTCATGGATTATCAACGGCATCGAATGGGCCATCGCGAATAACATGGATGTAATCAAC
ATGAGCCTGGGAGCACCAAGCGGCAGTGCGGCACTTAAAGCAGCAGTTGATAAAGCTGTTG
CATCTGGTGTCGTCGTAGTAGCGGCAGCTGGGAATGAGGGAACATCCGGATCATCGAGTAC
CGTCGGTTATCCAGGCAAGTACCCTTCAGTGATTGCAGTGGGCGCTGTAGACTCTTCAAATC
AACGTGCCTCTTTTTCCTCCGTGGGACCGGAGCTGGATGTCATGGCCCCTGGCGTTTCTATTC
AATCGACGCTTCCAGGGAACAAGTATGGTGCGCAAAACGGGACTTCCATGGCCTCGCCGCA
TGTAGCTGGGGCGGCCGCATTGATTCTTTCTAAGCACCCGAACTGGACAAACACTCAAGTCC
GCAGCAGTTTAGAAAACACCACTACAAAACTTGGTGATTCTTTCTACTATGGAAAAGGGCTG
ATCAACGTACAGGCGGCAGCTCAG (SEQ ID NO:3)

[0261] The protein sequence of the mature BPN'-v3 protease variant is shown below (the signal sequence and propeptide sequence used in the BPN'-v3 expression cassette is the same as that for BPN' shown in SEQ ID NO:168):
AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETNPFQDNNSH
GTHVAGTVAALNNSIGVLGVAPSASLYAVKVLAADGSGQYSWIINGIEWAIANNMDVINMSLG
APSGSAALKAAVDKAVASGVVVVAAAGNEGTSGSSSTVGYPGKYPSVIAVGAVDSSNQRASFS
SVGPELDVMAPGVSIQSTLPGNKYGAQNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTT
TKLGDSFYYGKGLINVQAAAQ (SEQ ID NO:4)

b) <u>Construction of combinatorial library using pHPLT-BPN'-v3 plasmid</u>

[0262] The pHPLT-BPN'-v3 plasmid (see Figure 1) containing the BPN'-v3 expression cassette described above served as template DNA for cloning to provide variants derived from BPN'-v3. The vector pHPLT (Figure 4 in US Patent No. 6,566,112) contains the *B. licheniformis* LAT promoter ("Plat"); a sequence encoding the LAT signal peptide ("preLAT"). Additional plasmid elements from plasmid pUB110 disclosed in McKenzie et al., Plasmid 15(2): 93-103 (1986): "ori-pUB" is the origin of replication from pUB110; "neo" is the neomycin/kanamycin resistance gene from pUB110; "Terminator" is the transcriptional terminator from *B. licheniformis* amylase.

[0263] A combinatorial DNA library was synthesized at DNA 2.0 and delivered as individual ligation reactions. In some instances for efficient transformation of *B. subtilis*, the DNA from the ligation reaction mixtures was amplified by rolling circle amplification (RCA) using the Illustra Templiphi kit (GE Healthcare). The reaction was performed according to the manufacturer's protocol. One microliter of tenfold diluted amplified DNA was used to transform 50 µl of competent *B. subtilis* cells (ΔaprE, ΔnprE, amyE::xylRPxylAcomK-phleo). The transformation mixture was shaken at 37°C for 1 hour. Ten microliter aliquots of the transformation mixture were plated on skim milk (1.6%) Luria agar plates supplemented with 10 µg/ml of neomycin (Teknova).

[0264] The transformants that formed halos on the skim milk plates were picked into microtiter plates containing 150µl Luria broth (LB) medium supplemented with 10µg/ml neomycin. Plates were grown overnight at 37°C with 250-300 rpm shaking and 70-80% humidity using Enzyscreen lids for microtiter plates (Enzyscreen). Using a 96 pin replicating tool, (Enzyscreen) the overnight

culture plate was used to inoculate a new microtiter plate containing 180 µl of MBD medium (a MOPS based defined medium) with 2.5 µg/ml neomycin. MBD medium was prepared essentially as known in the art (see Neidhardt et al., J. Bacteriol. 119:736-747 [1974]), except that NH_4Cl_2, $FeSO_4$ and $CaCl_2$ were omitted from the base medium, 3 mM K_2HPO_4 was used, and the base medium was supplemented with 60 mM urea, and 100ml of a solution made of 210 g/L glucose, and 350 g/L maltodextrin. 1 g/L of BD Bacto Yeast Extract was added and the pH was adjusted to 7.4 with KOH. The micronutrients were made up as a 100X stock solution containing in one liter, 400 mg $FeSO_4 \cdot 7H_2O$, 100 mg $MnSO_4 \cdot H_2O$, 100 mg $ZnSO_4 \cdot 7H_2O$, 50 mg $CuCl_2 \cdot 2H_2O$, 100 mg $CoCl_2 \cdot 6H_2O$, 100 mg $NaMoO_4 \cdot 2H_2O$, 100 mg $Na_2B_4O_7 \cdot 10H_2O$, 10 ml of 1M $CaCl_2$, and 10 ml of 0.5 M sodium citrate. The MBD medium containing microtiter plates were grown for 64 hours at 37°C, 250-300 rpm, and 70-80% humidity using Enzyscreen lids (Enzyscreen) for protease variant expression. The next day, cultures were filtered through a micro-filter plate (0.22um; Millipore) and the resulting filtrates containing protease variants were used for biochemical analysis.

[0265] The protease variants were tested for cleaning performance using a BMI microswatch assay in Detergent Composition 1 at 16°C and pH 8 and BMI microswatch assay in Detergent Composition 2 at 16°C and pH 8. Protein content was determined using the TCA assay. Assays were performed as described in Example 1 and Performance Indices were calculated relative to BPN'-v3 (with a PI value of 1.0).

[0266] The following BPN' subtilisin protease variant was determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2 to about 5, or from greater than 1.0 to about 5 relative to BPN'-v3 (SEQ ID NO:4) in a BMI microswatch cleaning assay in Detergent Composition 1 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising the set of amino acid substitutions G097A-G 128A-P210S-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variant has a PI value of 1.1 relative to BPN'-v3 in this BMI microswatch cleaning assay, and enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0267] The following BPN' variants were determined to have a PI value of about 1.0 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 1 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128A-Y217Q, G097A-G128A-E156S-P210S-Y217Q, G097A-G128A-P210S-Y217Q-N218A, G097A-G128A-P210S-Y217Q-N218S, and G097A-Y104F-G128A-E156S-P210I-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0268] The following BPN' variants were determined to have a PI value of about 0.9 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 1 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128A-E156A-P210S-Y217Q-N218S and G097A-G128A-Y217Q-N218A, wherein positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0269] The following BPN' variants were determined to have a PI value of greater than 1.0 to about 5 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 2 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128A-P210S-Y217Q-N218A and G097A-G128A-P210S-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0270] The following BPN' variants were determined to have a PI value of about 1.0 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 2 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128A-Y217Q (i.e., BPN'-v3), G097A-G128A-E156S-P210S-Y217Q, and G097A-G128A-P210S-Y217Q-N218S, wherein positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0271] The following BPN' variants were determined to have a PI value of about 0.9 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 2 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128A-E156A-P210S-Y217Q-N218S, G097A-G128A-Y217Q-N218A, and G097A-Y104F-G128A-E156S-P210I-Y217Q, wherein positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

EXAMPLE 3

Generation of Combinatorial Libraries and Cleaning Performance of Variants of BPN'-v3 + S78N

a) **Description of BPN'-v3 + S78N variant and synthetic gene sequences derived from this variant**

[0272] Gene Oracle synthesized and cloned eight genes into the pHPLT-BPN'-v3 + S78N (BPN'-S78N-G97A-G128A-Y217Q) parent plasmid (see Figure 2). Some of these genes were used as templates (parents) to create combinatorial libraries. The BPN'-v3 + S78N variant was generated using standard molecular biology methods known in the art. The nucleotide sequence encoding the BPN'-v3 + S78N variant is shown below:

GCGCAGTCCGTGCCTTACGGCGTATCACAAATTAAAGCCCCTGCTCTGCACTCTCAAGGCTA
CACTGGATCAAATGTTAAAGTAGCGGTTATCGACAGCGGTATTGATTCGAGCCATCCAGATC
TTAAAGTCGCTGGAGGGGCTTCTATGGTGCCGTCCGAAACAAACCCGTTTCAAGATAACAAT
TCTCATGGCACACACGTCGCAGGAACGGTTGCGGCGTTAAACAATAATATTGGCGTGCTTCG
TGTAGCCCCGTCTGCTTCGCTCTACGCCGTTAAAGTTCTTGCAGCAGACGGATCAGGCCAAT
ACTCATGGATTATCAACGGCATCGAATGGGCCATCGCGAATAACATGGATGTAATCAACAT
GAGCCTGGGAGCACCAAGCGGCAGTGCGGCACTTAAAGCAGCAGTTGATAAAGCTGTTGCA
TCTGGTGTCGTCGTAGTAGCGGCAGCTGGGAATGAGGGAACATCCGGATCATCGAGTACCG
TCGGTTATCCAGGCAAGTACCCTTCAGTGATTGCAGTGGGCGCTGTAGACTCTTCAAATCAA
CGTGCCTCTTTTTCCTCCGTGGGACCGGAGCTGGATGTCATGGCCCCTGGCGTTTCTATTCAA
TCGACGCTTCCAGGGAACAAGTATGGTGCGCAAAACGGGACTTCCATGGCCTCGCCGCATG
TAGCTGGGGCGGCCGCATTGATTCTTTCTAAGCACCCGAACTGGACAAACACTCAAGTCCGC
AGCAGTTTAGAAAACACCACTACAAAACTTGGTGATTCTTTCTACTATGGAAAAGGGCTGAT
CAACGTACAGGCGGCAGCTCAG (SEQ ID NO:7)

[0273] The amino acid sequence of the BPN'-v3 + S78N variant is shown below:

AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETNPFQDNNSH
GTHVAGTVAALNNNIGVLGVAPSASLYAVKVLAADGSGQYSWIINGIEWAIANNMDVINMSLG
APSGSAALKAAVDKAVASGVVVVAAAGNEGTSGSSSTVGYPGKYPSVIAVGAVDSSNQRASFS
SVGPELDVMAPGVSIQSTLPGNKYGAQNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTT
TKLGDSFYYGKGLINVQAAAQ (SEQ ID NO:8)

[0274] The nucleotide and protein sequences of genes GcM90-96, and GcM100 are shown below. The nucleotide sequence of synthesized gene GcM90 is:

GCGCAGTCCGTGCCTTACGGCGTATCACAAATTAAAGCCCCTGCTCTGCACTCTCAAGGCTA
CACTGGATCAAATGTTAAAGTAGCGGTTATCGACAGCGGTATCGACTCGAGCCATCCAGAT
CTTAAAGTCGCTGGAGGGGCTTCTATGGTGCCGGGAGAAACAAACCCGTTTCAAGATAACA
ATTCTCATGGCACACACGCAGCAGGAACGGTTGCGGCGTTAAACAATAATATTGGCGTGCTT
GGTGTAGCCCCGTCTGCTTCGCTCTACGCCGTTAAAGTTCTTGCAGCAGACGGATCAGCACA
ATACTCATGGATTATCAACGGCATCGAATGGGCCATCGCGAATAACATGGATGTAATCAAC
ATGAGCCTGGGAGCAACAAGCGGCAGTGCGGCACTTAAAGCAGCAGTTGATAAAGCTGTTG
CATCTGGTGTCGTCGTAGTAGCGGCAGCTGGGAATGAGGGAACATCCGGATCATCGAGTAC
CGTCGGTTATCCAGGCAAGTACCCTTCAGTGATTGCAGTGGGCGCTGTAGACTCTTCAAATA
CACGTGCCTCTTTTTCCTCCGTGGGACCGGAGCTGGATGTCATGGCCCCTGGCGTTTCTATTC
AATCGACGCTTCCAGGGAACAAGTATGGTGCGCAAAACGGGACTTCCATGGCCTCGCCGCA
TGTAGCTGGGGCGGCCGCATTGATTCTTTCTAAGCACCCGAACTGGACAAACACTCAAGTCC
GCAGCAGTTTAGAAAACACCACTACAAAACTTGGTGATTCTTTCTACTATGGAAAAGGGCTG
ATCAACGTACAGGCGGCAGCTCAGTAA (SEQ ID NO:9)

[0275] The amino acid sequence of GcM90 is provided below:

AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPGETNPFQDNNSH
GTHAAGTVAALNNNIGVLGVAPSASLYAVKVLAADGSAQYSWIINGIEWAIANNMDVINMSLG
ATSGSAALKAAVDKAVASGVVVVAAAGNEGTSGSSSTVGYPGKYPSVIAVGAVDSSNTRASFS
SVGPELDVMAPGVSIQSTLPGNKYGAQNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTT
TKLGDSFYYGKGLINVQAAAQ (SEQ ID NO:10)

[0276] The nucleotide sequence of synthesized gene GcM91 is provided below:

GCGCAGTCCGTGCCTTACGGCGTATCACAAATTAAAGCCCCTGCTCTGCACTCTCAAGGCTA
CACTGGATCAAATGTTAAAGTAGCGGTTATCGACAGCGGTATCGACTCGAGCCATCCAGAT
CTTAAAGTCGCTGGAGGGGCTTCTATGGTGCCGTCCGAAACAAACCCGTTTGTCGATAACAA
TTCTCATGGCACACACGTCGCAGGAACGGTTGCGGCGTTAAACAATAATATTGGCGTGCTTG
GTGTAGCCCCGTCTGCTTCGCTCTACGCCGTTAAAGTTCTTGCAGCAGACGGATCAGGCCAA

TACTCATGGATTGTCAACGGCATCGAATGGGCCATCGCGAATAACATGGATGTAATCAACA
TGAGCCTGGGAGCACCAAGCGGCAGTGCGGCACTTAAAGCAGCAGTTGATAAAGCTGTTGC
ATCTGGTCAAGTCGTAGTAGCGGCAGCTGGGAATGAGGGAACATCCGGATCATCGAGTACC
GTCGGTTATCCAGGCAAGTACCCTTCAGTGATTGCAGTGGGCGCTGTAGACTCTTCAAATCA
ACGTGCCTCTTTTTCCTCCGTGGGACCGGAGCTGGATGTCATGGCCCCTGGCGTTTCTATTCA
ATCGACGCTTCCAGCAAACAAGTATGGTGCGCAAAACGGGACTTCCATGGCCTCGCCGCAT
GTAGCTGGGGCGGCCGCATTGATTCTTTCTAAGCACCCGAACTGGACAAACACTCAAGTCCG
CAGCAGTTTAGAACAAACCACTACAAAACTTGGTGATTCTTTCTACTATGGAAAAGGGCTGA
TCAACGTACAGGCGGCAGCTCAGTAA (SEQ ID NO:11)

[0277] The amino acid sequence of GcM91 is provided below:
AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETNPFVDNNSH
GTHVAGTVAALNNNIGVLGVAPSASLYAVKVLAADGSGQYSWIVNGIEWAIANNMDVINMSL
GAPSGSAALKAAVDKAVASGQVVVAAAGNEGTSGSSSTVGYPGKYPSVIAVGAVDSSNQRASF
SSVGPELDVMAPGVSIQSTLPANKYGAQNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLEQT
TTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO:12)

[0278] The nucleotide sequence of synthesized gene GcM92 is provided below:
GCGCAGTCCGTGCCTTACGGCGTATCACAAATTAAAGCCCCTGCTCTGCACTCTCAAGGCTA
CACTGGATCAAATGTTAAAGTAGCGGTTATCGACAGCGGTATCGACTCGAGCCATCCAGAT
CTTAAAGTCGCTGGAGGGGCTTCTATGGTGCCGTCCGAAACAAACCCGTTTCAAGATGCAA
ATTCTCATGGCACACACGTCGCAGGAACGGTTGCGGCGTTAAACAATAATATTGGCGTGCTT
GGTGTAGCCCCGGAAGCTTCGCTCTACGCCGTTAAAGTTCTTGCAGCAGACGGATCAGGCCA
ATACTCATGGATTATCAACGGCATCGAATGGGCCATCGCGAATAACATGGATGTAATCAAC
ATCAGCCTGGGAGCACCAAGCGGCAGTGCGGCACTTAAAGCAGCAGTTGATAAAGCTGTTG
CATCTGGTGTCGTCGTAGTAGCGGCAGCTGGGAATGAGGGAACATCCGGACCTTCGAGTAC
CGTCGGTTATCCAGGCAAGTACCCTTCAGTGATTGCAGTGGGCGCTGTAGACTCTTCAAATC
AACGTGCCTCTTTTTCCTCCGTGGGACCGGAGCTGGATGTCATGGCCCCTGGCGTTTCTATTC
AATCGACGCTTCCAGGGAACAAGTATGGTGCGCAAAACGGGACTTCCATGGCCGCACCGCA
TGTAGCTGGGGCGGCCGCATTGATTCTTTCTAAGCACCCGAACTGGACAAACACTCAAGTCC
GCAGCAGTTTAGAAAACACCACTACAAAACTTGGTGATTCTTTCTACTATGGAAAAGGGCTG
ATCAACGTACAGGCGGCAGCTCAGTAA (SEQ ID NO:13)

[0279] The amino acid sequence of GcM92 is provided below:
AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETNPFQDANSH
GTHVAGTVAALNNNIGVLGVAPEASLYAVKVLAADGSGQYSWIINGIEWAIANNMDVINISLGA
PSGSAALKAAVDKAVASGVVVVAAAGNEGTSGPSSTVGYPGKYPSVIAVGAVDSSNQRASFSS
VGPELDVMAPGVSIQSTLPGNKYGAQNGTSMAAPHVAGAAALILSKHPNWTNTQVRSSLENTT
TKLGDSFYYGKGLINVQAAAQ (SEQ ID NO:14)

[0280] The nucleotide sequence of synthesized gene of GcM93 is provided below:
GCGCAGTCCGTGCCTTACGGCGTATCACAAATTAAAGCCCCTGCTCTGCACTCTCAAGGCTA
CACTGGATCAAATGTTAAAGTAGCGGTTATCGACAGCGGTATCGACTCGAGCCATCCAGAT
CTTAAAGTCGCTGGAGGGGCTTCTATGGTGCCGTCCGAAACAAACCCGTTTCAAGATAACCA
ATCTCATGGCACACACGTCGCAGGAACGGTTGCGGCGTTAAACAATAATATTGGCGTGCTTG
GTGTAGCCCCGTCTGCTTCGCTCTACGCCGTTAAAGTTCTTGCAGCAGACAACTCAGGCCAA
TACTCATGGATTATCAACGGCATCGAATGGGCCATCGCGAATAACATGGATGTAATCAACA
TGGCACTGGGAGCACCAAGCGGCAGTGCGGCACTTAAAGCAGCAGTTGATAAAGCTGTTGC
ATCTGGTGTCGTCGTAGTAGCGGCAGCTGGGAATGAGGGAACAGATGGATCATCGAGTACC
GTCGGTTATCCAGGCAAGTACCCTTCAGTGATTGCAGTGGGCGCTGTAGACTCTTCAAATCA
ACGTGCCTCTTTTTCCTCCGTGGGACCGGAGCTGGATGTCATGGCCCCTGGCGTTTCTATTCA
ATCGACGCTTCCAGGGAACAAGTATGGTGCGCAAAACGGGACTTCCATGGCCTCGCCGCAT
GTAGCTGGGGCGGCCGCATTGATTCTTTCTAAGCACCCGTCATGGACAAACACTCAAGTCCG
CAGCAGTTTAGAAAACACCACTACAAAACTTGGTGATTCTTTCTACTATGGAAAAGGGCTGA
TCAACGTACAGGCGGCAGCTCAGTAA (SEQ ID NO:15)

[0281] The amino acid sequence of GcM93 is provided below:
AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETNPFQDNQSH
GTHVAGTVAALNNNIGVLGVAPSASLYAVKVLAADNSGQYSWIINGIEWAIANNMDVINMALG
APSGSAALKAAVDKAVASGVVVVAAAGNEGTDGSSSTVGYPGKYPSVIAVGAVDSSNQRASFS
SVGPELDVMAPGVSIQSTLPGNKYGAQNGTSMASPHVAGAAALILSKHPSWTNTQVRSSLENTT
TKLGDSFYYGKGLINVQAAAQ (SEQ ID NO:16)

[0282] The nucleotide sequence of synthesized gene GcM94 is provided below:

GCGCAGTCCGTGCCTTACGGCGTATCACAAATTAAAGCCCCTGCTCTGCACTCTCAAGGCTA
CACTGGATCAAATGTTAAAGTAGCGGTTATCGACAGCGGTATCGACTCGAGCCATCCAGAT
CTTAAAGTCGCTGGAGGGGCTTCTATGGTGCCGTCCGAAACAAACCCGTTTCAAGATAACA
ATACACATGGCACACACGTCGCAGGAACGGTTGCGGCGTTAAACAATAATATTGGCGTGCT
TGGTGTAGCCCCGTCTGCTTCGCTCTACGCCGTTAAAGTTCTTGCAGCAGACGGAGCAGGCC
AATACTCATGGATTATCAACGGCATCGAATGGGCCATCGCGAATAACATGGATGTAATCAA
CATGAGCGTCGGAGCACCAAGCGGCAGTGCGGCACTTAAAGCAGCAGTTGATAAAGCTGTT
GCATCTGGTGTCGTCGTAGTAGCGGCAGCTGGGAATGAGGGAACATCCGGATCATCGAGTA
CCGTCGGTTATCCAGGCAAGTACCCTTCAGTGATTGCAGTGGGCGCTGTAGACTCTACAAAT
CAACGTGCCTCTTTTTCCTCCGTGGGACCGGAGCTGGATGTCATGGCCCCTGGCGTTTCTATT
CAATCGACGCTTCCAGGGAACAAGTATGGTGCGCAAAACGGGACTTCCATGGCCTCGCCGC
ATGTAGCTGGGGCGGCCGCATTGATTCTTTCTAAGCACCCGAACTGGACAAACAACCAAGT
CCGCAGCAGTTTAGAAAACACCACTACAAAACTTGGTGATTCTTTCTACTATGGAAAAGGGC
TGATCAACGTACAGGCGGCAGCTCAGTAA (SEQ ID NO:17)

[0283] The amino acid sequence of GcM94 is provided below:

AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETNPFQDNNTH
GTHVAGTVAALNNNIGVLGVAPSASLYAVKVLAADGAGQYSWIINGIEWAIANNMDVINMSVG
APSGSAALKAAVDKAVASGVVVVAAAGNEGTSGSSSTVGYPGKYPSVIAVGAVDSTNQRASFS
SVGPELDVMAPGVSIQSTLPGNKYGAQNGTSMASPHVAGAAALILSKHPNWTNNQVRSSLENT
TTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO:18)

[0284] The nucleotide sequence of synthesized gene GcM 95 is provided below:

GCGCAGTCCGTGCCTTACGGCGTATCACAAATTAAAGCCCCTGCTCTGCACTCTCAAGGCTA
CACTGGATCAAATGTTAAAGTAGCGGTTATCGACAGCGGTATCGACTCGAGCCATCTGGATC
TTAAAGTCGCTGGAGGGGCTTCTATGGTGCCGGGAGAAACAAACCCGTTTGTCGATGCACA
AACACATGGCACACACGTCGCAGGAACGGTTGCGGCGTTAAACAATAATATTGGCGTGCTT
GGTGTAGCCCCGGAAGCTTCGCTCTACGCCGTTAAAGTTCTTGCAGCAGACAACGCAGCAC
AATACTCATGGATTGTCAACGGCATCGAATGGGCCATCGCGAATAACATGGATGTAATCAA
CATGAGCCTGGGAGCACCAAGCGGCAGTGCGGCACTTAAAGCAGCAGTTGATAAAGCTGTT
GCATCTGGTGTCGTCGTAGTAGCGGCAGCTGGGAATGAGGGAACATCCGGATCATCGAGTA
CCGTCGGTTATCCAGGCAAGTACCCTTCAGTGATTGCAGTGGGCGCTGTAGACTCTTCAAAT
CAACGTGCCTCTTTTTCCTCCGTGGGACCGGAGCTGGATGTCATGGCCCCTGGCGTTTCTATT
CAATCGACGCTTCCAGGGAACAAGTATGGTGCGCAAAACGGGACTTCCATGGCCTCGCCGC
ATGTAGCTGGGGCGGCCGCATTGATTCTTTCTAAGCACCCGAACTGGACAAACACTCAAGTC
CGCAGCAGTTTAGAAAACACCACTACAAAACTTGGTGATTCTTTCTACTATGGAAAAGGGCT
GATCAACGTACAGGCGGCAGCTCAGTAA (SEQ ID NO:19)

[0285] The amino acid sequence of GcM 95 is provided below:

AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHLDLKVAGGASMVPGETNPFVDAQTH
GTHVAGTVAALNNNIGVLGVAPEASLYAVKVLAADNAAQYSWIVNGIEWAIANNMDVINMSL
GAPSGSAALKAAVDKAVASGVVVVAAAGNEGTSGSSSTVGYPGKYPSVIAVGAVDSSNQRASF
SSVGPELDVMAPGVSIQSTLPGNKYGAQNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENT
TTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO:20)

[0286] The nucleotide sequence of synthesized gene GcM96 is provided below:

GCGCAGTCCGTGCCTTACGGCGTATCACAAATTAAAGCCCCTGCTCTGCACTCTCAAGGCTA
CACTGGATCAAATGTTAAAGTAGCGGTTATCGACAGCGGTATCGACTCGAGCCATCCAGAT
CTTAAAGTCGCTGGAGGGGCTTCTATGGTGCCGTCCGAAACAAACCCGTTTCAAGATAACA
ATTCTCATGGCACACACGTCGCAGGAACGGTTGCGGCGTTAAACAATAATATTGGCGTGCTT

GGTGTAGCCCCGTCTGCTTCGCTCTACGCCGTTAAAGTTCTTGCAGCAGACGGATCAGGCCA
ATACTCATGGATTATCAACGGCATCGAATGGGCCATCGCGAATAACATGGATGTAATCAAC
ATGAGCCTGGGAGCAACAAGCGGCAGTGCGGCACTTAAAGCAGCAGTTGATAAAGCTGTTG
CATCTGGTCAAGTCGTAGTAGCGGCAGCTGGGAATGAGGGAACAGATGGACCTTCGAGTAC
CGTCGGTTATCCAGGCAAGTACCCTTCAGTGATTGCAGTGGGCGCTGTAGACTCTACAAATA
CACGTGCCTCTTTTTCCTCCGTGGGACCGGAGCTGGATGTCATGGCCCCTGGCGTTTCTATTC
AATCGACGCTTCCAGCAAACAAGTATGGTGCGCAAAACGGGACTTCCATGGCCGCACCGCA
TGTAGCTGGGGCGGCCGCATTGATTCTTTCTAAGCACCCGTCATGGACAAACAACCAAGTCC
GCAGCAGTTTAGAACAAACCACTACAAAACTTGGTGATTCTTTCTACTATGGAAAAGGGCTG
ATCAACGTACAGGCGGCAGCTCAGTAA (SEQ ID NO:21)

[0287] The amino acid sequence of GcM96 is provided below:

AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETNPFQDNNSH
GTHVAGTVAALNNNIGVLGVAPSASLYAVKVLAADGSGQYSWIINGIEWAIANNMDVINMSLG
ATSGSAALKAAVDKAVASGQVVVAAAGNEGTDGPSSTVGYPGKYPSVIAVGAVDSTNTRASFS
SVGPELDVMAPGVSIQSTLPANKYGAQNGTSMAAPHVAGAAALILSKHPSWTNNQVRSSLEQT
TTKLGDSFYYGKGLINVQAAAQ (SEQ ID NO:22)

[0288] The nucleotide sequence of synthesized gene GcM100 is provided below:

GCGCAGTCCGTGCCTTACGGCGTATCACAAATTAAAGCCCCTGCTCTGCACTCTCAAGGCTA
CACTGGATCAAATGTTAAAGTAGCGGTTATCGACAGCGGTATCGACTCGAGCCATCCAGAT
CTTAAAGTCGCTGGAGGGGCTTCTATGGTGCCGTCCGAAACAAACCCGTTTCAAGATAACA
ATTCTCATGGCACACACGCAGCAGGAACGGTTGCGGCGTTAAACAATAATATTGGCGTGCTT
GGTGTAGCCCCGTCTGCTTCGCTCTACGCCGTTAAAGTTCTTGCAGCAGACGGATCAGCACA
ATACTCATGGATTATCAACGGCATCGAATGGGCCATCGCGAATAACATGGATGTAATCAAC
ATGGCACTGGGAGCACCAAGCGGCAGTGCGGCACTTAAAGCAGCAGTTGATAAAGCTGTTG
CATCTGGTGTCGTCGTAGTAGCGGCAGCTGGGAATGAGGGAACATCCGGATCATCGAGTAC
CGTCGGTTATCCAGGCAAGTACCCTTCAGTGATTGCAGTGGGCGCTGTAGACTCTTCAAATC
AACGTGCCTCTTTTTCCTCCGTGGGACCGGAGCTGGATGTCATGGCCCCTGGCGTTTCTATTC
AATCGACGCTTCCAGCAAACAAGTATGGTGCGCAAAACGGGACTTCCATGGCCTCGCCGCA
TGTAGCTGGGGCGGCCGCATTGATTCTTTCTAAGCACCCGAACTGGACAAACACTCAAGTCC
GCAGCAGTTTAGAAAACACCACTACAAAACTTGGTGATTCTTTCTACTATGGAAAAGGGCTG
ATCAACGTACAGGCGGCAGCTCAGTAA (SEQ ID NO:23)

[0289] The amino acid sequence of GcM100 is provided below:

AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETNPFQDNNSH
GTHAAGTVAALNNNIGVLGVAPSASLYAVKVLAADGSAQYSWIINGIEWAIANNMDVINMALG
APSGSAALKAAVDKAVASGVVVVAAAGNEGTSGSSSTVGYPGKYPSVIAVGAVDSSNQRASFS
SVGPELDVMAPGVSIQSTLPANKYGAQNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTT
TKLGDSFYYGKGLINVQAAAQ (SEQ ID NO:24)

b) <u>Construction of combinatorial libraries CG1-CGS and CG8 using the synthetic genes GcM90-94 and GcM100</u>

[0290]

Table 3-1. List of Possible Substitutions Introduced and Primers Used for the Construction of Combinatorial Libraries CG1-CG5 and CG8

Synthesized Genes (template or parent)	Library Name	Substitutions Introduced	Primer Name	Primer Sequence
GcM90	CG1	G53S	1 S53 f	/5Phos/CTTCTATGGTGCCGTCCGAAACA AACCCGTTTCAAG (SEQ ID NO:25)
		A68V	1 V68 f	/5Phos/TCATGGCACACACGTCGCAGGAA CGGTTGCGGCG (SEQ ID NO:26)
		A102G	1 G102 f	/5Phos/AGCAGACGGATCAGGCCAATACT CATGGATTATCAAC (SEQ ID NO:27)
		T129P	1 P129 f	/5Phos/TGAGCCTGGGAGCACCAAGCGGC AGTGCGGCACTTAAAG (SEQ ID NO:28)
		T185Q	1 Q185 f	/5Phos/TAGACTCTTCAAATCAACGTGCC TCTTTTTCCTCCGTG (SEQ ID NO:29)
GcM91	CG2	V59Q	2 Q59 f	/5Phos/GAAACAAACCCGTTTCAAGATAA CAATTCTCATG (SEQ ID NO:30)
		V108I	2 I108 f	/5Phos/ATACTCATGGATTATCAACGGCA TCGAATGGGCCATC (SEQ ID NO:31)

Table 3-1. List of Possible Substitutions Introduced and Primers Used for the Construction of Combinatorial Libraries CG1-CG5 and CG8

Synthesized Genes (template or parent)	Library Name	Substitutions Introduced	Primer Name	Primer Sequence
		Q147V	2 V147 f	/5Phos/TGTTGCATCTGGTGTCGTCGTAGT AGCGGCAGCTGG (SEQ ID NO:32)
		A211G	2 G211 f	/5Phos/ATCGACGCTTCCAGGGAACAAGT ATGGTGCGCAAAAC (SEQ ID NO:33)
		Q252N	2 N252 f	/5Phos/CAGCAGTTTAGAAAACACCACTA CAAAACTTGGTG (SEQ ID NO:34)
GcM92	CG3	A61N	3 N61 f	/5Phos/CAAACCCGTTTCAAGATAACAAT TCTCATGGCACACAC (SEQ ID NO:35)
		E87S	3 S87 f	/5Phos/TTGGTGTAGCCCCGTCTGCTTCGC TCTACGCCGTTAAAG (SEQ ID NO:36)
		I124M	3 M 124 f	/5Phos/TGGATGTAATCAACATGAGCCTG GGAGCACCAAGCG (SEQ ID NO:37)
		P161S	3 S161 f	/5Phos/AGGGAACATCCGGATCATCGAGT ACCGTCGGTTATCCAG (SEQ ID NO:38)
		A224S	3 S224 f	/5Phos/GACTTCCATGGCCTCGCCGCATG TAGCTGGGGCGGC (SEQ ID NO:39)
GcM93	CG4	Q62N	4 N62 f	/5Phos/GTTTCAAGATAACAATTCTCATG GCACACACGTCGC (SEQ ID NO:40)
		N100G	4 G100 f	/5Phos/GTTCTTGCAGCAGACGGATCAGG CCAATACTCATG (SEQ ID NO:41)
		A125S	4 S125 f	/5Phos/ATGTAATCAACATGAGCCTGGGA GCACCAAGCGGCAG (SEQ ID NO:42)
		D159S	4 S159 f	/5Phos/GGAATGAGGGAACATCCGGATCA TCGAGTACCGTCGG (SEQ ID NO:43)
		S240N	4 N240 f	/5Phos/CTTTCTAAGCACCCGAACTGGAC AAACACTCAAGTCCG (SEQ ID NO:44)

Table 3-1. List of Possible Substitutions Introduced and Primers Used for the Construction of Combinatorial Libraries CG1-CG5 and CG8

Synthesized Genes (template or parent)	Library Name	Substitutions Introduced	Primer Name	Primer Sequence
GcM94	CG5	T63S	5 S63 f	/5Phos/TCAAGATAACAATTCTCATGGCA CACACGTCGCAGG (SEQ ID NO:45)
		A101S	5 S101 f	/5Phos/TGCAGCAGACGGATCAGGCCAAT ACTCATGGATTATC (SEQ ID NO:46)
		V126L	5 L126 f	/5Phos/AATCAACATGAGCCTGGGAGCAC CAAGCGGCAGTG (SEQ ID NO:47)
		T183S	5 S183 f	/5Phos/CGCTGTAGACTCTTCAAATCAAC GTGCCTCTTTTTCC (SEQ ID NO:48)
		N244T	5 T244 f	/5Phos/GAACTGGACAAACACTCAAGTCC GCAGCAGTTTAG (SEQ ID NO:49)
GcM100	CG8	A68V	1 V68 f	/5Phos/TCATGGCACACACGTCGCAGGAA CGGTTGCGGCG (SEQ ID NO:50)
		A102G	1 G102 f	/5Phos/AGCAGACGGATCAGGCCAATACT CATGGATTATCAAC (SEQ ID NO:51)
		A211G	2 G211 f	/5Phos/ATCGACGCTTCCAGGGAACAAGT ATGGTGCGCAAAAC (SEQ ID NO:52)
		A125S	4 S125 f	/5Phos/ATGTAATCAACATGAGCCTGGGA GCACCAAGCGGCAG (SEQ ID NO:53)

[0291] Each synthesized gene was built into the pHPLT-BPN'-S78N-G97A-G128A-Y217Q parent molecule. Resulting plasmids containing the six synthesized genes GcM90-94, and GcM100 served as templates to make combinatorial libraries at the respective positions (Table 3-1). Two additional genes, GcM95 and GcM96, were also synthesized for analysis, but did not serve as parental DNA for libraries. These genes each have nine mutations on top of the pHPLT-BPN'- S78N-G97A-G128A-Y217Q parent molecule.

[0292] The parent plasmids (template DNA) containing the synthetic genes GcM90-94, and GcM100 were methylated were methylated using two micrograms of DNA and methylase (NEB), according to the NEB protocol. Methylated DNA was then purified using DNA Clean and Concentrator kit (Zymo Research). Combinatorial libraries CG1-5 and CG8 were made using a QUIKCHANGE® Multi Site-Directed Mutagenesis kit ("QCMS kit"; Stratagene) following the manufacturer's protocol (see Table 3-1 for respective template and primer combinations), with the exception of libraries CG3 and CG4, which used 86.5ng of each primer in place of the 50ng suggested in the protocol. All primers used for introducing the desired substitutions in each library are listed in Table 3-1. They were synthesized and provided by Integrated DNA Technologies. After the QCMS reactions were completed for each library, the template DNA was digested by the addition of 0.5 -1 µl *Dpn*I (from the QCMS kit) and incubated at 37°C for 1 - 4 hours, followed by another addition of 0.5 - 1 µl *Dpn*I and another incubation at 37°C for 1-4 hours. For efficient transformation of *B. subtilis,* DNA from the QCMS reaction mixtures were amplified before transformation and transformants grown as described in

Example 2.

[0293] Additional variants of BPN'-v3+S78N were produced by DNA2.0. The following substitutions were introduced individually into the BPN'-v3+S78N parent molecule: Q59G, N62Q, V68A, S89Y, A92G, I108V, I115V, M124T, P129L, A138T, V147L, S161P, Y167A, P172V, G211T, L267V, and A273S.

[0294] All of the combinatorial library variants described above and the variants synthesized at DNA2.0 were tested for cleaning performance using a BMI microswatch assay in Detergent Composition 1 at 16°C and pH 8 and BMI microswatch assay in Detergent Composition 2 at 16°C and pH 8. Protein content was determined using the TCA assay. Assays were performed as described in Example 1 and Performance Indices were calculated relative to BPN'-v3 (with a PI value of 1.0).

[0295] The following BPN' variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 1 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of S063T-S078N-G097A-S101A-G128A-S183T-Y217Q-T244N, N061A-S078N-G097A-G128A-Y217Q-S224A, S053G-S078N-G097A-G128A-P129T-Q185T-Y217Q, S063T-S078N-G097A-S101A-G128A-S183T-Y217Q, S063T-S078N-G097A-S101A-G128A-Y217Q, S063T-S078N-G097A-S101A-G128A-Y217Q-T244I, and S078N-G097A-G128A-P129T-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' and BPN'-v3 in this assay.

[0296] The following BPN' variants were determined to have a PI value of about 1.0 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 1 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128A-Y217Q, N061A-S078N-S087E-G097A-G128A-Y217Q-S224A, Q059V-S078N-G097A-G128A-G211A-Y217Q, Q059V-S078N-G097A-G128A-V147Q-Y217Q, Q059V-S078N-G097A-G128A-Y217Q, Q059V-S078N-G097A-I108V-G128A-Y217Q-N252Q, S053G-S078N-G097A-G128A-P129T-Y217Q, S078N-G097A-G128A-G211A-Y217Q, S078N-G097A-G128A-Q185T-Y217Q, S078N-G097A-G 128A-V 147Q-Y217Q, S078N-G097A-G128A-Y217Q, S078N-G097A-G128A-Y217Q-S224A, and S078N-G097A-G128A-Y217Q-S224A-A274D, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0297] The following BPN' variant was determined to have a PI value of about 0.9 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 1 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising the set of amino acid substitutions S078N-G097A-I108V-G128A-V 147Q-Y217Q, wherein positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0298] The following BPN' variants were determined to a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 1 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of Q059V-S078N-G097A-I108V-G128A-V147Q-G211A-Y217Q-N252Q, S078N-G097A-I108V-G128A-V147Q-G211A-Y217Q, S078N-G097A-I108V-G128A-V147Q-G211A-Y217Q-N252Q, S078N-G097A-I108V-G128A-V147Q-Y217Q-N252Q, and S078N-S087E-G097A-M124I-G128A-Y217Q-S224A, wherein amino acid positions of the variant are numbered by correspondence with positions of the SEQ ID NO:2 sequence.

[0299] The following BPN' variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 2 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of S063T-S078N-G097A-S101A-G128A-S183T-Y217Q, S063T-S078N-G097A-S101A-G128A-S183T-Y217Q-T244N, S063T-S078N-G097A-S101A-G128A-Y217Q, and S063T-S078N-G097A-S101A-G128A-Y217Q-T244I, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0300] The following BPN' variants were determined to have a PI value of about 1.0 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 2 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128A-Y217Q, N061A-S078N-G097A-G128A-Y217Q-S224A, N061A-S078N-S087E-G097A-G128A-Y217Q-S224A, Q059V-S078N-G097A-G128A-G211A-Y217Q, Q059V-

S078N-G097A-G128A-V147Q-Y217Q, Q059V-S078N-G097A-G128A-Y217Q, Q059V-S078N-G097A-I108V-G128A-Y217Q-N252Q, S053G-S078N-G097A-G128A-P129T-Q185T-Y217Q, S053G-S078N-G097A-G128A-P129T-Y217Q, S078N-G097A-G128A-G211A-Y217Q, S078N-G097A-G128A-P129T-Y217Q, S078N-G097A-G128A-Q185T-Y217Q, S078N-G097A-G128A-Y217Q, S078N-G097A-G128A-Y217Q-S224A, and S078N-G097A-G128A-Y217Q-S224A-A274D, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0301] The following BPN' variants were determined to have a PI value of about 0.9 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 2 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of S078N-G097A-G128A-V147Q-Y217Q and S078N-G097A-I108V-G128A-V147Q-Y217Q, wherein positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0302] The following BPN' variants were determined to a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 2 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of Q059V-S078N-G097A-I108V-G128A-V147Q-G211A-Y217Q-N252Q, S078N-G097A-I108V-G128A-V147Q-G211A-Y217Q, S078N-G097A-I108V-G128A-V147Q-G211A-Y217Q-N252Q, S078N-G097A-I108V-G128A-V147Q-Y217Q-N252Q, and S078N-S087E-G097A-M124I-G128A-Y217Q-S224A, wherein amino acid positions of the variant are numbered by correspondence with positions of the SEQ ID NO:2 sequence.

EXAMPLE 4

Generation and Cleaning Performance of BPN' Variants

Generation of BPN' variants LC1-LC4 via QUIKCHANGE® Multi Site-Directed Mutagenesis

[0303] BPN' variants were constructed from different parental plasmids using QUIKCHANGE® Multi Site-Directed Mutagenesis kits. The parental plasmids (Table 4-1) were methylated using a NEB Dam Methylase Kit in a reaction containing 77.5 µL H20 + 10 µL Buffer 10X + 0.25 µL SAM + 2 µL DAM methylase + 10 µL miniprep DNA (~150 ng/µL) at 37°C overnight. The methylated plasmid DNA was purified using a QIAGEN® PCR purification kit. QUIKCHANGE® Multi Site-Directed Mutagenesis reactions were set up for each of the DNA templates in a reaction mix containing 2.5 µL Buffer 5X + 0.5 µL primer 1 (25 µM) + 0.5 µL primer 2 (25 µM) + 1 µL dNTP's + 1 µL enzyme blend + 18 µL H_2O + 1.5 µL DNA. The PCR program used was: 95°C for 1 min; (95°C for 1 min, 53°C for 1 min, 65°C for 9:39 min) x 29 cycles; 65°C for 10 min, 4°C hold. Primer sequences are shown in Table 4-2. In all reactions, PCR was performed using a MJ Research PTC-200 Peltier thermal cycler. Parental DNA from the PCR samples was removed by addition of 1 µL of *Dpn*I to QUIKCHANGE® Multi Site-Directed Mutagenesis reactions at 37°C overnight. To increase transformation frequency, the *Dpn*I-digested reactions were amplified using rolling circle amplification (RCA) using the Illustra TempliPhi kit according to the manufacturer's protocol. *B. subtilis* cells (ΔaprE, ΔnprE, amyE::xylRPxylAcomK-phleo) were transformed with 1 µL each of the RCA reaction and the transformed cells were plated onto LA + 1.6% skim milk plates containing 10 ppm neomycin and incubated at 37°C overnight. Colonies from overnight growth were selected to perform colony PCR for sequencing using "puReTaq Ready-To-Go PCR Beads" (Amersham). The PCR and sequencing primers used were pHPLT F1 (/5PHOS/TACATATGAGTTATGCAGTTTG (SEQ ID NO:54)) and pHPLT seq R1 (/5PHOS/TTATCCTTTACCTTGTCTC (SEQ ID NO:55)). Clones with appropriate sequences were frozen. BPN' variant proteins were produced by growing *B. subtilis* transformants in 96 well microtiter plates at 37°C for 68 hours in a MOPS based medium containing urea as described in Example 2.

Table 4-1. Parental Plasmids and Primers Used for Generation of BPN' Variants LC1-LC4

Parental Plasmid	Mutations Introduced	Primers Used
BPN'-G097A-G128A-Y217Q-S024G-N025G-N061P-S101N (termed LC1)	A128S	A128Sf, A128Sr
BPN'-G097A-G128A-Y217Q-S053G-N061P-S101N-V203Y (termed LC2)	A128S	A128Sf, A128Sr

Table 4-1. Parental Plasmids and Primers Used for Generation of BPN' Variants LC1-LC4		
Parental Plasmid	**Mutations Introduced**	**Primers Used**
BPN'-G097A-G128A-Y217Q-S024G-N025G-S053G-T055P-N061P-S101N-V203Y (termed LC3)	A128S	A128Sf, A128Sr
BPN'-G097A-G 128A-Y217Q-S024G-N025G-S053G-T055P-N061P-S101N-V203Y (termed LC4)	P55T	P55Tf, P55Tr

Table 4-2. Sequences of Primers Used for QUIKCHANGE® Multi Site-Directed Mutagenesis Reactions to Make BPN' variants LC1-LC4	
Primer Name	**Primer Sequence (5' to 3')**
A128Sf	/5Phos/CAACATGAGCCTGGGATCACCAAGCGGCAGTGCGG (SEQ ID NO:56)
A128Sr	/5Phos/CCGCACTGCCGCTTGGTGATCCCAGGCTCATGTTG (SEQ ID NO:57)
P55Tf	/5Phos/CTATGGTGCCGGGCGAAACAAACCCGTTTCAAGATCCG (SEQ ID NO:58)
P55Tr	/5Phos/CGGATCTTGAAACGGGTTTGTTTCGCCCGGCACCATAG (SEQ ID NO:59)

Generation of Additional BPN' Variants LC5-LC37

[0304] An additional 33 BPN' variants termed successively LC5 through LC37 were produced by DNA 2.0 using the BPN' nucleic acid as the parent gene contained in the expression plasmid pHPLT-BPN' partial opt (see Figure 3). LC5 through LC37 BPN' variants are as follows, respectively: BPN'-P52L-V68A-G97A-I111V, BPN'-I111V-M124V-Y167A-Y217Q, BPN'-Y104N-G128A-Y217Q, BPN'-M124V-Y167A-Y217Q, BPN'-I111V-M124V-Y217Q, BPN'-P52L-V68A-G97A, BPN'-G97A-I111V-M124V, BPN'-V68A-A92G-G97A, BPN'-G97A-I111V-M124V-Y167A-Y217Q, BPN'-P52L-V68A-I111V-Y217Q, BPN'-P52L-V68A-I111V, BPN'-V68A-A92G-I111V, BPN'-P52L-V68A-G97A-I111V-Y217Q, BPN'-V68A-G97A-I111V, BPN'-G97A-I111V-Y217Q, BPN'-G97A-I111V-M124V-Y167A, BPN'-S89Y-I111V-M124V, BPN'-V68A-S89Y-I111V, BPN'-V68A-A92G-Y217Q, BPN'-I111V-Y167A-Y217Q, BPN'-G97A-I111V-Y167A-Y217Q, BPN'-G97A-I111V-M124V-Y217Q, BPN'-V68A-I111V-Y167A-Y217Q, BPN'-I111V-G128A-Y217Q, BPN'-G97A-M124V-Y217Q, BPN'-V68A-Y167A-Y217Q, BPN'-I111V-M124V-Y167A, BPN'-N62Q-G97A-I111V, BPN'-G97A-M124V-Y167A-Y217Q, BPN'-G97A-L126A-Y217Q, BPN'-V68A-I111V-Y217Q, BPN'-S89Y-M124V-Y217Q, and BPN'-L96T-G97A-Y217Q. Plasmid pHPLT-BPN' partial opt was also created by DNA 2.0.

[0305] Transformants were picked into microtiter plates and grown as described in Example 2. The variants were assayed for cleaning performance using a BMI microswatch assay in Detergent Composition 2 at 16°C and pH 8. Protein content was determined using the TCA assay. The assays were performed as described in Example 1 and the Performance Indices were calculated relative to BPN'-v3 (with a PI value of 1.0).

[0306] The following BPN' variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 2 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-I111V-M124V-Y217Q, G097A-I111V-Y167A-Y217Q, S024G-N025G-N061P-G097A-S101N-G128S-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-G128A-V203Y-Y217Q, S024G-N025G-S053G-T055P-N061P-G097A-S101N-G128S-V203Y-Y217Q, and V068A-A092G-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0307] The following BPN' variants were determined to have a PI value of about 1.0 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 2 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128A-Y217Q, G097A-G128S-Y217Q, G097A-I111V-Y217Q, I111V-G128AY217Q, I111V-M124V-Y167A, I111V-M124V-Y217Q, L096T-G097A-Y217Q, N062Q-G097A-I111V, S053G-N061P-G097A-S101N-G128S-V203Y-Y217Q, S089Y-M124V-Y217Q, and V068A-I111V-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0308] The following BPN' variants were determined to have a PI value of about 0.9 relative to BPN'-v3 in a BMI microswatch

cleaning assay in Detergent Composition 2 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-I111V-M124V, G097A-L126A-Y217Q, G097A-M124V-Y217Q, I111V-Y167AY217Q, M124V-Y167A-Y217Q, P052L-V068A-G097A, S089Y-I111V-M124V, V068A-A092G-G097A, V068A-A092G-I111V, V068A-G097A-I111V, V068A-S089Y-I111V, and Y104N-G128AY217Q, wherein positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0309] The following BPN' variants were determined to a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 2 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-M124V-Y167A-Y217Q, V068A-Y167A-Y217Q, G097A-I111V-M124V-Y167A, I111V-M124V-Y167A-Y217Q, V068A-I111V-Y167A-Y217Q, G097A-I111V-M124V-Y167A-Y217Q, and P052L-V068A-I111V, wherein positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

EXAMPLE 5

Cleaning Performance of BPN'Variants

[0310] Variants based on parent BPN' were made by DNA 2.0. The variants were grown as described in Example 2 and tested for cleaning performance on BMI microswatch assay in Detergent Composition 1 at 16°C and pH 8, BMI microswatch assay in Detergent Composition 4 at 16°C and pH 8, and egg microswatch assay in Detergent Composition 4 at 16°C and pH 8. The protein content was determined using the TCA assay. The assays were performed as described in Example 1 and the Performance Indices were calculated relative to BPN'-v3 (with a PI value of 1.0).

[0311] The following BPN' variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of N061P-G097A-S101N-G128A-P210S-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-G128A-P210S-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-G128S-Y217Q, and S024G-N025G-S053G-N061P-S078N-G097A-S101N-I111V-G128S-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0312] The following BPN' variants were determined to have a PI value of about 1.0 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128A-Y217Q, G097A-G128A-P210S-Y217Q, G097A-G128S-P210S-Y217Q, G097A-I111V-M124I-Y217Q, G097A-I111V-M124V-P210S-Y217Q, G097A-N123Q-P210S-Y217Q, G097A-N123Q-Y217Q, N061P-G097A-G128A-P210S-Y217Q, N061P-G097A-G128S-Y217Q, N061P-G097A-I111V-M124V-Y217Q, N061P-G097A-N123Q-Y217Q, N061P-G097A-S101N-I111V-M124V-Y217Q, N061P-G097A-S101N-N123Q-Y217Q, N061P-G102A-P129S-Y217Q, N061P-N062Q-G097A-G100N-S101N-Y217Q, N061P-N062Q-G097A-G100N-Y217Q, N061P-N062Q-G097A-G100Q-P210S-Y217Q, N061P-N062Q-G097A-I111V-Y217Q, N061P-N062Q-G097A-S101N-I111V-Y217Q, N061P-S078N-G097A-I111V-M124I-Y217Q, N061P-S078N-G102A-I111V-PI29S-Y217Q, N062Q-G097A-I111V-P210S-Y217Q, N062Q-G097A-I111V-Y217Q, N062Q-S078N-G097A-I111V-Y217Q, S024G-N025G-N061P-G097A-S101N-G128A-P210S-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-I111V-M124V-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-N123Q-Y217Q, S024G-N025G-S053G-N061P-N062Q-G097A-G100N-S101N-Y217Q, S024G-N025G-S053G-N061P-N062Q-G097A-S101N-I111V-Y217Q, S024G-N025G-S053G-N061P-S101N-G102A-P129S-Y217Q, S053G-N061P-G097A-G128S-Y217Q, S053G-N061P-G097A-M124I-Y217Q, S053G-N061P-G097A-S101N-I111V-M124V-Y217Q, S053G-N061P-G102A-P129S-P210S-Y217Q, S053G-N061P-G102A-P129S-Y217Q, S053G-N061P-N062Q-G097A-G100N-S101N-Y217Q, S053G-N061P-N062Q-G097A-S101N-I111V-Y217Q, S053-N061P-SIOIN-GI02A-PI29S-Y217Q, S053G-S078N-G097A-1111V-G128S-Y217Q, S078N-G097A-G128S-Y217Q, and S078N-G097A-I111V-M124V-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0313] The following BPN' variants were determined to have a PI value of about 0.9 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of N061P-G097A-M124I-Y217Q, N061P-G097A-M124V-Y217Q,

N061P-N062Q-G097A-G100D-Y217Q, N061P-N062Q-G097A-G100Q-S101N-Y217Q, N061P-N062Q-G097A-G100Q-Y217Q, N061P-N062Q-G 100N-G 102A-Y217Q, N061P-N062Q-S078N-G097A-G 1 OON-1111V-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-M124I-Y217Q, S053G-N061P-G097A-S101N-M124I-Y217Q, and S053G-N061P-G097A-S101N-N123Q-Y217Q, wherein positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0314] The following BPN' variants were determined to have a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-N123A-Y217Q, G097A-N123V-Y217Q, N061P-G102A-G128S-Y217Q, N061P-S101N-G102A-G128S-Y217Q, Y217Q, S078N-G097A-I111V-N123Q-Y217Q, and G102A-N123Q-Y217Q, wherein positions of the variant are numbered by correspondence with positions of the SEQ ID NO:2 sequence.

[0315] The following BPN' variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 1 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of N061P-G097A-G128S-Y217Q, N061P-G097A-S101N-G128A-P210S-Y217Q, N061P-N062Q-G097A-S 101N-1111V-Y217Q, S024G-N025G-N061 P-G097A-S 101N-G128A-P210S-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-G128A-P210S-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-G128S-Y217Q, and S024G-N025G-S053G-N061P-S078N-G097A-S101N-I111V-G128S-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0316] The following BPN' variants were determined to have a PI value of about 1.0 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 1 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128A-Y217Q, G097A-G128A-P210S-Y217Q, G097A-G128S-P210S-Y217Q, G097A-I111V-M124I-Y217Q, G097A-I111V-M124V-P210S-Y217Q, G097A-N123Q-P21OS-Y217Q, G097A-N123Q-Y217Q, N061P-G097A-G128A-P210S-Y217Q, N061P-GO97A-It I IV-MI24V-Y217Q, N061P-G097A-M124V-Y217Q, N061P-G097A-N123Q-Y217Q, N061P-G097A-S101N-I111V-M124V-Y217Q, N061P-G102A-P129S-Y217Q, N061P-N062Q-G097A-G100N-S101N-Y217Q, N061P-N062Q-G097A-G100Q-Y217Q, N061P-N062Q-G097A-I111V-Y217Q, N061P-N062Q-S078N-G097A-G100N-I111V-Y217Q, N061P-S078N-G097A-I111V-M124I-Y217Q, N061P-S078N-G102A-I111V-P129S-Y217Q, N062Q-G097A-I111V-P210S-Y217Q, N062Q-G097A-I111V-Y217Q, N062Q-S078N-G097A-I111V-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-I111V-M124V-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-N123Q-Y217Q, S024G-N025G-S053G-N061P-N062Q-G097A-G100N-S101N-Y217Q, S024G-N025G-S053G-N061P-N062Q-G097A-S101N-I111V-Y217Q, S024G-N025G-S053G-N061P-S101N-G102A-P129S-Y217Q, S053G-N061P-G097A-G128S-Y217Q, S053G-N061P-G102A-P129S-P210S-Y217Q, S053G-N061P-G102A-P129S-Y217Q, S053G-NO61P-NO62Q-GO97A-S101N-I111V-Y217Q, S053G-NO61P-S101N-G102A-P129S-Y217Q, S053G-S078N-G097A-I111V-G128S-Y217Q, S078N-G097A-G128S-Y217Q, and S078N-G097A-I111V-M124V-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0317] The following BPN' variants were determined to have a PI value of about 0.9 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 1 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of N061P-G097A-M124I-Y217Q, N061P-G097A-S101N-N123Q-Y217Q, N061P-N062Q-G097A-G100N-Y217Q, N061P-N062Q-G097A-G100Q-P210S-Y217Q, N061P-N062Q-G097A-G100Q-S101N-Y217Q, N061P-N062Q-GIOON-G102A-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-M124I-Y217Q, S053G-NO61P-GO97A-MI241-Y217Q, S053G-N061P-G097A-S101N-IU IV-MI24V-Y217Q, S053G-N061P-G097A-S101N-M124I-Y217Q, S053G-N061P-G097A-S101N-N123Q-Y217Q, and S053G-N061P-N062Q-G097A-G100N-S101N-Y217Q, wherein positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0318] The following BPN' variants were determined to have a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 1 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-N123A-Y217Q, G097A-N123V-Y217Q, N061P-N062Q-G097A-G100D-Y217Q, N061P-S101N-G102A-G128S-Y217Q, Y217Q, N061P-G102A-G128S-Y217Q, S078N-G097A-I111V-N123Q-Y217Q, and G102A-N123Q-Y217Q, wherein positions of the variant are numbered by correspondence with positions of the SEQ ID NO:2 sequence.

[0319] The following BPN' variants were determined to have a PI value greater than 1.0 to about 5 relative to BPN'-v3 in an egg microswatch cleaning assay in Detergent Composition 4 at 16°C and pH 8: BPN' amino acid sequence (SEQ ID NO:2) comprising

the set of amino acid substitutions N061P-G097A-S101N-G128A-P210S-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0320] The following BPN' variants were determined to have a PI value of about 1.0 relative to BPN'-v3 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128A-Y217Q, N061P-G102A-P129S-Y217Q, N062Q-G097A-I111V-P210S-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-G128A-P210S-Y217Q, S024G-N025G-N061P-G097A-S101N-G128A-P210S-Y217Q, N061P-G097A-G128A-P210S-Y217Q, G097A-G128S-P210S-Y217Q, S024G-N025G-S053G-N061P-S078N-G097A-S101N-I111V-G128S-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-G128S-Y217Q, N061P-G097A-G128S-Y217Q, and G097A-G128A-P210S-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0321] The following BPN' variants were determined to have a PI value equal to or greater than 0.5 and equal to or less than 0.9 relative to BPN'-v3 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of N061P-G097A-M124I-Y217Q, S053G-N061P-G097A-S101N-N123Q-Y217Q, S053G-N061P-G102A-PI29S-P210S-Y217Q, G097A-I111V-M124V-P210S-Y217Q, G097A-N123Q-P210S-Y217Q, S053G-N061P-S101N-G102A-P129S-Y217Q, S053G-N061P-N062Q-G097A-S101N-I111V-Y217Q, N061P-N062Q-G097A-S101N-I111V-Y217Q, N061P-N062Q-G097A-I111V-Y217Q, N062Q-G097A-I111V-Y217Q, N061P-G097A-S101N-I111V-M124V-Y217Q, G097A-N123Q-Y217Q, N061P-G097A-I111V-M124V-Y217Q, S078N-G097A-I111V-M124V-Y217Q, S053G-S078N-G097A-I111V-G128S-Y217Q, S078N-G097A-G128S-Y217Q, S053G-N061P-G097A-G128S-Y217Q, N061P-N062Q-G097A-G100N-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-N123Q-Y217Q, N061P-G097A-S101N-N123Q-Y217Q, N061P-N062Q-G097A-G100Q-P210S-Y217Q, N061P-G097A-N123Q-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-M124I-Y217Q, S053G-N061P-G097A-S101N-M124I-Y217Q, S053G-N061P-G097A-M124I-Y217Q, N061P-S078N-G097A-I111V-M124I-Y217Q, N061P-G097A-M124V-Y217Q, S024G-N025G-S053G-N061P-N062Q-G097A-G100N-S101N-Y217Q, S024G-N025G-S053G-N061P-S101N-G102A-P129S-Y21'7Q, N061P-S078N-G102A-I111V-P129S-Y217Q, S053G-N061P-GI02A-P129S-Y217Q, S024G-N025G-S053G-N061P-N062Q-G097A-S101N-I111V-Y217Q, N062Q-S078N-G097A-I111V-Y217Q, S024G-N025G-S053G-N061P-G097A-S101N-I111V-M124V-Y217Q, S053G-N061P-G097A-S101N-I111V-M124V-Y217Q, G097A-I111V-M124I-Y217Q, Y217Q, N061P-N062Q-G100N-G102A-Y217Q, S053G-N061P-N062Q-G097A-G100N-S101N-Y217Q, N061P-N062Q-G097A-G100N-S101N-Y217Q, N061P-N062Q-S078N-G097A-G100N-I111V-Y217Q, N061P-N062Q-G097A-G100Q-Y217Q, N061P-S101N-G102A-G128S-Y217Q, G097A-N123V-Y217Q, G097A-N123A-Y217Q, G102A-N123Q-Y217Q, N061P-N062Q-G097A-G100Q-S101N-Y217Q, S078N-G097A-I111V-N123Q-Y217Q, N061P-N062Q-G097A-G100D-Y217Q, andN061P-G102A-G128S-Y217Q, wherein positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

EXAMPLE 6

Construction and Cleaning Performance of BPN' variants

[0322] A BPN' combinatorial library based on BPN' parent was made by DNA2.0 and delivered as a ligation reaction. For efficient transformation of *B. subtilis,* DNA from the ligation reaction mixtures was amplified before transformation and transformants grown as described in Example 2. These variants were tested for cleaning performance using BMI microswatch assay in Detergent Composition 1 and Detergent Composition 4 at 16°C and pH 8 as well as egg microswatch assay in Detergent Composition 4 at 16°C and pH 8. Protein content was determined using the TCA assay and protease activity was assayed using the AAPF assay. The assays were performed as described in Example 1 and the Performance Indices were calculated relative to BPN'-v3 (with a PI value of 1.0).

[0323] The following BPN' variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 1 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of S024G-N025G-S053G-T055P-N061P-G097A-S101N-G128AY217Q, N025G-G097A-S101N-G128A-Y217Q, N025G-S038G-S053G-N061P-S078N-G097A-S 101N-G128A-Y217Q, N025G-S053G-N061P-S078N-G128A-Y217Q, N025G-S053G-N061P-S078N-S101N-G128A-Y217Q, N025G-S053G-T055P-N061P-S078N-G097A-SIO1N-G128A-Y217Q, N025G-S053G-T055P-S078N-G097A-S101N-G128A-Y217Q, N025G-S078N-G097A-S101N-G128A-

Y217Q, N025G-T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, N025G-T055P-N061P-S078N-S101N-G128A-Y217Q, N061P-S101N-G128A-Y217Q, S024G-N025G-N061P-G097A-G128A-Y217Q, S024G-N025G-N061P-G097A-S101N-G128A-Y217Q, S024G-N025G-S053G-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-N025G-S053G-N061P-S078N-S101N-G128A-Y217Q, S024G-N025G-S053G-T055P-G097A-S101N-G128A-Y217Q, S024G-N025G-S053G-T055P-N061P-G128A-Y217Q, S024G-N025G-T055P-G097A-G128A-Y217Q, S024G-N025G-T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-S053G-N061P-G097A-G128A-Y217Q, S024G-S053G-N061P-S078N-G097A-G128A-Y217Q, S024G-S053G-T055P-G097A-S101N-G128A-Y217Q, S024G-S053G-T055P-N061P-G097A-S101N-G128A-Y217Q, S024G-S053G-T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-S053G-T055P-N061P-S101N-G128A-Y217Q, S024G-T055P-N061P-G097A-G128A-Y217Q, S053G-G097A-S101N-G128A-Y217Q, S053G-N061P-G097AS101N-G128A-Y217Q-S249N, S053G-N061P-S078N-G097A-G128A-Y217Q, S053G-S078N-G097A-S101N-G128A-Y217Q, S053G-T055P-G097A-S101N-G128A-Y217Q, S053G-T055P-N061P-S101N-G128A-Y217Q, S053G-T055P-S078N-G097A-S101N-G128A-Y217Q, T055P-G097A-S101N-G128A-Y217Q, and T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0324] The following BPN' variants were determined to have a PI value of about 1.0 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 1 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128S-Y217Q, G097A-G128A-Y217Q, N025G-S078N-G097A-G128A-Y217Q, N025G-T055P-G097A-G128A-Y217Q, S024G-G097A-S101N-G128A-Y217Q, S024G-I035V-T055P-N061P-S078N-G097A-Y217Q, S024G-N025G-N061P-S078N-G097A-S101N-G128A, S024G-N025G-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-N025G-N061P-S078N-S101N-G128A-Y217Q, S024G-N025G-S053G-N061P-G097A-G128A-S130G-Y217Q, S024G-N025G-S053G-N061P-G128A-Y217Q, S024G-N025G-S053G-T055P-G097A-G128A-Y217Q, S024G-N025G-S053G-T055P-N061P-S078N-G097A-G128A-Y217Q, S024G-N025G-S053G-T055P-N061P-S078N-G128A-Y217Q, S024G-N025G-S053G-T055P-S078N-S101N-G128A-Y217Q, S024G-N025G-S053G-T055P-S101N-G128AY217Q, S024G-N025G-T055P-G097A-S101N-G128A-Y217Q, S024G-N025G-T055P-N061P-S078N-G097A-Y217Q, S024G-N061P-G097A-S101N-G128A-Y217Q, S024G-S038G-S053G-S078N-S101N-G128A-Y217Q, S024G-SO53G-SO78N-GO97A-SIOIN-GI28A.-Y217Q, S024G-S053G-S078N-S101N-G128A-Y217Q, S024G-S053G-T055P-N061P-S078N-G097A-G128A-Y217Q, S024G-T055P-G097A-G128A-Y217Q, S024G-T055P-N061P-G097A-S101N-G128A, S024G-T055P-N061P-S078N-S101N-G128A-Y217Q, S024G-T055P-S078N-G097A-S101N-G128A-Y217Q, S101N-G128A-Y217Q, T055P-N061P-G097A-A116S-G128A, and T055P-N061P-S078N-G128A-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0325] The following BPN' variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of S024G-N025G-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-N025G-S053G-N061P-S078N-S101N-G128A-Y217Q, S024G-N025G-S053G-T055P-N061P-G097A-S101N-G128A-Y217Q, S024G-S053G-T055P-N061P-G097A-S101N-G128A-Y217Q, S024G-S053G-T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-T055P-N061P-S078N-S101N-G128A-Y217Q, S053G-G097A-S101N-G128A-Y217Q, and T055P-N061P-S078N-G128A-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0326] The following BPN' variants were determined to have a PI value of about 1.0 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128S-Y217Q, G097A-G128A-Y217Q, N02SG-G097A-S 101N-G 128A-Y217Q, N025G-S038G-S053G-N061P-S078N-G097A-S101N-G128A-Y217Q, N025G-S053G-NO61P-SO78N-G128A-Y217Q, N025G-S053G-N061P-S078N-S101N-G128A-Y217Q, N025G-S053G-T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, N025G-S053G-T055P-S078N-G097A-S101N-G128A-Y217Q, N025G-S078N-G097A-G128A-Y217Q, N025G-S078N-G097A-S101N-G128A-Y217Q, N025G-T055P-G097A-G128A-Y217Q, N025G-T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, N025G-T055P-N061P-S078N-S101N-G128A-Y217Q, N061P-S101N-G128A-Y217Q, S024G-G097A-S101N-G128AY217Q, S024G-I035V-T055P-N061P-S078N-G097A-Y217Q, S024G-N025G-N061P-G097A-G128A-Y217Q, S024G-N025G-N061P-G097A-S101N-G128A-Y217Q, S024G-N025G-N061P-S078N-G097AS101N-G128A, S024G-NO25G-NO61P-SO78N-SIOIN-GI28A-Y217Q, S024G-N025G-S053G-N061P-G097A-G128A-S130G-Y217Q, S024G-N025G-S053G-N061P-G128A-Y217Q, S024G-N025G-S053G-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-N025G-S053G-T055P-G097A-G128A-1'217Q, S024G-N025G-S053G-T055P-G097A-S101N-G128A-Y217Q, S024G-N025G-S053G-T055P-N061P-G128A-Y217Q, S024G-N025G-S053G-T055P-N061P-S078N-G097A-G128A-Y217Q, S024G-N025G-S053G-T055P-N061P-

S078N-G128A-Y217Q, S024G-N025G-S053G-T055P-S078N-S101N-G128A-Y217Q, S024G-N025G-S053G-T055P-S101N-G128A-Y217Q, S024G-NO25G-TO55P-GO97A-GI28A-Y217Q, S024G-N025G-T055P-G097A-S101N-G128A-Y217Q, S024G-N025G-T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-N061P-G097A-S101N-G128A-Y217Q, S024G-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-S038G-S053G-S078N-S101N-G128A-Y217Q, S024G-S053G-N061P-G097A-G128A-Y217Q, S024G-S053G-N061P-S078N-G097A-G128A-Y217Q, S024G-S053G-S078N-G097A-S101N-G128A-Y217Q, S024G-S053G-S078N-S101N-G128A-Y217Q, S024G-S053G-T055P-G097A-S101N-G128A-Y217Q, S024G-S053G-T055P-N061P-S078N-G097A-G128A-Y217Q, S024G-S053G-T055P-N061P-S101N-G128A-Y217Q, S024G-T055P-G097A-G128A-Y217Q, S024G-T055P-N061P-G097A-G128A-Y217Q, S024G-T055P-N061P-G097A-S101N-G128A, S024G-T055P-S078N-G097A-S101N-G128A-Y217Q, S053G-N061P-G097A-S101N-G128A-Y217Q-S249N, S053G-N061P-S078N-G097A-G128A-Y217Q, S053G-S078N-G097A-S101N-G128A-Y217Q, S053G-T055P-G097A-S101N-G128A-Y217Q, S053G-T055P-N061P-S101N-G128A-Y217Q, S053G-T055P-S078N-G097A-S101N-G128A-Y217Q, S101N-G128A-Y217Q, T055P-G097A-S101N-G128A-Y217Q, and T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0327] The following BPN' variants were determined to have a PI value of about 0.9 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of T055P-N061P-G097A-A116S-G128A and S024G-N025G-TO55P-N061P-S078N-G097A-Y217Q, wherein positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0328] The following BPN' variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v3 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of N025G-T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, N061P-S101N-G128A-Y217Q, S024G-N025G-S053G-N061P-S078N-S101N-G128A-Y217Q, S024G-N025G-S053G-TOSSP-S101N-G128A-Y217Q, S024G-N025G-S053G-T055P-N061P-G128A-Y217Q, S024G-N025G-T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, N025G-S053G-N061P-S078N-G128A-Y217Q,S024G-N025G-S053G-T055P-G097A-S 101N-G128A-Y217Q, S024G-N025G-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-S053G-T055P-G097A-S101N-G128A-Y217Q, S053G-N061P-S078N-G097A-G128A-Y217Q, S024G-N025G-T055P-G097A-G128A-Y217Q, and S024G-N025G-S053G-T055P-G097A-G128A-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0329] The following BPN' variants were determined to have a PI value of about 1.0 relative to BPN'-v3 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of G097A-G128S-Y217Q, G097A-G128A-Y217Q, S024G-G097A-S101N-G128A-Y217Q, N025G-TO55P-N061P-S078N-S101N-G128A-Y217Q, S053G-T055P-N061P-S101N-G128A-Y217Q, S053G-T055P-S078N-G097A-S101N-G128A-Y217Q, N025G-S053G-N061P-S078N-S101N-G128A-Y217Q, S024G-S053G-T055P-N061P-S101N-G128A-Y217Q, S024G-N025G-S053G-T055P-N061P-G097A-S101N-G128A-Y217Q, N025G-S053G-T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-N025G-N061P-S078N-S101N-G128A-Y217Q, S024G-S053G-T055P-N061P-G097A-S 101N-G128A-Y217Q,T055P-N061P-S078N-G128A-Y217Q, N025G-S038G-S053G-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-N025G-S053G-N061P-G128A-Y217Q, S024G-N025G-S053G-T055P-S078N-S101N-G128A-Y217Q, T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-N025G-S053G-TOSSP-N061P-S078N-G128A-Y217Q, S024G-N025G-N061P-G097A-S101N-G128A-Y217Q, S024G-T055P-G097A-G128A-Y217Q, T055P-N061P-G097A-A116S-G128A, S053G-T055P-G097AS101N-G128A-Y217Q, T055P-G097A-S101N-G128A-Y217Q, S024G-N061P-G097A-S101N-G128A-Y217Q, S024G-N025G-N061P-G097A-G128A-Y217Q, S024G-S053G-S078N-S101N-G128A-Y217Q, S024G-N025G-S053G-T055P-N061P-S078N-G097A-G128A-Y217Q, S053G-G097A-S101N-G128A-Y217Q, N025G-T055P-G097A-G128A-Y217Q, S024G-T055P-S078N-G097A-S101N-G128A-Y217Q, N025G-S078N-G097A-S101N-G128A-Y217Q, N025G-G097A-S101N-G128A-Y217Q, S024G-S053G-N061P-S078N-G097A-G128A-Y217Q, S024G-N025G-T055P-N061P-S078N-G097A-Y217Q, and S024G-I035V-T055P-N061P-S078N-G097A-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0330] The following BPN' variants were determined to have a PI value of about 0.9 relative to BPN'-v3 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of S101N-G128A-Y217Q, S024G-T055P-N061P-G097A-S101N-G128A, S024G-N025G-N061P-S078N-G097A-S101N-G128A, S024G-T055P-N061P-S078N-S101N-G128A-Y217Q,

S024G-N025G-S053G-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-S053G-T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, S053G-N061P-G097A-S101N-G128A-Y217Q-S249N, N025G-S053G-T055P-S078N-G097A-S101N-G128A-Y217Q, S024G-N025G-T055P-G097A-S101N-G128A-Y217Q, S024G-S053G-N061P-G097A-G128A-Y217Q, S024G-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-S053G-T055P-N061P-S078N-G097A-G128A-Y217Q, S024G-T055P-N061P-G097A-G128AY217Q, S024G-S038G-S053G-S078N-S101N-G128A-Y217Q, S053G-S078N-G097A-S101N-G128AY217Q, N025G-S078N-G097A-G128A-Y217Q, and S024G-N025G-S053G-N061P-G097A-G128A-S130G-Y217Q, wherein positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0331] The following BPN' variant was determined to have a PI value of about 0.8 relative to BPN'-v3 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising amino acid substitutions S024G-S053G-S078N-G097A-S101N-G 128A-Y217Q, wherein positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0332] The following BPN' variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1 to about 12, from greater than 4 to about 12, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v3 in an AAPF proteolytic assay: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of S024G-G097A-S101N-G128A-Y217Q, S101N-G128A-Y217Q, N025G-T055P-N061P-S078N-S101N-G128A-Y217Q, S053G-T055P-N061P-S101N-G128A-Y217Q, S024G-T055P-N061P-G097A-S101N-G128A, S053G-T055P-S078N-G097A-S101N-G128A-Y217Q, N025G-S053G-N061P-S078N-S101N-G128A-Y217Q, S024G-S053G-T055P-N061P-S101N-G128A-Y217Q, S024G-N025G-S053G-TOSSP-N061P-G097A-S101N-G128A-Y217Q, S024G-N025G-N061P-S078N-G097A-S101N-G128A, N061P-SIOIN-GI28A-Y217Q, S024G-N025G-S053G-N06IP-S078N-SIOIN-G 128A-Y217Q, S024G- T055P-N061P-S078N-S101N-G 128A-Y217Q, N025G-S053G-TOSSP-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-N025G-S053G-T055P-S101N-G128A-Y217Q, N025G-T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-N025G-N061P-S078N-S101N-G128A-Y217Q, S024G-N025G-S053G-T055P-N061P-G128A-Y217Q, S024G-S053G-T055P-N061P-G097A-S101N-G128A-Y217Q, S024G-N025G-S053G-N061P-S078N-G097A-S101N-G128A-Y217Q, T055P-N06IP-S078N-G128A-Y217Q, S024G-S053G-T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, N025G-S038G-S053G-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-N025G-T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-N025G-S053G-N061P-G128A-Y217Q, N025G-S053G-N061P-S078N-G128A-Y217Q, S024G-N025G-S053G-T055P-G097A-S101N-G128A-Y217Q, S024G-N025G-S053 G-T055P-S078N-S101N-G128A-Y217Q, T055P-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-N025G-S053G-T055P-N061P-S078N-G128A-Y217Q, S024G-N025G-N061P-G097A-S101N-G128A-Y217Q, S053G-N061P-G097A-S101N-G128A-Y217Q-S249N, N025G-S053G-T055P-S078N-G097A-S101N-G128A-Y217Q, S024G-T055P-G097A-G128A-Y217Q, T055P-N061P-G097A-A116S-G128A, S024G-N025G-T055P-G097A-S101N-G128A-Y217Q, S024G-N025G-N061P-S078N-G097A-S101N-G128A-Y217Q, S053G-T055P-G097A-S101N-G128A-Y217Q, T055P-G097A-S101N-G128A-Y217Q, S024G-NO61P-G097A-S101N-G128A-Y217Q, S024G-S053G-T055P-G097A-S101N-G128A-Y217Q, G097A-G128S-Y217Q, S024G-S053G-N061P-G097A-G128A-Y217Q, S024G-N025G-N061P-G097A-G128A-Y217Q, S024G-N061P-S078N-G097A-S101N-G128A-Y217Q, S024G-S053G-T055P-NO61P-S078N-G097A-G128A-Y217Q, S024G-S053G-S078N-S101N-G128A-Y217Q, S024G-N025G-S053G-T055P-N061P-S078N-G097A-G128A-Y217Q, S053G-N061P-S078N-G097A-G 128A-Y217Q, S024G-T055P-N061P-G097A-G 128A-Y217Q, S024G-S038G-S053G-S078N-S101N-G128A-Y217Q, S053G-G097A-S101N-G128A-Y217Q, N025G-T055P-G097A-G128AY217Q, S024G-T055P-S078N-G097A-S101N-G128A-Y217Q, S053G-S078N-G097A-S101N-G128A-Y217Q, S024G-N025G-T055P-G097A-G128A-Y217Q, S024G-N025G-S053G-T055P-G097A-G128AY217Q, N025G-S078N-G097A-S101N-G128A-Y217Q, N025G-G097A-S101N-G128A-Y217Q, S024G-S053G-N061P-S078N-G097A-G128A-Y217Q, S024G-S053G-S078N-G097A-S 101N-G128AY217Q, N025G-S078N-G097A-G128A-Y217Q, and S024G-N025G-S053G-N061P-G097A-G128AS130G-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' protease (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0333] The following BPN' variant was determined to have a PI value of about 1.0 relative to BPN'-v3 in an AAPF proteolytic assay: BPN' amino acid sequence (SEQ ID NO:2) comprising amino acid substitutions G097A-G128A-Y217Q, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

EXAMPLE 7

Construction of Site Evaluation Libraries of BPN'-v36 and Cleaning Performance of BPN'-v36 Variants

Construction of the site evaluation libraries of BPN'-v36

[0334] The amino acid sequence of BPN'-v36 is set forth in SEQ ID NO:6 below:
AQSVPYGVSQIKAPALHSQGYTGGNVKVAVIDSGIDSSHPDLKVAGGASMVPGETNPFQDNNSH
GTHVAGTVAALNNNIGVLGVAPSASLYAVKVLGADGNGQYSWIINGIEWAIANNMDVINMSLG
APSGSAALKAAVDKAVASGVVVVAAAGNEGTSGSSSTVGYPGKYPSVIAVGAVDSSNQRASFS
SVGPELDVMAPGVSIQSTLPGNKYGAQNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTT
TKLGDSFYYGKGLINVQAAAQ (SEQ ID NO:6)

[0335] The nucleic acid sequence encoding the BPN'-v36 protease variant is:
Gcgcagtccgtgccttacggcgtatcacaaattaaagcccctgctctgcactctcaaggctacactggaggcaatgttaaagtagcggttatcgacagc
ggtatcgactcgagccatccagatcttaaagtcgctggaggggcttctatggtgcccgggcgaaacaaacccgtttcaagataacaattctcatggcacac
acgtcgcaggaacggttgcggcgttaaacaataatattggcgtgcttggtgtagccccgtctgcttcgctctacgccgttaaagttcttggcgcagacgga
aatggccaatactcatggattatcaacggcatcgaatgggccatcgcgaataacatggatgtaatcaacatgagcctgggagcaccaagcggcagtgc
ggcacttaaagcagcagttgataaagctgttgcatctggtgtcgtcgtagtagcggcagctgggaatgagggaacatccggatcatcgagtaccgtcgg
ttatccaggcaagtacccttcagtgattgcagtgggcgctgtagactcttcaaatcaacgtgcctctttttcctccgtgggaccggagctggatgtcatggc
ccctggcgtttctattcaatcgacgcttccagggaacaagtatggtgcgcaaaacgggacttccatggcctcgccgcatgtagctggggcggccgcatt
gattctttctaagcacccgaactggacaaacactcaagtccgcagcagtttagaaaacaccactacaaaacttggtgattctttctactatggaaaagggct
gatcaacgtacaggcggcagctcag (SEQ ID NO:5)

[0336] The amino acid sequence of BPN'-v36 may be represented by reference to the subtilisin BPN' amino acid sequence of SEQ ID NO:2. That is, BPN'-v36 may be represented as the subtilisin BPN' sequence of SEQ ID NO:2 with the six amino acid substitutions S024G-S053G-S078N-S101N-G128A-Y217Q. The BPN'-v36 amino acid sequence may be conveniently designated as BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q or BPN'+S024G+S053G+S078N+S101N+G128A+Y217Q. Throughout this specification, unless otherwise indicated, each amino acid position of an amino acid sequence is numbered according to the numbering of a corresponding amino acid position in the amino acid sequence of *Bacillus amyloliquefaciens* subtilisin BPN' shown in SEQ ID NO:2 as determined by alignment of the variant amino acid sequence with the *Bacillus amyloliquefaciens* subtilisin BPN' amino acid sequence.

[0337] Site evaluation libraries (SELs) were created at every single amino acid position in mature BPN'-v36 (i.e., BPN'-S24G-S53G-S78N-S101N-G128A-Y217Q) protein by PCR fusion.

[0338] For each codon to be mutated in the BPN'-v36 protease, a pair of partially overlapping, complementary (mutagenic forward and reverse) primers were designed. Each mutagenic primer contained the NNS (N=A,C,G, or T and S=G or C) mutagenic codon in the center flanked by at least 15 nucleotides on each side. To create a library at a given position, two PCR reactions were carried out using either a common forward gene-flanking primer (P4974, GCCTCACATTTGTGCCACCTA; SEQ ID NO:60) and a mutagenic NNS reverse primer, or the common reverse gene-flanking primer (P4976, CCTCTCGGTTATGAGTTAGTTC; SEQ ID NO:61) and a mutagenic NNS forward primer. These PCR reactions generated two PCR fragments, one encoding the 5' half of the mutant BPN'-v36 gene (5' gene fragment) and the other encoding the 3' half of the mutant BPN'-v36 gene (3' gene fragment).

[0339] Each PCR amplification reaction contained 30 pmol of each primer and 100 ng of the BPN'-v36 parent template DNA (plasmid pHPLT-BPN'-v36, see Figure 4). Amplifications were carried out using Vent DNA polymerase (NEB). The PCR reaction (20 µL) was initially heated at 95°C for 2.5 min followed by 30 cycles of denaturation at 94°C for 15 sec., annealing at 55°C for 15 sec. and extension at 72°C for 40 sec. Following amplification, the 5' and 3' gene fragments were gel-purified by the QIAGEN® gel-band purification kit, mixed (50 ng of each fragment), mixed and amplified by PCR once again using the primers P4973 (AAAGGATCCTAATCGGCGCTTTTC; SEQ ID NO:62) and P4950 (CTTGTCTCCAAGCTTAAAATAAAA; SEQ ID NO:63) to generate the full-length gene fragment. The PCR conditions were same as described above, except the extension phase, which was carried out at 72°C for 2 min. The full-length DNA fragment was gel-purified by the QIAGEN® gel-band purification kit, digested by the *Bam*HI and *Hind*III restriction enzymes and ligated with the pHPLT-BPN' partial opt vector that also was digested with the same restriction enzymes. Ligation mixtures were amplified using rolling circle amplification in an Illustra Templiphi kit according to the manufacturer's recommendation (GE Healthcare) to generate multimeric DNA for transformation into *Bacillus subtilis*. For this purpose, µl of the ligation mixture was mixed with 5 µl of the sample buffer, heated to 95°C for 3 min and cooled on ice. Next, 5 µl of the reaction buffer and 0.2 µl of the enzyme were added to each tube, followed by incubation at 30°C for 10

hours. Products of the rolling circle amplification were diluted 100 times and used to transform *B. subtilis* cells (ΔaprE, ΔnprE, amyE::xylRPxylAcomK-phleo). An aliquot of the transformation mix was plated on LB plates containing 1.6% skim milk and 10 µg/mL neomycin and incubated overnight at 37°C. Subsequently, the colonies with halos were inoculated in 150 µl of LB media containing 10 µg/mL neomycin. The next day, cultures were either frozen with 15% glycerol or grown in MBD medium for biochemical analysis as described in Example 2.

Cleaning Performance of the BPN'-v36 Variants

[0340] Protein variants from BPN'-v36 SEL were tested for cleaning performance using a BMI microswatch assay in Detergent Composition 4 at 16°C and pH 8 and egg microswatch assay in Detergent Composition 4 at 16°C and pH 8. Protein content was determined using the TCA assay. The assays were performed as described in Example 1 and the Performance Indices were calculated relative to BPN'-v36 (i.e., BPN'-S24G-S53G-S78N-S101N-G128A-Y217Q) (with a PI value of 1.0).

[0341] The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q (SEQ ID NO:6) (i.e., BPN'-v36) comprising at least one amino acid substitution selected from the group consisting of A116V, G160S, I111L, I115V, N109S, N117M, P005G, Q059V, T164S, Y262M, A015Q, A015S, A098E, A098N, A098S, A098T, A098V, A098Y, A114S, A114T, A116G, A116L, A116S, A116T, A116W, A133G, A133H, A133T, A133V, A137G, A137I, A137L, A137S, A137T, A138S, A216E, A216F, A216V, D099S, D181E, F261A, F261Q, G024F, G024I, G024Q, G024Y, G097S, G160T, G211L, G211V, H017F, H017W, H039V, H226A, I031V, I111V, I268V, K170R, K265R, L016Q, L016T, L135M, L209T, L209V, L233M, L257T, L257V, L267A, L267V, N025A, N025I, N025Q, N025R, N025T, N025V, N101I, N101Q, N101S, N109A, N109G, N109H, N109L, N109M, N109Q, N109T, N117Q, N184A, N184L, N184T, N184W, N212G, N212L, N212V, N243P, N252G, N252M, P005T, P014S, P040G, P040L, P040Q, P129A, P129S, P172G, P172S, P194Q, P210A, P210S, Q185F, Q185G, Q185I, Q185M, Q185N, Q185S, Q275H, R186K, S009A, S009G, S009H, S009M, S018T, S130T, S132N, S145K, S159T, S161I, S161K, S161N, S161T, S162I, S162M, S162Y, S163G, S182F, S182G, S182V, S182W, S183F, S183L, S 183M, S183T, S183V, S183W, S224A, S236T, S249V, T022A, T022G, T022Q, T022V, T208V, T242S, T253N, T253S, T254A, T254S, T255L, T255S, T255V, V004A, V004P, V004W, V084C, V139C, V165M, V203F, Y021K, Y021N, Y021T, Y021V, Y167F, Y171F, Y214F, Y262F, and Y262T, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0342] The following BPN'-v36 variants were determined to have a PI value of about 1.0 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one amino acid substitution selected from the group consisting of A001G, A001Y, A013G, A013V, A015F, A015G, A015K, A015M, A015P, A015T, A015W, A015Y, A029G, A073S, A088C, A088I, A088L, A088T, A088V, A098D, A098K, A098P, A098R, A098W, A116D, A116E, A116R, A128S, A133L, A133M, A133S, A134G, A134S, A137N, A137V, A144M, A144Q, A144S, A144T, A144V, A151C, A176S, A176T, A179S, A216G, A216L, A216P, A216Q, A216S, A216T, A216Y, A228T, A230C, A231C, A272I, A272L, A272Q, A272S, A272T, A272W, A273S, A274G, A274L, A274Q, A274T, A274V, D041E, D099G, D099N, D120A, D120K, D120Q, D120R, D120S, D140E, D181S, D259E, E054D, E156D, E156T, E251V, F261G, F261H, F261L, F261R, F261S, F261T, F261V, F261W, G007A, G007S, G020A, G020D, G020S, G024N, G024R, G024S, G024T, G024V, G024W, G053H, G053K, G053N, G053T, G097A, G097D, G097T, G131A, G131H, G131P, G131Q, G131T, G131V, G160H, G160P, G166A, G166S, G166T, G211A, G211D, G211M, G211N, G211P, G211Q, G211R, G211W, G215A, G215N, G215V, H017L, H017M, H017T, H017V, H017Y, H039A, H039C, H039N, H226F, H226I, H226M, H226S, H226V, H226Y, I035V, I079A, I079S, I079T, I079V, I079W, I108V, I115L, I122A, I234L, I234V, K012R, K027R, K136R, K141F, K213W, K237R, K256R, K265Q, L016A, L016F, L016I, L016S, L016V, L042I, L075A, L075M, L075Q, L075V, L075Y, L082K, L082M, L082Q, L082V, L090I, L196I, L196V, L209Q, L209W, L233A, L233Q, L233V, L235I, L235K, L250I, L257A, L257H, L257Q, L257S, L257Y, L267Q, L267R, L267S, L267T, M199V, N025F, N025G, N025H, N025K, N025L, N025M, N025S, N025Y, N061F, N061H, N061P, N061S, N061T, N061V, N061W, N076G, N076W, N078S, N078T, N078V, N101A, N101H, N101L, N101T, N109K, N109R, N117A, N117E, N117H, N117K, N118G, N184G, N184H, N184I, N184S, N184V, N212A, N212F, N212I, N212K, N212P, N212Q, N212S, N212Y, N218A, N218H, N218L, N218S, N240E, N240H, N240L, N240R, N240T, N243A, N243Q, N243T, N243V, N252A, N252K, N252L, N252Q, N252R, N252S, N252T, N269Q, N269S, P014G, P014Q, P014T, P040A, P040H, P040S, P040T, P040V, P040Y, P086A, P086C, P086F, P086H, P086S, P129D, P129G, P129K, P129T, P172A, P172Q, P194A, P194G, P194L, P194M, P194S, P194V, P194Y, P210G, P210R, P210V, Q002A, Q002S, Q010A, Q010F, Q010H, Q010I, Q010L, Q010N, Q010S, Q010T, Q019A, Q019G, Q019N, Q019S, Q019T, Q019V, Q019W, Q059I, Q103L, Q103S, Q185A, Q185H, Q185L, Q185T, Q185Y, Q206P, Q206S, Q206Y, Q217I, Q217N, Q217S, Q217T, Q245K, Q275D, Q275S, Q275W, S003A, S003G,

S003H, S003M, S003P, S003Q, S003T, S003V, S009I, S009L, S009P, S009T, S009W, S018A, S018G, S018I, S018L, S018M, S018N, S018P, S018V, S018W, S033T, S037Q, S037T, S037V, S038G, S038H, S038K, S038Q, S038T, S063K, S063N, S063Q, S063T, S087A, S087F, S087G, S087Q, S087T, S089L, S089M, S089N, S089Q, S089T, S089W, S130A, S130F, S130G, S130L, S130V, S145A, S145H, S145M, S145V, S159A, S159G, S159H, S159Q, S159R, S161A, S161G, S161H, S161L, S161M, S161P, S161Q, S161W, S162A, S162F, S162G, S162L, S162N, S162P, S162R, S162V, S163P, S173A, S173G, S182A, S182H, S182K, S182L, S182N, S182P, S182Q, S182T, S183A, S183G, S183H, S183Q, S188A, S188G, S188T, S188V, S191A, S204A, S204I, S204L, S204Q, S204V, S224C, S236A, S236N, S236Q, S248A, S248F, S248G, S248I, S248K, S248L, S248M, S248N, S248Q, S248T, S248V, S249A, S249C, S249H, S249Q, S249T, S249W, S249Y, S260H, S260N, S260P, S260T, T022H, T022K, T022N, T022R, T022S, T022Y, T055A, T055G, T055L, T055N, T055P, T055Q, T071S, T158H, T158S, T164N, T208C, T208L, T220S, T242N, T244A, T244G, T244H, T244I, T244Q, T244S, T244V, T244W, T253A, T253G, T253H, T253Q, T254V, T255A, T255G, T255H, T255I, T255Q, T255Y, V004G, V004N, V004R, V008A, V008C, V008M, V026I, V044I, V044L, V045H, V045K, V045L, V045M, V045Q, V045S, V045V, V045W, V045Y, V051I, V081L, V081Q, V081T, V084A, V084S, V084T, V093I, V121I, V143N, V143S, V143Y, V147C, V147I, V147L, V147T, V180I, V180L, V180T, V192A, V192S, V192T, V198I, V198L, V198M, V203H, V203I, V203L, V203N, V203Q, V203T, V203W, V203Y, V270A, V270S, V270T, W241M, W241Y, Y006G, Y006H, Y006I, Y006K, Y006L, Y006P, Y006Q, Y006T, Y006V, Y006W, Y021A, Y021D, Y021E, Y021L, Y021Q, Y021R, Y021S, Y104F, Y104I, Y214L, Y214V, Y214W, Y262A, Y262G, Y262L, Y262N, Y262S, Y262W, Y263G, and Y263W, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0343] The following BPN'-v36 variants were determined to have a PI value of about 0.9 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one amino acid substitution selected from the group consisting of A001F, A001K, A001L, A001M, A001Q, A001R, A001S, A001T, A001V, A013C, A013S, A015D, A015E, A015L, A015R, A048S, A073N, A073T, A074G, A074S, A085C, A085G, A085S, A085V, A088M, A088S, A092S, A098G, A114G, A133P, A137E, A137H, A144G, A144H, A144K, A144L, A144N, A153S, A153V, A176C, A179G, A187G, A187S, A200G, A216W, A223S, A228S, A230T, A230V, A231V, A232C, A232V, A272E, A272G, A272K, A272P, A273G, A273L, A273V, A274M, A274R, D036E, D099A, D099Q, D120E, D181A, D181G, D259A, D259G, D259Q, D259T, E156A, E156S, E251I, E251L, E251Q, E251T, F058Y, F261C, F261D, F261K, F261P, G020E, G020F, G020H, G020L, G020N, G020Q, G020R, G020T, G020Y, G024A, G024P, G053A, G053D, G053E, G053F, G053L, G053Q, G053S, G053Y, G097K, G097M, G157A, G157S, G160A, G160L, G166C, G166I, G166Q, G169A, G211K, G215H, G215L, G215S, G215T, G215W, G258S, H017I, H039S, H226L, H238N, H238Y, I011L, I011V, I031L, I079F, I079K, I079L, I079M, I079Q, I205A, I205V, I268L, I268M, K012G, K043F, K043H, K043I, K043N, K043Q, K043T, K141A, K141R, K141W, K170A, K213A, K213G, K213H, K213I, K213L, K213N, K213Q, K213R, K213S, K213T, K213V, K237A, K237H, K237I, K237L, K237N, K237S, K256A, K256G, K256H, K256M, K256P, K256Q, K256W, K265H, L016E, L042V, L075G, L075H, L075I, L075T, L082A, L082F, L082H, L082R, L082S, L082T, L090M, L135F, L196M, L209C, L209H, L209S, L233S, L235M, L235R, L235W, L257C, L257G, L267F, M050Y, M119C, M119I, M124L, N025C, N025E, N025P, N061A, N061G, N061I, N061K, N061L, N061Q, N061R, N062S, N062T, N076A, N076P, N076Q, N076S, N076T, N076V, N078G, N078H, N078K, N078P, N078Q, N078R, N101F, N117R, N117S, N118D, N118H, N118Q, N118R, N118S, N118T, N184C, N184E, N184R, N212D, N212R, N212W, N218F, N218G, N218M, N218P, N218T, N218V, N218W, N240A, N240G, N240Q, N240S, N240W, N243C, N243G, N243S, N252V, N269H, P005A, P005D, P005M, P005Q, P014A, P014M, P014R, P014V, P040F, P040R, P040W, P129E, P129R, P172E, P172K, P194H, P194R, P194W, P201A, P201G, P210L, P239K, P239R, Q002D, Q002E, Q002G, Q002I, Q002P, Q002V, Q010D, Q010R, Q019C, Q019D, Q019E, Q019H, Q019L, Q019P, Q019R, Q059A, Q059E, Q059L, Q059S, Q059T, Q103W, Q185D, Q185K, Q185R, Q185W, Q206G, Q206H, Q206L, Q206V, Q206W, Q217E, Q217F, Q217H, Q217L, Q217V, Q245M, Q271A, Q271D, Q271G, Q271L, Q271P, Q271T, Q271Y, Q275F, Q275L, Q275P, Q275R, S003D, S003F, S003K, S003R, S009K, S018D, S018R, S037A, S037G, S037K, S037L, S037P, S038M, S063A, S063F, S063G, S063M, S063R, S063Y, S087C, S087K, S087L, S087M, S087N, S087Y, S089A, S089D, S089F, S089G, S089H, S089I, S089K, S089R, S089V, S089Y, S130D, S130E, S130K, S130W, S145G, S145L, S145R, S145T, S159D, S159L, S159W, S161E, S161R, S162C, S162E, S162W, S163A, S182E, S182R, S183C, S183D, S183P, S183R, S188D, S188P, S204G, S204Y, S207G, S224G, S224T, S236C, S236G, S248D, S248H, S248R, S249E, S249L, S249R, S260A, S260G, S260K, S260Q, S260V, S260Y, T022L, T055D, T055E, T055I, T055K, T055M, T055S, T055V, T055Y, T158A, T158G, T158L, T158Q, T158V, T164K, T164Q, T208S, T244D, T244E, T244R, T253E, T253R, T253Y, T254G, T255D, T255E, T255K, T255R, V026A, V028I, V028L, V030I, V044C, V044P, V045E, V045G, V045N, V072L, V081A, V081G, V081H, V081S, V084I, V084M, V095A, V095C, V143A, V143F, V143H, V143Q, V143T, V143W, V147A, V147Q, V147S, V148I, V148L, V149C, V149I, V149L, V165L, V180A, V180C, V180M, V192C, V192F, V192I, V192Q, V192Y, V203A, V203G, V203K, V203S, V270C, V270L, V270P, W241F, Y006A, Y006M, Y006N, Y006R, Y006S, Y021C, Y091W, Y104V, Y104W, Y262C, Y262D, Y262E, Y262H, Y262I, Y262R, and Y262V, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants may have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and/or a greater PI value than that of BPN' in this assay.

[0344] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.5 and less than 0.9 relative

to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S24G-S53G-S78N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one amino acid substitution selected from the group consisting of A001D, A001H, A001N, A015C, A048C, A048E, A085T, A133R, A137R, A142C, A144D, A144R, A152S, A153G, A187P, A187Q, A187T, A187V, A216R, A230S, A272R, A273H, A273T, A274H, D036N, D036S, D181H, D181T, D259N, D259P, D259S, E156G, E156H, E156L, E156Q, E156V, E251C, F189S, F189T, F189W, F189Y, F261E, G020C, G024D, G053M, G053R, G097R, G131D, G131R, G157N, G160R, G160V, G166L, G166W, G211E, G215D, G258A, G258D, G258P, I011T, I031C, I079E, I079R, I175L, I205C, K012H, K012N, K027A, K027N, K027S, K043A, K043D, K043E, K043G, K043L, K043M, K043P, K043V, K043W, K043Y, K136H, K141H, K141L, K141M, K141N, K141Q, K141T, K141V, K170G, K170S, K237T, K237V, K256D, K256S, K256T, K256V, K265N, K265S, L042F, L042M, L082E, L209A, L209E, L209G, L209R, L233G, L235V, L257D, L257E, L257P, L257R, L257W, L267E, M050L, N056D, N056S, N061C, N061D, N062A, N062H, N062L, N062V, N062Y, N076D, N076L, N076M, N078D, N078F, N101D, N101R, N118A, N212C, N212E, N218C, N218D, N218E, N252D, N252E, P014F, P014K, P057A, P057W, P172R, P194E, P201T, P210E, Q059C, Q059D, Q059R, Q185E, Q206D, Q217A, Q217K, Q217R, Q245A, Q245D, Q245E, Q245H, Q245R, Q271E, Q271F, Q271R, Q271W, Q275G, Q275I, R186I, R186L, R186V, R186W, S003E, S009C, S009E, S018C, S037D, S037E, S037H, S037R, S037Y, S038D, S038P, S038R, S038Y, S063L, S087D, S087R, S089C, S089E, S130C, S130R, S145D, S159C, S159P, S161C, S173T, S182C, S188E, S188F, S188K, S188L, S188R, S188W, S190A, S190G, S190T, S204R, S236D, S236E, S248C, S248E, S260C, S260E, S260R, T022P, T055C, T055W, T071A, T158D, T158E, T158P, T158R, T158Y, T164R, T242D, T242G, T255C, V004E, V004T, V045C, V045D, V045R, V045T, V051H, V081R, V143C, V143E, V143G, V192G, V203C, V203D, V203E, V203M, V203R, V270G, W241L, Y214H, Y214Q, A001E, A133E, A187L, A187N, A216C, A216H, A273Q, D099H, D259H, E156C, E195G, F189H, G131C, G146A, G166V, G215C, G215E, I107L, K012A, K012S, K012T, K043C, K170C, K256C, K256E, K265G, K265Y, L233E, M222F, M222S, N062Q, N076E, N078E, N184P, N218R, P005V, P014D, Q002K, Q002L, Q002R, Q010W, Q271C, R186H, S049C, S063C, S063D, S105T, S188C, S190C, S204E, T055R, T164G, V004D, V044T, V045I, V165C, V180S, Y006C, Y006D, Y006E, Y104T, A001C, A187C, A230G, A273D, A273P, D036Q, F189G, F189L, F189R, G157T, G178A, I031F, I111M, K012F, K012L, K027T, K043R, K136G, K141G, K170Q, M222A, M222L, N062R, N117G, N269C, P005W, P129V, P239A, P239H, P239T, Q059W, Q217G, Q275A, R186A, S191G, T164A, T220A, A001P, A187F, A187W, A273R, D041C, D060G, D197T, F189A, G046D, G157P, K012C, K012E, K012W, L042C, M222T, N062C, P239G, P239N, Q217C, R186M, S049T, S089P, S125A, S173V, and V044A, wherein amino acid positions of the variant are numbered by correspondence with amino acid positions of the SEQ ID NO:2 sequence.

[0345] The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at 16°C and pH 8: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one amino acid substitution selected from the group consisting of A216E, L090I, A098R, A098W, A098Y, A116G, A116R, A116S, A133M, I107L, I115V, M124L, N101I, N109H, N109S, N109T, N117R, P005G, Q185L, S089V, V095A, A015Y, A029G, A098D, A098E, A098G, A098N, A098S, A098T, A098V, A114S, A114T, A116E, A116L, A116T, A116V, A133H, A133L, A133S, A137G, A137I, A137L, A137S, A137V, A138S, A144S, A144V, A176S, A176T, A187T, A216F, A216P, A216Q, A216R, A216S, A216T, A216V, A216Y, D041E, D120A, D120E, D120Q, D120R, D120S, D181S, G020A, G020S, G024A, G097A, G097D, G097S, G131Q, G160S, G166I, G211L, G215N, H039N, H238N, I111L, I111V, I122A, L075I, L075Q, L135M, L209T, L209V, L233V, L235M, L235R, L257A, M119I, N025A, N025G, N025T, N061K, N101F, N101H, N101L, N101Q, N101R, N101S, N101T, N109A, N109G, N109K, N109L, N117E, N117H, N117K, N117S, N212G, N212S, N218F, N218G, N218H, N218L, N218S, N218W, N240Q, N252M, N252R, N252S, P005T, P040A, P040G, P040T, P129D, P129S, P194S, P210R, Q019R, Q019W, Q103L, Q103W, Q185A, Q185G, Q185M, Q185R, Q185T, Q206G, Q206Y, Q217A, Q217E, Q217R, Q217S, Q217T, S003Q, S009H, S018M, S033T, S130A, S130F, S130G, S130T, S130V, S145T, S159A, S161N, S161T, S162V, S162Y, S182L, S182W, S183F, S183L, S183V, S183W, S188K, S188W, S236Q, S236T, S248L, T022H, T022K, T208C, T253H, T255V, V044I, V121I, V139C, V143H, V143Q, V143T, V143W, V143Y, Y006K, Y021A, Y104W, A001F, A001G, A001H, A001K, A001L, A001Q, A001S, A001Y, A013V, A015G, A015K, A015R, A015S, A015T, A015W, A048S, A073N, A073S, A092S, A098K, A098P, A116D, A116W, A128S, A133P, A133T, A133V, A134G, A134S, A137H, A137N, A137T, A144D, A144K, A144L, A144M, A144N, A144R, A179G, A179S, A187V, A216G, A216L, A216W, A223S, A230C, A272K, A272L, A272P, A272S, A272T, A272W, A273G, A273S, A274G, A274M, A274T, D120K, D140E, D181A, D181E, D181G, D181H, D181T, D259E, D259N, D259Q, E054D, E156D, E156T, E251L, E251T, E251V, F058Y, F189W, F261K, F261Q, F261R, G007A, G007S, G020F, G020H, G020N, G020Q, G020T, G020Y, G024F, G024Q, G024R, G024T, G024V, G024W, G024Y, G053T, G097K, G097M, G097R, G097T, G131A, G131H, G131P, G131R, G131T, G131V, G160H, G160T, G166C, G166Q, G166S, G166T, G211A, G211D, G211K, G211M, G211N, G211Q, G211R, G211V, G211W, G215S, G215T, G215W, H017T, H017W, H017Y, H039V, H226A, H226F, H226I, H226L, H226M, H226V, I035V, I079A, I079S, I108V, I205V, I234L, I234V, I268V, K012S, K043P, K136H, K136R, K141A, K141F, K141T, K141W, K170A, K170G, K170R, K213A, K213R, K213S, K237A, K237H, K237L, K237S, K237V, K256A, K256G, K256H, K256M, K256P, K256Q, K256R, L016A, L016Q, L016T, L016V, L042V, L075M, L075T, L082M, L082V, L135F, L196I, L209H, L209Q, L209R, L209S, L209W, L233A, L233M, L233Q, L235I, L235K, L250I, L257S, L257T, L257V, L267A, L267Q, L267T, L267V,

M119C, M199V, N025C, N025E, N025F, N025I, N025K, N025L, N025M, N025Q, N025V, N025Y, N061F, N061P, N061S, N061T, N076G, N078S, N101A, N109M, N109Q, N109R, N117A, N117M, N117Q, N118D, N118G, N118H, N118Q, N118R, N118S, N184A, N184C, N184G, N184L, N184R, N1845, N184T, N184V, N184W, N212C, N212F, N212I, N212K, N212L, N212P, N212Q, N212R, N212V, N212W, N212Y, N218A, N218P, N218T, N240A, N240E, N240G, N240H, N240L, N240R, N240S, N240T, N243C, N243Q, N243T, N243V, N252A, N252G, N252K, N252Q, P014G, P014Q, P014R, P014S, P014T, P040F, P040L, P040Q, P040S, P040V, P086C, P086H, P086S, P129A, P129E, P129G, P129K, P129R, P172A, P172K, P172Q, P172S, P194A, P194G, P194H, P194L, P194M, P194Q, P194R, P194V, P194W, P194Y, P210A, P210G, P210L, P210S, P239K, P239R, Q002A, Q002S, Q010A, Q010N, Q010R, Q010T, Q019A, Q019C, Q019D, Q019G, Q019S, Q019T, Q019V, Q059I, Q059V, Q103S, Q185F, Q185H, Q185I, Q185K, Q185N, Q185S, Q185Y, Q206H, Q206L, Q206P, Q206W, Q217F, Q217H, Q217I, Q217K, Q217L, Q217N, Q217V, Q271G, Q271R, Q271T, Q275F, Q275P, Q275R, R186A, R186I, R186K, S003A, S003F, S003G, S003H, S003K, S003R, S003T, S009T, S018N, S018T, S037G, S037T, S037V, S038G, S038Q, S063N, S063Q, S063T, S089M, S089N, S130K, S130L, S130R, S130W, S132N, S145G, S145K, S145M, S145R, S145V, S159C, S159H, S159L, S159Q, S159R, S159T, S159W, S161A, S161C, S161G, S161H, S161I, S161K, S161P, S161Q, S161R, S162F, S162G, S162I, S162L, S162M, S162N, S162P, S162R, S163G, S173A, S173G, S182F, S182G, S182K, S182N, S182Q, S182V, S183A, S183M, S183Q, S183R, S183T, S188A, S188F, S188G, S188P, S188R, S188T, S188V, S190C, S204A, S204G, S204I, S204L, S204Q, S204R, S204V, S207G, S224A, S224T, S236C, S236D, S236E, S236G, S236N, S248A, S248F, S248K, S248M, S248T, S249A, S249R, S249T, S249V, S249W, S249Y, S260G, S260H, S260K, S260N, T022A, T022G, T022Q, T022S, T022V, T022Y, T055A, T055K, T158A, T158S, T208L, T208S, T208V, T220S, T242D, T242N, T242S, T244E, T244G, T244I, T244R, T244V, T244W, T253A, T253G, T253N, T253S, T254S, T254V, T255H, T255I, T255K, T255L, T255Q, T255R, T255S, T255Y, V004A, V004N, V004P, V004W, V008A, V008M, V026I, V045S, V045W, V051I, V081Q, V081T, V084C, V093I, V095C, V143A, V143E, V143F, V143N, V143S, V147I, V147L, V147S, V147T, V148I, V149C, V149I, V165M, V180A, V180C, V180I, V180L, V180T, V192A, V192C, V192I, V1925, V192T, V192Y, V198L, V203A, V203F, V203K, V203L, V203M, V203N, V203Y, W241Y, Y006A, Y006G, Y006H, Y006L, Y006N, Y006P, Y006Q, Y006T, Y021E, Y021K, Y021L, Y021N, Y021Q, Y021R, Y021S, Y021T, Y104F, Y104I, Y104V, Y171F, Y214F, Y214L, Y214W, Y262F, and Y262S, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0346] The following BPN'-v36 variants were determined to have a PI value of about 1.0 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one amino acid substitution selected from the group consisting of A001D, A001M, A001N, A001R, A001T, A001V, A013C, A013G, A013S, A015D, A015F, A015L, A015M, A015P, A015Q, A074G, A074S, A085S, A085T, A085V, A088C, A088L, A088S, A088T, A088V, A133E, A133G, A133R, A137E, A144G, A144H, A144Q, A144T, A151C, A152S, A153G, A153S, A153V, A176C, A187G, A187Q, A187S, A200G, A228S, A228T, A230T, A230V, A231C, A231V, A232C, A232V, A272E, A272G, A272I, A272Q, A272R, A273L, A273V, A274L, A274Q, A274R, A274V, D036E, D259A, D259G, D259P, D259S, D259T, E156A, E156S, E156V, E195G, E251C, E251I, E251Q, F189Y, F261A, F261G, F261H, F261L, F261P, F261S, F261T, F261V, F261W, G020C, G020D, G020E, G020L, G020R, G024D, G024I, G024N, G024P, G024S, G053A, G053D, G053F, G053H, G053K, G053N, G053S, G053Y, G157A, G157S, G160A, G160L, G160P, G160R, G166A, G166L, G166V, G166W, G169A, G211E, G211P, G215A, G215D, G215E, G215H, G215V, G258D, G258S, H017F, H017I, H017L, H017M, H017V, H039C, H226S, H226Y, 101IT, 101IV, I031C, I031L, I031V, I079F, I079K, I079L, I079M, I079Q, I079R, I079T, 1079V, I079W, I115L, I205A, I205C, I268L, K012R, K012T, K027R, K043A, K043E, K043F, K043H, K043I, K043M, K043N, IC043Q, K043T, K043V, K043Y, K141H, K141Q, K141V, K170S, K213G, K213H, K213I, K213L, K213N, K213Q, K213T, K213W, K237I, K237N, K237R, K237T, K256C, K256E, K256S, K256T, K256V, K256W, K265H, K265R, L016E, L016F, L016I, L016S, L042I, L042M, L075A, L075H, L075Y, L082K, L082Q, L082T, L196M, L196V, L209A, L209C, L209E, L209G, L235W, L257C, L257H, L257Q, L257Y, L267E, L267F, L267R, L267S, M050Y, N025H, N025P, N025S, N056D, N061A, N061C, N061D, N061G, N061H, N061I, N061L, N061Q, N061R, N061V, N061W, N062A, N062S, N062V, N076A, N076E, N076L, N076M, N076Q, N076S, N076T, N076W, N078G, N078H, N078K, N078P, N078Q, N078T, N101D, N118A, N118T, N184E, N184H, N184I, N212A, N212D, N212E, N218C, N218D, N218E, N218M, N218R, N218V, N240W, N243A, N243G, N243P, N243S, N252D, N252E, N252L, N252T, N252V, N269S, P005A, P005D, P005M, P005Q, P014A, P014D, P014F, P014M, P014V, P040H, P040R, P040W, P040Y, P086A, P129T, P172E, P172G, P172R, P194E, P201A, P201G, P210E, P210V, Q002E, Q002G, Q010D, Q010F, Q010H, Q010I, Q010L, Q010S, Q019E, Q019H, Q019N, Q059A, Q059L, Q059R, Q059S, Q059T, Q185D, Q185E, Q206D, Q206S, Q206V, Q217G, Q245D, Q245E, Q245K, Q245M, Q245R, Q271A, Q271F, Q271P, Q271Y, Q275D, Q275H, Q275I, Q275L, Q275S, Q275W, R186H, R186L, R186V, R186W, S003D, S003E, S003M, S003P, S003V, S009A, S009E, S009G, S009I, S009K, S009M, S009P, S009W, S018A, S018G, S018I, S018L, S018P, S018R, S018V, S018W, S037A, S037D, S037Q, S037R, S037Y, S038D, S038H, S038K, S038M, S038R, S038T, S063A, S063G, S063K, S063M, S063R, S087A, S087D, S087F, S087G, S087Q, S087T, S089A, S089C, S089H, S089I, S089K, S089L, S089Q, S089R, S089T, S089Y, S130D, S130E, S145A, S145H, S145L, S159G, S161E, S161L, S161M, S161W, S162A, S162C, S162E, S162W, S163P, S173T, S182A, S182C, S182E, S182H, S182R, S182T, S183C, S183D, S183G, S183H, S188C, S188D, S188E, S188L, S190T, S191A, S204Y, S224C, S236A, S248C, S248D, S248G, S248I, S248N, S248Q, S248R, S248V, S249C, S249H, S249L, S249Q, S260A, S260C, S260E, S260P, S260Q, S260R, S260T, T022L,

T022N, T022R, T055C, T055D, T055G, T055I, T055L, T055N, T055Q, T055S, T055V, T055Y, T071A, T071S, T158G, T158H, T158L, T158P, T158Q, T158R, T158V, T158Y, T164N, T164S, T244A, T244D, T244H, T244Q, T244S, T253Q, T253R, T253Y, T254A, T254G, T255A, T255D, T255E, V004E, V004G, V004R, V008C, V026A, V028L, V030I, V044C, V045D, V045E, V045H, V045M, V045Q, V045Y, V072L, V081L, V081S, V084A, V084I, V084M, V084T, V143C, V147C, V147Q, V149L, V165L, V180M, V192F, V192G, V192Q, V198I, V198M, V203C, V203E, V203H, V203I, V203Q, V203R, V203T, V203W, V270A, V270L, V270T, W241F, W241M, Y006C, Y006D, Y006E, Y006I, Y006M, Y006R, Y006S, Y006V, Y006W, Y021C, Y021D, Y021V, Y091W, Y167F, Y214V, Y262A, Y262C, Y262I, Y262M, Y262R, Y262T, Y262V, and Y262W, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0347] The following BPN'-v36 variants were determined to have a PI value of about 0.9 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one amino acid substitution selected from the group consisting of A001E, A015C, A015E, A048C, A048E, A073T, A085C, A085G, A088I, A088M, A114G, A137R, A187L, A187N, A187P, A187W, A216C, A230S, A273D, A273H, A273T, D036N, D099N, D259H, E156C, E156G, E156H, E156Q, F189H, F189R, F189S, F189T, F261C, F261D, F261E, G053E, G053M, G053Q, G131C, G131D, G157N, G160V, G215C, G215L, G258A, H039A, I011 L, I079E, I268M, K012A, K012G, K012H, K012N, K027N, K043C, K043D, K043G, K043L, K043W, K136G, K141G, K141L, K141M, K141N, K141R, K170C, K170Q, K213V, K256D, K265G, K265N, K265Q, K265S, L082A, L082F, L082H, L082R, L082S, L090M, L233S, L235V, L257E, L257G, L257R, L257W, M222S, N025R, N062H, N062T, N076D, N076P, N078D, N078E, N078F, N078R, N078V, N269H, N269Q, P014K, P057A, P086F, P201T, Q002D, Q002I, Q002P, Q002V, Q019L, Q019P, Q059C, Q059D, Q059E, Q185W, Q271C, Q271D, Q271E, Q271L, Q271W, Q275G, R186M, S009C, S009L, S018D, S037E, S037H, S037K, S037L, S037P, S038P, S063C, S063D, S063F, S063L, S063Y, S087L, S087N, S087R, S087Y, S089D, S089F, S089G, S089W, S105T, S125A, S130C, S159D, S159P, S163A, S182P, S183P, S190A, S190G, S204E, S224G, S248E, S248H, S249E, S260V, S260Y, T055M, T055R, T055W, T158D, T158E, T164G, T164K, T164Q, T220A, T242G, T253E, T255C, T255G, V004D, V044L, V044P, V045C, V045G, V045L, V045N, V045R, V045V, V081A, V081G, V081H, V084S, V147A, V203D, V203G, V270C, V270P, V270S, W241L, Y104T, Y214Q, Y262D, Y262E, Y262G, Y262H, Y262L, Y262N, Y263G, and Y263W, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0348] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one amino acid substitution selected from the group consisting of A001C, A142C, A187C, A216H, A273Q, A274H, D036Q, D036S, D099S, D197T, E156L, F189A, F189L, G053L, G053R, G157P, G178A, G258P, H039S, H238Y, K012C, K012E, K012L, K012W, K136E, K265Y, L075G, L075V, L082E, L126W, L257D, L257P, M050L, M222A, M222F, M222L, N056S, N062C, N062L, N062Y, N269C, P057W, Q002K, Q002L, Q217C, Q245A, Q245H, S018C, S038Y, S049C, S087C, S087K, S145D, S191G, T022P, T055E, T164A, T164R, V045K, V051H, V081R, V143G, V148L, V180S, V203S, V270G, Y214H, A187F, A273P, F189G, G046D, G146A, G157T, I031F, I175L, K012F, K027T, L042F, L233E, L233G, M222T, N062R, N184P, P005V, P005W, P129V, P239N, P239T, Q010W, Q059W, Q275A, V004T, V165C, A128H, A230G, D041C, H067T, K027S, K043R, L090T, N062Q, N117G, P225G, P225S, P239G, P239H, Q002R, S089E, V044A, V045I, A001P, A273R, D041N, D099A, D099H, D099Q, F058G, I111M, L042C, N118L, P239A, S049N, S089P, S173V, T242P, V044T, and V045T, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

EXAMPLE 8

Cleaning Performance of Additional Combinatorial Variants Based on BPN'-v36 Parent

[0349] Additional combinatorial variants based on parent BPN'-v36 (BPN'-S24G-S53G-S78N-S101N-G128A-Y217Q) were made and provided by DNA 2.0. These variants were tested for their cleaning performance using BMI microswatch assay in Detergent Composition 4 at 16°C and pH 8, BMI microswatch assay in Detergent Composition 4 at 16°C and pH 7, Egg microswatch assay in Detergent Composition 4 at 16°C and pH 8, and Grass microswatch assay in Detergent Composition 4 at 16°C and pH 8. Protein content was determined using TCA assay and protease activity was assayed using AAPF assay. All assays were performed as described in Example 1 and the Performance Indices were calculated relative to BPN'-v36 (with a PI value of 1.0).

[0350] The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from

greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A088T-L257G, A116T-A128S, N061S-N109G-A128S-N243V-S260P, S009T-N109G-A128S-K141R-N243V, S009T-S018T-Y021N-N109G-A128S-K141R, and S162G-K256R, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN', BPN'-v3, and BPN'-v36, and a greater PI value than that of BPN', BPN'-v3 and BPN'-v36 in this assay.

[0351] The following BPN'-v36 variants were determined to have a PI value of about 1.0 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A088T, A088T-A116T, A088T-G131H, A088T-K256R, A088T-N109G, A088T-N243V, A088T-Q103H, A088T-S162G, A088T-S248N, A088T-S249A, A088T-T158S, A116T, A116T-G131H, A116T-K256R, A116T-L257G, A116T-N243V, A116T-S162G, A116T-S248N, A116T-S249A, A116T-T158S, A128S-K256R, A128S-L257G, A128S-N243V-S248N-K256R, A128S-S162G, A128S-S248N, A128S-S249A, A128S-T158S, G024E-A116T, G024E-K256R, G024E-L257G, G024E-N109G, G024E-N243V, G024E-T158S, G131H, G131H-K256R, G131H-L257G, G131H-N243V-K256R, G131H-S162G, G131H-S248N, G131H-S249A, G131H-T158S, K043Y-A088T, K043Y-K256R, K043Y-N243V, K256R, K256R-L257G, L257G, N061G-N109G-N243V, N061P-N109G-G131H-N243V, N061P-N109G-N243V, N061S-A128S-N243V-S260P, N061S-N109G-A128S-N243V-S248N-K256R-S260P, N061S-N109G-A128S-S260P, N061S-N109G-N243V, N076D-K256R, N076D-L257G, N076D-N109G, N076D-T158S, N109A-A128S-N243V-K256R, N109G, N109G-A116T, N109G-A128S, N109G-A128S-G131H-N243V-S248N-K256R, N109G-A128S-N243V-K256R, N109G-A128S-N243V-S248A, N109G-A128S-N243V-S248A-K256R, N109G-A128S-N243V-S248N, N109G-A128S-N243V-S248N-K256R, N109G-A128S-N243V-S248N-K256R-L257G, N109G-A128S-S162G-N243V-S248N-K256R, N109G-A128S-S248N-K256R, N109G-A128S-T158S-N243V-S248N-K256R, N109G-G131H, N109G-K256R, N109G-L257G, N109G-N218S, N109G-N243P-S248A-K256R, N109G-N243P-S248N-K256R, N109G-N243V, N109G-N243V-K256R, N109G-N243V-S248A-K256R, N109G-N243V-S248N, N109G-N243V-S248N-K256R, N109G-S162G, N109G-S248N-K256R, N109G-S249A, N109G-T158S, N109Q-A128S-N243V-K256R, N109S-A128S-N243V-K256R, N218S-N243V, N243V, N243V-K256R, N243V-L257G, N243V-S248N, N243V-S248N-K256R, N243V-S249A, P040A-N109G-A128S-N243V-S248N-K256R, Q103H-A116T, Q103H-A128S, Q103H-G131H, Q103H-K256R, Q103H-L257G, Q103H-N109G, Q103H-N218S, Q103H-N243V, Q103H-S162G, Q103H-S248N, Q103H-S249A, Q103H-T158S, S009T-A128S-K141R-N243V, S009T-N109G-A128S-K141R, S009T-N109G-A128S-K141R-N243V-S248N-K256R, S009T-S018T-Y021N-A128S-K141R-N243V, S018T-Y021N-A128S-N243V, S018T-Y021N-N061S-A128S-N243V-S260P, S018T-Y021N-N061S-N109G-A128S-S260P, S018T-Y021N-N109G-A128S, S018T-Y021N-N109G-A128S-N243V, S018T-Y021N-,N109G-A128S-N243V-S248N-K256R, S033T-N109G-A128S-N243P-S248N-K256R, S033T-N243V, S033T-Q103H, S033T-T158S, S063G, S063G-A088T, S063G-A128S, S063G-K256R, S063G-L257G, S063G-N076D, S063G-N109G, S063G-Q103H, S063G-S162G, S063G-S248N, S063G-T158S, S162G, S162G-L257G, S162G-N243V, S162G-S248N, S248N, S248N-L257G, S249A, T158S, T158S-L257G, T158S-N218S, T158S-N243V, T158S-S248N, and T158S-S249A, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0352] The following BPN'-v36 variants were determined to have a PI value of about 0.9 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A001E-A088T, A001E-A116T, A001E-A128S-G131H-N243V, A001E-G131H-G169A-N243V, A001E-K256R, A001E-N109G, A001E-N243V, A001E-S033T, A001E-S033T-N109G-N218S, A001E-S033T-N109G-N243V, A001E-S162G, A001E-T158S, A088T-A128S, A088T-G169A, A088T-N218S, A088T-Q206D, A116T-G169A, A116T-N218S, A116T-Q206D, A128S, A128S-G131H, A128S-G169A, A128S-N218S, A128S-N243V, A128S-Q206D, G024E, G024E-A088T, G024E-A128S, G024E-G131H, G024E-K043Y, G024E-N218S, G024E-Q103H, G024E-S033T, G024E-S063G, G024E-S162G, G024E-S248N, G024E-S249A, G131H-G169A, G131H-N218S, G131H-N243V, G131H-Q206D, G169A, G169A-K256R, G169A-L257G, G169A-N218S, G169A-N243V, G169A-Q206D, G169A-S248N, G169A-S249A, K043Y, K043Y-A116T, K043Y-A128S, K043Y-G131H, K043Y-G169A, K043Y-L257G, K043Y-N109G, K043Y-N218S, K043Y-Q103H, K043Y-S063G, K043Y-S162G, K043Y-S248N, K043Y-S249A, K043Y-T158S, N076D, N076D-A088T, N076D-A128S, N076D-G131H, N076D-N218S, N076D-N243V, N076D-Q103H, N076D-S162G, N076D-S248N, N076D-S249A, N109G-G169A, N109G-Q206D, N109G-S248N, N218S, N218S-K256R, N218S-L257G, N218S-S248N, N218S-S249A, P040E-N109G-A128S-G131H, Q103H, Q103H-G169A, Q206D, Q206D-K256R, Q206D-L257G, Q206D-N218S, Q206D-N243V, Q206D-S248N, Q206D-S249A, S018T-Y021N-S033T-N109G-A128S-N243V-S248N-K256R, S033T, S033T-A088T, S033T-A116T, S033T-A128S, S033T-A128S-G131H-N243P, S033T-G131H, S033T-K043Y, S033T-K256R, S033T-L257G, S033T-N076D, S033T-N076D-A128S-N218S, S033T-N076D-N109G-A128S-N218S-N243V-S248N-K256R, S033T-N109G, S033T-N109G-A128S-N243V-S248N-K256R, S033T-N218S, S033T-P040E-Q103H-N109G, S033T-Q103H-A128S-G131H, S033T-Q206D, S033T-S063G, S033T-S162G, S033T-S248N, S033T-S249A, S063G-A116T, S063G-

G131H, S063G-G169A, S063G-N109G-A128S-G131H, S063G-N218S, S063G-N243V, S063G-Q206D, S063G-S249A, S162G-G169A, S162G-N218S, S162G-Q206D, S162G-S249A, S248N-K256R, S248N-S249A, S249A-K256R, S249A-L257G, T158S-G169A, T158S-K256R, T158S-Q206D, and T158S-S162G, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0353] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A001E, A001E-A128S, A001E-G024E, A001E-G131H, A001E-G169A, A001E-L257G, A001E-N218S, A001E-Q103H, A001E-S063G, A001E-S248N, A001E-S249A, G024E-N076D, K043Y-N076D, K043Y-Q206D, N076D-A116T, N076D-G169A, N076D-Q206D, Q103H-Q206D, S033T-G169A, S033T-S063G-Q103H-N109Q-A128S-G131H-G169A-N243P, S033T-S063G-Q103H-N109Q-A128S-G131H-G169A-N243V, A001E-K043Y, A001E-N076D, A001E-N076D-N109G-A128S, A001E-Q206D, and G024E-Q206D, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0354] The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 7 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A116T, A088T-N243V, G024E-A116T, K043Y, N076D-A116T, N218S-S248N, S033T-N243V, S033T-S063G, S248N-L257G, A001E-S249A, A088T-A116T, A088T-A128S, A088T-G131H, A088T-L257G, A088T-N109G, A088T-S248N, A088T-S249A, A116T-N243V, A116T-T158S, A128S, A128S-K256R, A128S-L257G, A128S-N243V, A128S-S248N, A128S-T158S, G024E-A088T, G024E-A128S, G024E-G131H, G024E-K256R, G024E-L257G, G024E-N218S, G024E-N243V, G024E-S162G, G024E-S249A, G024E-T158S, G131H, G131H-K256R, G131H-S249A, K043Y-A088T, K043Y-A116T, K256R, N076D-K256R, N109G, N109G-A116T, N109G-A128S, N109G-A128S-N243V-K256R, N109G-A128S-N243V-S248A, N109G-G131H, N109G-K256R, N109G-L257G, N109G-N218S, N109G-N243V, N109G-S248N, N218S-L257G, N243V, N243V-K256R, N243V-L257G, N243V-S248N, N243V-S249A, Q103H-A128S, Q103H-G131H, Q103H-K256R, Q103H-L257G, Q103H-N243V, Q103H-S248N, Q103H-S249A, Q103H-T158S, Q206D-N243V, S033T-A128S, S033T-K256R, S033T-N076D, S033T-N218S, S033T-S248N, S033T-T158S, S063G-A128S, S063G-K256R, S063G-N243V, S063G-S162G, S063G-T158S, S162G-K256R, S248N-K256R, S249A, T158S-N243V, and T158S-S249A, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN', BPN'-v3, and BPN'-v36, and a greater PI value than that of BPN', BPN'-v3 and BPN'-v36 in this assay.

[0355] The following BPN'-v36 variants were determined to have a PI value of about 1.0 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 7 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A001E-A128S, A001E-G131H, A001E-K256R, A001E-N218S, A001E-N243V, A001E-S033T, A001E-S063G, A001E-S162G, A088T, A088T-K256R, A088T-N218S, A088T-Q103H, A088T-S162G, A088T-T158S, A116T-A128S, A116T-G131H, A116T-K256R, A116T-L257G, A116T-S162G, A116T-S248N, A116T-S249A, A128S-G169A, A128S-N218S, A128S-S162G, A128S-S249A, G024E, G024E-N109G, G024E-Q103H, G024E-S033T, G024E-S063G, G024E-S248N, G131H-L257G, G131H-N243V, G131H-S162G, G131H-T158S, G169A, G169A-L257G, G169A-S248N, K043Y-A128S, K043Y-G131H, K043Y-K256R, K043Y-L257G, K043Y-N109G, K043Y-N243V, K043Y-Q103H, K043Y-S063G, K043Y-S162G, K043Y-S248N, K043Y-S249A, K043Y-T158S, K256R-L257G, L257G, N061G-N109G-N243V, N061S-A128S-N243V-S260P, N061S-N109G-A128S-N243V-S260P, N061S-N109G-A128S-S260P, N076D-A088T, N076D-A128S, N076D-G169A, N076D-N218S, N076D-N243V, N076D-S162G, N076D-S248N, N076D-T158S, N109A-A128S-N243V-K256R, N109G-A128S-G131H-N243V-S248N-K256R, N109G-A128S-N243V-S248A-K256R, N109G-A128S-N243V-S248N-K256R-L257G, N109G-A128S-S162G-N243V-S248N-K256R, N109G-A128S-T158S-N243V-S248N-K256R, N109G-Q206D, N109G-S162G, N109G-S249A, N109G-T158S, N109Q-A128S-N243V-K256R, N109S-A128S-N243V-K256R, N218S, N218S-K256R, N218S-N243V, P040A-N109G-A128S-N243V-S248N-K256R, Q103H, Q103HA116T, Q103H-G169A, Q103H-N109G, Q103H-N218S, Q103H-S162G, S009T-A128S-K141R-N243V, S009T-N109G-A128S-K141R, S009T-N109G-A128S-K141R-N243V, S009T-S018T-Y021N-N109G-A128S-K141R, S018T-Y021N-N109G-A128S, S018T-Y021N-N109G-A128S-N243V, S018T-Y021N-N109G-A128S-N243V-S248N-K256R, S033T-A088T, S033T-A116T, S033T-G131H, S033T-K043Y, S033T-L257G, S033T-N109G, S033T-Q103H, S033T-Q206D, S033T-S162G, S033T-S249A, S063G, S063G-A088T, S063G-A116T, S063G-L257G, S063G-N076D, S063G-N109G, S063G-N218S, S063G-Q103H, S063G-S248N, S063G-S249A, S162G, S162G-G169A, S162G-L257G, S162G-N218S, S162G-N243V, S162G-S248N, S162G-S249A, S248N, S248N-S249A, S249A-K256R, S249A-L257G, T158S, T158S-G169A, T158S-K256R, T158S-L257G, T158S-N218S, and T158S-S248N, wherein amino acid positions of the variant are numbered by correspondence with the

sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' protease (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0356] The following BPN'-v36 variants were determined to have a PI value of about 0.9 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 7 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A001E, A001E-A088T, A001E-A116T, A001E-G169A, A001E-L257G, A001E-N109G, A001E-S033T-N109G-N243V, A001E-T158S, A088T-G169A, A088T-Q206D, A116T-N218S, A128S-G131H, A128S-N243V-S248N-K256R, A128S-Q206D, G024E-K043Y, G024E-N076D, G024E-Q206D, G131H-G169A, G131H-N218S, G131H-N243V-K256R, G131H-Q206D, G131H-S248N, G169A-K256R, G169A-N218S, G169A-N243V, G169A-Q206D, K043Y-N076D, K043Y-N218S, N061P-N109G-G131H-N243V, N061P-N109G-N243V, N061S-N109G-A128S-N243V-S248N-K256R-S260P, N061S-N109G-N243V, N076D, N076D-G131H, N076D-L257G, N076D-N109G, N076D-Q103H, N076D-S249A, N109G-A128S-N243V-S248N, N109G-A128S-N243V-S248N-K256R, N109G-A128S-S248N-K256R, N109G-N243P-S248A-K256R, N109G-N243P-S248N-K256R, N109G-N243V-K256R, N109G-N243V-S248A-K256R, N109G-N243V-S248N, N109G-N243V-S248N-K256R, N109G-S248N-K256R, N218S-S249A, N243V-S248N-K256R, Q103H-Q206D, Q206D, Q206D-K256R, Q206D-N218S, Q206D-S248N, Q206D-S249A, S009T-N109G-A128S-K141R-N243V-S248N-K256R, S009T-S018T-Y021N-A128S-K141R-N243V, S018T-Y021N-A128S-N243V, S018T-Y021N-N061S-A128S-N243V-S260P, S018T-Y021N-N061S-N109G-A128S-S260P, S018T-Y021N-S033T-N109G-A128S-N243V-S248N-K256R, S033T, S033T-G169A, S033T-N076D-A128S-N218S, S033T-N076D-N109G-A128S-N218S-N243V-S248N-K256R, S033T-N109G-A128S-N243P-S248N-K256R, S033T-N109G-A128S-N243V-S248N-K256R, S063G-G131H, S063G-G169A, S162G-Q206D, and T158S-S162G, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have proteolytic activity.

[0357] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 7 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A001E-A1285-G131H-N243V, A001E-G024E, A001E-G131H-G169A-N243V, A001E-Q103H, A001E-S033T-N109G-N218S, A001E-S248N, A116T-G169A, A116T-Q206D, G169A-S249A, K043Y-G169A, N109G-G169A, P040E-N109G-A128S-G131H, Q206D-L257G, S033T-A128S-G131H-N243P, S033T-A128S-G131H-N243V, S033T-P040E-Q103H-N109G, S033T-Q103H-A128S-G131H, S063G-N109G-A128S-G131H, S063G-Q206D, T158S-Q206D, A001E-K043Y, A001E-N076D, A001E-Q206D, S033T-S063G-Q103H-N109Q-A128S-G131H-G169A-N243P, S033T-S063G-Q103H-N109Q-A128S-G131H-G169A-N243V, A001E-N076D-N109G-A128S, K043Y-Q206D, and N076D-Q206D, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO: 2.

[0358] The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A088T-L257G, G024E-K256R, G024E-L257G, N109G-A116T, N109G-L257G, N243V-K256R, S033T-N109G, S033T-T158S, S063G-L257G, A001E-L257G, A088T-A128S, A088T-G169A, A088T-K256R, A088T-N109G, A088T-N218S, A088T-N243V, A088T-S248N, A088T-T158S, A116T, A116T-A128S, A116T-G131H, A116T-K256R, A116T-L257G, A116T-N218S, A116T-S162 A116T-T158S, A128S, A128S-G169A, A128S-K256R, A128S-L257G, A128S-N218S, G024E, G024E-A128S, G024E-G131H, G024E-N109G, G024E-N243V, G024E-S033T, G024E-S063G, G024E-S248N, G024E-S249A, G024E-T158S, G131H, G131H-G169A, G131H-K256R, G131H-N218S, G131H-S249A, G169A, G169A-L257G, G169A-N243V, K043Y-A088T, K043Y-N109G, K256R, K256R-L257G, N061G-N109G-N243V, N076D-N109G, N109G, N109G-A128S, N109G-G131H, N109G-K256R, N109G-N218S, N109G-S162G, N109G-S248N, N109G-S249A, N109G-T158S, N218S, N218S-K256R, N218S-L257G, N218S-S248N, N243V, N243V-L257G, N243V-S248N, N243V-S249A, P040A-N109G-A128S-N243V-S248N-K256R, Q103H-K256R, Q103H-L257G, Q103H-N109G, S009T-S018T-Y021N-N109G-A128S-K141R, S033T-A088T, S033T-A116T, S033T-A128S, S033T-G131H, S033T-K043Y, S033T-K256R, S033T-L257G, S033T-N076D, S033T-N218S, S033T-N243V, S033T-Q103H, S033T-S063G, S033T-S162G, S033T-S248N, S033T-S249A, S063G, S063G-A088T, S063G-A116T, S063G-A128S, S063G-G131H, S063G-K256R, S063G-N109G, S063G-N218S, S063G-N243V, S063G-S248N, S063G-S249A, S063G-T158S, S162G-K256R, S162G-N218S, S162G-N243V, S162G-S248N, S162G-S249A, S248N, S249A, S249A-L257G, T158S, T158S-L257G, and T158S-N243V, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN', BPN'-v3, and BPN'-v36, and a greater PI value than that of BPN', BPN'-v3 and BPN'-v36 in this assay.

[0359] The following BPN'-v36 variants were determined to have a PI value of about 1.0 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q

amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A001E, A001E-A116T, A001E-G131H, A001E-G169A, A001E-K256R, A001E-N109G, A001E-S033T-N109G-N243V, A001E-S063G, A001E-S248N, A001E-S249A, A001E-T158S, A088T, A088T-A116T, A088T-G131H, A088T-Q103H, A088T-Q206D, A088T-S162G, A088T-S249A, A116T-G169A, A116T-N243V, A116T-S248N, A116T-S249A, A128S-G131H, A128S-N243V, A128S-S162G, A128S-S248N, A128S-S249A, A128S-T158S, G024E-A088T, G024E-A116T, G024E-K043Y, G024E-N076D, G024E-N218S, G024E-Q103H, G024E-S162G, G131H-L257G, G131H-N243V, G131H-N243V-K256R, G131H-S162G, G131H-S248N, G131H-T158S, G169A-K256R, G169A-N218S, G169A-Q206D, G169A-S248N, G169AS249A, K043Y, K043Y-A116T, K043Y-A128S, K043Y-G169A, K043Y-K256R, K043Y-L257G, K043Y-N076D, K043Y-N218S, K043Y-N243V, K043Y-S063G, K043Y-S248N, K043Y-S249A, K043Y-T158S, L257G, N061P-N109G-G131H-N243V, N061P-N109G-N243V, N061S-A128S-N243V-S260P, N061S-N109G-A128S-N243V-S248N-K256R-S260P, N061S-N109G-A128S-S260P, N061S-N109G-N243V, N076D, N076D-A088T, N076D-A116T, N076D-G131H, N076D-G169A, N076D-K256R, N076D-L257G, N076D-N218S, N076D-N243V, N076D-Q103H, N076D-S249A, N076D-T158S, N109A-A128S-N243V-K256R, N109G-A128S-G131H-N243V-S248N-K256R, N109G-A128S-N243V-K256R, N109G-A128S-N243V-S248A, N109G-A128S-N243V-S248A-K256R, N109G-A128S-N243V-S248N, N109G-A128S-N243V-S248N-K256R, N109G-A128S-N243V-S248N-K256R-L257G, N109G-A128S-S162G-N243V-S248N-K256R, N109G-G169A, N109G-N243P-S248A-K256R, N109G-N243V-K256R, N109G-N243V-S248A-K256R, N109G-N243V-S248N, N109G-N243V-S248N-K256R, N109G-S248N-K256R, N109Q-A128S-N243V-K256R, N109S-A128S-N243V-K256R, N218S-N243V, N218S-S249A, Q103H, Q103H-A116T, Q103H-A128S, Q103H-G131H, Q103H-G169A, Q103H-N218S, Q103H-N243V, Q103H-S162G, Q103H-S248N, Q103H-S249A, Q103H-T158S, Q206D, Q206D-L257G, Q206D-N218S, S009T-A128S-K141R-N243V, S009T-N109G-A128S-K141R, S009T-N109G-A128S-K141R-N243V, S009T-N109G-A128S-K141R-N243V-S248N-K256R, S009T-S018T-Y021N-A128S-K141R-N243V, S018T-Y021N-A128S-N243V, S018T-Y021N-N109G-A128S, S018T-Y021N-N109G-A128S-N243V, S018T-Y021N-N109G-A128S-N243V-S248N-K256R, S018T-Y021N-S033T-N109G-A128S-N243V-S248N-K256R, S033T, S033T-A128S-G131H-N243V, S033T-G169A, S033T-N109G-A128S-N243P-S248N-K256R, S033T-N109G-A128S-N243V-S248N-K256R, S033T-Q103H-A128S-G131H, S033T-Q206D, S033T-S063G-Q103H-N109Q-A128S-G131H-G169A-N243V, S063G-G169A, S063G-N076D, S063G-N109G-A128S-G131H, S063G-Q103H, S063G-S162G, S162G, S162G-G169A, S162G-L257G, S248N-K256R, S248N-L257G, S248N-S249A, S249A-K256R, T158S-G169A, T158S-K256R, T158S-N218S, T158S-S162G, T158S-S248N, and T158S-S249A, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' protease (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0360] The following BPN'-v36'variants were determined to have a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A001E-A088T, A001E-A128S, A001E-A128S-0131H-N243V, A001E-G024E, A001E-G024E-S204E-Q206D, A001E-G131H-G169A-N243V, A001E-K043Y, A001E-N076D, A001E-N076D-N109G-A128S, A001E-N218S, A001E-N243V, A001E-Q103H, A001E-Q206D, A001E-S033T, A001E-S033T-N109G-N218S, A001E-S162G, A116T-Q206D, A128S-N243V-S248N-K256R, A128S-Q206D, G024E-Q206D, G131H-Q206D, K043Y-G131H, K043Y-Q103H, K043Y-Q206D, K043Y-S162G, N061S-N109G-A128S-N243V-S260P, N076D-A128S, N076D-Q206D, N076D-S162G, N076D-S248N, N109G-A128S-S248N-K256R, N109G-A128S-T158S-N243V-S248N-K256R, N109G-N243P-S248N-K256R, N109G-Q206D, N243V-S248N-K256R, P040E-N109G-A128S-G131H, Q103H-Q206D, Q206D-K256R, Q206D-N243V, Q206D-S248N, Q206D-S249A, S018T-Y021N-N061S-A128S-N243V-S260P, S018T-Y021N-N061S-N109G-A128S-S260P, S033T-A128S-G131H-N243P, S033T-N076D-A128S-N218S, S033T-N076D-N109G-A128S-N218S-N243V-S248N-K256R, S033T-P040E-Q103H-N109G, S033T-S063G-Q103H-N109Q-A128S-G131H-G169A-N243P, S063G-Q206D, S162G-Q206D, and T158S-Q206D, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0361] The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in a grass microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of T158S-L257G, K256R, L257G, S033T-N109G, S162G-K256R, S162G-L257G, G024E-K256R, G024E-L257G, G024E-S033T, N109G-A116T, N218S-L257G, S033T-A088T, S033T-A116T, S033T-N243V, S033T-Q103H, S162G-N218S, S162G-N243V, T158S, T158S-N218S, T158S-N243V, A088T, A088T-G169A, A088T-K256R, A088T-L257G, A088T-S162G, A088T-T158S, A116T-K256R, A116T-L257G, A116T-N243V, A128S-L257G, A128S-N218S, A128S-N243V, A128S-S248N, G024E-A116T, G024E-A128S, G024E-G131H, G024E-N243V, G024E-S248N, G024E-S249A, G024E-T1585, G131H-N243V, G131H-T158S, G169A-N218S, G169A-N243V, G169A-S248N, K256R-L257G, N109G-A128S, N109G-G131H, N109G-N218S, N109G-N243V, N109G-S249A, N218S, N218S-K256R, N218S-N243V, N218S-S249A, N243V, N243V-K256R, N243V-L257G, N243V-S248N, Q103H-N109G, Q103H-N218S, S033T-A128S, S033T-L257G, S033T-N218S, S033T-S162G, S033T-S248N, S033T-T158S, S063G-K256R, S063G-

L257G, S162G, S162G-G169A, S162G-S248N, S248N, S248N-K256R, S248N-L257G, S249A, T158S-S162G, and T158S-S248N, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN', BPN'-v3, and BPN'-v36, and a greater PI value than that of BPN', BPN'-v3 and BPN'-v36 in this assay.

[0362] The following BPN'-v36 variants were determined to have a PI value of about 1.0 relative to BPN'-v36 in a grass microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A001E-A088T, A001E-A116T, A088T-A128S, A088T-N243V, A088T-Q103H, A088T-S248N, A088T-S249A, A116T, A116T-G169A A116T-N218S, A116T-S162G, A116T-S249A, A116T-T158S, A128S-G169A, A128S-K256R, A128S-S162G, A128S-S249A, A128S-T158S, G024E-A088T, G024E-K043Y, G024E-N218S, G024E-Q103H, G024E-S063G, G024E-S162G, G131H-G169A, G131H-K256R, G131H-N218S, G131H-S162G, G131H-S248N, G131H-S249A, G169A, G169A-L257G, G169A-S249A, N076D, N076D-K256R, N076D-L257G, N076D-S162G, N076D-S249A, N109G-K256R, N109G-L257G, N109G-S248N, N243V-S249A, Q103H-A116T, Q103H-G169A, Q103H-K256R, Q103H-L257G, Q103H-N243V, Q103H-S162G, S033T-G131H, S033T-G169A, S033T-K043Y, S033T-N076D, S033T-Q206D, S063G, S063G-A116T, S063G-A128S, S063G-N243V, S063G-S162G, S063G-S248N, S063G-S249A, S063G-T158S, S249A-L257G, T158S-G169A, and T158S-K256R, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0363] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v36 in a grass microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO: 6) comprising at least one set of amino acid substitutions selected from the group consisting of A001E-G169A, A001E-K256R, A001E-N109G, A001E-N218S, A088T-A116T, A088T-G131H, A088T-N109G, A088T-N218S, A116T-A128S, A116T-G131H, A116T-S248N, A128S, A128S-G131H, G024E, G024E-N109G, G131H, G131H-L257G, G169A-K256R, G169A-Q206D, K043Y, K043Y-A088T, K043Y-L257G, K043Y-N109G, N076D-A088T, N076D-G131H, N076D-G169A, N076D-N243V, N076D-T158S, N109G, N109G-S162G, N109G-T158S, N218S-S248N, Q103H-A128S, Q103H-S248N, Q103H-S249A, Q103H-T158S, Q206D-K256R, Q206D-L257G, Q206D-N218S, Q206D-N243V, Q206D-S248N, S033T, S033T-K256R, S033T-S063G, S033T-S249A, S063G-A088T, S063G-G131H, S063G-G169A, S063G-N109G, S162G-Q206D, S162G-S249A, S248N-S249A, S249A-K256R, T158S-Q206D, T158S-S249A, A001E-L257G, A001E-N243V, A001E-Q103H, A001E-S063G, A001E-S162G, A001E-T158S, G024E-N076D, G131H-Q206D, K043Y-A116T, K043Y-G169A, K043Y-K256R, K043Y-N076D, K043Y-S063G, K043Y-S162G, K043Y-S248N, K043Y-S249A, K043Y-T158S, N076D-A116T, N076D-A128S, N076D-S248N, N109G-G169A, Q103H, Q103H-G131H, Q206D-S249A, S063G-N076D, S063G-N218S, S063G-Q103H, A001E-A128S, A001E-G024E, A001E-G131H, A001E-N076D, A001E-Q206D, A001E-S033T, A001E-S248N, A001E-S249A, A088T-Q206D, A116T-Q206D, A128S-Q206D, G024E-Q206D, K043Y-A128S, K043Y-G131H, K043Y-N218S, K043Y-Q103H, N076D-N109G, N076D-N218S, N076D-Q103H, N109G-Q206D, Q103H-Q206D, Q206D, A001E, A001E-K043Y, K043Y-N243V, and S063G-Q206D, N076D-Q206D, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0364] The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in an AAPF proteolytic assay: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of S033T-N076D-A128S-N218S, A001E-S033T-N109G-N218S, S033T-N218S, S033T-S063G-Q103H-N109Q-A128S-G131H-G169A-N243V, A128S-G169A, S033T-S063G-Q103H-N109Q-A128S-G131H-G169A-N243P, S018T-Y021N-S033T-N109G-A128S-N243V-S248N-K256R, S033T-A128S-G131H-N243P, 'P040E-N109G-A128S-G131H, S033T-A128S, S033T-N109G-A128S-N243V-S248N-K256R, N109G-G169A, S063G-N109G-A128S-G131H, G169A, N109G-A128S-G131H-N243V-S248N-K256R, S033T-A128S-G131H-N243V, A128S-N218S, A001E-G169A, A088T-G169A, G169A-L257G, N109G-N218S, S033T-N109G-A128S-N243P-S248N-K256R, G169A-K256R, N076D-G169A, A001E-G131H-G169A-N243V, G169A-S249A, S033T-N109G, G169A-S248N, K043Y-G169A, K043Y-N218S, N218S-L257G, N218S-N243V, S063G-G169A, A001E-A128S-G131H-N243V, A001E-S033T-N109G-N243V, A088T-N218S, G024E-N218S, G024E-S033T, G169A-Q206D, N076D-N218S, S033T-L257G, S162G-G169A, A001E-N218S, A116T-N218S, G169A-N243V, N218S, P040A-N109G-A128S-N243V-S248N-K256R, S033T-N076D, A001E-S033T, A128S-G131H, N218S-S248N, S018T-Y021N-N109G-A128S, S033T-K043Y, S033T-N243V, S033T-Q206D, S063G-N218S, S162G-N218S, T158S-G169A, A116T-G169A, G131H-G169A, N061S-N109G-A128S-S260P, N109G-A128S-N243V-K256R, N109G-A128S-N243V-S248A, N109G-A128S-N243V-S248A-K256R, N109G-A128S-N243V-S248N-K256R-L257G, N218S-K256R, S009T-N109G-A128S-K141R, S009T-S018T-Y021N-N109G-A128S-K141R, S033T-A088T, S033T-S063G, S033T-S162G, T158S-N218S, A001E-N076D-N109G-A128S, N109G-A128S-N243V-S248N-K256R, N109G-A128S-S248N-K256R, S009T-N109G-A128S-K141R-N243V, S018T-Y021N-N061S-N109G-A128S-S260P, S033T-A116T, S033T-S248N, S033T-S249A, S033T-T158S, G131H-N218S, N109A-A128S-N243V-

K256R, N109G-A128S, N109G-A128S-S162G-N243V-S248N-K256R, N109G-A128S-T158S-N243V-S248N-K256R, N218S-S249A, Q206D-N218S, S018T-Y021N-N109G-A128S-N243V, S018T-Y021N-N109G-A128S-N243V-S248N-K256R, S033T-K256R, A116T-A128S, N061S-N109G-A128S-N243V-S260P, N109G-A128S-N243V-S248N, S009T-N109G-A128S-K141R-N243V-S248N-K256R, G024E-A128S, N061S-N109G-A128S-N243V-S248N-K256R-S260P, N109S-A128S-N243V-K256R, S033T, S033T-G131H, A001E-A128S, A128S, A128S-L257G, A128S-Q206D, N109Q-A128S-N243V-K256R, S009T-A128S-K141R-N243V, S009T-S018T-Y021N-A128S-K141R-N243V, A088T-A128S, A128S-K256R, A128S-N243V, N061P-N109G-N243V, N061S-A128S-N243V-S260P, S018T-Y021N-A128S-N243V, A128S-N243V-S248N-K256R, A128S-S248N, A128S-S249A, N076D-A128S, S063G-A128S, A128S-S162G, A128S-T158S, S018T-Y021N-N061S-A128S-N243V-S260P, S033T-Q103HA128S-G131H, N061S-N109G-N243V, K043Y-A128S, N061P-N109G-G131H-N243V, N109G-L257G, A001E-G024E-S204E-Q206D, A001E-L257G, A088T-N109G, G024E-N109G, K043Y-N109G, N061G-N109G-N243V, N076D-N109G, N109G, N109G-A116T, N109G-K256R, N109G-N243V-K256R, N109G-N243V-S248A-K256R, N109G-Q206D, S063G-N109G, A001E-A116T, A001EN109G, A001E-Q206D, A088T-A116T, A088T-N243V, A116T-L257G, G024E-A116T, G024EL257G, G024E-N243V, G024E-Q206D, N109G-G131H, N109G-N243V, N109G-S162G, N109G-S248N, N109G-S248N-K256R, N109G-S249A, N109G-T158S, N243V-L257G, A001E-A088T, A001E-G024E, A001E-K256R, A001E-N076D, A001E-N243V, A088T, A088T-L257G, A088T-Q206D, A116T, A116T-K256R, A116T-N243V, G024E-A088T, G024E-K043Y, G024E-K256R, G024EN076D, G024E-S162G, G024E-S248N, K043Y-A088T, K043Y-A116T, K043Y-L257G, K043Y-N243V, K043Y-Q206D, K256R-L257G, N076D-A116T, N076D-L257G, N076D-N243V, N076D-Q206D, N109G-N243V-S248N, N109G-N243V-S248N-K256R, N243V-K256R, Q206D, Q206D-L257G, Q206D-N243V, Q206D-S248N, S063G-K256R, S063G-L257G, T158S-L257G, A001E, A001E-K043Y, A001E-S162G, A001E-S248N, A001E-S249A, A001E-T158S, A088T-K256R, A088T-S162G, A088T-S248N, A088T-S249A, A116T-Q206D, A116T-S248N, A116T-S249A, G024E, G024EG131H, G024E-S249A, G024E-T158S, G131H, G131H-K256R, G131H-L257G, K043Y-K256R, K043Y-N076D, K256R, L257G, N076D-A088T, N076D-K256R, N076D-S162G, N076D-S248N, N076D-S249A, N109G-N243P-S248A-K256R, N109G-N243P-S248N-K256R, N243V, Q206D-K256R, S033T-P040E-Q103H-N109G, S063G, S063G-A116T, S063G-Q206D, S162G-K256R, S162G-L257G, S162G-N243V, S162G-Q206D, S162G-S248N, S248N, S248N-L257G, S249A, S249A-L257G, T158S, T158S-N243V, and T158S-Q206D, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN', BPN'-v3, and BPN'-v36, and a greater PI value than that of BPN', BPN'-v3 and BPN'-v36 in this assay.

[0365] The following BPN'-v36 variants were determined to have a PI value of about 1.0 relative to BPN'-v36 in an AAPF proteolytic assay: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A001E-G131H, A001E-S063G, A088T-G131H, A088T-T158S, A116T-G131H, A116T-S162G, A116T-T158S, G024E-S063G, G131H-N243V, G131H-N243V-K256R, G131H-Q206D, G131H-S249A, K043Y, K043Y-S063G, K043Y-S248N, K043Y-S249A, K043Y-T158S, N076D, N076D-G131H, N076D-T158S, N243V-S248N, N243V-S248N-K256R, N243V-S249A, Q103H-G169A, Q206D-S249A, S063G-N076D, S063G-N243V, S063G-S162G, S063G-S249A, S063G-T158S, S162G, S162G-S249A, S248N-K256R, S248N-S249A, S249A-K256R, T158S-K256R, T158S-S248N, and T158S-S249A, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0366] The following BPN'-v36 variants were determined to have a PI value of about 0.9 relative to BPN'-v36 in an AAPF proteolytic assay: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of G131H-S162G, G131H-S248N, G131H-T158S, K043Y-G131H, K043Y-S162G, S063G-A088T, S063G-G131H, S063G-S248N, T158S-S162G, Q103H-N218S, S033T-Q103H, and Q103H-A128S, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

EXAMPLE 9

Construction and Cleaning Performance of Variants from a Combinatorial Library Based on BPN'-v36 Parent

[0367] A BPN' combinatorial library based on the BPN'-v36 parent molecule was made by DNA 2.0 and delivered as a ligation reaction. For efficient transformation of *B. subtilis*, DNA from the ligation reaction mixture was amplified before transformation and transformants grown as described in Example 2. The variants were tested for cleaning performance using BMI microswatch assay in Detergent Composition 4 at 16°C and pH 8 and egg microswatch assay in Detergent Composition 4 at 16°C and pH 8. Protein content was determined using the TCA assay. Assays were performed as in Example 1 and Performance Indices were calculated relative to BPN'-v36 (i.e., BPN'-S24G-S53G-S78N-S101N-G128A-Y217Q) (with a PI value of 1.0).

[0368] The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A088TA116T-N243V-K256R-L257G, A088T-A116T-N243V-L257G, A088T-T158S-N218S-K256R, A088T-T158S-N218S-N243V-L257G, A088T-A116T-T158S-N218S-N243V-K256R-L257G, A088T-N109G-A116T-G131H-A153S-N218S-S248N-L257G, A088T-N109G-A116T-T158S-S248N-K256R-L257G, A088T-N109G-T158S-L257G, A114S-A116T-N218S-N243V-S248N-K256R-L257G, A116T-T158S-K256R, A088T-A116T-G131H-T158S-S248N-L257G, A088T-A116T-T158S, A088T-N109G-A116T-G131H-L257G, A088T-N109G-A116T-T158S-N243V-S248N-L257G, A088T-N109G-N243V-L257G, A088T-N109G-N243V-S248N, A088T-N109G-T158S-N243V-L257G, A088T-N109G-T158S-N243V-S248N-L257G, A116T-T158S-S248N-L257G, Y006H-A116T-G131H-S248N, A088T-A116T-G131H-T158S-N218S-N243V, A088T-A116T-G131H-T158S-N243V, A088T-A116T-G131H-T158S-N243V-K256R-L257G, A088T-A116T-N218S-N243V-K256R-L257G, A088T-A116T-S248N-K256R-L257G, A088T-A116T-T158S-N218S-N243V, A088T-A116T-T158S-N243V-K256R-L257G, A088T-A116T-T158S-N243V-S248N-L257G, A088T-G131H-N243V-S248N-K256R-L257G, A088T-N109G-A116T-T158S-L257G, A088T-N109G-A116T-T158S-N212D-N243V-K256R-L257G, A088T-N109G-A116T-T158S-N218S-N243V-S248N-K256R, A088T-N109G-A116T-T158S-S248N-L257G, A088T-N109G-G131H-V148A-N218S-N243V-K256R-L257G, A088T-N109G-K256R, A088T-N109G-N243V-S248N-L257G, A088T-N109G-T158S-K256R, A088T-N109G-T158S-N243V, A088T-T158S-N243V-K256R-L257G, A116T, A116T-N218S-N243V-L257G-N269S, A116T-T158S-K256R-L257G, N109G-A116T-K256R-L257G, N109G-A116T-N243V, N109G-AI16T-T158S-N243V-K256R-L257G, N109G-G131H-L257G, N109G-G131H-S248N-K256R-L257G, N109G-G131H-T158S-K256R-L257G, S003P-A116T-T158S-S248N-K256R, T158S-S248N-K256R, A088T-A116T-G131H-N243V-K256R, A088T-A116T-G131H-S248N-K256R-L257G, A088T-A116T-G131H-V147A-T158S-N218S-N243V-S248N-L257G, A088T-A116T-S248N-L257G, A088T-A116T-T158S-N218S, A088T-A116T-T158S-N218S-K256R-L257G, A088T-A116T-T158S-N218S-L257G, A088T-G131H-N243V-L257G, A088T-G131H-T158S-S248N-L257G, A088T-L257G, A088T-N109G-A116T, A088T-N109G-A116T-G131H-N218S, A088T-N109G-A116T-G131H-N218S-S248N-L257G, A088T-N109G-A116T-G131H-N243V-S248N-K256R-L257G, A088T-N109G-A116T-G131H-T158S-S248N-K256R-L257G, A088T-N109G-A116T-N218S-N243V-K256R, A088T-N109G-A116T-N218S-N243V-L257G, A088T-N109G-A116T-N243V-S248N-K256R, A088T-N109G-A116T-N243V-S248N-K256R-L257G, A088T-N109G-A116T-T158S-L257G, A088T-N109G-A116T-T158S-N243V-L257G, A088T-N109G-G131H-T158S-N243V-S248N-K256R, A088T-N109G-G131H-T158S-W241R-S248N-K256R, A088T-N109G-K256R-L257G, A088T-N109G-L257G, A088T-N109G-N243V, A088T-N109G-N243V-K256R, A088T-N109G-N243V-K256R-L257G, A088T-N109G-S248N-K256R, A088T-N109G-T158S-N218S-K256R-L257G, A088T-N109G-T158S-N218S-N243V-S248N-K256R, A088T-N109G-T158S-N243V-K256R, A088T-N109G-T158S-N243V-K256R-L257G, A088T-N109G-T158S-N243V-S248N-A274D, A088T-N109G-T158S-S248N-L257G, A088T-T158S-K256R, A088T-T158S-N218S-N243V-K256R-L257G, A088T-T158S-N243V-L257G, A116T-G131H-N218S-N243V-S248N, A116T-G131H-S248N-L257G, A116T-S248N-K256R-L257G, A116T-T158S-N218S-N243V-K256R, A116T-T158S-N218S-S248N-L257G-Q271R, A116T-T158S-N243V-K256R-L257G, A116T-T158S-N243V-S248N-L257G, G131H-S248N, G131H-T158S-I234T-N243V-K256R, G131H-W241L-N243V-S248N-K256R, N109G-A116T-G131H-A137V-T158S-S248N-K256R-L257G, N109G-A116T-G131H-A151S-N218S-K256R-L257G, N109G-A116T-G131H-T158S-N218S-N243V-K256R, N109G-A116T-G131H-T158S-N218S-S248N, N109G-A116T-G131H-T158S-N243V-S248N, N109G-A116T-S248N, N109G-A116T-T158S-L257G, N109G-A116T-T158S-N218S-W241R-N243V, N109G-A116T-T158S-N243V-S248N-L257G, N109G-A116T-T158S-S248N-K256R-L257G, N109G-A116T-T158S-S248N-L257G, N109G-G131H-N218S-L257G, N109G-G131H-N218S-S248N-K256R-L257G, N109G-G131H-T158S-N218S-S248N-K256R-L257G-A274T, N109G-K256R, N109G-N243V-L257G, N109G-T158S-N218S-K256R-L257G, N109G-T158S-N218S-L257G, N109G-T158S-S248N-K256R, P014L-A015L-L016C-H017T-S018L-Q019K-G020A-Y021T-T022L-G023E, S003F-A088T-N109G-A116T-T158S-N243V-K256R-L257G, V004A-A088T-A116T-T158S-N218S, V004A-N109G-A116T-G131H-S248N-K256R-L257G, V004L-A116T-N218S-N243V-S248N-L257G, Y006H-N109G-N218S-N243V-S248N, A001T-A116T-T158S-N243V-L257G, A088T-A116T, A088T-A116T-G131H-L257G, A088T-A116T-G131H-N218S-L257G, A088T-A116T-G131H-N218S-S248N-K256R-L257G, A088T-A116T-G131H-N218S-S248N-L257G, A088T-A116T-G131H-N243V-K256R-L257G, A088T-A116T-G131H-N243V-L257G, A088T-A116T-G131H-N243V-S248N, A088TA116T-G131H-T158S-K256R-L257G, A088T-A116T-G131H-T158S-L257G, A088T-A116T-G131H-T158S-N218S, A088T-A116T-G131H-T158S-N218S-N243V-K256R-A273T, A088T-A116T-G131H-T158S-N218S-S248N-K256R, A088T-A116T-G131H-T158S-N218S-S248N-L257G, A088T-A116T-G131H-T158S-N243V-S248N-K256R, A088T-A116T-G131H-T158S-S248N, A088T-A116T-G131H-T158S-S248N-L257G, A088T-A116T-K256R, A088T-A116T-K256R-L257G, A088T-A116T-N218S-N243V-L257G, A088T-A116T-N243V-K256R, A088T-A116T-N243V-L257G, A088T-A116T-N243V-S248N-K256R-L257G, A088T-A116T-S248N-K256R, A088T-A116T-T158S-K256R, A088T-A116T-T158S-N218S, A088T-A116T-T158S-N218S-N243V-K256R, A088T-A116T-T158S-N218S-N243V-K256R-N269S, A088T-A116T-T158S-N218S-N243V-S248N, A088T-A116T-T158S-N218S-N243V-S248N, A088T-A116T-T158S-N218S-N243V-S248N-K256R-L257G, A088T-A116T-T158S-N243V-K256R, A088T-A116T-T158S-N243V-L257G, A088T-A116T-T158S-N243V-S248N-K256R, A088T-A116T-T158S-N243V-S248N-K256R-L257G, A088T-A116T-T158S-S248N-K256R, A088T-A116T-V143A-N218S-S248N-K256R, A088T-A116T-V1471-T158S-N218S-N243V-L257G, A088T-G131H-K256R-L257G, A088T-G131H-N218S-N243V-S248N, A088T-G131H-N218S-S248N-L257G, A088T-G131H-S248N-K256R-L257G, A088T-G131H-T158S-L257G, A088T-G131H-

T158S-N218S-K256R, A088T-G131H-T158S-N218S-N243V-K256R-L257G, A088T-G131H-T158S-N218S-N243V-L257G, A088T-G131H-T158S-N218S-S248N, A088T-G131H-T158S-N243V, A088T-G131H-T158S-N243V, A088T-G131H-T158S-N243V-S248N, A088T-G131H-T158S-N243V-S248N-K256R, A088T-G131H-T158S-N243V-S248N-L257G, A088T-I107T-N109G-A116T-G131H-T158S-N218S-N243V-S248N, A088T-N109G-A116T-G131H-L257G, A088T-N109G-A116T-G131H-N218S, A088T-N109G-A116T-G131H-N218S-L257G, A088T-N109G-A116T-G131H-N218S-N243V, A088T-N109G-A116T-G131H-N218S-N243V-K256R-L257G, A088T-N109G-A116T-G131H-N218S-N243V-L257G, A088T-N109G-A116T-G131H-N218S-S248N-K256R-L257G, A088T-N109G-A116T-G131H-N243V, A088T-N109G-A116T-G131H-N243V-L257G, A088T-N109G-A116T-G131H-N243V-S248N-L257G, A088T-N109G-A116T-G131H-S248N, A088T-N109G-A116T-G131H-S248N-K256R, A088T-N109G-A116T-G131H-S248N-L257G, A088T-N109G-A116T-G131H-T158S-L257G, A088T-N109G-A116T-G131H-T158S-N218S, A088T-N109G-A116T-G131H-T158S-N218S-S248N-K256R, A088T-N109G-A116T-G131H-T158S-N218T-N243V, A088T-N109G-A116T-G131H-T158S-N243V-K256R, A088T-N109G-A116T-G131H-T158S-N243V-K256R-L257G, A088T-N109G-A116T-G131H-T158S-N243V-S248N, A088T-N109G-A116T-G131H-T158S-N243V-S248N-K256R, A088T-N109G-A116T-G131H-T158S-S248N-K256R-L257G, A088T-N109G-A116T-G131H-T158S-S248N-L257G, A088T-N109G-A116T-N218S, A088T-N109G-A116T-N218S-L257G, A088T-N109G-A116T-N218S-N243V, A088T-N109G-A116T-N218S-N243V-S248N-L257G, A088T-N109G-A116T-N218S-S248N-K256R, A088T-N109G-A116T-N218T-K256R, A088T-N109G-A116T-N218T-K256R-L257G, A088T-N109G-A116T-N243V, A088T-N109G-A116T-N243V-K256R-L257G, A088T-N109G-A116T-N243V-K256R-L257G-N269D, A088T-N109G-A116T-S248N-K256R, A088T-N109G-A116T-T158S, A088T-N109G-A116T-T158S-N218S-L257G, A088T-N109G-A116T-T158S-N218S-N243V, A088T-N109G-A116T-T158S-N218S-N243V-K256R, A088T-N109G-A116T-T158S-N218S-N243V-K256R-L257G, A088T-N109G-A116T-T158S-N218S-N243V-K256R-L257G, A088T-N109G-A116T-T158S-N218S-N243V-L257G, A088T-N109G-A116T-T158S-N218S-S248N, A088T-N109G-A116T-T158S-N243V, A088T-N109G-A116T-T158S-N243V-K256R, A088T-N109G-A116T-T158S-N243V-K256R-L257G, A088T-N109G-A116T-T158S-S248N-L257G, A088T-N109G-G131H-L257G, A088T-N109G-G131H-N218S-K256R-L257G, A088T-N109G-G131H-N218S-N243V-K256R, A088T-N109G-G131H-N218S-N243V-L257G, A088T-N109G-G131H-N218S-N243V-S248N-K256R-L257G, A088T-N109G-G131H-N243V, A088T-N109G-G131H-N243V-L257G, A088T-N109G-G131H-N243V-S248N-K256R, A088T-N109G-G131H-N243V-S248N-L257G, A088T-N109G-G131H-S248N-L257G, A088T-N109G-G131H-T158S-L257G, A088T-N109G-G131H-T158S-N218S-N243V-S248N-K256R, A088T-N109G-G131H-T158S-N243V, A088T-N109G-G131H-T158S-N243V-K256R, A088T-N109G-G131H-T158S-N243V-K256R-L257G, A088T-N109G-G131H-T158S-N243V-L257G, A088T-N109G-L257G, A088T-N109G-N218S-K256R, A088T-N109G-N218S-N243V-S248N-L257G, A088T-N109G-N218S-S248N-K256R-L257G, A088T-N109G-N243V-K256R-L257G, A088T-N109G-N243V-S248N-K256R-L257G, A088T-N109G-N243V-S248N-L257G-I268V, A088T-N109G-S248N-K256R-L257G, A088T-N109G-T158S-N218S-K256R, A088T-N109G-T158S-N218S-N243V-L257G, A088T-N109G-T158S-N218S-N243V-L257G, A088T-N109G-T158S-N243V-K256R-I268V, A088T-N109G-T158S-N243V-S248N-Q275R, A088T-N218S-N243V, A088T-N218S-N243V-S248N-K256R-L257G, A088T-N218S-S248N, A088T-N218S-S248N-L257G, A088T-N243V, A088T-N243V, A088T-N243V-K256R, A088T-N243V-L257G, A088T-S145T-T158S-S248N, A088T-T158S-L257G, A088T-T158S-N218S-S248N-L257G, A088T-T158S-N243V-K256R-L257G-Q271H, A088T-T158S-S248N, A088T-V143A-T158S-K256R, A116T-G131H-K256R, A116T-G131H-N218S, A116T-G131H-N243V, A116T-G131H-N243V-K256R, AI16T-G131H-N243V-L257G, A116T-G131H-S248N-K256R, A116T-G131H-T158S-N218S-I234T-N243V-S248N-K256R, A116T-G131H-T158S-N243V-L257G, A116T-G131H-T158S-N243V-S248N-K256R, A116T-G131H-V143F-T158S-N218S, A116T-L257G, A116T-N218S, A116T-N218S-L257G, A116T-N218S-N243V-L257G, A116T-N243V, A116T-N243V-K256R, A116T-N243V-S248N, A116T-N243V-S248N-K256R-L257G, A116T-S248N, A116T-T158S, A116T-T158S-N218S-N243V, A116T-T158S-N218S-S248N, A116T-T158S-N243V, A116T-T158S-N243V-K256R, A116T-T158S-N243V-L257G, A116T-T158S-N243V-S248N, A116T-T158S-S248N-K256R-L257G, A116T-V1491-T158S-N243V-S248N-K256R-Q271H, G131H-N218S-N243V-L257G, G131H-N243V, G131H-N243V-S248N-K256R, G131H-T158S, G131H-T158S-N218S-N243V-K256R, G131H-T158S-N243V-K256R-L257G, G131H-T158S-N243V-S248N-L257G, N109G-A116T-G131H-N218S-K256R-L257G, N109G-A116T-G131H-N218S-L257G, N109G-A116T-G131H-N218S-N243V-K256R-L257G, N109G-A116T-G131H-N218S-S248N-K256R, N109G-A116T-G131H-N243V-K256R, N109G-A116T-G131H-N243V-L257G, N109G-A116T-G131H-N243V-S248N-K256R-L257G, N109G-A116T-G131H-S248N, N109G-A116T-G131H-S248N-I268V, N109G-A116T-G131H-T158S-N218S-N243V-S248N-K256R, N109G-A116T-G131H-T158S-N218S-S248N-L257G, N109G-A116T-G131H-T158S-S248N, N109G-A116T-G131H-T158S-S248N-K256R, N109G-A116T-N218S, N109G-A116T-N218S-N243V-K256R, N109G-A116T-N218S-N243V-K256R-L257G, N109G-A116T-N218S-S248N-L257G, N109G-A116T-N243V-K256R, N109G-A116T-N243V-S248N-K256R-L257G, N109G-A116T-S248N-L257G, N109G-A116T-T158S-G211V-N243V-S248N-K256R, N109G-A116T-T158S-K256R-L257G, N109G-A116T-T158S-N218S, N109G-A116T-T158S-N218S-N243V-K256R-L257G, N109G-A116T-T158S-N218S-N243V-L257G, N109G-A116T-T158S-N218S-N243V-S248N-L257G, N109G-A116T-T158S-N218S-S248N-K256R-L257G, N109G-A116T-T158S-N243V, N109G-A116T-T158S-Q275R, N109G-G131H-A137V-T158S-N218S-S248N, N109G-G131H-N218S-K237N, N109G-G131H-N218S-N243V-K256R-L257G, N109G-G131H-N218S-S248N-K256R, N109G-G131H-N243V-K256R-L257G, N109G-G131H-S145F-N218S-N243V-K256R-L257G, N109G-G131H-S248N-K256R, N109G-G131H-S248N-L257G, N109G-G131H-T158S-K256R, N109G-G131H-T158S-N218S-N243V-K256R, N109G-G131H-T158S-N243V, N109G-G131H-T158S-N243V-K256R-L257G, N109G-G131H-T158S-N243V-L257G, N109G-G131H-T158S-S248N-L257G, N109G-G131H-T158S-S248N-Q271R, N109G-N218S-

L257G, N109G-N218S-N243V, N109G-N243V-K256R-L257G, N109G-N243V-S248N-K256R-L257G, N109G-T158S-I268V, N109G-T158S-K256R, N109G-T158S-N218S-N243V-K256R-L257G, N109G-T158S-N218S-S248N-L257G, N109G-T158S-N243V, N109G-T158S-N243V-K256R-L257G, N109G-T158S-N243V-S248N, N109S-A116T-S248N, N218S, N218S-N243V-S248N-K256R-L257G, N218S-S248N-L257G, N243V, N243V-K256R, N243V-S248N-K256R, N243V-S248N-K256R-L257G, S105P-A116T-T158S-N218S-N243V-S248N-K256R, S248N, T158S-N243V-K256R, and T158S-N243V-L257G, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN', BPN'-v3, and BPN'-v36, and a greater PI value than that of BPN', BPN'-v3 and BPN'-v36 in this assay.

[0369] The following BPN'-v36 variants were determined to have a PI value of about 1.0 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A088T, A088T-A116T-G131H-L257G, A088T-A116T-G131H-N218S-A274T, A088T-A116T-G131H-N218S-K256R, A088T-A116T-G131H-N218S-K256R-L257G, A088T-A116T-G131H-N218S-N243V, A088T-A116T-G131H-N218S-N243V-L257G, A088T-A116T-G131H-N218S-N243V-S248N-K256R, A088T-A116T-G131H-N218S-N243V-S248N-K256R-L257G, A088T-A116T-G131H-N218S-N243V-S248N-L257G, A088T-A116T-G131H-N218S-S248N, A088T-A116T-G131H-N218S-S248N-L257G, A088T-A116T-G131H-N243V, A088T-A116T-G131H-N243V-K256R, A088T-A116T-G131H-N243V-S248N, A088T-A116T-G131H-N243V-S248N-A274V, A088T-A116T-G131H-N243V-S248N-K256R-L257G, A088T-A116T-G131H-N243V-S248N-K256R-L257G, A088T-A116T-G131H-N243V-S248N-L257G, A088T-A116T-G131H-S248N-K256R, A088T-A116T-G131H-S248N-K256R-L257G, A088T-A116T-G131H-T158S-K256R, A088T-A116T-G131H-T158S-K256R-L257G, A088T-A116T-G131H-T158S-N218S-K256R, A088TA116T-G131H-T158S-N218S-K256R-L257G, A088T-A116T-G131H-T158S-N218S-L257G, A088TA116T-G131H-T158S-N218S-N243V-K256R, A088T-A116T-G131H-T158S-N218S-N243V-K256R-L257G, A088T-A116T-G131H-T158S-N218S-N243V-L257G, A088T-A116T-G131H-T158S-N218S-N243V-S248N, A088T-A116T-G131H-T158S-N218S-N243V-S248N, A088T-A116T-G131H-T158S-N218S-N243V-S248N-K256R, A088T-A116T-G131H-T158S-N243V-K256R, A088T-A116T-G131H-T158S-N243V-K256R, A088T-A116T-G131H-T158S-N243V-K256R-L257G, A088T-A116T-G131H-T158S-N243V-L257G, A088T-A116T-G131H-T158S-N243V-S248N, A088T-A116T-G131H-T158S-N243V-S248N-K256R-L257G, A088T-A116T-G131H-T158S-N243V-S248N-K256R-L257G, A088TA116T-G131H-T158S-N243V-S248N-L257G, A088T-A116T-G131H-T158S-S248N-K256R, A088TA116T-G131H-T158S-S248N-K256R-L257G, A088T-A116T-K256R, A088T-A116T-L257G, A088TA116T-N218S, A088T-A116T-N218S-N243V-K256R, A088T-A116T-N218S-N243V-N269D, A088TA116T-N218S-N243V-S248N, A088T-A116T-N218S-N243V-S248N-K256R-L257G, A088T-A116T-N218S-N243V-S248N-L257G, A088T-A116T-N218S-S248N, A088T-A116T-N218S-S248N-L257G, A088T-A116T-N243V-K256R-L257G, A088T-A116T-N243V-S248N-K256R, A088T-A116T-S248N, A088T-A116T-S248N-K256R, A088T-A116T-T158S-A216S-N218S-N243V-K256R-L257G, A088TA116T-T158S-N218S-K256R, A088T-A116T-T158S-N218S-N243V-K256R, A088T-A116T-T158S-N218S-N243V-S248N-K256R-L257G, A088T-A116T-T158S-N218S-S248N, A088T-A116T-T158S-N243V-K256R, A088T-A116T-T158S-N243V-S248N, A088T-A116T-T158S-N243V-S248N-K256R, A088T-A116T-T158S-N243V-S248N-L257G, A088T-A116T-T158S-S248N, A088T-G131D-T158S-N243V-S248N, A088T-G131H-A138V-N218S-L257G, A088T-G131H-K256R, A088T-G131H-N218S-N243V-K256R, A088T-G131H-N218S-N243V-K256R, A088T-G131H-N218S-S248N, A088T-G131H-N218S-S248N-K256R-L257G, A088T-G131H-N218T-L257G, A088T-G131H-N243V-L257G, A088T-G131H-N243V-S248N-K256R, A088T-G131H-S248N, A088T-G131H-S248N-L257G, A088T-G131H-T158S-K256R, A088T-G131H-T158S-K256R-L257G, A088T-G131H-T158S-N218S, A088T-G131H-T158S-N218S, A088T-G131H-T158S-N218S-K256R-L257G, A088T-G131H-T158S-N218S-N243V-K256R, A088T-G131H-T158S-N218S-N243V-K256R, A088T-G131H-T158S-N218S-N243V-S248N, A088T-G131H-T158S-N218S-S248N, A088T-G131H-T158S-N218S-S248N-K256R, A088T-G131H-T158S-N218S-S248N-L257G-I268V, A088T-G131H-T158S-N243V-K256R, A088T-G131H-T158S-N243V-K256R-L257G, A088T-G131H-T158S-N243V-S248N, A088T-G131H-T158S-N243V-S248N-K256R-L257G, A088T-G131H-T158S-S248N, A088T-G131H-T158S-S248N-K256R, A088T-N109G-A116T-G131H-K256R, A088T-N109G-A116T-G131H-K256R-L257G, A088T-N109G-A116T-G131H-K256R-L257G, A088T-N109G-A116T-G131H-N218S-K256R, A088T-N109G-A116T-G131H-N218S-K256R, A088T-N109G-A116T-G131H-N218S-K256R-L257G, A088T-N109G-A116T-G131H-N218S-N243V-L257G, A088T-N109G-A116T-G131H-N218S-S248N, A088T-N109G-A116T-G131H-N218S-S248N-L257G, A088T-N109G-A116T-G131H-N243V-K256R, A088T-N109G-A116T-G131H-N243V-K256R-L257G, A088T-N109G-A116T-G131H-N243V-S248N-K256R, A088T-N109G-A116T-G131H-N243V-S248N-K256R, A088T-N109G-A116T-G131H-S248N-K256R-L257G, A088T-N109G-A116T-G131H-S248N-K256R-L257G, A088T-N109G-A116T-G131H-S248N-L257G, A088T-N109G-A116T-G131H-T158S-K256R, A088T-N109G-A116T-G131H-T158S-L257G, A088T-N109G-A116T-G131H-T158S-N218S, A08ST-N109G-A116T-G131H-T158S-N218S-L257G, A088T-N109G-A116T-G131H-T158S-N218S-N243V, A088T-N109G-A116T-G131H-T158S-N218S-N243V-K256R, A088T-N109G-A116T-G131H-T158S-N218S-N243V-L257G, A088T-N109G-A116T-G131H-T158S-N218S-N243V-S248N-K256R, A088T-N109G-A116T-G131H-T158S-N218S-N243V-S248N-K256R, A088T-N109G-A116T-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A088T-N109G-A116T-G131H-T158S-N218S-S248N, A088T-N109G-A116T-G131H-T158S-N218T-K256R, A088T-N109G-A116T-G131H-T158S-N243V, A088T-N109G-A116T-G131H-T158S-N243V-S248N, A088T-N109G-A116T-G131H-T158S-N243V-S248N-K256R-L257G, A088T-N109G-A116T-G131H-T158S-N243V-S248N-L257G, A088T-N109G-A116T-G131H-V149A-T158S-N218S-K256R, A088T-N109G-A116T-K256R, A088T-N109G-A116T-N218S-K256R, A088T-

N109G-A116T-N218S-N243V, A088T-N109G-A116T-N218S-N243V-K256R-L257G, A088T-N109G-A116T-N218S-N243V-L257G, A088T-N109G-A116T-N218S-N243V-S248N-K256R, A088T-N109G-A116T-N218S-S248N, A088T-N109G-A116T-N218S-S248N-K256R-L257G, A088T-N109G-A116T-N243V-K256R, A088T-N109G-A116T-N243V-S248N-K256R-L257G, A088T-N109G-A116T-N243V-S248N-L257G, A088T-N109G-A116T-N243V-S248N-L257G, A088T-N109G-A116T-S248N, A088T-N109G-A116T-S248N-K256R-L257G, A088T-N109G-A116T-S248N-L257G, A088T-N109G-A116T-T158S, A088T-N109G-A116T-T158S-K256R, A088T-N109G-A116T-T158S-N218S, A088T-N109G-A116T-T158S-N218S-L257G, A088T-N109G-A116T-T158S-N218S-N243V-S248N, A088T-N109G-A116T-T158S-N218S-N243V-S248N, A088T-N109G-A116T-T158S-N218S-N243V-S248N-K256R, A088T-N109G-A116T-T158S-N218S-N243V-S248N-K256R-L257G, A088T-N109G-A116T-T158S-N218S-S248N, A088T-N109G-A116T-T158S-N218S-S248N-K256R, A088T-N109G-A116T-T158S-N218S-S248N-L257G, A088T-N109G-A116T-T158S-N218S-S248N-L257G, A088T-N109G-A116T-T158S-N243V-K256R, A088T-N109G-A116T-T158S-N243V-S248N, A088T-N109G-A116T-T158S-S248N, A088T-N109G-A116T-T158S-S248N-K256R, A088T-N109G-A137E-T158S-N218S-N243V-S248N-K256R-L257G, A088T-N109G-G131H-A152S-T158S-N218S-S248N-K256R, A088T-N109G-G131H-K256R-L257G, A088T-N109G-G131H-N218S, A088T-N109G-G131H-N218S, A088T-N109G-G131H-N218S-K256R, A088T-N109G-G131H-N218S-K256R, A088T-N109G-G131H-N218S-L257G, A088T-N109G-G131H-N218S-N243V, A088T-N109G-G131H-N218S-N243V-K256R, A088T-N109G-G131H-N218S-N243V-K256R-L257G, A088T-N109G-G131H-N218S-N243V-S248N-K256R, A088T-N109G-G131H-N218S-S248N, A088T-N109G-G131H-N218S-S248N-K256R, A088T-N109G-G131H-N218S-S248N-K256R, A088T-N109G-G131H-N218S-S248N-K256R-L257G, A088T-N109G-G131H-N243V-K256R, A088T-N109G-G131H-N243V-K256R-L257G, A088T-N109G-G131H-N243V-K256R-L257G, A088T-N109G-G131H-N243V-L257G, A088T-N109G-G131H-N243V-S248N-K256R, A088T-N109G-G131H-S248N-K256R, A088T-N109G-G131H-S248N-L257G, A088T-N109G-G131H-T158S, A088T-N109G-G131H-T158S-K256R, A088T-N109G-G131H-T158S-K256R-L257G, A088T-N109G-G131H-T158S-N218S-K256R, A088T-N109G-G131H-T158S-N218S-K256R, A088T-N109G-G131H-T158S-N218S-L257G, A088T-N109G-G131H-T158S-N218S-L257G, A088T-N109G-G131H-T158S-N218S-N243V, A088T-N109G-G131H-T158S-N218S-N243V-K256R, A088T-N109G-G131H-T158S-N218S-N243V-S248N, A088T-N109G-G131H-T158S-N218S-S248N-L257G, A088T-N109G-G131H-T158S-N243V-K256R-L257G, A088T-N109G-G131H-T158S-N243V-S248N, A088T-N109G-G131H-T158S-N243V-S248N-K256R-L257G, A088T-N109G-G131H-T158S-N243V-S248N-K256R-L257G, A088T-N109G-G131H-T158S-N243V-S248N-L257G, A088T-N109G-G131H-T158S-N243V-S248N-L257G, A088T-N109G-G131H-T158S-S248N, A088T-N109G-G131H-T158S-S248N-L257G, A088T-N109G-G131H-V149A-K256R-L257G, A088T-N109G-G154A-N155P-E156T-G157L-T158M-S159E-G160E-S161L, A088T-N109G-K256R-L257G, A088T-N109G-N218S-K256R, A088T-N109G-N218S-N243V-L257G, A088T-N109G-N218S-N243V-S248N-K256R, A088T-N109G-N218S-N243V-S248N-K256R, A088T-N109G-N218S-S248N, A088T-N109G-N218S-S248N-L257G, A088T-N109G-N218S-S248N-L257G, A088T-N109G-N243V-S248N-K256R, A088T-N109G-S248N, A088T-N109G-S248N, A088T-N109G-T158S-N218S, A088T-N109G-T158S-N218S-K256R-Q271H, A088T-N109G-T158S-N218S-N243V, A088T-N109G-T158S-N218S-N243V-K256R-Q275R, A088T-N109G-T158S-N218S-N243V-S248N-K256R-L257G, A088T-N109G-T158S-N218S-S248N, A088T-N109G-T158S-N218S-S248N-K256R, A088T-N109G-T158S-N218S-S248N-N269D, A088T-N109G-T158S-N243V-K256R, A088T-N109G-T158S-N243V-S248N-K256R, A088T-N109G-T158S-N243V-S248N-K256R-L257G-N269D, A088T-N109G-T158S-N243V-S248N-L257G, A088T-N109G-T158S-S248N-K256R-L257G, A088T-N109G-T158S-S248N-L257G, A088T-N109G-V147A-N218S-N243V-K256R, A088T-N218S, A088T-N218S-K256R, A088T-N218S-L257G-I268V, A088T-N218S-N243V, A088T-N218S-N243V-K256R, A088T-N218S-N243V-K256R-L257G, A088T-N218S-N243V-L257G, A088T-N218S-N243V-S248N-K256R-L257G, A088T-N218S-N243V-S248N-L257G, A088T-N218S-N243V-S248N-N269S, A088T-N218S-S248N-K256R, A088T-N243V-S248N, A088T-N243V-S248N-K256R, A088T-N243V-S248N-K256R, A088T-N243V-S248N-K256R-L257G, A088T-N243V-S248N-L257G, A088T-S248N, A088T-S248N, A088T-S248N-K256R-L257G, A088T-S248N-L257G, A088T-S248N-L257G-I268V, A088T-T158S, A088T-T158S, A088T-T158S-N218S, A088T-T158S-N218S-K256R, A088T-T158S-N218S-L257G, A088T-T158S-N218S-N243V-K256R-I268V, A088T-T158S-N218S-N243V-K256R-L257G, A088T-T158S-N218S-N243V-S248N-L257G, A088T-T158S-N218S-S248N, A088T-T158S-N243V-K256R, A088T-T158S-N243V-K256R-L257G, A088T-T158S-N243V-S248N-K256R, A088T-T158S-N243V-S248N-L257G, A088T-T158S-N243V-S248N-L257G, A088T-T158S-S248N, A088T-T158S-S248N-L257G, A088T-V147A-K256R, A098S-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A116T-G131H-N218S-K256R, A116T-G131H-N218S-K256R-L257G, A116T-G131H-N218S-L257G, A116T-G131H-N218S-N243V-S248N-L257G, A116T-G131H-N218S-S248N-K256R-L257G, A116T-G131H-N243V-S248N, A116T-G131H-N243V-S248N-L257G, A116T-G131H-T158S-A231V-N243V-L257G, A116T-G131H-T158S-K256R, A116T-G131H-T158S-K256R-L257G, A116T-G131H-T158S-N218S-K256R, A116T-G131H-T158S-N218S-K256R-L257G, A116T-G131H-T158S-N218S-N243V, A116T-G131H-T158S-N218S-N243V-K256R, A116T-G131H-T158S-N218S-N243V-K256R-L257G, A116T-G131H-T158S-N218S-N243V-L257G, A116T-G131H-T158S-N218S-N243V-S248N-K256R, A116T-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A116T-G131H-T158S-N218S-N243V-S248N-L257G, A116T-G131H-T158S-N218S-S248N-K256R, A116T-G131H-T158S-N218T-L257G, A116T-G131H-T158S-S248N-K256R, A116T-G131H-T158S-S248N-L257G, A116T-N218S-K256R, A116T-N218S-K256R-L257G, A116T-N218S-N243V-S248N-K256R, A116T-N218S-N243V-S248N-K256R-L257G, A116T-N218S-N243V-S248N-L257G, A116T-N218S-S248N, A116T-N218S-S248N-K256R, A116T-N218S-S248N-L257G, A116T-N243V-S248N-L257G, A116T-S248N-L257G, A116T-T158S-L257G-Q271R, A116T-T158S-N218S-L257G, A116T-T158S-N218S-N243V-K256R-L257G, A116T-T158S-N218S-S248N-K256R, A116T-T158S-N218S-S248N-K256R-L257G, A116T-T158S-

N243V-S248N-K256R, A116T-T158S-N243V-S248N-K256R-L257G, G024S-G053S-N078S-G097A-N101S-A128S, G131H, G131H-N218S, G131H-N218S-K256R, G131H-N218S-N243V-K256R, G131H-N218S-N243V-K256R-L257G, G131H-N218S-N243V-S248N, G131H-N218S-N243V-S248N-K256R, G131H-N218S-S248N-K256R-L257G, G131H-N218S-S248N-L257G, G131H-N243V-K256R, G131H-N243V-K256R-L257G, G131H-N243V-S248N-K256R-L257G, G131H-S248N-K256R, G131H-T158S-K256R, G131H-T158S-K256R-L257G, G131H-T158S-N218S-K256R, G131H-T158S-N218S-K256R-L257G, G131H-T158S-N218S-N243V-K256R-L257G, G131H-T158S-N218S-N243V-S248N, G131H-T158S-N218S-S248N-K256R-L257G, G131H-T158S-N218S-S248N-L257G, G131H-T158S-N243V-K256R, G131H-T158S-N243V-S248N-K256R, G131H-T158S-S248N-L257G, G131H-V147I-N218S-S248N-K256R, I107T-N109G-A116T-G131H-T158S-N218S-N243V-S248N-K256R-L257G, I107T-N109G-G131H-N218S-N243V-K256R-L257G, N109G, N109G-A116T-G131H, N109G-A116T-G131H-A144V-T158S-S248N-K256R-L257G, N109G-A116T-G131H-K256R-L257G, N109G-A116T-G131H-L257G, N109G-A116T-G131H-N218S-N243V-K256R, N109G-A116T-G131H-N218S-N243V-S248N-K256R, N109G-A116T-G131H-N243V, N109G-A116T-G131H-N243V-K256R-L257G, N109G-A116T-G131H-N243V-S248N, N109G-A116T-G131H-N243V-S248N-K256R, N109G-A116T-G131H-S248N-K256R, N109G-A116T-G131H-T158S-K256R, N109G-A116T-G131H-T158S-K256R-L257G, N109G-A116T-G131H-T158S-L257G, N109G-A116T-G131H-T158S-N218S, N109G-A116T-G131H-T158S-N218S-K256R-L257G, N109G-A116T-G131H-T158S-N218S-L257G, N109G-A116T-G131H-T158S-N218S-N243V-S248N-K256R-L257G, N109G-A116T-G131H-T158S-N218S-S248N-K256R, N109G-A116T-G131H-T158S-N218S-S248N-K256R-L257G, N109G-A116T-G131H-T158S-N243V-L257G, N109G-A116T-G131H-T158S-S248N-L257G, N109G-A116T-G131H-V147A-T158S-N218S-K256R-L257G, N109G-A116T-G131H-V149AT158S-N218S-N243V-S248N-L257G, N109G-A116T-K256R, N109G-A116T-N218S-K256R, N109G-A116T-N218S-N243V, N109G-A116T-N218S-N243V-L257G, N109G-A116T-N218S-N243V-S248N-I268V, N109G-A116T-N218S-N243V-S248N-K256R-L257G, N109G-A116T-N218S-N243V-S248N-L257G, N109G-A116T-N243V-S248N, N109G-A116T-N243V-S248N-K256R, N109G-A116T-N243V-S248N-L257G, N109G-A116T-S248N-K256R, N109G-A116T-T158S, N109G-A116T-T158S-N218S-N243V-S248N-K256R, N109G-A116T-T158S-N218S-N243V-S248N-K256R-L257G, N109G-A116T-T158S-N218S-S248N-K256R, N109G-A116T-T158S-N243V-L257G, N109G-A116T-T158S-N243V-S248N, N109G-A116T-T158S-S248N, N109G-A116T-T158S-S248N-K256R, N109G-G131H, N109G-G131H-K256R, N109G-G131H-N218S-K256R, N109G-G131H-N218S-K256R-L257G, N109G-G131H-N218S-N243V-L257G, N109G-G131H-N218S-N243V-S248N-K256R, N109G-G131H-N218S-N243V-S248N-K256R-L257G, N109G-G131H-N218S-N243V-S248N-L257G, N109G-G131H-N218S-S248N-L257G, N109G-G131H-N243V, N109G-G131H-N243V-K256R, N109G-G131H-N243V-S248N, N109G-G131H-N243V-S248N-K256R-L257G, N109G-G131H-N243V-S248N-L257G, N109G-G131H-T158S-N218S-K256R-L257G, N109G-G131H-T158S-N218S-L257G, N109G-G131H-T158S-N218S-N243V, N109G-G131H-T158S-N218S-N243V-K256R-L257G, N109G-G131H-T158S-N218S-N243V-S248N, N109G-G131H-T158S-N218S-N243V-S248N-K256R-L257G, N109G-G131H-T158S-N218S-N243V-S248N-L257G, N109G-G131H-T158S-N218S-S248N-K256R-L257G, N109G-G131H-T158S-N243V-K256R-I268V, N109G-G131H-T158S-N243V-S248N, N109G-G131H-T158S-N243V-S248N-K256R, N109G-G131H-T158S-N243V-S248N-L257G, N109G-G131H-T158S-S248N, N109G-G131H-T158S-S248N-K256R-L257G, N109G-K141E-N218S-S248N-L257G, N109G-N218S, N109G-N218S-N243V-K256R, N109G-N218S-N243V-L257G, N109G-N218S-N243V-S248N-S260F, N109G-N218S-S248N, N109G-N218S-S248N-K256R, N109G-N243V-K256R, N109G-N243V-S248N, N109G-N243V-S248N-K256R, N109G-N243V-S248N-L257G, N109G-N243V-S248N-L257G-Q275R, N109G-S182F-S204F-S207L-N218S-S236F-S248N-L257G, N109G-S248N-K256R, N109G-T158S-K256R-L257G, N109G-T158S-L257G, N109G-T158S-N218S-N243V-K256R, N109G-T158S-N218S-N243V-S248N, N109G-T158S-N218S-N243V-S248N-L257G, N109G-T158S-N243V-K256R, N109G-T158S-N243V-S248N-K256R, N109G-T158S-N243V-S248N-L257G, N109G-T158S-S248N-L257G, N218S-N243V-L257G, N218S-N243V-S248N-K256R, N243V-K256R-L257G, N243V-S248N-L257G-Q271R, P057Q-A088T-N109G-A116T-G131H-T158S-N218S-S248N, S003P-A116T-N218S-K256R, S003P-N109G-G131H-N218S-N243V-S248N-K256R-L257G, S248N-K256R-L257G, T158S-K256R-L257G, T158S-N218S-A272V, T158S-N218S-K256R-L257G, T158S-N218S-L233S, T158S-N218S-N243V, T158S-N218S-N243V-K256R-L257G, T158S-N218S-N243V-L257G, T158S-N218S-N243V-S248N-K256R, T158S-N218S-S248N-K256R, T158S-N243V, T158S-N243V-K256R-L257G, T158S-N243V-S248N, T158S-N243V-S248N-K256R-N269D, and V004A-N109G-A116T-T158S-N218S-S248N-L257G, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0370] The following BPN'-v36 variants were determined to have a PI value of about 0.9 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A088T-A098S-N218S-K256R, A088T-A116T-G131H-K256R, A088T-A116T-G131H-K256R-L257G-L267M, A088T-A116T-G131H-N218S-N243V-K256R, A088T-A116T-G131H-N218S-N243V-K256R-L257G, A088T-A116T-G131H-N218S-N243V-S248N, A088T-A116T-G131H-N218S-S248N, A088T-A116T-G131H-N218S-S248N-K256R, A088T-A116T-G131H-N218S-S248N-K256R, A088T-A116T-G131H-N243V, A088T-A116T-G131H-S248N, A088T-A116T-G131H-S248N-L257G, A088T-A116T-G131H-S248N-L257G, A088T-A116T-G131H-T158S-N218S, A088T-A116T-G131H-T158S-N218S-K256R-L257G, A088T-A116T-G131H-T158S-N218S-N243V-S248N-K256R, A088T-A116T-G131H-T158S-N218S-N243V-S248N-L257G, A088T-A116T-G131H-T158S-N218S-S248N, A088T-A116T-G131H-T158S-N218S-S248N-K256R, A088T-A116T-K256R-L257G, A088T-A116T-N218S-I268V, A088T-A116T-N218S-K256R, A088T-A116T-N218S-N243V-Q271R, A088T-A116T-N218S-N243V-S248N-K256R, A088T-

A116T-N218S-N243V-S248N-K256R-Q275R, A088T-A116T-N218S-S248N, A088T-A116T-N218S-S248N-K256R, A088T-A116T-N243V-S248N-K256R, A088T-A116T-T158S, A088T-A116T-T158S-N218S-K256R, A088T-A116T-T158S-N218S-N243V-L257G, A088T-A116T-T158S-N218S-N243V-S248N-L257G, A088T-A116T-T158S-N218S-S248N, A088T-A116T-T158S-N218S-S248N-K256R, A088TA116T-T158S-N218S-S248N-K256R-L257G, A088T-A116T-T158S-N218S-S248N-L257G, A088TA116T-T158S-S248N-L257G, A088T-G131H, A088T-G131H, A088T-G131H-N218S-K237R-K256R-L257G, A088T-G131H-N218S-K256R, A088T-G131H-N218S-K256R-L257G, A088T-G131H-N218S-N243V-K256R-L257G, A088T-G131H-N218S-N243V-L257G, A088T-G131H-N218S-N243V-L257G, A088T-G131H-N218S-N243V-S248N, A088T-G131H-N218S-N243V-S248N-K256R, A088T-G131H-N218S-N243V-S248N-K256R-L257G, A088T-G131H-N218S-N243V-S248N-K256R-L257G, A088T-G131H-N218S-S248N, A088T-G131H-N243V, A088T-G131H-N243V-K256R, A088T-G131H-N243V-K256R-L257G, A088T-G131H-N243V-S248N, A088T-G131H-N243V-S248N, A088T-G131H-N243V-S248N-K256R, A088T-G131H-S248N, A088T-G131H-S248N-K256R, A088T-G131H-T158S-N218S-K256R-L257G, A088T-G131H-T158S-N218S-L257G, A088T-G131H-T158S-N218S-N243V, A088T-G131H-T158S-N218S-N243V-K256R-L257G, A088T-G131H-T158S-N218S-N243V-S248N-K256R, A088T-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A088T-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A088T-G131H-T158S-N218S-N243V-S248N-L257G, A088T-G131H-T158S-N218S-S248N-K256R, A088T-G131H-T158S-N218S-S248N-K256R-L257G, A088T-G131H-T158S-S248N-K256R-L257G, A088T-L257G, A088T-N109G-A116T-G131H-N218S-L257G, A088T-N109G-A116T-G131H-N218S-N243V-S248N-K256R, A088T-N109G-AI16T-G131H-N218S-N243V-S248N-K256R-L257G, A088T-N109G-A116T-G131H-N218S-N243V-S248N-N269D, A088T-N109G-A116T-G131H-N218S-N243V-S248N-Q275R, A088T-N109G-A116T-G131H-N218S-S248N-K256R-L257G, A088T-N109G-A116T-G131H-N243V-S248N, A088T-N109G-A116T-G131H-T158S, A088T-N109G-A116T-G131H-T158S-N218S-L257G-I268V, A088T-N109G-A116T-G131H-T158S-N218S-N243V-K256R, A088T-N109G-A116T-G131H-T158S-N218S-N243V-K256R-L257G, A088T-N109G-A116T-G131H-T158S-N218S-N243V-S248N, A088T-N109G-A116T-G131H-T158S-N218S-N243V-S248N-L257G, A088T-N109G-A116T-G131H-T158S-N218S-S248N-L257G, A088T-N109G-A116T-G131H-T158S-S248N, A088T-N109G-A116T-G131H-T158S-S248N, A088T-N109G-A116T-G131H-W241L-S248N-K256R-L257G, A088T-N109G-A116T-K256R, A088T-N109G-A116T-N218S-K256R-L257G, A088T-N109G-A116T-N218S-N243V-S248N-K256R, A088T-N109G-A116T-N218S-S248N, A088T-N109G-A116T-T158S-N218S, A088T-N109G-A116T-T158S-N218S-K256R-L257G, A088T-N109G-A116T-T158S-N218S-N243V-S248N-L257G, A088T-N109G-A116T-T158S-N243V-S248N, A088T-N109G-A116T-T158S-N243V-S248N-K256R, A088T-N109G-G131H-A138V-T158S-N218S-N243V-S248N-L257G, A088T-N109G-G131H-K256R-L257G, A088T-N109G-G131H-N218S-N243V, A088T-N109G-G131H-N218S-N243V-S248N-L257G, A088T-N109G-G131H-N218S-S248N-K256R-L257G, A088T-N109G-G131H-N218S-S248N-K256R-L257G-Q275R, A088T-N109G-G131H-N243V-S248N, A088T-N109G-G131H-N243V-S248N-K256R-L257G, A088T-N109G-G131H-T158S, A088T-N109G-G131H-T158S-L233S-N243V-S248N, A088T-N109G-G131H-T158S-N218S-N243V-K256R, A088T-N109G-G131H-T158S-N218S-N243V-K256R-L257G, A088T-N109G-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A088T-N109G-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A088T-N109G-G131H-T158S-N218S-N243V-S248N-L257G, A088T-N109G-G131H-T158S-N218S-S248N-K256R, A088T-N109G-G131H-T158S-N243V-S248N-K256R, A088T-N109G-G131H-T158S-S248N-K256R, A088T-N109G-G131H-T158S-S248N-K256R-L257G, A088T-N109G-G131H-V149A-K256R-L257G, A088T-N109G-N218S-K256R-L257G, A088T-N109G-N218S-N243V-K256R, A088T-N109G-N218S-N243V-L257G, A088T-N109G-N218S-N243V-S248N, A088T-N109G-N218S-N243V-S248N-K256R-L257G, A088T-N109G-N218S-S248N-K256R, A088T-N109G-N243V-K256R, A088T-N109G-N243V-S248N-K256R, A088T-N109G-S248N-K256R-L257G, A088T-N109G-T158S, A088T-N109G-T158S-K256R-L257G, A088T-N109G-T158S-N218S-N243V-K256R, A088T-N109G-T158S-N218S-N243V-K256R-L257G, A088T-N109G-T158S-N218S-N243V-S248N-K256R, A088T-N109G-T158S-N218S-N243V-S248N-L257G, A088T-N109G-T158S-N218S-S248N, A088T-N109G-T158S-S248N, A088T-N109G-T158S-S248N, A088T-N218S-N243V-K256R, A088T-N218S-N243V-L257G, A088T-N218S-N243V-S248N, A088T-N218S-N243V-S248N-K256R, A088T-N218S-N243V-S248N-K256R, A088T-N218S-S248N, A088T-N218S-S248N-L257G, A088T-S248N-K256R-L257G, A088T-T158S-K256R, A088T-T158S-N218S-N243V-K256R, A088T-T158S-N218S-N243V-L257G, A088T-T158S-N218S-N243V-S248N, A088T-T158S-N218S-N243V-S248N-K256R, A088T-T158S-N218S-N243V-S248N-K256R-L257G, A088T-T158S-N218S-N243V-S248N-K256R-L257G, A088T-T158S-N218S-N243V-S248N-L257G, A088T-T158S-N218S-S248N, A088T-T158S-N218S-S248N-K256R, A088T-T158S-N218S-S248N-L257G, A088T-T158S-N218S-S248N-L257G-Q275K, A088T-T158S-N243V, A088T-T158S-N243V-K256R, A088T-T158S-N243V-S248N, A088T-T158S-S248N-K256R-L257G, A088T-T158S-S248N-L257G, A088T-V147I-N218S-N243V-K256R-L257G, A116T-G131H-L257G, A116T-G131H-N218S-N243V, A116T-G131H-N218S-N243V-K256R-L257G, A116T-G131H-N218S-N243V-S248N-K256R-L257G, A116T-G131H-N218S-S248N, A116T-G131H-N218S-S248N-K256R, A116T-G131H-N243V-S248N-K256R, A116T-G131H-T158S-N218S-N243V-S248N, A116T-G131H-T158S-N218S-S248N, A116T-G131H-T158S-N243V-K256R, A116T-G131H-V139I-N218S-N243V-S248N, A116T-K141E-N218S-N243V-S248N-K256R-L257G, A116T-K256R, A116T-N218S-N243V-S248N, A116T-N218T-N243V-S248N, A116T-N243V-K256R-L257G, A116T-S248N-K256R, A116T-T158S-N218S-K256R, A116T-T158S-N218S-K256R-L257G, A116T-T158S-N218S-N243V-L257G, A116T-T158S-N218S-N243V-S248N, A116T-T158S-N218S-S248N-L257G, G024S-G053S-N078S-G097A-N101S, G053S-A088T-N109G-A116T-G131H-T158S-G169S-N218S-S248N-K256R-L257G, G131H-K141R-T158S-N218S-K256R, G131H-K256R, G131H-N218S-K256R-L257G, G131H-N218S-N243V-S248N-L257G, G131H-N218S-S248N-K256R, G131H-N243V-S248N, G131H-N243V-S248N-L257G, G131H-T158S-N218S, G131H-T158S-N218S-N240H-N243V-S248N-K256R-L257G, G131H-T158S-N218S-N243V, G131H-

T158S-N218S-S248N-K256R-L257G-N269S, G131H-T158S-N243V-L257G, G131H-T158S-N243V-S248N, G131H-T158S-S248N, K256R, K256R-L257G, N109G-A116T-G131H-N218S-N243V, N109G-A116T-G131H-N218S-N243V-L257G, N109G-A116T-G131H-N218S-S248N-L257G, N109G-A116T-G131H-N218S-W241R-N243V-K256R, N109G-A116T-G131H-S248N-L257G, N109G-A116T-G131H-T158S-N218S-K256R, N109G-A116T-G131H-T158S-N218S-N243V-K256R-L257G, N109G-A116T-G131H-T158S-N218S-N243V-S248N, N109G-A116T-G131H-T158S-N218S-N243V-S248N-L257G, N109G-A116T-G131H-T158S-N243V-K256R-L257G, N109G-A116T-G131H-T158S-N243V-S248N-K256R, N109G-A116T-G131H-T158S-S248N-K256R-L257G, N109G-A116T-I234T-N243V-S248N-K256R-L257G, N109G-A116T-N218S-N243V-S248N, N109G-A116T-N243V-K256R-L257G, N109G-A116T-T158S-N218S-K237R-N243V-S248N, N109G-G131H-N218S-N243V-S248N, N109G-G131H-S248N, N109G-G131H-T158S, N109G-G131H-T158S-L257G, N109G-G131H-T158S-N218S-N243V-S248N-K256R, N109G-G131H-T158S-N218S-S248N-K256R, N109G-G131H-T158S-N218S-S248N-L257G, N109G-G131H-T158S-N243V-S248N-K256R-L257G, N109G-G131H-T158S-S248N-K256R, N109G-N218S-K256R-L257G, N109G-N218S-N243V-S248N-K256R, N109G-N218S-S248N-L257G, N109G-S248N, N109G-T158S-N218S, N109G-T158S-N218S-N243V, N109G-T158S-N243V-L257G, N218S-K256R, N218S-N243V-K256R, N218S-N243V-S248N, N218S-S248N, N218S-S248N-K256R, N243V-L257G, S003P-N109G-A116T-G131H-N218S-N243V-S248N, S003P-N109G-A116T-G131H-T158S-N218S-K256R, S105H-W106G-I107L-I108S-N109A-G110A-I111S-E112N-W113G-A114P, S248N-L257G, T158S, T158S-K256R, T158S-N218S, T158S-N218S-K256R, T158S-N218S-L233S-S248N, T158S-N218S-L257G, T158S-N218S-N243V-K256R, T158S-N218S-N243V-S248N, T158S-N218S-N243V-S248N-L257G, T158S-N218S-S248N-K256R-L257G, T158S-N218S-S248N-L257G, T158S-N243V-S248N-L257G, T158S-S248N, and V004L-A088T-G131H-T158S-N218S-S248N-L257G, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0371] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A015S-A088T-N109G-G131H-T158S-N218S-S248N, A088T-A098S-G131H-S248N-K256R-L257G, A088T-A116T-G131H-N218S-N243V-K256R-L257G, A088T-A116T-G131H-T158S-L257G, A088T-A116T-G131H-T158S-N218S-L257G, A088T-A116T-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A088T-A116T-G131H-T158S-N218S-N243V-S248N-L257G, A088T-A116T-N218S-L257G, A088T-A116T-T158S-K256R, A088T-A116T-T158S-S248N-K256R-L257G, A088T-G131H-K141E-N218S-N243V-S248N-L257G, A088T-G131H-K256R, A088T-G131H-N218S-K256R, A088T-G131H-N218S-N243V-S248N-K256R, A088T-G131H-N218S-N243V-S248N-L257G, A088T-G131H-N218S-S248N-K256R, A088T-G131H-N218S-S248N-K256R, A088T-G131H-T158S-S248N-K256R, A088T-G131H-T158S-S248N-K256R-L257G, A088T-I107T-N109G-G131H-N218S-S248N-K256R, A088T-N109G-A116T-G131H-D140G-T158S-N218S-N243V-K256R, A088T-N109G-A116T-G131H-N218S-N243V-K256R, A088T-N109G-A116T-G131H-N218S-N243V-S248N-K256R, A088T-N109G-A116T-G131H-T158S-N218S-S248N-L257G, A088T-N109G-A116T-G131H-T158S-N243V-S248N-K256R-I268V, A088T-N109G-A116T-G131H-V149A-N218S-S248N-K256R-L257G, A088T-N109G-A116T-N218S-N243V-S248N-K256R-L257G, A088T-N109G-A116T-T158S-K256R-L257G, A088T-N109G-A116T-T158S-N218S-N243V-L257G, A088T-N109G-D140G-N243V, A088T-N109G-G131H-D140G-T158S-N243V-S248N-K256R, A088T-N109G-G131H-K141E-T158S-N218S-K256R, A088T-N109G-G131H-N218S-S248N, A088T-N109G-G131H-N218S-S248N-K256R-Q271R, A088T-N109G-G131H-N218S-S248N-L257G, A088T-N109G-G131H-T158S-K256R, A088T-N109G-G131H-T158S-N218S-S248N-K256R, A088T-N109G-G131H-V149L-T158S-K256R-L257G, A088T-N109G-T158S-N218S, A088T-N109G-T158S-N218S-K256R-L257G-Q271K, A088T-N109G-T158S-N218S-L257G, A088T-N109G-T158S-S248N-K256R, A088T-N218S-S248N-L257G-Q271R, A088T-T158S-N218S-K256R-L257G, A088T-T158S-N218S-N243V-K256R, A088T-Y104H-A116T-G131H-N218S-N243V, A116T-G131H-K141E-N218S-N243V-S248N-L257G, A116T-G131H-N218S-N243V-S248N-K256R, A116T-G131H-T158S-N218S-S248N-L257G-N269D, A116T-G131H-T158S-N218S-S248N-Q271R, A116T-G131H-T158S-N243V-S248N, A116T-G157E-T158S-N243V-S248N-K256R, A116T-T158S-N218S, G131H-N218S-L257G, G131H-N218S-S248N, G131H-T158S-N218S-N243V-S248N-K256R-L257G, G131H-T158S-N218S-N243V-S248N-L257G, G131H-T158S-N218S-S248N-I268V, I107T-N109G-G131H-N218S-L257G, L090I-N109G-T158S-N243V, L257G, N109G-A116T-G131H-T158S-N218S-K256R-L257G-Q271R, N109G-A116T-N218S-W241R-N243V-S248N-K256R-L257G, N109G-G131H-K141E-L257G, N109G-G131H-N218S-N243V, N109G-T158S-N218S-N243V-L257G, N109G-T158S-N218S-S248N-K256R, N109G-T158S-N243V-S248N-K256R-L257G, N218S-S248N-K256R-L257G, S003P-N109G-G131H-T158S-L257G, S003P-S248N-L257G, T158S-S248N-K256R-L257G, V004A-A088T-G131H-N218S-N243V-S248N-L257G, Y006H-N218S-N243V-S248N, Y104H-N109G-G131H-N243V-S248N, A088T-A116T-T158S-N218S-N243V-S248N-K256R, A088T-A116T-T158S-N243V, A088T-G131H-T158S-N218S-I234T-S248N-L257G, A088T-G131H-T158S-N218S-N243V-S248N-K256R, A088T-G131H-V149L-T158S-N243V-S248N-K256R-L257G, A088T-I107T-N109G-G131H-N218S-A223G-S248N-K256R, A088T-K213N-N243V-S248N-K256R, A088T-K256R-L257G, A088T-N109G-A116T-G131H-A232S-N243V-K256R, A088T-N109G-A116T-G131H-D140G-S248N-L257G, A088T-N109G-A116T-G131H-N218S-N243V-S248N-K256R-L257G, A088T-N109G-A116T-G131H-T158S-N218S-N243V-S248N, A088T-N109G-A116T-G131H-T158S-N243V-S248N-L257G, A088T-N109G-A116T-M124I-G131H-T158S-N218S-S248N-L257G, A088T-N109G-A116T-V148A-N218S-N243V, A088T-N109G-G131H-N218S-N243V-S248N, A088T-N109G-N218S-S248N-T255K-K256R-L257G, A088T-T158S-N218S-L257G, A088T-T158S-N218S-Q245K-S248N-K256R, A088T-T158S-N218S-S248N-K256R, A116T-G131H-N218S-N243V-K256R, A116T-G131H-N218S-W241R-N243V-

S248N-K256R-L257G, A116T-G131H-T158S-N218S-L257G, A116T-G131H-V150A-T158S-N243V-S248N-K256R-L257G, I107T-G131H-T158S-N243V-S248N-K256R-L257G, N109G-A116T-K141E-T158S-N218S-N243V-L257G, N109G-A116T-T158S-N218S-N243V-S248N, T158S-N243V-S248N-K256R, T158S-N243V-S248N-K256R-L257G, A088T-A116T-G131H-G146C, A088T-A116T-N218S, A088T-A116T-T158S-N243V-K256R-L257G, A088T-A138E-N218S-N243V-K256R, A088T-N109G-A116T-G131H-T158S-N218S-N243F-S248N, A088T-T158S-V203I-N218S-K256R-L257G, A116T-D140G-T158S-N218S-N243V-S248N, A088T-A116T-T158S-K256R-L257G, A088T-A116T-T158S-N218S-N243V-S248N-E251K-K256R-L257G, A088T-I108T-N109G-G131H-T158S-N218S-S248N-K256R-L257G, A088T-N109G-A116T-G131H-K141E-N218S, A088T-N109G-W241R-S248N-K256R, and G065D-A088T-G131H-N243V-S248N, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0372] The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A088T-N109G-A116T-T158S-N243V-L257G, A116T-N218S-N243V-L257G-N269S, A088T-A116T-K256R, A088T-G131H-K256R, A088T-N109G-A116T-T158S-S248N-K256R-L257G, A088T-N109G-T158S-L257G, A088T-A116T-G131H-T158S-N218S-N243V-K256R-A273T, A088T-A116T-N243V-L257G, A088T-A116T-S248N-K256R-L257G, A088T-A116T-T158S-N243V-L257G, A088T-A116T-T158S-N243V-S248N-L257G, A088T-N109G-A116T-G131H-N218S-N243V-S248N-K256R-L257G, A088T-N109G-A116T-G131H-N243V-L257G, A088T-N109G-A116T-G131H-T158S-L257G, A088T-N109G-A116T-T158S-N218S-L257G, A088T-N109G-A116T-T158S-N218S-N243V-S248N-K256R-L257G, A088T-N109G-G131H-N218S-K256R-L257G, A088T-N109G-N218S-S248N-L257G, A088T-T158S-N218S-N243V-K256R-I268V, A088T-T158S-N218S-S248N-L257G, A116T-N218S-K256R-L257G, N109G-A116T, N109G-A116T-G131H-T158S-L257G, N109G-A116T-N243V, N109G-A116T-N243V-K256R, N109G-A116T-T158S-L257G, N109G-K256R, N109G-N243V-K256R-L257G, S003P-N109G-G131H-T158S-K256R, A088T-A116T, A088T-A116T-G131H-N218S-K256R-L257G, A088T-A116T-G131H-N218S-L257G, A088T-A116T-G131H-N218S-N243V-S248N-L257G, A088T-A116T-G131H-N243V-K256R-L257G, A088T-A116T-G131H-N243V-S248N-K256R-L257G, A088T-A116T-G131H-T158S-N218S-N243V, A088T-A116T-G131H-T158S-N218S-N243V-K256R-L257G, A088T-A116T-G131H-T158S-N218S-N243V-S248N, A088T-A116T-G131H-T158S-S248N-K256R-L257G, A088TA116T-G131H-T158S-S248N-L257G, A088T-A116T-N218S-N243V-L257G, A088T-A116T-N218S-N243V-S248N-K256R-L257G, A088T-A116T-N218S-N243V-S248N-K256R-Q275R, A088T-A116T-T158S-A216S-N218S-N243V-K256R-L257G, A088T-A116T-T158S-K256R, A088T-A116T-T158S-N218S-L257G, A088T-A116T-T158S-N218S-N243V, A088T-A116T-T158S-N218S-N243V-K256R, A088T-A116T-T158S-N218S-N243V-K256R-L257G, A088T-A116T-T158S-N218S-N243V-K256R-N269S, A088T-A116T-T158S-N243V, A088T-A116T-T158S-N243V-K256R, A088T-A116T-V147IT158S-N218S-N243V-L257G, A088T-G131H-K256R-L257G, A088T-G131H-N218S-N243V-S248N-K256R-L257G, A088T-G131H-S248N-K256R-L257G, A088T-G131H-T158S-N218S-L257G, A088T-G131H-T158S-N218S-N243V-L257G, A088T-I107T-N109G-A116T-G131H-T158S-N218S-N243V-S248N, A088T-I107T-N109G-G131H-N218S-S248N-K256R, A088T-N109G-A116T-G131H-A153S-N218S-S248N-L257G, A088T-N109G-A116T-G131H-K256R-L257G, A088T-N109G-A116T-G131H-N218S-K256R-L257G, A088T-N109G-A116T-G131H-N218S-L257G, A088T-N109G-A116T-G131H-N218S-N243V-K256R, A088T-N109G-A116T-G131H-N218S-N243V-L257G, A088T-N109G-A116T-G131H-N243V-L257G, A088T-N109G-A116T-G131H-S248N-L257G, A088T-N109G-A116T-G131H-T158S-N218S, A088T-N109G-A116T-G131H-T158S-N218S-N243V-S248N-K256R, A088T-N109G-A116T-G131H-T158S-N218S-N243V-S248N-L257G, A088T-N109G-A116T-N218S-K256R-L257G, A088T-N109G-A116T-N218S-L257G, A088T-N109G-A116T-N218S-N243V, A088T-N109G-A116T-N218S-N243V-L257G, A088T-N109G-A116T-N218T-K256R, A088T-N109G-A116T-N218T-K256R-L257G, A088T-N109G-A116T-N243V, A088T-N109G-A116T-N243V-K256R-L257G, A088T-N109G-A116T-N243V-K256R-L257G-N269D, A088T-N109G-A116T-T158S, A088T-N109G-A116T-T158S, A088T-N109G-A116T-T158S-L257G, A088T-N109G-A116T-T158S-N218S-N243V-K256R-L257G, A088T-N109G-A116T-T158S-N218S-N243V-K256R-L257G, A088T-N109G-A116T-T158S-N218S-N243V-S248N, A088T-N109G-A116T-T158S-N243V-S248N-L257G, A088T-N109G-G131H-A138V-T158S-N218S-N243V-S248N-L257G, A088T-N109G-G131H-L257G, A088T-N109G-G131H-N218S, A088T-N109G-G131H-N218S-N243V-L257G, A088T-N109G-G131H-N218S-N243V-S248N-K256R-L257G, A088T-N109G-G131H-N218S-S248N-K256R-L257G, A088T-N109G-G131H-T158S-N218S-K256R, A088T-N109G-G131H-T158S-N218S-N243V, A088T-N109G-G131H-T158S-N243V-K256R-L257G, A088T-N109G-G131H-T158S-N243V-L257G, A088T-N109G-G131H-T158S-N243V-S248N-L257G, A088T-N109G-G131H-V149A-K256R-L257G, A088T-N109G-N218S-N243V-L257G, A088T-N109G-N218S-N243V-S248N-L257G, A088T-N109G-N218S-S248N-K256R-L257G, A088T-N109G-N243V, A088T-N109G-N243V-K256R-L257G, A088T-N109G-N243V-L257G, A088T-N109G-N243V-S248N-L257G, A088T-N109G-S248N-K256R-L257G, A088T-N109G-T158S-K256R, A088T-N109G-T158S-N218S-N243V-K256R-Q275R, A088T-N109G-T158S-N243V, A088T-N109G-T158S-N243V-K256R-I268V, A088T-N109G-T158S-N243V-L257G, A088T-N109G-T158S-N243V-S248N-L257G, A088T-N218S-N243V-L257G, A088T-N218S-N243V-S248N-K256R-L257G, A088T-N218S-S248N, A088T-T158S-N218S-N243V-K256R-L257G, A088T-V143A-T158S-K256R, A088T-V147IN218S-N243V-K256R-L257G, A114S-A116T-N218S-N243V-S248N-K256R-L257G, A116T-G131H-N218S-N243V-S248N-K256R-L257G, A116T-G131H-N218S-

N243V-S248N-L257G, A116T-G131H-N243V-K256R, A116T-G131H-N243V-L257G, A116T-G131H-N243V-S248N-K256R, A116T-G131H-S248N-L257G, A116T-G131H-T158S-N218S-I234T-N243V-S248N-K256R, A116T-G131H-T158S-N218S-N243V-S248N-L257G, A116T-N218S-L257G, A116T-T158S-N218S-K256R-L257G, A116T-T158S-N218S-S248N, A116T-T158S-N218S-S248N-L257G-Q271R, A116T-T158S-N243V-S248N-L257G, A116T-T158S-S248N-L257G, G131H-N218S-S248N-K256R-L257G, I107T-N109G-A116T-G131H-T158S-N218S-N243V-S248N-K256R-L257G, N109G-A116T-G131H-A137V-T158S-S248N-K256R-L257G, N109G-A116T-G131H-A151S-N218S-K256R-L257G, N109G-A116T-G131H-N218S-K256R-L257G, N109G-A116T-G131H-N218S-N243V-L257G, N109G-A116T-G131H-N243V-L257G, N109G-A116T-G131H-S248N-L257G, N109G-A116T-G131H-T158S-N218S-N243V-K256R-L257G, N109G-A116T-G131H-T158S-N218S-N243V-S248N-K256R-L257G, N109G-A116T-K256R-L257G, N109G-A116T-N218S-N243V-K256R, N109G-A116T-N218S-N243V-S248N-K256R-L257G, N109G-A116T-N218S-N243V-S248N-L257G, N109G-A116T-N243V-S248N-K256R, N109G-A116T-S248N-L257G, N109G-A116T-T158S-G211V-N243V-S248N-K256R, N109G-A116T-T158S-N218S, N109G-A116T-T158S-N218S-N243V-L257G, N109G-A116T-T158S-N218S-N243V-S248N, N109G-G131H-N218S-K237N, N109G-G131H-N218S-K256R-L257G, N109G-G131H-N218S-L257G, N109G-G131H-N218S-N243V-L257G, N109G-G131H-N243V-K256R-L257G, N109G-G131H-N243V-S248N-L257G, N109G-G131H-S248N-K256R, N109G-G131H-T158S-K256R-L257G, N109G-G131H-T158S-N218S-K256R-L257G, N109G-G131H-T158S-N243V, N109G-N218S, N109G-N218S-N243V-L257G, N109G-T158S-N218S-K256R-L257G, N109G-T158S-N218S-L257G, N109G-T158S-N218S-N243V-K256R, N109G-T158S-N243V-L257G, N243V-K256R-L257G, N243V-L257G, S003F-A088T-N109G-A116T-T158S-N243V-K256R-L257G, T158S-N218S-L233S, T158S-N218S-N243V-S248N-K256R, V004A-N109G-A116T-T158S-N218S-S248N-L257G, Y006H-A116T-G131H-T158S-N218S-N243V-S248N-K256R-A272G, A088T-A098S-N218S-K256R, A088T-A116T-G131H-K256R-L257G-L267M, A088T-A116T-G131H-L257G, A088T-A116T-G131H-N218S-A274T, A088T-A116T-G131H-N218S-K256R, A088T-A116T-G131H-N218S-N243V-K256R, A088T-A116T-G131H-N218S-N243V-K256R-L257G, A088T-A116T-G131H-N218S-N243V-K256R-L257G, A088T-A116T-G131H-N218S-N243V-L257G, A088T-A116T-G131H-N218S-N243V-S248N-K256R, A088T-A116T-G131H-N218S-N243V-S248N-K256R-L257G, A088T-A116T-G131H-N218S-S248N-L257G, A088T-A116T-G131H-N243V-K256R, A088T-A116T-G131H-N243V-L257G, A088T-A116T-G131H-N243V-S248N, A088T-A116T-G131H-N243V-S248N-A274V, A088T-A116T-G131H-N243V-S248N-K256R-L257G, A088T-A116T-G131H-S248N-K256R-L257G, A088T-A116T-G131H-S248N-L257G, A088T-A116T-G131H-T158S-K256R-L257G, A088T-A116T-G131H-T158S-K256R-L257G, A088T-A116T-G131H-T158S-L257G, A088T-A116T-G131H-T158S-N218S, A088T-A116T-G131H-T158S-N218S-K256R, A088T-A116T-G131H-T158S-N218S-K256R-L257G, A088T-A116T-G131H-T158S-N218S-K256R-L257G, A088TA116T-G131H-T158S-N218S-L257G, A088T-A116T-G131H-T158S-N218S-N243V-K256R, A088TA116T-G131H-T158S-N218S-N243V-S248N-K256R, A088T-A116T-G131H-T158S-N218S-S248N-K256R, A088T-A116T-G131H-T158S-N218S-S248N-L257G, A088T-A116T-G131H-T158S-N243V-K256R-L257G, A088T-A116T-G131H-T158S-N243V-L257G, A088T-A116T-G131H-T158S-N243V-S248N-K256R, A088T-A116T-G131H-T158S-N243V-S248N-K256R-L257G, A088T-A116T-K256R, A088T-A116T-N218S-K256R, A088T-A116T-N218S-N243V-K256R, A088T-A116T-N218S-N243V-K256R-L257G, A088T-A116T-N218S-N243V-S248N-K256R, A088T-A116T-N218S-N243V-S248N-L257G, A088T-A116T-N243V-K256R, A088T-A116T-N243V-L257G, A088T-A116T-N243V-S248N-K256R, A088T-A116T-N243V-S248N-K256R-L257G, A088T-A116T-S248N, A088T-A116T-S248N-K256R, A088T-A116T-S248N-L257G, A088T-A116T-T158S-N218S, A088T-A116T-T158S-N218S-K256R, A088T-A116T-T158S-N218S-K256R, A088T-A116T-T158S-N218S-K256R-L257G, A088T-A116T-T158S-N218S-N243V-K256R, A088T-A116T-T158S-N218S-N243V-S248N, A088T-A116T-T158S-N218S-N243V-S248N, A088T-A116T-T158S-N218S-N243V-S248N-K256R, A088T-A116T-T158S-N218S-N243V-S248N-K256R-L257G, A088T-A116T-T158S-N218S-N243V-S248N-L257G, A088T-A116T-T158S-N243V-K256R-L257G, A088T-G131H, A088T-G131H-N218S-K256R-L257G, A088T-G131H-N218S-N243V-K256R, A088T-G131H-N218S-N243V-K256R, A088T-G131H-N218S-N243V-L257G, A088T-G131H-N218S-N243V-L257G, A088T-G131H-N218S-S248N, A088T-G131H-N218S-S248N-K256R, A088T-G131H-N218S-S248N-K256R-L257G, A088T-G131H-N218S-S248N-L257G, A088T-G131H-N218T-L257G, A088T-G131H-N243V-L257G, A088T-G131H-N243V-S248N-K256R, A088T-G131H-S248N, A088T-G131H-S248N-L257G, A088T-G131H-T158S-N218S-K256R, A088T-G131H-T158S-N218S-K256R-L257G, A088T-G131H-T158S-N218S-N243V-K256R, A088T-G131H-T158S-N218S-N243V-K256R, A088T-G131H-T158S-N218S-N243V-K256R-L257G, A088T-G131H-T158S-N218S-N243V-K256R-L257G, A088T-G131H-T158S-N218S-S248N, A088T-G131H-T158S-N218S-S248N-K256R, A088T-G131H-T158S-N218S-S248N-K256R, A088T-G131H-T158S-N218S-S248N-L257G-I268V, A088T-G131H-T158S-N243V, A088T-G131H-T158S-N243V-S248N, A088T-G131H-T158S-N243V-S248N, A088T-G131H-T158S-N243V-S248N-K256R-L257G, A088T-N109G-A116T, A088T-N109G-A116T-G131H-D140G-T158S-N218S-N243V-K256R, A088T-N109G-A116T-G131H-K256R, A088T-N109G-A116T-G131H-N218S, A088T-N109G-A116T-G131H-N218S, A088T-N109G-A116T-G131H-N218S-K256R, A088T-N109G-A116T-G131H-N218S-L257G, A088T-N109G-A116T-G131H-N218S-N243V, A088T-N109G-A116T-G131H-N218S-N243V-K256R-L257G, A088T-N109G-A116T-G131H-N218S-N243V-L257G, A088T-N109G-A116T-G131H-N218S-N243V-S248N-K256R, A088T-N109G-A116T-G131H-N218S-S248N-K256R-L257G, A088T-N109G-A116T-G131H-N218S-S248N-L257G, A088T-N109G-A116T-G131H-N218S-S248N-L257G, A088T-N109G-A116T-G131H-N243V-K256R-L257G, A088T-N109G-A116T-G131H-N243V-S248N, A088T-N109G-A116T-G131H-N243V-S248N-K256R-L257G, A088T-N109G-A116T-G131H-N243V-S248N-L257G, A088T-N109G-A116T-G131H-S248N-K256R, A088T-N109G-A116T-G131H-S248N-K256R-L257G, A088T-N109G-A116T-G131H-S248N-L257G, A088T-N109G-A116T-G131H-T158S, A088T-N109G-A116T-G131H-T158S-N218S, A088T-N109G-A116T-G131H-T158S-N218S-L257G, A088T-N109G-A116T-G131H-T158S-N218S-N243V-K256R, A088T-N109G-A116T-G131H-T158S-N218S-N243V-K256R, A088T-

N109G-A116T-G131H-T158S-N218S-N243V-K256R-L257G, A088T-N109G-A116T-G131H-T158S-N218S-N243V-S248N-K256R, A088T-N109G-A116T-G131H-T158S-N218S-S248N, A088T-N109G-A116T-G131H-T158S-N218S-S248N-K256R, A088T-N109G-A116T-G131H-T158S-N218S-S248N-L257G, A088T-N109G-A116T-G131H-T158S-N218S-S248N-L257G, A088T-N109G-A116T-G131H-T158S-N218T-N243V, A088T-N109G-A116T-G131H-T158S-N243V-K256R-L257G, A088T-N109G-A116T-G131H-T158S-N243V-S248N, A088T-N109G-A116T-G131H-T158S-N243V-S248N-K256R, A088T-N109G-A116T-G131H-T158S-N243V-S248N-L257G, A088T-N109G-A116T-G131H-T158S-S248N-K256R-L257G, A088T-N109G-A116T-G131H-T158S-S248N-L257G, A088T-N109G-A116T-K256R, A088T-N109G-A116T-N218S, A088T-N109G-A116T-N218S-N243V, A088T-N109G-A116T-N218S-N243V-K256R, A088T-N109G-A116T-N218S-N243V-K256R-L257G, A088T-N109G-A116T-N218S-N243V-L257G, A088T-N109G-A116T-N218S-N243V-S248N-K256R-L257G, A088T-N109G-A116T-N243V-S248N, A088T-N109G-A116T-N243V-S248N-K256R, A088T-N109G-A116T-N243V-S248N-K256R-L257G, A088T-N109G-A116T-N243V-S248N-K256R-L257G, A088T-N109G-A116T-N243V-S248N-L257G, A088T-N109G-A116T-S248N-K256R, A088T-N109G-A116T-S248N-K256R-L257G, A088T-N109G-A116T-T158S-K256R, A088T-N109G-A116T-T158S-N218S, A088T-N109G-A116T-T158S-N218S-K256R-L257G, A088T-N109G-A116T-T158S-N218S-L257G, A088T-N109G-A116T-T158S-N218S-N243V, A088T-N109G-AI 16T-T158S-N218S-N243V-L257G, A088T-N109G-A116T-T158S-N218S-N243V-S248N, A088T-N109G-A116T-T158S-N218S-N243V-S248N-K256R, A088T-N109G-A116T-T158S-N218S-N243V-S248N-L257G, A088T-N109G-A116T-T158S-N218S-S248N, A088T-N109G-A116T-T158S-N218S-S248N-K256R, A088T-N109G-A116T-T158S-N218S-S248N-L257G, A088T-N109G-A116T-T158S-N243V-K256R, A088T-N109G-A116T-T158S-N243V-S248N, A088T-N109G-A116T-T158S-S248N, A088T-N109G-A116T-T158S-S248N-K256R, A088T-N109G-A116T-T158S-S248N-L257G, A088T-N109G-G131H-N218S-K256R, A088T-N109G-G131H-N218S-K256R, A088T-N109G-G131H-N218S-N243V-K256R, A088T-N109G-G131H-N218S-N243V-K256R, A088T-N109G-G131H-N218S-N243V-K256R-L257G, A088T-N109G-G131H-N218S-N243V-S248N-K256R, A088T-N109G-G131H-N218S-S248N, A088T-N109G-G131H-N218S-S248N-L257G, A088T-N109G-G131H-N243V-K256R-L257G, A088T-N109G-G131H-N243V-K256R-L257G, A088T-N109G-G131H-N243V-L257G, A088T-N109G-G131H-N243V-L257G, A088T-N109G-G131H-N243V-S248N-K256R, A088T-N109G-G131H-N243V-S248N-K256R-L257G, A088T-N109G-G131H-N243V-S248N-L257G, A088T-N109G-G131H-S248N-L257G, A088T-N109G-G131H-T158S-K256R-L257G, A088T-N109G-G131H-T158S-N218S-L257G, A088T-N109G-G131H-T158S-N218S-L257G, A088T-N109G-G131H-T158S-N218S-N243V-S248N, A088T-N109G-G131H-T158S-N218S-N243V-S248N-K256R, A088T-N109G-G131H-T158S-N218S-S248N-K256R, A088T-N109G-G131H-T158S-N218S-S248N-L257G, A088T-N109G-G131H-T158S-N243V, A088T-N109G-G131H-T158S-N243V-S248N, A088T-N109G-G131H-T158S-N243V-S248N-K256R, A088T-N109G-G131H-T158S-N243V-S248N-K256R, A088T-N109G-G131H-T158S-N243V-S248N-K256R-L257G, A088T-N109G-G131H-T158S-S248N-K256R-L257G, A088T-N109G-G131H-T158S-W241R-S248N-K256R, A088T-N109G-G131H-V148A-N218S-N243V-K256R-L257G, A088T-N109G-G154A-N155P-E156T-G157L-T158M-S159E-G160E-S161L, A088T-N109G-N218S-K256R, A088T-N109G-N218S-N243V-L257G, A088T-N109G-N218S-N243V-S248N, A088T-N109G-N243V-K256R, A088T-N109G-N243V-S248N, A088T-N109G-N243V-S248N-K256R-L257G, A088T-N109G-S248N, A088T-N109G-T158S-N218S, A088T-N109G-T158S-N218S-K256R, A088T-N109G-T158S-N218S-K256R-L257G, A088T-N109G-T158S-N218S-L257G, A088T-N109G-T158S-N218S-N243V-S248N-K256R, A088T-N109G-T158S-N218S-N243V-S248N-K256R-L257G, A088T-N109G-T158S-N218S-S248N-K256R, A088T-N109G-T158S-N218S-S248N-K256R-L257G, A088T-N109G-T158S-N243V-K256R, A088T-N109G-T158S-N243V-K256R, A088T-N109G-T158S-N243V-S248N-L257G, A088T-N109G-T158S-S248N-K256R-L257G, A088T-N109G-T158S-S248N-L257G, A088T-N109G-T158S-S248N-L257G, A088T-N109G-V147A-N218S-N243V-K256R, A088T-N218S-L257G-I268V, A088T-N218S-N243V, A088T-N218S-N243V-S248N, A088T-N218S-N243V-S248N-N269S, A088T-N218S-S248N-K256R, A088T-N218S-S248N-L257G-Q271R, A08T-N243V, A088T-N243V, A088T-N243V-K256R, A088T-N243V-S248N-K256R, A088T-N243V-S248N-K256R-L257G, A088T-S248N, A088T-T158S-N218S-K256R-L257G, A088T-T158S-N218S-N243V-K256R, A088T-T158S-N218S-N243V-L257G, A088T-T158S-N218S-N243V-S248N-K256R-L257G, A088T-T158S-N218S-N243V-S248N-K256R-L257G, A088T-T158S-N243V-K256R-L257G, A088T-T158S-N243V-K256R-L257G-Q271H, A088T-T158S-N243V-S248N, A088T-T158S-N243V-S248N-L257G, A088T-T158S-S248N, A088T-V147A-K256R, A116T-G131H-K256R, A116T-G131H-N218S, A116T-G131H-N218S-K256R-L257G, A116T-G131H-N218S-L257G, A116T-G131H-N218S-N243V, A116T-G131H-N218S-S248N-K256R, A116T-G131H-N218S-S248N-K256R-L257G, A116T-G131H-N243V-S248N, A116T-G131H-N243V-S248N-L257G, A116T-G131H-S248N-K256R, A116T-G131H-T158S-A231V-N243V-L257G, A116T-G131H-T158S-N218S-K256R, A116T-G131H-T158S-N218S-K256R-L257G, A116T-G131H-T158S-N218S-N243V-K256R-L257G, A116T-G131H-T158S-N218S-N243V-S248N-K256R, A116T-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A116T-G131H-T158S-N218S-S248N, A116T-G131H-T158S-N243V-L257G, A116T-G131H-T158S-N243V-S248N-K256R, A116T-G131H-T158S-S248N-K256R, A116T-G131H-T158S-S248N-L257G, A116T-G131H-V143F-T158S-N218S, A116T-K256R, A116T-N218S, A116T-N218S-K256R, A116T-N218S-N243V-L257G, A116T-N218S-N243V-S248N-K256R, A116T-N218S-N243V-S248N-K256R-L257G, A116T-N218S-N243V-S248N-L257G, A116T-N218S-S248N-L257G, A116T-N243V, A116T-N243V-K256R-L257G, A116T-N243V-S248IV, A116T-N243V-S248N-K256R-L257G, A116T-S248N-K256R-L257G, A116T-T158S-K256R-L257G, A116T-T158S-N218S, A116T-T158S-N218S-K256R, A116T-T158S-N218S-N243V, A116T-T158S-N218S-N243V-S248N, A116T-T158S-N218S-S248N-K256R, A116T-T158S-N218S-S248N-K256R-L257G, A116T-T158S-N243V-K256R, A116T-T158S-N243V-L257G, A116T-T158S-N243V-S248N, A116T-T158S-S248N-K256R-L257G, G131H-K141R-T158S-N218S-K256R, G131H-N218S, G131H-N218S-K256R, G131H-N218S-N243V-K256R-L257G, G131H-N218S-N243V-S248N, G131H-N218S-

N243V-S248N-L257G, G131H-N243V-S248N-K256R, G131H-N243V-S248N-K256R-L257G, G131H-S248N, G131H-T158S-I234T-N243V-K256R, G131H-T158S-N218S-K256R-L257G, G131H-T158S-N218S-N243V, G131H-T158S-N218S-N243V-S248N-L257G, G131H-T158S-N218S-S248N-K256R-L257G, G131H-T158S-N218S-S248N-L257G, G131H-T158S-N243V-K256R, G131H-T158S-N243V-S248N-L257G, N109G, N109G-A116T-G131H-A144V-T158S-S248N-K256R-L257G, N109G-A116T-G131H-K256R-L257G, N109G-A116T-G131H-N218S-N243V-K256R, N109G-A116T-G131H-N218S-N243V-K256R-L257G, N109G-A116T-G131H-N218S-S248N-K256R, N109G-A116T-G131H-N218S-S248N-L257G, N109G-A116T-G131H-N243V-K256R, N109G-A116T-G131H-N243V-S248N, N109G-A116T-G131H-N243V-S248N-K256R-L257G, N109G-A116T-G131H-S248N, N109G-A116T-G131H-S248N-K256R, N109G-A116T-G131H-T158S-K256R, N109G-A116T-G131H-T158S-K256R-L257G, N109G-A116T-G131H-T158S-N218S-K256R, N109G-A116T-G131H-T158S-N218S-K256R-L257G, N109G-A116T-G131H-T158S-N218S-L257G, N109G-A116T-G131H-T158S-N218S-N243V-K256R, N109G-A116T-G131H-T158S-N218S-N243V-S248N-L257G, N109G-A116T-G131H-T158S-N218S-S248N, N109G-A116T-G131H-T158S-N243V-L257G, N109G-A116T-G131H-T158S-N243V-S248N, N109G-A116T-G131H-T158S-S248N, N109G-A116T-G131H-T158S-S248N-K256R, N109G-A116T-G131H-T158S-S248N-K256R-L257G, N109G-A116T-G131H-V149A-T158S-N218S-N243V-S248N-L257G, N109G-A116T-N218S, N109G-A116T-N218S-K256R, N109G-A116T-N218S-N243V-K256R-L257G, N109G-A116T-N218S-N243V-S248N-I268V, N109G-A116T-N243V-K256R-L257G, N109G-A116T-N243V-S248N, N109G-A116T-N243V-S248N-L257G, N109G-A116T-T158S, N109G-A116T-T158S-N218S-N243V-K256R-L257G, N109G-A116T-T158S-N218S-N243V-S248N-K256R-L257G, N109G-A116T-T158S-N218S-N243V-S248N-L257G, N109G-A116T-T158S-N218S-S248N-K256R-L257G, N109G-A116T-T158S-N243V-K256R-L257G, N109G-A116T-T158S-N243V-L257G, N109G-A116T-T158S-N243V-S248N-L257G, N109G-A116T-T158S-Q275R, N109G-A116T-T158S-S248N-K256R-L257G, N109G-G131H-L257G, N109G-G131H-N218S-N243V-S248N-K256R-L257G, N109G-G131H-N218S-N243V-S248N-L257G, N109G-G131H-N218S-S248N-K256R-L257G, N109G-G131H-N243V-K256R, N109G-G131H-S145F-N218S-N243V-K256R-L257G, N109G-G131H-S248N-K256R-L257G, N109G-G131H-S248N-L257G, N109G-G131H-T158S-K256R, N109G-G131H-T158S-N218S-L257G, N109G-G131H-T158S-N218S-N243V, N109G-G131H-T158S-N218S-N243V-K256R, N109G-G131H-T158S-N218S-N243V-K256R-L257G, N109G-G131H-T158S-N218S-N243V-S248N-L257G, N109G-G131H-T158S-N218S-S248N-K256R, N109G-G131H-T158S-N218S-S248N-K256R-L257G-A274T, N109G-G131H-T158S-N218S-S248N-L257G, N109G-G131H-T158S-N243V-S248N, N109G-G131H-T158S-N243V-S248N-K256R-L257G, N109G-G131H-T158S-S248N-K256R-L257G, N109G-G131H-T158S-S248N-L257G, N109G-N218S-K256R-L257G, N109G-N218S-L257G, N109G-N218S-N243V-K256R, N109G-N218S-N243V-S248N-S260F, N109G-N218S-S248N, N109G-N243V-K256R, N109G-N243V-L257G, N109G-N243V-S248N, N109G-N243V-S248N-K256R-L257G, N109G-S182F-S204F-S207L-N218S-S236F-S248N-L257G, N109G-S248N-K256R, N109G-T158S-K256R, N109G-T158S-N218S-N243V-K256R-L257G, N109G-T158S-N218S-S248N-L257G, N109G-T158S-N243V, N109G-T158S-N243V-K256R, N109G-T158S-N243V-S248N, N109G-T158S-N243V-S248N-K256R, N109G-T158S-S248N-K256R, N109G-T158S-S248N-L257G, N218S, N218S-N243V-S248N-K256R, N218S-S248N-L257G, N243V-K256R, N243V-S248N-K256R, N243V-S248N-K256R-L257G, N243V-S248N-L257G-Q271R, S003P-A116T-T158S-S248N-K256R, S248N, T158S-N218S, T158S-N218S-A272V, T158S-N218S-L257G, T158S-N218S-N243V-K256R-L257G, T158S-N218S-N243V-L257G, T158S-N218S-S248N-K256R-L257G, T158S-N243V-K256R, T158S-N243V-K256R-L257G, T158S-N243V-S248N-K256R, V004A-N109G-A116T-G131H-S248N-K256R-L257G, and Y006H-A116T-G131H-S248N, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN', BPN'-v3, and BPN'-v36, and a greater PI value than that of BPN', BPN'-v3 and BPN'-v36 in this assay.

[0373] The following BPN'-v36 variants were determined to have a PI value of about 1.0 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A015S-A088T -N109G-G131H-T158S-N218S-S248N, A088T, A088T-A116T-G131H-N218S-N243V-S248N, A088T-A116T-G131H-N218S-S248N, A088T-A116T-G131H-N218S-S248N, A088T-A116T-G131H-N218S-S248N-K256R-L257G, A088T-A116T-G131H-N243V, A088T-A116T-G131H-N243V-K256R, A088T-A116T-G131H-N243V-S248N, A088T-A116T-G131H-S248N-K256R, A088T-A116T-G131H-T158S-N218S-N243V-S248N, A088T-A116T-G131H-T158S-N218S-N243V-S248N-K256R, A088T-A116T-G131H-T158S-N218S-N243V-S248N-L257G, A088T-A116T-G131H-T158S-N218S-N243V-S248N-L257G, A088TA116T-G131H-T158S-N218S-S248N, A088T-A116T-G131H-T158S-N218S-S248N-K256R, A088T-AI16T-G131H-T158S-N243V, A088T-A116T-G131H-T158S-N243V-K256R, A088T-A116T-G131H-T158S-N243V-S248N, A088T-A116T-G131H-T158S-N243V-S248N-K256R-L257G, A088T-A116T-G131H-T158S-S248N, A088T-A116T-G131H-T158S-S248N-K256R, A088T-A116T-G131H-T158S-S248N-L257G, A088T-A116T-G131H-V147A-T158S-N218S-N243V-S248N-L257G, A088T-A116T-N218S-I268V, A088T-A116T-N218S-L257G, A088T-A116T-N218S-N243V-N269D, A088T-A116T-N218S-S248N, A088T-A116T-N218S-S248N, A088T-A116T-N218S-S248N-L257G, A088T-A116T-N243V-K256R-L257G, A088T-A116T-N243V-S248N-K256R, A088T-A116T-S248N-K256R, A088TA116T-T158S-N218S, A088T-A116T-T158S-N218S-N243V-S248N-K256R-L257G, A088T-A116T-T158S-N218S-S248N, A088T-A116T-T158S-N218S-S248N-K256R-L257G, A088T-A116T-T158S-N243V-S248N, A088T-A116T-T158S-N243V-S248N-K256R, A088T-A116T-T158S-N243V-S248N-K256R-L257G, A088T-A116T-T158S-N243V-S248N-L257G, A088T-A116T-T158S-S248N, A088TA116T-T158S-S248N-K256R-L257G, A088T-A116T-T158S-S248N-L257G, A088T-A116T-V143A-N218S-S248N-K256R, A088T-G131H-A138V-N218S-L257G, A088T-G131H-K141E-N218S-N243V-S248N-L257G, A088T-G131H-

K256R, A088T-G131H-N218S-K256R, A088T-G131H-N218S-N243V-K256R-L257G, A088T-G131H-N218S-N243V-S248N, A088T-G131H-N218S-N243V-S248N, A088T-G131H-N218S-N243V-S248N-K256R, A088T-G131H-N218S-N243V-S248N-K256R-L257G, A088T-G131H-N218S-S248N, A088T-G131H-N218S-S248N-K256R, A088T-G131H-N243V-K256R-L257G, A088T-G131H-N243V-S248N, A088T-G131H-N243V-S248N-K256R-L257G, A088T-G131H-S248N, A088T-G131H-S248N-K256R, A088T-G131H-T158S-K256R, A088T-G131H-T158S-L257G, A088T-G131H-T158S-N218S, A088T-G131H-T158S-N218S-N243V-S248N, A088T-G131H-T158S-N218S-N243V-S248N-K256R, A088T-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A088T-G131H-T158S-N218S-N243V-S248N-L257G, A088T-G131H-T158S-N218S-S248N, A088T-G131H-T158S-N218S-S248N-K256R-L257G, A088T-G131H-T158S-N243V, A088T-G131H-T158S-N243V-K256R, A088T-G131H-T158S-N243V-S248N-K256R, A088T-G131H-T158S-N243V-S248N-L257G, A088T-G131H-T158S-S248N-K256R-L257G, A088T-G131H-T158S-S248N-L257G, A088T-I107T-N109G-G131H-N218S-A223G-S248N-K256R, A088T-L257G, A088T-L257G, A088T-N109G-A116T-G131HA232S-N243V-K256R, A088T-N109G-A116T-G131H-K256R-L257G, A088T-N109G-A116T-G131H-L257G, A088T-N109G-A116T-G131H-N218S-K256R, A088T-N109G-A116T-G131H-N218S-N243V-S248N-K256R, A088T-N109G-A116T-G131H-N218S-N243V-S248N-N269D, A088T-N109G-A116T-G131H-N243V-K256R, A088T-N109G-A116T-G131H-S248N, A088T-N109G-A116T-G131H-T158S-L257G, A088T-N109G-A116T-G131H-T158S-N218S-N243F-S248N, A088T-N109G-A116T-G131H-T158S-N218S-N243V, A088T-N109G-A116T-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A088T-N109G-A116T-G131H-T158S-N218T-K256R, A088T-N109G-A116T-G131H-T158S-N243V, A088T-N109G-A116T-G131H-T158S-N243V-K256R, A088T-N109G-A116T-G131H-T158S-N243V-S248N, A088T-N109G-A116T-G131H-T158S-N243V-S248N-K256R-I268V, A088T-N109G-A116T-G131H-T158S-N243V-S248N-L257G, A088T-N109G-A116T-G131H-T158S-S248N, A088T-N109G-A116T-G131H-T158S-S248N-K256R-L257G, A088T-N109G-A116T-G131H-V149A-N218S-S248N-K256R-L257G, A088T-N109G-A116T-K256R, A088T-N109G-A116T-N218S-K256R, A088T-N109G-A116T-N218S-N243V-S248N-K256R, A088T-N109G-A116T-N218S-N243V-S248N-L257G, A088T-N109G-A116T-N218S-S248N, A088T-N109G-A116T-N218S-S248N-K256R, A088T-N109G-A116T-S248N, A088T-N109G-A116T-T158S-L257G, A088T-N109G-Al 16T-T158S-N218S, A088T-N109G-A116T-T158S-N243V, A088T-N109G-A116T-T158S-N243V-K256R, A088T-N109G-A116T-T158S-N243V-S248N-K256R, A088T-N109G-G131H-N218S-N243V-S248N, A088T-N109G-G131H-N218S-S248N-K256R, A088T-N109G-G131H-N218S-S248N-K256R-L257G, A088T-N109G-G131H-N218S-S248N-K256R-L257G-Q275R, A088T-N109G-G131H-N218S-S248N-K256R-Q271R, A088T-N109G-G131H-N243V, A088T-N109G-G131H-N243V-K256R, A088T-N109G-G131H-N243V-S248N-K256R, A088T-N109G-G131H-S248N-K256R, A088T-N109G-G131H-S248N-L257G, A088T-N109G-G131H-T158S, A088T-N109G-G131H-T158S, A088T-N109G-G131H-T158S-K256R, A088T-N109G-G131H-T158S-K256R, A088T-N109G-G131H-T158S-N218S-N243V-K256R, A088T-N109G-G131H-T158S-N218S-N243V-K256R, A088T-N109G-G131H-T158S-N218S-N243V-K256R-L257G, A088T-N109G-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A088T-N109G-G131H-T158S-N218S-N243V-S248N-L257G, A088T-N109G-G131H-T158S-N243V-K256R, A088T-N109G-G131H-T158S-N243V-K256R-L257G, A088T-N109G-G131H-T158S-N243V-S248N-K256R-L257G, A088T-N109G-G131H-T158S-S248N, A088T-N109G-G131H-T158S-S248N-L257G, A088T-N109G-G131H-V149A-K256R-L257G, A088T-N109G-G131H-V149L-T158S-K256R-L257G, A088T-N109G-K256R, A088T-N109G-K256R-L257G, A088T-N109G-K256R-L257G, A088T-N109G-L257G, A088T-N109G-N218S-K256R, A088T-N109G-N218S-N243V-K256R, A088T-N109G-N218S-N243V-S248N-K256R, A088T-N109G-N218S-N243V-S248N-K256R, A088T-N109G-N218S-S248N, A088T-N109G-N218S-S248N-L257G, A088T-N109G-N243V-K256R, A088T-N109G-N243V-S248N-L257G-I268V, A088T-N109G-S248N, A088T-N109G-S248N-K256R, A088T-N109G-T158S-K256R-L257G, A088T-N109G-T158S-N218S, A088T-N109G-T158S-N218S-K256R-Q271H, A088T-N109G-T158S-N218S-N243V-K256R, A088T-N109G-T158S-N218S-N243V-K256R-L257G, A088T-N109G-T158S-N218S-N243V-L257G, A088T-N109G-T158S-N218S-N243V-S248N-K256R, A088T-N109G-T158S-N218S-S248N-N269D, A088T-N109G-T158S-N243V-K256R-L257G, A088T-N109G-T158S-N243V-S248N-A274D, A088T-N109G-T158S-N243V-S248N-K256R-L257G-N269D, A088T-N218S-K256R, A088T-N218S-N243V, A088T-N218S-N243V-K256R, A088T-N218S-N243V-K256R-L257G, A088T-N218S-N243V-S248N-K256R, A088T-N218S-N243V-S248N-L257G, A088T-N218S-S248N-L257G, A088T-N243V-S248N, A088T-N243V-S248N-K256R, A088T-N243V-S248N-L257G, A088T-S145T-T158S-S248N, A088T-S248N-K256R-L257G, A088T-S248N-L257G, A088T-T158S, A088T-T158S, A088T-T158S-K256R, A088T-T158S-L257G, A088T-T158S-N218S, A088T-T158S-N218S-K256R, A088T-T158S-N218S-N243V-K256R, A088T-T158S-N218S-N243V-K256R-L257G, A088T-T158S-N218S-N243V-L257G, A088T-T158S-N218S-S248N, A088T-T158S-N218S-S248N, A088T-T158S-N243V, A088T-T158S-N243V-K256R, A088T-T158S-N243V-S248N-L257G, A116T, A116T-G131H-L257G, A116T-G131H-N218S-K256R, A116T-G131H-N218S-N243V-K256R, A116T-G131H-N218S-N243V-K256R-L257G, A116T-G131H-N218S-N243V-S248N, A116T-G131H-N218S-N243V-S248N-K256R, A116T-G131H-N218S-S248N, A116T-G131H-T158S-K256R, A116T-G131H-T158S-N218S-L257G, A116T-G131H-T158S-N218S-N243V-K256R, A116T-G131H-T158S-N218S-N243V-L257G, A116T-G131H-T158S-N218S-N243V-S248N, A116T-G131H-T158S-N218S-S248N-L257G-N269D, A116T-G131H-T158S-N218S-S248N-Q271R, A116T-G131H-T158S-N218T-L257G, A116T-G131H-T158S-N243V-K256R, A116T-G131H-V150A-T158S-N243V-S248N-K256R-L257G, A116T-K141E-N218S-N243V-S248N-K256R-L257G, A116T-L257G, A116T-N218S-N243V-S248N, A116T-N218T-N243V-S248N, A116T-N243V-S248N-L257G, A116T-S248N, A116T-S248N-L257G, A116T-T158S-K256R, A116T-T158S-N218S-L257G, A116T-T158S-N218S-N243V-K256R, A116T-T158S-N218S-N243V-L257G, A116T-T158S-N243V, A116T-T158S-N243V-K256R-L257G, A116T-T158S-N243V-S248N-K256R, A116T-T158S-N243V-S248N-K256R-L257G, A116T-V149I-T158S-N243V-S248N-K256R-Q271H, G024S-G053S-N078S-G097A-N101S, G024S-

G053S-N078S-G097A-N101S-A128S, G131H-K256R, G131H-N218S-K256R-L257G, G131H-N218S-N243V-L257G, G131H-N218S-N243V-S248N-K256R, G131H-N218S-S248N-K256R, G131H-N218S-S248N-L257G, G131H-N243V, G131H-N243V-S248N, G131H-N243V-S248N-L257G, G131H-T158S-K256R-L257G, G131H-T158S-N218S, G131H-T158S-N218S-K256R, G131H-T158S-N218S-N240H-N243V-S248N-K256R-L257G, G131H-T158S-N218S-N243V-K256R, G131H-T158S-N218S-N243V-K256R-L257G, G131H-T158S-N218S-N243V-S248N, G131H-T158S-N218S-N243V-S248N-K256R-L257G, G131H-T158S-N218S-S248N-I268V, G131H-T158S-N218S-S248N-K256R-L257G-N269S, G131H-T158S-N243V-K256R-L257G, G131H-T158S-N243V-S248N, G131H-T158S-N243V-S248N-K256R, G131H-T158S-S248N, G131H-T158S-S248N-L257G, G131H-W241L-N243V-S248N-K256R, 1107T-G131H-T158S-N243V-S248N-K256R-L257G, 1107T-N109G-G131H-N218S-L257G, N109G-A116T-G131H-L257G, N109G-A116T-G131H-N218S-L257G, N109G-A116T-G131H-N218S-N243V, N109G-A116T-G131H-N218S-N243V-S248N-K256R, N109G-A116T-G131H-N218S-W241R-N243V-K256R, N109G-A116T-G131H-N243V-S248N-K256R, N109G-A116T-G131H-S248N-I268V, N109G-A116T-G131H-T158S-N218S, N109G-A116T-G131H-T158S-N218S-S248N-K256R, N109G-A116T-G131H-T158S-N218S-S248N-K256R-L257G, N109G-116T-G131H-T158S-N218S-S248N-L257G, N109G-A116T-G131H-T158S-N243V-K256R-L257G, N109G-A116T-G131H-T158S-S248N-L257G, N109G-A116T-K141E-T158S-N218S-N243V-L257G, N109G-A116T-K256R, N109G-A116T-N218S-N243V, N109G-A116T-N218S-N243V-S248N, N109G-A116T-N218S-S248N-L257G, N109G-A116T-N243V-S248N-K256R-L257G, N109G-A116T-S248N, N109G-A116T-T158S-N218S-N243V-S248N-K256R, N109G-A116T-T158S-N218S-W241R-N243V, N109G-Al 16T-T158S-N243V-S248N, N109G-A116T-T158S-S248N-L257G, N109G-G131H-K256R, N109G-G131H-N218S-K256R, N109G-G131H-N218S-N243V, N109G-G131H-N218S-N243V-K256R-L257G, N109G-G131H-N218S-N243V-S248N-K256R, N109G-G131H-N218S-S248N-K256R, N109G-G131H-N243V, N109G-G131H-N243V-S248N, N109G-G131H-N243V-S248N-K256R-L257G, N109G-G131H-S248N, N109G-G131H-T158S-L257G, N109G-G131H-T158S-N218S-N243V-S248N, N109G-G131H-T158S-N218S-N243V-S248N-K256R, N109G-G131H-T158S-N218S-N243V-S248N-K256R-L257G, N109G-G131H-T158S-N243V-L257G, N109G-G131H-T158S-N243V-S248N-K256R, N109G-G131H-T158S-S248N-K256R, N109G-K141E-N218S-S248N-L257G, N109G-N218S-N243V, N109G-N218S-N243V-S248N-K256R, N109G-N218S-S248N-K256R-L257G, N109G-N218S-S248N-L257G, N109G-N243V-S248N-L257G-Q275R, N109G-T158S-1268V, N109G-T158S-N218S, N109G-T158S-N218S-N243V, N109G-T158S-N218S-N243V-S248N, N109G-T158S-N218S-S248N-K256R, N109S-A116T-S248N, N218S-K256R, N218S-N243V-K256R, N218S-N243V-L257G, N218S-N243V-S248N, N218S-S248N, N218S-S248N-K256R, N218S-S248N-K256R-L257G, S003P-N109G-G131H-N218S-N243V-S248N-K256R-L257G, S248N-K256R-L257G, T158S-K256R, T158S-K256R-L257G, T158S-N218S-K256R-L257G, T158S-N218S-L233S-S248N, T158S-N243V, T158S-N243V-S248N-K256R-N269D, T158S-N243V-S248N-L257G, V004L-A116T-N218S-N243V-S248N-L257G, and Y006H-N218S-N243V-S248N, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0374] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A088T-A098S-G131H-S248N-K256R-L257G, A088T-A116T-G131H-K256R, A088T-A116T-G131H-N218S-S248N-K256R, A088T-A116T-G131H-N243V-S248N-L257G, A088T-A116T-G131H-S248N, A088T-A116T-G131H-S248N-K256R-L257G, A088T-A116T-G131H-T158S-K256R, A088T-A116T-G131H-T158S-L257G, A088T-A116T-G131H-T158S-N218S-L257G, A088T-A116T-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A088T-A116T-K256R-L257G, A088T-A116T-K256R-L257G, A088T-A116T-N218S-N243V-Q271R, A088T-A116T-N218S-S248N-K256R, A088T-A116T-T158S, A088T-A116T-T158S, A088T-A116T-T158S-K256R, A088T-A116T-T158S-N218S-N243V-L257G, A088T-A116T-T158S-N218S-S248N-K256R, A088T-G131H, A088T-G131H-L257G, A088T-G131H-N218S-N243V-S248N-L257G, A088T-G131H-N243V-K256R, A088T-G131H-N243V-L257G, A088T-G131H-N243V-S248N, A088T-G131H-N243V-S248N-K256R, A088T-G131H-T158S-N218S-I234T-S248N-L257G, A088T-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A088T-G131H-T158S-N243V-K256R-L257G, A088T-G131H-T158S-S248N, A088T-G131H-T158S-S248N-K256R, A088T-G131H-T158S-S248N-K256R-L257G, A088T-G131H-V149L-T158S-N243V-S248N-K256R-L257G, A088T-N109G-A116T-G131H-K141E-N218S, A088T-N109G-A116T-G131H-N218S-N243V-S248N-Q275R, A088T-N109G-A116T-G131H-N218S-S248N, A088T-N109G-A116T-G131H-N243V-S248N-K256R, A088T-N109G-A116T-G131H-S248N-K256R-L257G, A088T-N109G-A116T-G131H-T158S-K256R, A088T-N109G-A116T-G131H-T158S-N218S-N243V-S248N, A088T-N109G-A116T-G131H-T158S-N243V-S248N-K256R-L257G, A088T-N109G-A116T-G131H-T158S-S248N, A088T-N109G-A116T-G131H-V149A-T158S-N218S-K256R, A088T-N109G-A116T-N218S-S248N-K256R-L257G, A088T-N109G-A116T-N243V-K256R, A088T-N109G-A116T-N243V-S248N-L257G, A088T-N109G-A116T-S248N-L257G, A088T-N109G-A116T-T158S-N212D-N243V-K256R-L257G, A088T-N109G-A137E-T158S-N218S-N243V-S248N-K256R-L257G, A088T-N109G-D140G-N243V, A088T-N109G-G131H-A152S-T158S-N218S-S248N-K256R, A088T-N109G-G131H-D140G-T158S-N243V-S248N-K256R, A088T-N109G-G131H-K256R-L257G, A088T-N109G-G131H-N218S, A088T-N109G-G131H-N218S-N243V, A088T-N109G-G131H-T158S-L257G, A088T-N109G-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A088T-N109G-G131H-T158S-N218S-W241R-S248N-L257G, A088T-N109G-G131H-T158S-S248N-K256R, A088T-N109G-N218S-S248N-K256R, A088T-N109G-N243V-S248N-K256R, A088T-N109G-T158S, A088T-N109G-T158S-N243V-S248N-K256R, A088T-N109G-T158S-N243V-S248N-Q275R, A088T-N109G-T158S-S248N, A088T-N218S-N243V-S248N-K256R-L257G, A088T-N243V-L257G, A088T-S248N, A088T-T158S-K256R, A088T-T158S-N218S-K256R, A088T-

T158S-N218S-L257G, A088T-T158S-N218S-L257G, A088T-T158S-N218S-N243V-S248N, A088T-T158S-N218S-N243V-S248N-L257G, A088T-T158S-N218S-Q245K-S248N-K256R, A088T-T158S-N218S-S248N-L257G-Q275K, A088T-T158S-N243V-K256R, A088T-T158S-N243V-K256R-L257G, A088T-T158S-N243V-S248N-K256R, A088T-T158S-S248N, A088T-T158S-S248N-K256R-L257G, A088T-T158S-S248N-L257G, A088T-T158S-S248N-L257G, A098S-G131H-T158S-N218S-N243V-S248N-K256R-L257G, A116T-G131H-K141E-N218S-N243V-S248N-L257G, A116T-G131H-N218S-W241R-N243V-S248N-K256R-L257G, A116T-G131H-N243V, A116T-G131H-T158S-N218S-S248N-K256R, A116T-G131H-V139I-N218S-N243V-S248N, A116T-N218S-S248N-K256R, A116T-T158S-L257G-Q271R, A116T-T158S-N218S-N243V-K256R-L257G, G053S-A088T-N109G-A116T-G131H-T158S-G169S-N218S-S248N-K256R-L257G, G131H-N218S-L257G, G131H-T158S, G131H-T158S-K256R, K256R, L0901-NI09G-T158S-N243V, L257G, N109G-A116T-G131H, N109G-A116T-G131H-N243V, N109G-A116T-G131H-T158S-N218S-N243V-S248N-K256R, N109G-A116T-S248N-K256R, N109G-A116T-T158S-N218S-K237R-N243V-S248N, N109G-A116T-T158S-S248N, N109G-G131H-T158S, N109G-G131H-T158S-N243V-K256R-L257G, N109G-G131H-T158S-S248N-Q271R, N109G-N218S-S248N-K256R, N109G-N243V-S248N-L257G, N109G-S248N, N109G-T158S-N218S-N243V-L257G, N109G-T158S-N243V-K256R-L257G, N109G-T158S-N243V-S248N-K256R-L257G, N218S-N243V-S248N-K256R-L257G, S003P-N109G-A116T-G131H-T158S-N218S-K256R, S003P-N109G-G131H-T158S-L257G, S105H-W106G-I107L-I108S-N109A-G110A-I111S-E112N-W113G-A114P, T158S-N218S-S248N-K256R, T158S-N243V-S248N, T158S-S248N, T158S-S248N-K256R-L257G, V004A-A088T-A116T-T158S-N218S, V004A-A088T-G131H-N218S-N243V-S248N-L257G, Y006H-N109G-N218S-N243V-S248N, Y104H-A116T-T158S-S248N, A088T-A116T-G131H-N218S-S248N-L257G, A088T-A116T-G131H-T158S-N218S-N243V-L257G, A088T-A116T-G131 H-T158S-N243V-K256R-L257G, A088T-A116T-N218S, A088T-G131D-T158S-N243V-S248N, A088T-G131H-N218S-K237R-K256R-L257G, A088T-G131H-N218S-K256R, A088T-K213N-N243V-S248N-K256R, A088T-K256R-L257G, A088T-N109G-A116T-G131H-D140G-S248N-L257G, A088T-N109G-A116T-G131H-L257G, A088T-N109G-A116T-G131H-N218S-N243V-S248N-K256R-L257G, A088T-N109G-A116T-G131H-T158S-N218S-N243V-S248N, A088T-N109G-A116T-M124I-G131H-T158S-N218S-S248N-L257G, A088T-N109G-A116T-T158S-N218S-N243V-K256R, A088T-N109G-A116T-T158S-N218S-N243V-L257G, A088T-N109G-A116T-T158S-N218S-S248N, A088T-N109G-G131H-K256R-L257G, A088T-N109G-G131H-N218S-N243V-S248N-L257G, A088T-N109G-G131H-N243V-S248N, A088T-N109G-G131H-T158S-L233S-N243V-S248N, A088T-N109G-G131H-T158S-N218S-S248N-K256R, A088T-N109G-L257G, A088T-N109G-N218S-K256R-L257G, A088T-N109G-N218S-S248N-T255K-K256R-L257G, A088T-N109G-S248N-K256R-L257G, A088T-N109G-T158S-N218S-N243V, A088T-N109G-T158S-N218S-N243V-L257G, A088T-N109G-T158S-IV218S-N243V-S248N-L257G, A088T-N109G-T158S-N218S-S248N, A088T-S248N-K256R-L257G, A088T-S248N-L257G-I268V, A088T-T158S-N243V-L257G, A088T-T158S-V203I-N218S-K256R-L257G, A088T-Y104H-A116T-G131H-N218S-N243V, A116T-D140G-T158S-N218S-N243V-S248N, AI16T-G131H-T158S-K256R-L257G, A116T-G131H-T158S-N243V-S248N, A116T-G157E-T158S-N243V-S248N-K256R, A116T-S248N-K256R, G131H-N218S-S248N, G131H-N243V-K256R, G131H-T158S-N243V-L257G, K256R-L257G, N109G-A116T-G131H-T158S-N218S-N243V-S248N, N109G-A116T-N218S-N243V-L257G, N109G-A116T-N218S-W241R-N243V-S248N-K256R-L257G, N109G-A116T-T158S-N243V, N109G-A116T-T158S-S248N-K256R, N109G-N243V, N109G-T158S-K256R-L257G, N243V, P014L-A015L-L016C-H017T-S018L-Q019K-G020A-Y021T-T022L-G023E, S003P-S248N-L257G, T158S-N218S-N243V-S248N, T158S-S248N-K256R, Y104H-N109G-G131H-N243V-S248N, A088T-A116T-G131H-L257G, A088T-A116T-G131H-N218S-N243V, A088T-A116T-G131H-N218S-S248N-K256R, A088T-A116T-G131H-T158S-N243V-S248N-L257G, A088T-A116T-L257G, A088T-A116T-N218S, A088TA116T-N218S-N243V-S248N, A088T-A116T-T158S-N218S-S248N, A088T-A116T-T158S-N243V-S248N-K256R, A088T-A138E-N218S-N243V-K256R, A088T-G131H-N218S-N243V-S248N-K256R, A088T-G131H-T158S-S248N-K256R, A088T-N109G-A116T-G131H-N218S-S248N-K256R-L257G, A088T-N109G-A116T-G131H-N243V-S248N-K256R, A088T-N109G-A116T-G131H-T158S-N218S-L257G-I268V, A088T-N109G-A116T-G131H-W241L-S248N-K256R-L257G, A088T-N109G-A116T-N218S-N243V-S248N-K256R, A088T-N109G-A116T-T158S-N243V-K256R-L257G, A088T-N109G-A116T-T158S-N243V-S248N, A088T-N109G-G131H-N218S-L257G, A088T-N109G-G131H-N218S-S248N, A088T-N109G-G131H-T158S-N243V-S248N-L257G, A088T-N109G-N243V-K256R-L257G, A088T-N109G-T158S-S248N-K256R, A088T-N109G-W241R-S248N-K256R, A088T-N218S-S248N-L257G, A088T-T158S-N218S-S248N-K256R, A088T-T158S-N218S-S248N-L257G, A116T-G131H-T158S-N218S-N243V, A116T-N218S-S248N, A116T-N243V-K256R, A116T-T158S, G131H-S248N-K256R, N109G-A116T-G131H-N243V-K256R-L257G, N109G-A116T-I234T-N243V-S248N-K256R-L257G, N109G-A116T-T158S-K256R-L257G, N109G-A116T-T158S-N218S-S248N-K256R, N109G-G131H, N109G-G131H-A137V-T158S-N218S-S248N, N109G-G131H-K141E-L257G, N109G-G131H-T158S-N243V-S248N-L257G, T158S-N218S-N243V-K256R, A088T-A116T-T158S-N243V-K256R-L257G, A088T-N109G-A116T-T158S-K256R-L257G, A088T-N109G-T158S-N218S-S248N, A088T-S248N-L257G, A088T-Y104H-N109G-G131H-A137E-T158S-N218S-N243V-S248N-K256R, G065D-A088T-G131H-N243V-S248N, Y104H-N218S-L257G, A088T-A116T-G131H-V150A-N218S-S248N-L257G, A088T-A116T-T158S-K256R-L257G, A088T-I108T-N109G-G131H-T158S-N218S-S248N-K256R-L257G, A088T-N109G-A116T-T158S-S248N-L257G-Q271P, A088T-N109G-A116T-V148AN218S-N243V, A088T-Y104H-N109G-AI16T-A153S-N218S-N243V-S248N-L257G-N269D, and V004M-A116T-V148A-T158S-N243V-S248N-K256R, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

EXAMPLE 10

Construction and Cleaning Performance of Additional Variants of BPN'-v36

[0375] The DNA from the site evaluation libraries of the BPN'-v36 (described in Example 7) was further mutagenized by error-prone PCR. These libraries were amplified with primers P4973 and P4950 (described in Example 7) using *Taq* DNA polymerase (Promega). Each PCR amplification reaction contained 30 pmol of each primer, 100 ng of the template DNA (SELs of the BPN'-v36) and various amount of $MnCl_2$. The PCR reaction (20 (µL) was initially heated at 95°C for 2.5 min followed by 30 cycles of denaturation at 94°C for 15 sec., annealing at 55°C for 15 sec. and extension at 72°C for 2 min. The DNA fragment was gel-purified by the QIAGEN® gel-band purification kit, digested by the *Bam*HI and *Hind*III restriction enzymes and ligated with the pHPLT-BPN' partial opt vector that also was digested with the same restriction enzymes. Ligation mixtures were amplified using rolling circle amplification in an Illustra Templiphi kit according to the manufacturer's recommendation (GE Healthcare) to generate multimeric DNA for transformation into *Bacillus subtilis*. For this purpose, 1µl of the ligation mixture was mixed with 5 µl of the sample buffer, heated to 95°C for 3 min and cooled on ice. Next, 5 µl of the reaction buffer and 0.2 µl of the enzyme were added to each tube, followed by incubation at 30°C for 10 hours. Products of the rolling circle amplification were diluted 100 times and used to transform *B. subtilis* cells (ΔaprE, ΔnprE, amyE::xylRPxylAcomK-phleo). An aliquot of the transformation mix was plated on LB plates containing 1.6% skim milk and 10 µg/mL neomycin and incubated overnight at 37°C.

[0376] About 500,000 clones were pre-screened on skim milk plates. Very few of them formed halos (i.e., indicative of the presence of functional protease). Colonies with halos were picked, inoculated in 150 µl of LB media containing 10µg/mL neomycin and sequenced (Quintara). Sequences of these clones were analyzed by looking for combination of mutations, which occurred in this pool multiple times and might provide performance benefits. In order to assess the performance of these mutation combinations, double mutants were created in the BPN'-v36 background by PCR fusion as described below. For this purpose, two or three partially overlapping fragments were amplified by mutagenic primers. Primer combinations used to generate the respective variants are shown in Table 10-1 and primer sequences are shown in Table 10-2.

Table 10-1. Primers Pairs Used to Amplify Fragments

Variant	Mutation 1	Mutation 2	Fragment 1	Fragment 2	Fragment 3
1	A45S	S236Y	P4974, P6645	P6644, P6647	P6646, P4976
2	A45S	S236G	P4974, P6645	P6644, P6649	P6648, P4976
3	I115T	S183T	P4974, P6651	P6650, P6655	P6654, P4976
4	I115V	N184Y	P4974, P6653	P6652, P6657	P6656, P4976
5	I31T	S37P	P4974, P6659	P6658, P4976	
6	I31T	I35L	P4974, P6661	P6660, P4976	
7	I31V	S38W	P4974, P6663	P6662, P4976	
8	*N25K*	P129R	P4974, P6665	P6664, P6667	P6666, P4976
9	*N25K*	P129K	P4974, P6665	P6664, P6669	P6668, P4976
10	P14T	S37T	P4974, P6671	P6670, P6673	P6672, P4976
11	P5L	Q217K	P4974, P6679	P6678, P6681	P6680, P4976
12	P5L	Q217G	P4974, P6679	P6678, P6683	P6682, P4976
13	QI0L	S37P	P4974, P6685	P6684, P6675	P6674, P4976
14	Q10R	S37T	P4974, P6687	P6686, P6673	P6672, P4976
15	S37P	T254S	P4974, P6675	P6674, P6689	P6688, P4976
16	*N25K*	S37P	P4974, P6665	P6664, P6675	P6674, P4976
17	G24A	S37W	P4974, P6691	P6690, P6677	P6676, P4976
18	*N25K*	P129R	P4974, P6665	P6664, P6667	P6666, P4976
20	S161P	S162L	P4974, P6695	P6694, P4976	
21	S161P	T253A	P4974, P6693	P6692, P6701	P6700, P4976
22	S161P	S260P	P4974, P6693	P6692, P6703	P6702, P4976
23	S162L	D181H	P4974, P6697	P6696, P6711	P6710, P4976
24	S162L	D181G	P4974, P6697	P6696, P6713	P6712, P4976

Table 10-1. Primers Pairs Used to Amplify Fragments

Variant	Mutation 1	Mutation 2	Fragment 1	Fragment 2	Fragment 3
25	S18F	S162L	P4974, P6715	P6714, P6697	P6696, P4976
26	S18T	S162P	P4974, P6717	P6716, P6699	P6698, P4976
27	S18P	D120N	P4974, P6719	P6718, P6727	P6726, P4976
28	S18Y	K213R	P4974, P6721	P6720, P6729	P6728, P4976
29	S18L	Y21S	P4974, P6731	P6730, P4976	
30	S18T	Y21N	P4974, P6733	P6732, P4976	
31	S9T	K141F	P4974, P6635	P6734, P6737	P6736, P4976
32	S9T	K141R	P4974, P6635	P6734, P6739	P6738, P4976
33	Q19L	S260N	P4974, P6725	P6724, P6705	P6704, P4976
34	Q19L	S260P	P4974, P6725	P6724, P6703	P6702, P4976
35	N61S	S260P	P4974, P6741	P6740, P6703	P6702, P4976
36	N61D	S260I	P4974, P6743	P6742, P6707	P6706, P4976
37	T253A	S260P	P4974, P6701	P6700, P6703	P6702, P4976
38	A134T	S260G	P4974, P6745	P6744, P6709	P6708, P4976
39	A133V	S260N	P4974, P6648	P6746, P6705	P6704, P4976

Table 10-2. Primer Sequences Used for Generation of Double Mutants of BPN'-v36

Primer Name	**Primer Sequence**	**SEQ ID NO:**
P6644	CAGATCTTAAAGTCTCTGGAGGGGCTTCTATGGTGC	SEQ ID NO:64
P6645	CATAGAAGCCCCTCCAGAGACTTTAAGATCTGGATGGCTC	SEQ ID NO:65
P6646	GCATTGATTCTTTACAAGCACCCGAACTGGACAAAC	SEQ ID NO:66
P6647	CAGTTCGGGTGCTTGTAAAGAATCAATGCGGCCGCCCCA	SEQ ID NO:67
P6648	GCATTGATTCTTGGTAAGCACCCGAACTGGACAAAC	SEQ ID NO:68
P6649	CCAGTTCGGGTGCTTACCAAGAATCAATGCGGCCGCCCCA	SEQ ID NO:69
P6650	CATCGAATGGGCCACAGCGAATAACATGGATGTAATCAAC	SEQ ID NO:70
P6651	CATCCATGTTATTCGCTGTGGCCCATTCGATGCCGTTGAT	SEQ ID NO:71
P6652	CATCGAATGGGCCGTAGCGAATAACATGGATGTAATCAAC	SEQ ID NO:72
P6653	CATCCATGTTATTCGCTACGGCCCATTCGATGCCGTTGAT	SEQ ID NO:73
P6654	CTGTAGACTCTACAAATCAACGTGCCTCTTTTTCCT	SEQ ID NO:74
P6655	AAAGAGGCACGTTGATTTGTAGAGTCTACAGCGCCCACTG	SEQ ID NO:75
P6656	CTGTAGACTCTTCATACCAACGTGCCTCTTTTTCCTCC	SEQ ID NO:76
P6657	GAAAAAGAGGCACGTTGGTATGAAGAGTCTACAGCGCCCA	SEQ ID NO:77
P6658	TAGCGGTTACAGACAGCGGTATCGACCCAAGCCATCCAGATC TTAAAGTCG	SEQ ID NO:78
P6659	ATGGCTTGGGTCGATACCGCTGTCTGTAACCGCTACTTTAACA TTGCCTC	SEQ ID NO:79
P6660	TAAAGTAGCGGTTACAGACAGCGGTTTAGACTCGAGCCATCC AGATCTT	SEQ ID NO:80

Table 10-2. Primer Sequences Used for Generation of Double Mutants of BPN'-v36		
Primer Name	**Primer Sequence**	**SEQ ID NO:**
P6661	ATGGCTCGAGTCTAAACCGCTGTCTGTAACCGCTACTTTAACA TTGCCTC	SEQ ID NO:81
P6662	GGTTGTAGACAGCGGTATCGACTCGTGGCATCCAGATCTTAA AGTCGCTG	SEQ ID NO:82
P6663	ATGCCACGAGTCGATACCGCTGTCTACAACCGCTACTTTAACA TTGCCTC	SEQ ID NO:83
P6664	CTACACTGGAGGCAAAGTTAAAGTAGCGGTTATCGACA	SEQ ID NO:84
P6665	ATAACCGCTACTTTAACTTTGCCTCCAGTGTAGCCTTGAG	SEQ ID NO:85
P6666	GAGCCTGGGAGCACGTAGCGGCAGTGCGGCACTTAAA	SEQ ID NO:86
P6667	GTGCCGCACTGCCGCTACGTGCTCCCAGGCTCATGTTGAT	SEQ ID NO:87
P6668	TGAGCCTGGGAGCAAAGAGCGGCAGTGCGGCACTTAAA	SEQ ID NO:88
P6669	GTGCCGCACTGCCGCTCTTTGCTCCCAGGCTCATGTTGAT	SEQ ID NO:89
P6670	ATCACAAATTAAAGCCACAGCTCTGCACTCTCAAGGCTAC	SEQ ID NO:90
P6671	AGAGTGCAGAGCTGTGGCTTTAATTTGTGATACGCCGTAA	SEQ ID NO:91
P6672	GACAGCGGTATCGACACAAGCCATCCAGATCTTAAAGTCG	SEQ ID NO:92
P6673	TAAGATCTGGATGGCTTGTGTCGATACCGCTGTCGATAAC	SEQ ID NO:93
P6674	GACAGCGGTATCGACCCAAGCCATCCAGATCTTAAAGTCG	SEQ ID NO:94
P6675	TAAGATCTGGATGGCTTGGGTCGATACCGCTGTCGATAAC	SEQ ID NO:95
P6676	GACAGCGGTATCGACTGGAGCCATCCAGATCTTAAAGTCG	SEQ ID NO:96
P6677	TAAGATCTGGATGGCTCCAGTCGATACCGCTGTCGATAAC	SEQ ID NO:97
P6678	ACGCGCAGTCCGTGTTATACGGCGTATCACAAATTAAAGC	SEQ ID NO:98
P6679	ATTTGTGATACGCCGTATAACACGGACTGCGCGTACGCAT	SEQ ID NO:99
P6680	ACAAGTATGGTGCGAAAAACGGGACTTCCATGGCCTC	SEQ ID NO:100
P6681	CATGGAAGTCCCGTTTTTCGCACCATACTTGTTCCCTG	SEQ ID NO:101
P6682	ACAAGTATGGTGCGGGAAACGGGACTTCCATGGCCTC	SEQ ID NO:102
P6683	CCATGGAAGTCCCGTTTCCCGCACCATACTTGTTCCCTG	SEQ ID NO:103
P6684	CTTACGGCGTATCATTAATTAAAGCCCCTGCTCTGCAC	SEQ ID NO:104
P6685	GAGCAGGGGCTTTAATTAATGATACGCCGTAAGGCACGGA	SEQ ID NO:105
P6686	CTTACGGCGTATCACGTATTAAAGCCCCTGCTCTGCAC	SEQ ID NO:106
P6687	GAGCAGGGGCTTTAATACGTGATACGCCGTAAGGCACGGA	SEQ ID NO:107
P6688	TTTAGAAAACACCTCTACAAAACTTGGTGATTCTTTCTAC	SEQ ID NO:108
P6689	TCACCAAGTTTTGTAGAGGTGTTTTCTAAACTGCTGCGGA	SEQ ID NO:109

Table 10-2. Primer Sequences Used for Generation of Double Mutants of BPN'-v36		
Primer Name	**Primer Sequence**	**SEQ ID NO:**
P6690	AGGCTACACTGGAGCAAATGTTAAAGTAGCGGTTATCGAC	SEQ ID NO:110
P6691	GCTACTTTAACATTTGCTCCAGTGTAGCCTTGAGAGTG	SEQ ID NO:111
P6692	GAGGGAACATCCGGACCATCGAGTACCGTCGGTTATCCA	SEQ ID NO:112
P6693	ACCGACGGTACTCGATGGTCCGGATGTTCCCTCATTCCCA	SEQ ID NO:113
P6694	AGGGAACATCCGGACCATTAAGTACCGTCGGTTATCCAGG	SEQ ID NO:114
P6695	ACCGACGGTACTTAATGGTCCGGATGTTCCCTCATTCCCA	SEQ ID NO:115
P6696	GAACATCCGGATCATTAAGTACCGTCGGTTATCCAGGCA	SEQ ID NO:116
P6697	ATAACCGACGGTACTTAATGATCCGGATGTTCCCTCATTC	SEQ ID NO:117
P6698	GAACATCCGGATCACCAAGTACCGTCGGTTATCCAGGCA	SEQ ID NO:118
P6699	ATAACCGACGGTACTTGGTGATCCGGATGTTCCCTCATTC	SEQ ID NO:119
P6700	GTTTAGAAAACGCAACTACAAAACTTGGTGATTCTTTC	SEQ ID NO:120
P6701	CACCAAGTTTTGTAGTTGCGTTTTCTAAACTGCTGCGGAC	SEQ ID NO:121
P6702	CAAAACTTGGTGATCCATTCTACTATGGAAAAGGGCTGAT	SEQ ID NO:122
P6703	TTTCCATAGTAGAATGGATCACCAAGTTTTGTAGTGGTGT	SEQ ID NO:123
P6704	CAAAACTTGGTGATAACTTCTACTATGGAAAAGGGCTGAT	SEQ ID NO:124
P6705	TTTCCATAGTAGAAGTTATCACCAAGTTTTGTAGTGGTGT	SEQ ID NO: 125
P6706	CAAAACTTGGTGATATCTTCTACTATGGAAAAGGGCTGAT	SEQ ID NO:126
P6707	TTTCCATAGTAGAAGATATCACCAAGTTTTGTAGTGGTGT	SEQ ID NO:127
P6708	CAAAACTTGGTGATGGATTCTACTATGGAAAAGGGCTGAT	SEQ ID NO:128
P6709	TTTCCATAGTAGAATCCATCACCAAGTTTTGTAGTGGTGT	SEQ ID NO:129
P6710	GTGGGCGCTGTACACTCTTCAAATCAACGTGCCTCTT	SEQ ID NO:130
P6711	CACGTTGATTTGAAGAGTGTACAGCGCCCACTGCAATCAC	SEQ ID NO:131
P6712	GTGGGCGCTGTAGGATCTTCAAATCAACGTGCCTCTT	SEQ ID NO:132
P6713	CACGTTGATTTGAAGATCCTACAGCGCCCACTGCAATCAC	SEQ ID NO:13
P6714	CCTGCTCTGCACTTCCAAGGCTACACTGGAGGCAATG	SEQ ID NO:134

Table 10-2. Primer Sequences Used for Generation of Double Mutants of BPN'-v36		
Primer Name	**Primer Sequence**	**SEQ ID NO:**
P6715	CTCCAGTGTAGCCTTGGAAGTGCAGAGCAGGGGCTTTAAT	SEQ ID NO:135
P6716	CCTGCTCTGCACACACAAGGCTACACTGGAGGCAATG	SEQ ID NO:136
P6717	CTCCAGTGTAGCCTTGTGTGTGCAGAGCAGGGGCTTTAAT	SEQ ID NO:137
P6718	CCTGCTCTGCACCCACAAGGCTACACTGGAGGCAATG	SEQ ID NO:138
P6719	CTCCAGTGTAGCCTTGTGGGTGCAGAGCAGGGGCTTTAAT	SEQ ID NO:139
P6720	CCTGCTCTGCACTACCAAGGCTACACTGGAGGCAATG	SEQ ID NO:140
P6721	CTCCAGTGTAGCCTTGGTAGTGCAGAGCAGGGGCTTTAAT	SEQ ID NO:141
P6722	CCTGCTCTGCACTTACAAGGCTACACTGGAGGCAATG	SEQ ID NO:142
P6723	CTCCAGTGTAGCCTTGTAAGTGCAGAGCAGGGGCTTTAAT	SEQ ID NO:143
P6724	TGCTCTGCACTCTTTAGGCTACACTGGAGGCAATGTTA	SEQ ID NO:144
P6725	TTGCCTCCAGTGTAGCCTAAAGAGTGCAGAGCAGGGGCTT	SEQ ID NO:145
P6726	ATCGCGAATAACATGAACGTAATCAACATGAGCCTGGGA	SEQ ID NO:146
P6727	CTCATGTTGATTACGTTCATGTTATTCGCGATGGCCCAT	SEQ ID NO:147
P6728	CTTCCAGGGAACCGTTATGGTGCGCAAAACGGGACTT	SEQ ID NO:148
P6729	GTTTTGCGCACCATAACGGTTCCCTGGAAGCGTCGATTG	SEQ ID NO:149
P6730	CTGCACTTACAAGGCTCTACTGGAGGCAATGTTAAAGTAG	SEQ ID NO:150
P6731	TAACATTGCCTCCAGTAGAGCCTTGTAAGTGCAGAGCAGGGG CTTTAAT	SEQ ID NO:151
P6732	GCTCTGCACTTACAAGGCAACACTGGAGGCAATGTTAAAGTA G	SEQ ID NO:152
P6733	AACATTGCCTCCAGTGTTGCCTTGTAAGTGCAGAGCAGGGGC TTTAAT	SEQ ID NO:153
P6734	CTTACGGCGTAACACAAATTAAAGCCCCTGCTCTG	SEQ ID NO:154
P6735	AGGGGCTTTAATTTGTGTTACGCCGTAAGGCACGGACT	SEQ ID NO:155
P6736	TTAAAGCAGCAGTTGATTTCGCTGTTGCATCTGGTGTCGT	SEQ ID NO:156

Table 10-2. Primer Sequences Used for Generation of Double Mutants of BPN'-v36		
Primer Name	**Primer Sequence**	**SEQ ID NO:**
P6737	AGATGCAACAGCGAAATCAACTGCTGCTTTAAGTGCCGCA	SEQ ID NO:157
P6738	TTAAAGCAGCAGTTGATCGTGCTGTTGCATCTGGTGTCGT	SEQ ID NO:158
P6739	AGATGCAACAGCACGATCAACTGCTGCTTTAAGTGCCGCA	SEQ ID NO:159
P6740	AAACCCGTTTCAAGATTCTAATTCTCATGGCACACACGTC	SEQ ID NO:160
P6741	TGTGCCATGAGAATTAGAATCTTGAAACGGGTTTGTTTCG	SEQ ID NO:161
P6742	AAACCCGTTTCAAGATGATAATTCTCATGGCACACACGTC	SEQ ID NO:162
P6743	TGTGCCATGAGAATTATCATCTTGAAACGGGTTTGTTTCG	SEQ ID NO:163
P6744	AAGCGGCAGTGCGACACTTAAAGCAGCAGTTGATAAAGC	SEQ ID NO:164
P6745	TCAACTGCTGCTTTAAGTGTCGCACTGCCGCTTGGTGCTC	SEQ ID NO:165
P6746	CAAGCGGCAGTGTTGCACTTAAAGCAGCAGTTGATAA	SEQ ID NO:166
P6747	ACTGCTGCTTTAAGTGCAACACTGCCGCTTGGTGCTCCCA	SEQ ID NO:167

[0377] Each PCR amplification reaction contained 30 pmol of each primer and 100 ng of the BPN'-v36 parent template DNA (plasmid pHPLT-BPN'-v36) (see Figure 4). Amplifications were carried out using Vent DNA polymerase (NEB). The PCR reaction (20 µL) was initially heated at 95°C for 2.5 min followed by 30 cycles of denaturation at 94°C for 15 sec., annealing at 55°C for 15 sec, and extension at 72°C for 40 sec. Following amplification, the 5' and 3' gene fragments were gel-purified by the QIAGEN® gel-band purification kit, mixed (50 ng of each fragment), mixed and amplified by PCR once again using the primers P4973 and P4950 to generate the full-length gene fragment. The PCR conditions were same as described above, except the extension phase, which was carried out at 72°C for 2 min. The full-length DNA fragment was gel-purified by the QIAGEN® gel-band purification kit, digested by the *Bam*HI and *Hind*III restriction enzymes and ligated with the pHPLT-BPN' partial opt vector that also was digested with the same restriction enzymes. Ligation mixtures were amplified using rolling circle amplification in an Illustra Templiphi kit according to the manufacturer's recommendation (GE Healthcare) to generate multimeric DNA for transformation into *Bacillus subtilis*. For this purpose, 1 µl of the ligation mixture was mixed with 5 µl of the sample buffer, heated to 95°C for 3 min and cooled on ice. Next, 5 µl of the reaction buffer and 0.2 µl of the enzyme were added to each tube, followed by incubation at 30°C for 10 hours. Products of the rolling circle amplification were diluted 100 times and used to transform *B. subtilis* cells (ΔaprE, ΔnprE, amyE::xylRPxylAcomK-phleo). An aliquot of the transformation mix was plated on LB plates containing 1.6% skim milk and 10 µg/mL neomycin and incubated overnight at 37°C. Subsequently, the colonies with halos were inoculated in 150 µl of LB media containing 10µg/mL neomycin. The next day, the cultures were either frozen with 15% glycerol or grown in MBD medium for biochemical analysis as described in Example 2.

[0378] The variants were tested for cleaning performance using BMI microswatch assay in Detergent Composition 4 at 16°C and pH 8, BMI microswatch assay in Detergent Composition 4 at 16°C and pH 7, and Egg microswatch assay in Detergent Composition 4 at 16°C and pH 8. Protein content was determined using TCA assay. All assays were performed as described in Example 1 and Performance Indices were calculated relative to BPN'-v36 (i.e., BPN'-S24G-S53G-S78N-S101N-G128A-Y217Q) (with a PI value of 1.0).

[0379] The following BPN'-v36 variants were determined to have a PI value of about 1.0 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A133V-S260N, N061S-S260P, P014T-S037T, S009T-K141F, S009T-K141R, S018F-S162L, S018L-Y021S, S018P-D120N,

S018T-S162P, S018T-Y021N, S018Y-K213R, S161P-S162L, S161P-S260P, and T253A-S260P, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0380] The following BPN'-v36 variants were determined to have a PI value of about 0.9 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A134T-S260G, I115V-N184Y, N025K-S037P, Q010L-S037P, Q019L-S260N, Q019L-S260P, S037P-T254S, and S161P-T253A, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0381] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A045S-S236G, G024A-S037W, I031V-S038W, N061D-S2601, Q010R-S037T, I115T-S183T, N025K-P129K, N025K-P129R, A045S-S236Y, and S162L-D181H, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0382] The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 7 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of S018F-S162L, S018P-D120N, P014T-S037T, S009T-K141R, and S161P-S162L, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN', BPN'-v3, and BPN'-v36, and a greater PI value than that of BPN', BPN'-v3 and BPN'-v36 in this assay.

[0383] The following BPN'-v36 variants were determined to have a PI value of about 1.0 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 7 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of N061S-S260P, Q010L-S037P, S009T-K141F, S018L-Y021S, S018T-S162P, S018T-Y021N, S018Y-K213R, S037P-T254S, S161P-S260P, S161P-T253A, and T253A-S260P, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0384] The following BPN' variants were determined to have a PI value of about 0.9 relative to BPN'-v3 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 7 and 16°C: BPN' amino acid sequence (SEQ ID NO:2) comprising at least one set of amino acid substitutions selected from the group consisting of A133V-S260N, A134T-S260G, I115T-S183T, I115V-N184Y, N061D-S260I, Q019L-S260N, and Q019L-S260P, wherein positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0385] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 7 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A045S-S236G, G024A-S037W, Q010R-S037T, A045S-S236Y, I031V-S038W, N025K-S037P, S162L-D181H, and N025K-P129R, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0386] The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of 1031V-S038W, P014T-S037T, S018F-S162L, S018P-D120N, and S162L-D181H, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN', BPN'-v3, and BPN'-v36, and a greater PI value than that of BPN', BPN'-v3 and BPN'-v36 in this assay.

[0387] The following BPN'-v36 variants were determined to have a PI value of about 1.0 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q

amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A133V-S260N, A134T-S260G, G024A-S037W, I115V-N184Y, N025K-P129K, N025K-P129R, N061D-S260I, Q019L-S260P, S009T-K141F, S009T-K141R, S018L-Y021S, S018T-S162P, S018T-Y021N, S018Y-K213R, S161P-S162L, S161P-T253A, and T253A-S260P, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0388] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.8 and equal to or less than 0.9 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of A045S-S236G, A045S-S236Y, I115T-S183T, N025K-S037P, N061S-S260P, Q010L-S037P, Q010R-S037T, Q019L-S260N, S037P-T254S, and S161P-S260P, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

EXAMPLE 11

Liquid Laundry Detergent Compositions

[0389] In this Example, various formulations for liquid laundry detergent compositions are provided. The following liquid laundry detergent compositions of the present invention are prepared as shown below. In each of these formulations, at least one protease variant provided herein is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative aspects, other concentrations will find use, as determined by the formulator, based on their needs.

Table 11-1: Liquid Laundry Detergent Composition

Compound	Formulations				
	I	II	III	IV	V
LAS	24.0	32.0	6.0	3.0	6.0
NaC 16-C_{17}HSAS	-	-	-	5.0	-
C_{12}-$C_{15}AE_{1.8}S$	-	-	8.0	7.0	5.0
C_8-C_{10} propyl dimethyl amine	2.0	2.0	2.0	2.0	1.0
C_{12}-C_{14} alkyl dimethyl amine oxide	-	-	-	-	2.0
C_{12}-C_{15} AS	-	-	17.0	-	8.0
CFAA	-	5.0	4.0	4.0	3.0
C_{12}-C_{14} Fatty alcohol ethoxylate	12.0	6.0	1.0	1.0	1.0
C_{12}-C_{18} Fatty acid	3.0	-	4.0	2.0	3.0
Citric acid (anhydrous)	4.5	5.0	3.0	2.0	1.0
DETPMP	-	-	1.0	1.0	0.5
Monoethanolamine	5.0	5.0	5.0	5.0	2.0
Sodium hydroxide	-	-	2.5	1.0	1.5
1 N HCl aqueous solution	#1	#1	-	-	-
Propanediol	12.7	14.5	13.1	10.	8.0
Ethanol	1.8	2.4	4.7	5.4	1.0
DTPA	0.5	0.4	0.3	0.4	0.5
Pectin Lyase	-	-	-	0.005	-
Amylase	0.001	0.002	-	-	-
Cellulase	-	-	0.0002	-	0.0001
Lipase	0.1	-	0.1	-	0.1

Table 11-1: Liquid Laundry Detergent Composition					
Compound	**Formulations**				
	I	**II**	**III**	**IV**	**V**
NprE (optional)	0.05	0.3	-	0.5	0.2
PMN	-	-	0.08	-	-
Protease A (optional)	-	-	-	-	0.1
Aldose Oxidase	-	-	0.3	-	0.003
ZnCl2	0.1	0.05	0.05	0.05	0.02
Ca formate	0.05	0.07	0.05	0.06	0.07
DETBCHD	-	-	0.02	0.01	-
SRP1	0.5	0.5	-	0.3	0.3
Boric acid	-	-	-	-	2.4
Sodium xylene sulfonate	-	-	3.0	-	-
Sodium cumene sulfonate	-	-	-	0.3	0.5
DC 3225C	1.0	1.0	1.0	1.0	1.0
2-butyl-octanol	0.03	0.04	0.04	0.03	0.03
Brightener 1	0.12	0.10	0.18	0.08	0.10
Balance to 100% perfume / dye and/or water					
#1: Add 1N HCl aqueous solution to adjust the neat pH of the formula in the range from about 3 to about 5.					

[0390] The pH of Formulations (I)-(II) in Table 11-1 is about 5 to about 7 and of Formulations (III)-(V) in Table 11-1 is about 7.5 to about 8.5.

EXAMPLE 12

Hand Dish Liquid Detergent Compositions

[0391] In this Example, various hand dish liquid detergent formulations are provided. The following hand dish liquid detergent compositions of the present invention are provided below. In each of these formulations, at least one protease variant provided herein is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative aspects, other concentrations will find use, as determined by the formulator, based on their needs.

Table 12-1: Hand Dish Liquid Detergent Compositions						
Compound	**Formulations**					
	I	**II**	**III**	**IV**	**V**	**VI**
C_{12}-$C_{15}AE_{1.8}S$	30.0	28.0	25.0	-	15.0	10.0
LAS	-	-	-	5.0	15.0	12.0
Paraffin Sulfonate	-	-	-	20.0	-	-
C_{10}-C_{18} Alkyl Dimethyl Amine Oxide	5.0	3.0	7.0	-	-	-
Betaine	3.0	-	1.0	3.0	1.0	-
C_{12} poly-OH fatty acid amide	-	-	-	3.0	-	1.0
C_{14} poly-OH fatty acid amide	-	1.5	-	-	-	-
$C_{11}E_9$	2.0	-	4.0	-	-	20.0
DTPA	-	-	-	-	0.2	-

Table 12-1: Hand Dish Liquid Detergent Compositions						
Compound	**Formulations**					
	I	**II**	**III**	**IV**	**V**	**VI**
Tri-sodium Citrate dehydrate	0.25	-	-	0.7	-	-
Diamine	1.0	5.0	7.0	1.0	5.0	7.0
$MgCl_2$	0.25	-	-	1.0	-	-
nprE (optional)	0.02	0.01	-	0.01	-	0.05
PMN	-	-	0.03	-	0.02	-
Protease A (optional)	-	0.01	-	-	-	-
Amylase	0.001	-	-	0.002	-	0.001
Aldose Oxidase	0.03	-	0.02	-	0.05	-
Sodium Cumene Sulphonate	-	-	-	2.0	1.5	3.0
PAAC	0.01	0.01	0.02	-	-	-
DETBCHD	-	-	-	0.01	0.02	0.01
Balance to 100% perfume / dye and/or water						

[0392] The pH of Formulations (I)-(VI) in Table 12-1 is about 8 to about 11.

EXAMPLE 13

Liquid Automatic Dishwashing Detergent Compositions

[0393] In this Example, various liquid automatic dishwashing detergent formulations are provided. The following hand dish liquid detergent compositions of the present invention are provided below. In each of these formulations, at least one protease variant provided herein is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative aspects, other concentrations will find use, as determined by the formulator, based on their needs.

Table 13-1: Liquid Automatic Dishwashing Detergent Compositions					
Compound	**Formulations**				
	I	**II**	**III**	**IV**	**V**
STPP	16	16	18	16	16
Potassium Sulfate	-	10	8	-	10
1,2 propanediol	6.0	0.5	2.0	6.0	0.5
Boric Acid	-	-	-	4.0	3.0
$CaCl_2$ dihydrate	0.04	0.04	0.04	0.04	0.04
Nonionic	0.5	0.5	0.5	0.5	0.5
nprE (optional)	0.1	0.03	-	0.03	-
PMN	-	-	0.05	-	0.06
Protease B (optional)	-	-	-	0.01	-
Amylase	0.02	-	0.02	0.02	-
Aldose Oxidase	-	0.15	0.02	-	0.01
Galactose Oxidase	-	-	0.01	-	0.01
PAAC	0.01	-	-	0.01	-
DETBCHD	-	0.01	-	-	0.01
Balance to 100% perfume / dye and/or water					

EXAMPLE 14

Granular and/or Tablet Laundry Compositions

[0394] This Example provides various formulations for granular and/or tablet laundry detergents. The following laundry compositions of present invention, which may be in the form of granules or tablet, are provided below. In each of these formulations, at least one protease variant provided herein is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative aspects, other concentrations will find use, as determined by the formulator, based on their needs.

Table 14-1: Granular and/or Tablet Laundry Compositions

Compound	Formulations				
	I	II	III	IV	V
Base Product					
C_{14}-C_{15}AS or TAS	8.0	5.0	3.0	3.0	3.0
LAS	8.0	-	8.0	-	7.0
C_{12}-$C_{15}AE_3S$	0.5	2.0	1.0	-	-
C_{12}-$C_{15}E_5$ or E_3	2.0	-	5.0	2.0	2.0
QAS	-	-	-	1.0	1.0
Zeolite A	20.0	18.0	11.0	-	10.0
SKS-6 (dry add)	-	-	9.0	-	-
MA/AA	2.0	2.0	2.0	-	-
AA	-	-	-	-	4.0
3Na Citrate $\cdot 2H_2O$	-	2.0	-	-	-
Citric Acid (Anhydrous)	2.0	-	1.5	2.0	-
DTPA	0.2	0.2	-	-	-
EDDS	-	-	0.5	0.1	-
HEDP	-	-	0.2	0.1	-
PB1	3.0	4.8	-	-	4.0
Percarbonate	-	-	3.8	5.2	-
NOBS	1.9	-	-	-	-
NACA OBS	-	-	2.0	-	-
TAED	0.5	2.0	2.0	5.0	1.00
BB1	0.06	-	0.34	-	0.14
BB2	-	0.14	-	0.20	-
Anhydrous Na Carbonate	15.0	18.0	-	15.0	15.0
Sulfate	5.0	12.0	5.0	17.0	3.0
Silicate	-	1.0	-	-	8.0
nprE (optional)	0.03	-	0.1	0.06	-
PMN	-	0.05	-	-	0.1
Protease B (optional)	-	0.01	-	-	-
Protease C (optional)	-	-	-	0.01	-
Lipase	-	0.008	-	-	-
Amylase	0.001	-	-	-	0.001

Table 14-1: Granular and/or Tablet Laundry Compositions					
Compound	**Formulations**				
	I	**II**	**III**	**IV**	**V**
Cellulase	-	0.0014	-	-	-
Pectin Lyase	0.001	0.001	0.001	0.001	0.001
Aldose Oxidase	0.03	-	0.05	-	-
PAAC	-	0.01	-	-	0.05
Balance to 100% Moisture and/or Minors*					
* Perfume, dye, brightener / SRP1 / Na carboxymethylcellulose / photobleach / $MgSO_4$ / PVPVI/ suds suppressor /high molecular PEG/clay.					

EXAMPLE 15

Liquid Laundry Detergents

[0395] This Example provides various formulations for liquid laundry detergents. The following liquid laundry detergent formulations of the present invention are provided below. In each of these formulations, at least one protease variant provided herein is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative aspects, other concentrations will find use, as determined by the formulator, based on their needs.

Table 15-1: Liquid Laundry Detergents						
Compound	**Formulations**					
	I	**II**	**III**	**IV**	**V**	**VI**
LAS	11.5	11.5	9.0	-	4.0	-
C_{12}-$C_{15}AE_{2.85}S$	-	-	3.0	18.0	-	16.0
C_{14}-$C_{15}E_{2.5}S$	11.5	11.5	3.0	-	16.0	-
C_{12}-$C_{13}E_9$	-	-	3.0	2.0	2.0	1.0
C_{12}-$C_{13}E_7$	3.2	3.2	-	-	-	-
CFAA	-	-	-	5.0	-	3.0
TPKFA	2.0	2.0	-	2.0	0.5	2.0
Citric Acid (Anhydrous)	3.2	3.2	0.5	1.2	2.0	1.2
Ca formate	0.1	0.1	0.06	0.1	-	-
Na formate	0.5	0.5	0.06	0.1	0.05	0.05
ZnCl2	0.1	0.05	0.06	0.03	0.05	0.05
Na Culmene Sulfonate	4.0	4.0	1.0	3.0	1.2	-
Borate	0.6	0.6	1.5	-	-	-
Na Hydroxide	6.0	6.0	2.0	3.5	4.0	3.0
Ethanol	2.0	2.0	1.0	4.0	4.0	3.0
1,2 Propanediol	3.0	3.0	2.0	8.0	8.0	5.0
Monoethanolamine	3.0	3.0	1.5	1.0	2.5	1.0
TEPAE	2.0	2.0	-	1.0	1.0	1.0
nprE (optional)	0.03	0.05	-	0.03	-	0.02
PMN	-	-	0.01	-	0.08	-
Protease A (optional)	-	-	0.01	-	-	-

Table 15-1: Liquid Laundry Detergents						
Compound	**Formulations**					
	I	**II**	**III**	**IV**	**V**	**VI**
Lipase	-	-	-	0.002	-	-
Amylase	-	-	-	-	0.002	-
Cellulase	-	-	-	-	-	0.0001
Pectin Lyase	0.005	0.005	-		-	-
Aldose Oxidase	0.05	-	-	0.05	-	0.02
Galactose oxidase	-	0.04				
PAAC	0.03	0.03	0.02	-	-	-
DETBCHD	-	-	-	0.02	0.01	-
SRP 1	0.2	0.2	-	0.1	-	-
DTPA	-	-	-	0.3	-	-
PVNO	-	-	-	0.3	-	0.2
Brightener 1	0.2	0.2	0.07	0.1	-	-
Silicone antifoam	0.04	0.04	0.02	0.1	0.1	0.1
Balance to 100% perfume/dye and/or water						

EXAMPLE 16

High Density Dishwashing Detergents

[0396] This Example provides various formulations for high density dishwashing detergents. The following compact high density dishwashing detergents of the present invention are provided below. In each of these formulations, at least one protease variant provided herein is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative aspects, other concentrations will find use, as determined by the formulator, based on their needs.

Table 16-1: High Density Dishwashing Detergents						
Compound	**Formulations**					
	I	**II**	**III**	**IV**	**V**	**VI**
STPP	-	45.0	45.0	-	-	40.0
3Na Citrate·$2H_2O$	17.0	-	-	50.0	40.2	-
Na Carbonate	17.5	14.0	20.0	-	8.0	33.6
Bicarbonate	-	-	-	26.0	-	-
Silicate	15.0	15.0	8.0	-	25.0	3.6
Metasilicate	2.5	4.5	4.5	-	-	-
PB1	-	-	4.5	-	-	-
PB4	-	-	-	5.0	-	-
Percarbonate	-	-	-	-	-	4.8
BB1	-	0.1	0.1	-	0.5	-
BB2	0.2	0.05	-	0.1	-	0.6
Nonionic	2.0	1.5	1.5	3.0	1.9	5.9
HEDP	1.0	-	-	-	-	-
DETPMP	0.6	-	-	-	-	-

Table 16-1: High Density Dishwashing Detergents						
Compound	**Formulations**					
	I	**II**	**III**	**IV**	**V**	**VI**
PAAC	0.03	0.05	0.02	-	-	-
Paraffin	0.5	0.4	0.4	0.6	-	-
nprE (optional)	0.072	0.053	-	0.026	-	0.01
PMN	-	-	0.053	-	0.059	-
Protease B (optional)	-	-	-	-	-	0.01
Amylase	0.012	-	0.012	-	0.021	0.006
Lipase	-	0.001	-	0.005	-	-
Pectin Lyase	0.001	0.001	0.001	-	-	-
Aldose Oxidase	0.05	0.05	0.03	0.01	0.02	0.01
BTA	0.3	0.2	0.2	0.3	0.3	0.3
Polycarboxylate	6.0	-	-	-	4.0	0.9
Perfume	0.2	0.1	0.1	0.2	0.2	0.2
Balance to 100% Moisture and/or Minors*						
*Brightener / dye / SRP 1 / Na carboxymethylcellulose/ photobleach / $MgSO_4$ / PVPVI/ suds suppressor /high molecular PEG/clay.						

[0397] The pH of Formulations (I) through (VI) in Table 16-1 is from about 9.6 to about 11.3.

EXAMPLE 17

Tablet Detergent Compositions

[0398] This Example provides various tablet detergent formulations. The following tablet detergent compositions of the present invention are prepared by compression of a granular dishwashing detergent composition at a pressure of 13KN/cm^2 using a standard 12 head rotary press. In each of these formulations, at least one protease variant provided herein is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative aspects, other concentrations will find use, as determined by the formulator, based on their needs.

Table 17-1: Tablet Detergent Compositions								
Compound	**Formulations**							
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
STPP	-	48.8	44.7	38.2	-	42.4	46.1	46.0
3Na Citrate·$2H_2O$	20.0	-	-	-	35.9	-	-	-
Na Carbonate	20.0	5.0	14.0	15.4	8.0	23.0	20.0	-
Silicate	15.0	14.8	15.0	12.6	23.4	2.9	4.3	4.2
Lipase	0.001	-	0.01	-	0.02	-	-	-
Protease B (optional)	0.01	-	-	-	-	-	-	-
Protease C (optional)	-	-	-	-	-	0.01	-	-
nprE (optional)	0.01	0.08	-	0.04	-	0.023	-	0.05
PMN	-	-	0.05	-	0.052	-	0.023	-
Amylase	0.012	0.012	0.012	-	0.015	-	0.017	0.002
Pectin Lyase	0.005	-	-	0.002	-	-	-	-

Table 17-1: Tablet Detergent Compositions								
Compound	**Formulations**							
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
Aldose Oxidase	-	0.03	-	0.02	0.02	-	0.03	-
PB1	-	-	3.8	-	7.8	-	-	4.5
Percarbonate	6.0	-	-	6.0	-	5.0	-	-
BB1	0.2	-	0.5	-	0.3	0.2	-	-
BB2	-	0.2	-	0.5	-	-	0.1	0.2
Nonionic	1.5	2.0	2.0	2.2	1.0	4.2	4.0	6.5
PAAC	0.01	0.01	0.02	-	-	-	-	-
DETBCHD	-	-	-	0.02	0.02	-	-	-
TAED	-	-	-	-	-	2.1	-	1.6
HEDP	1.0	-	-	0.9	-	0.4	0.2	-
DETPMP	0.7	-	-	-	-	-	-	-
Paraffin	0.4	0.5	0.5	0.5	-	-	0.5	-
BTA	0.2	0.3	0.3	0.3	0.3	0.3	0.3	-
Polycarboxylate	4.0	-	-	-	4.9	0.6	0.8	-
PEG 400-30,000	-	-	-	-	-	2.0	-	2.0
Glycerol	-	-	-	-	-	0.4	-	0.5
Perfume	-	-	-	0.05	0.2	0.2	0.2	0.2
Balance to 100% Moisture and/or Minors*								
*Brightener / SRP1 / Na carboxymethylcellulose/ photobleach / $MgSO_4$ / PVPVI/ suds suppressor /high molecular PEG/clay.								

[0399] The pH of Formulations (I) through (VII) in Table 17-1 is from about 10 to about 11.5 and the pH of Formulation (VIII) in Table 19-1 is from 8-10. The tablet weight of Formulations (I) through (VIII) in Table 17-1 is from about 20 grams to about 30 grams.

EXAMPLE 18

Liquid Hard Surface Cleaning Detergents

[0400] This Example provides various formulations for liquid hard surface cleaning detergents. The following liquid hard surface cleaning detergent compositions of the present invention are provided below. In each of these formulations, at least one protease variant provided herein is included at a concentration of from about 0.0001 to about 10 weight percent. In some alternative aspects, other concentrations will find use, as determined by the formulator, based on their needs.

Table 18-1: Liquid Hard Surface Cleaning Detergents							
Compound	**Formulations**						
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**
C_9-$C_{11}E_5$	2.4	1.9	2.5	2.5	2.5	2.4	2.5
C_{12}-$C_{14}E_5$	3.6	2.9	2.5	2.5	2.5	3.6	2.5
C_7-C_9E_6	-	-	-	-	8.0	-	-
C_{12}-$C_{14}E_{21}$	1.0	0.8	4.0	2.0	2.0	1.0	2.0
LAS	-	-	-	0.8	0.8	-	0.8

Table 18-1: Liquid Hard Surface Cleaning Detergents							
Compound	**Formulations**						
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**
Sodium culmene sulfonate	1.5	2.6	-	1.5	1.5	1.5	1.5
Isachem® AS	0.6	0.6	-	-	-	0.6	-
Na_2CO_3	0.6	0.13	0.6	0.1	0.2	0.6	0.2
3Na Citrate·$2H_2O$	0.5	0.56	0.5	0.6	0.75	0.5	0.75
NaOH	0.3	0.33	0.3	0.3	0.5	0.3	0.5
Fatty Acid	0.6	0.13	0.6	0.1	0.4	0.6	0.4
2-butyl octanol	0.3	0.3	-	0.3	0.3	0.3	0.3
PEG DME-2000®	0.4	-	0.3	0.35	0.5	-	-
PVP	0.3	0.4	0.6	0.3	0.5	-	-
MME PEG (2000)®	-	-	-	-	-	0.5	0.5
Jeffamine® ED-2001	-	0.4	-	-	0.5	-	-
PAAC	-	-	-	0.03	0.03	0.03	-
DETBCHD	0.03	0.05	0.05	-	-	-	-
nprE (optional)	0.07	-	0.08	0.03	-	0.01	0.04
PMN	-	0.05	-	-	0.06	-	-
Protease B (optional)	-	-	-	-	-	0.01	-
Amylase	0.12	0.01	0.01	-	0.02	-	0.01
Lipase	-	0.001	-	0.005	-	0.005	-
Pectin Lyase	0.001	-	0.001	-	-	-	0.002
ZnCl2	0.02	0.01	0.03	0.05	0.1	0.05	0.02
Calcium Formate	0.03	0.03	0.01	-	-	-	-
PB1	-	4.6	-	3.8	-	-	-
Aldose Oxidase	0.05	-	0.03	-	0.02	0.02	0.05
Balance to 100% perfume / dye and/or water							

[0401] The pH of Formulations (I) through (VII) in Table 18-1 is from about 7.4 to about 9.5.

EXAMPLE 19

Cleaning Performance of BPN'-v36 Polypeptide Variants

[0402] BPN'-v36 polypeptide variants comprising two amino acid substitutions were constructed by standard PCR fusion using the BPN'-v36 variant as a backbone or parent sequence. For this purpose, two or three partially overlapping fragments were amplified by mutagenic primers prepared such that the primer encoded a desired substitution. PCR amplification reactions were carried out as described in Example 7 *sura*. The following BPN'-v36 double mutant variants (i.e., BPN'-v36 with the following two amino acid substitution) were constructed: Q019R-N025D, A001Y-Q275R, V004A-S249N, V004E-S260P, V004A-T55A, Y006F-S249C, Y006D-T55A, V008L-Q275R, Q010R-Q275K, L016Q-Q217H, H017R-T158A, S183D-Q206R, P210S-N212D, S018Y-V203A, S018K-V203I, Y021H-D259G, Y021H-D259R, K027R-N269D, K027R-N269T, S037P-S260F, S037T-S260P, D041E-N077D, D041G-N077E, G166V-S183T, N252S-L257H, V044A-Q206H, V044A-Q206K, V044A-Q206R, N076T-N212D, N076P-N212S, N077D-N252D, N077D-N252T, K141I-S248N, T158I-D259N, T158A-D259P, S161E-Q185H, K237M-H238R, G160A-D259G, G160R-D259V, G215R-D259R, G215D-D259V, N061D-Q206R, N061L-Q206H, S009L-N218S, S161E-S260T, Q019A-N109S, T022S-G166V, Y021H-N252H, P129S-K136R, T022S-T242S, N025K-H238R, N025D-Q185R, S037G-Q275H, K043R-N076S, K043N-Q217R, K043N-S163T, T055A-V147A, N061K-N252K, N062Y-G097D, Y021H-V084E, Y021H-S037E, N062Y-

T244A, K027E-Y091F, A074S-P129Q, S249R-Q275R, I079V-Q217H, A098T-T158A, K027R-D120H, Q019R-Q185R, G131S-K265N, A133V-D259N, A144H-T244A, I035V-K043N, G160R-T244A, S161P-T253A, S163T-Q245L, K170R-D259G, S183T-S249R, N184Y-Y262N, V198L-D259G, A200T-H226L, Q206R-S260P, G211V-T244A, Q217R-T244A, L75I-N76D, S260P-Q275L, S260P-Q275R, Y262N-Q275R, V004A-Y006F, H017L-Q019A, N025D-V026A, N118G-V121A, V072F-L075I, S183T-R186K, V203A-Q217R, and S249R-Y262H. The cleaning performance of each of these variants was tested in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C and egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C as described in Example 1. Results are provided below.

[0403] The following BPN' protease variants were determined to have a PI value equal to or greater than 0.9 and equal or less than 1.0 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of S183T-S249R, N61D-Q206R, Y262N-Q275R, K43R-N76S, K170R-D259G, Y6F-S249C, Q19A-N109S, H17L-Q19A, Q19R-Q185R, S18Y-V203A, S161E-S260T, S18K-V203I, V4AT55A, N252S-L257H, S249R-Y262H, N61L-Q206H, N184Y-Y262N, Q19R-N25D, A74S-P129Q, K27R-D120H, Y21H-N252H, K27R-N269D, A98T-T158A, 179V-Q217H, S9L-N218S, V4A-Y6F, S161P-T253A, V203A-Q217R, T22S-T242S, N76P-N212S, S37T-S260P, T55A-V147A, G160R-T244A, N25D-Q185R, G211V-T244A, A144H-T244A, Y21H-N252H, A1Y-Q275R, V198L-D259G, K141I-S248N, S183T-R186K, S161E-Q185H, P129S-K136R, K43N-S163T, S37G-Q275H, N62Y-T244A, and S260P-Q275R, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to BPN' (SEQ ID NO:2) and a greater PI value than that of BPN' in this assay.

[0404] The following BPN' subtilisin protease variants were determined to have a PI value equal or greater than 0.5 and less than 0.9 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of Y21H-D259G, A133V-D259N, I79V-Q217H, S18K-V203I, T158A-D259P, N61K-N252K, K43N-Q217R, T158A-D259P, Q206R-S260P, A133V-D259N, V198L-D259G, N61K-N252K, S161E-S260T, G160A-D259G, K43N-Q217R, A1Y-Q275R, A200T-H226L, Q217R-T244A, S260P-Q275R, Q206R-S260P, T158I-D259N, Q217R-T244A, L75I-N76D, S161E-Q185H, Y21H-S37E, S249R-Q275R, T158I-D259N, Y21H-S37E, N76T-N212D, S260P-Q275L, G131S-K265N, V4A-S249N, N25D-Q185R, K43R-N76S, S183D-Q206R, Q10R-Q275K, K43N-S163T, Q10R-Q275K, N25D-V26A, G131S-K265N, S260P-Q275L, K141I-S248N, L16Q-Q217H, S249R-Q275R, K27R-N269T, P210S-N212D, L75I-N76D, S183D-Q206R, N118G-V121A, G215D-D259V, N76T-N212D, K27R-N269T, N62Y-G97D, V4E-S260P, G215D-D259V, K27E-Y91F, Y6D-T55A, N77D-N252T, V4E-S260P, Y6D-T55A, N25K-H238R, V44A-Q206H, L16Q-Q217H, V44A-Q206R, V44A-Q206H, and S37P-S260F, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0405] The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of Y21H-D259G, S183T-S249R, N61D-Q206R, Y262N-Q275R, K043R-N076S, A133V-D259N, and I079V-Q217H, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to the BPN', BPN'-v3, and BPN'-v36, and a greater PI value than that of BPN', BPN'-v3 and BPN'-v36 in this assay.

[0406] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.5 and less than or equal to 1.0 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of K170R-D259G, S18K-V203I, Y6F-S249C, Q19A-N109S, H17L-Q19A, Q19R-Q185R, S18Y-V203A, N61D-Q206R, S161E-S260T, S18K-V203I, V4A-T55A, N252S-L257H, S249R-Y262H, N61L-Q206H, N184Y-Y262N, Q19R-N25D, S249R-Y262H, A74S-P129Q, T158A-D259P, H17L-Q19A, K27R-D120H, V4A-T55A, N61K-N252K, Y21H-N252H, K27R-N269D, K43N-Q217R, T158A-D259P, Q206R-S260P, K27R-N269D, A98T-T158A, 179V-Q217H, S9L-N218S, V4A-Y6F, S161P-T253A, V203A-Q217R, T22S-T242S, N76P-N212S, A133V-D259N, S37T-S260P, T55A-V147A, V198L-D259G, Q19R-Q185R, V4A-Y6F, Q19A-N109S, Y262N-Q275R, G160R-T244A, Q19R-N25D, N25D-Q185R, N61K-N252K, S161E-S260T, A98T-T158A, N61L-Q206H, G211V-T244A, S9L-N218S, A144H-T244A, A144H-T244A, S18Y-V203A, Y21H-N252H, A74S-P129Q, G160A-D259G, K43N-Q217R, A1Y-Q275R, A1Y-Q275R, A200T-H226L, Q217R-T244A, S260P-Q275R, Q206R-S260P, K141I-S248N, S183T-R186K, T158I-D259N, S37T-S260P, K27R-D120H, T22S-T242S, Q217R-T244A, S161E-Q185H, P1295-K136R, G211V-T244A, N76P-N212S, L75I-N76D, S161E-Q185H, Y21H-S37E, S249R-Q275R, G160A-D259G, K43N-S163T, T158I-D259N, Y21H-S37E, S37G-Q275H, S161P-T253A, N76T-N212D, S260P-Q275L, Y6F-S249C, N184Y-Y262N, G131S-K265N, V4AS249N, N25D-Q185R, N252S-L257H, K43R-N76S, S183D-Q206R, G160R-T244A; Q10R-Q275K, S37G-Q275H, K43N-S163T, Q10R-Q275K, N25D-V26A, P129S-K136R, G131S-K265N, S260P-Q275L, K141I-S248N, T22S-G166V, N62Y-T244A,

L16Q-Q217H, S249R-Q275R, S260P-Q275R, K27R-N269T, P210S-N212D, L75I-N76D, S183D-Q206R, N118G-V121A, G215D-D259V, N76T-N212D, V4A-S249N, K27R-N269T, G166V-S183T, N62Y-G97D, V4E-S260P, G215D-D259V, K27E-Y91F, Y21H-D259R, Y6D-T55A, N77D-N252T, V4E-S260P, Y6D-T55A, N77D-N252T, Y21H-D259R, N25K-H238R, N77D-N252D, V44A-Q206H, L16Q-Q217H, V72F-L75I, S37P-S260F, V72F-L75I, N77D-N252D, V44A-Q206R, S163T-Q245L, V44A-Q206H, V44A-Q206R, S37P-S260F, G215R-D259R, V44A-Q206K, and V44A-Q206K, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

EXAMPLE 20

Cleaning Performance of Additional BPN'-v36 Polypeptide Variants

[0407] The following BPN'-v36 variants were synthesized at DNA2.0 (Menlo Park, CA) using the pHPLT-BPN'-v36 plasmid containing the BPN' expression cassette served as template DNA (parent plasmid) for cloning: N109G-A128S-S224A, N109G-A128S-S224A-N243V, A88T-N109G-A116T-A128S-S224A-N243V, N61G-N109G-A128S-S224A, N61G-N109G-A128S-S224A-N243V, N61G-A88T-N109G-A116T-A128S-S224A-N243V, N109Q-A128S-S224A, N109Q-A128S-S224A-N243V, A88T-N109Q-A116T-A128S-S224A-N243V, N109S-A128S-S224A, N109S-A128S-S224A-N243V, A88T-N109S-A116T-A128S-S224A-N243V, N109M-A128S-S224A, N109M-A128S-S224A-N243V, A88T-N109M-A116T-A128S-S224A-N243V, N109G-A114S-A128S, N109G-A114S-A128S-N243V, A88T-N109G-A114S-A116T-A128S-N243V, N109G-A114S-A128S-S224A, N109G-A114S-A128S-S224A-N243V, N109G-A128S-S183V, N109G-A128S-S183L, N109G-A128S-S183L-S224A, N109G-A114S-A128S-S183L-S224A, A88T-N109G-A114S-A116T-A128S-S183L-S224A-N243V, N76D-N109G-A128S-S224A, N101Q-N109Q-A128S-S224A-N243V, N101Q-N109Q-A128S-P129S-S130T-S224A-N243V, N109G-A128S-P129S-S130T-S224A-N243V, S33T-A128S-N218S, S33T-N109G-A128S-N218S-N243V, S33T-N61G-N109G-A128S-N218S-N243V, S33T-N109G-A128S-G169A-N218S-N243V, S33T-S63G-N109G-A128S-N218S-N243V, S33T-N76D-N109G-A128S-N218S-N243V, S33T-S63G-N109G-A128S-G169A-N218S-N243V, I31L-S33T-S63G-N109G-A128S-G169A-N218S-N243V, S33T-N61G-S63G-N109G-A128S-N218S-N243V, S33T-N61G-A88T-N109G-A116T-A128S-N218S-N243V, S33T-N61G-S63G-N109G-A128S-G131H-G169A-N218S-N243V, S33T-N61P-S63G-N109G-A128S-G131H-G169A-N218S-N243V, S33T-N109G-A128S-N218S-S224A-N243V, S63G-N109Q-A128S-S224A-N243V, S63G-N109Q-A128S-G131H-S224A-N243V, N61P-S63G-N109Q-A128S-S224A-N243V, N61P-S63G-N109Q-A128S-G131H-S224A-N243V, A1G-N61P-S63G-N109Q-A128S-G131H-S224A-N243V, I31L-N61P-S63G-N109Q-A128S-G131H-S224A-N243V, N61P-S63G-N109Q-A128S-G131H-S224A-N243V-S249Q, S33T-T55P-N61P-S63G-N109Q-A128S-G131H-S224A-N243V, S33T-T55P-N61P-S63G-A88T-N109G-A116T-A128S-G131H-S224A-N243V, S33T-T55P-N61P-S63G-A88T-N109G-A116T-A128S-G131H-S224A-N243V-S249Q, A1G-I31L-S33T-T55P-N61P-S63G-A88T-N109G-A116T-A128S-G131H-S224A-N243V-S249Q, N61P-S63G-N109Q-A128S-G131H-G169A-S224A-N243V-S249Q, and A1G-I31L-S33T-T55P-N61P-S63G-A88T-N109G-A116T-A128S-G131H-G169A-S224A-N243V-S249Q.

[0408] The variants were grown for protein expression as described in Example 11. These variants were tested for their performance in the BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C, the egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C, and the AAPF assay as described in Example 1. Results are provided below.

[0409] The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of N61P-S63G-N109Q-A128S-S224A-N243V, A88T-N109G-A114S-A116T-A128S-N243V, A88T-N109G-A114S-A116T-A128S-S183L-S224A-N243V, N109G-A128S-S183V, N109G-A128S-N243V-K256R, N109MA128S-S224A, A88T-N109S-A116T-A128S-S224A-N243V, N109Q-A128S-S224A-N243V, A88T-N109M-A116T-A128S-S224A-N243V, N109S-A128S-S224A-N243V, A88T-N109G-A116T-N243V, N101Q-N109Q-A128S-S224A-N243V, N109G-A116T-N243V-K256R, N109G-A128S-P129S-S130T-S224A-N243V, and A88T-N109Q-A116T-A128S-S224A-N243V, wherein amino acid positions of the variant are numbered by correspondence with positions of the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to the BPN', BPN'-v3, and BPN'-v36, and a greater PI value than that of BPN', BPN'-v3 and BPN'-v36 in this assay.

[0410] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.9 and equal to or less than 1.0 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of G24S-G53S-N78S-G97A-N101S-A128S, G24S-G53S-N78S-G97A-N101S, S33T-T55P-

N61P-S63G-A88T-N109G-A116T-A128S-G131H-S224A-N243V, S33T-N61G-S63G-N109G-A128S-N218S-N243V, S33T-S63G-N109G-A128S-N218S-N243V, S33T-T55P-N61P-S63G-N109Q-A128S-G131H-S224A-N243V, N61P-S63G-N109Q-A128S-G131H-G169A-S224A-N243V-S249Q, S33T-N61G-A88T-N109G-A116T-A128S-N218S-N243V, S33T-N109G-A128S-N218S-N243V, S33T-N76D-N109G-A128S-N218S-N243V, S33T-N76D-N109G-A128S-N218S-N243V-S248N-K256R, S33T-N61G-N109G-A128S-N218S-N243V, S33T-A128S-N218S, A1G-N61P-S63G-N109Q-A128S-G131H-S224A-N243V, N61P-S63G-N109Q-A128S-G131H-S224A-N243V-S249Q, N61P-S63G-N109Q-A128S-G131H-S224A-N243V, S63G-N109Q-A128S-G131H-S224A-N243V, N109G-A114S-A128S, N109G-A114S-A128S-S183L-S224A, N109G-A114S-A128S-S224A, N109G-A114S-A128S-S224A-N243V, A88T-N109G-A116T-A128S-S224A-N243V, N61G-A88T-N109G-A116T-A128S-S224AN243V, N109G-A114S-A128S-N243V, N109G-A128S-S224A-N243V, N109G-A128S-S224A, N109G-A128S-S183L-S224A, N61G-N109G-A128S-S224A, N109G-A128S-S183L, S33T-N76D, N109S-A128S-S224A, N101Q-N109Q-A128S-P129S-S130T-S224A-N243V, S63G-N109Q-A128S-S224A-N243V, N109M-A128S-S224A-N243V, S63G-N109G, N109G-K256R, S63G-N76D, S33T-N109G-A128S-G169A-N218S-N243V, and S33T-N109G-A128S-N218S-S224A-N243V, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0411] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v36 in a BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of I31L-S33T-S63G-N109G-A128S-G169A-N218S-N243V, A1G-I31L-S33T-T55P-N61P-S63G-A88T-N109G-A116T-A128S-G131H-G169A-S224A-N243V-S249Q, S33T-N61G-S63G-N109G-A128S-G131H-G169A-N218S-N243V, S33T-S63G-N109G-A128S-G169A-N218S-N243V, S33T-T55P-N61P-S63G-A88T-N109G-A116T-A128S-G131H-S224A-N243V-S249Q, S33T-N76D-A128S-N218S, N76D-N109G-A128S-S224A, and S33T-N61P-S63G-N109G-A128S-G131H-G169A-N218S-N243V, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in an egg microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of S33T-T55P-N61P-S63G-A88T-N109G-A116T-A128S-G131H-S224A-N243V, S33T-N61G-S63G-N109G-A128S-N218S-N243V, S33T-S63G-N109G-A128S-N218S-N243V, N61P-S63G-N109Q-A128S-G131H-G169A-S224A-N243V-S249Q, S33T-N61G-A88T-N109G-A116T-A128S-N218S-N243V, S33T-N109G-A128S-N218S-N243V, S33T-A128S-N218S, A1G-N61P-S63G-N109Q-A128S-G131H-S224A-N243V, N61P-S63G-N109Q-A128S-G131H-S224A-N243V-S249Q, S63G-N109Q-A128S-G131H-S224A-N243V, N61P-S63G-N109Q-A128S-S224A-N243V, A88T-N109G-A114S-A116T-A128S-N243V, A88T-N109G-A114S-A116T-A128S-S183L-S224A-N243V, N109G-A114S-A128S, N109G-A114S-A128S-S183L-S224A, N109G-A114S-A128S-S224A, N109G-A114S-A128S-S224A-N243V, A88T-N109G-A116T-A128S-S224A-N243V, N61G-A88T-N109G-A116T-A128S-S224A-N243V, N109G-A128S-S183V, N109G-A114S-A128S-N243V, N109G-A128S-N243V-S248A, N109G-A128S-S224A-N243V, N109G-A128S-N243V-K256R, N109G-A128S-S224A, N109G-A128S-S183L-S224A, N61G-N109G-A128S-S224A, N109M-A128S-S224A, A88T-N109S-A116T-A128S-S224A-N243V, N109M-A128S-S224A-N243V, S63G-A128S, A88T-N109G-A116T-N243V, N101Q-N109Q-A128S-S224A-N243V, N109G-A116T-N243V-K256R, N109G-A116T, S63G-N109G, A88T-N109G, N109G-K256R, N61G-N109G-N243V, S33T-N109G-A128S-G169A-N218S-N243V, S33T-N109G-A128S-N218S-S224A-N243V, N109G-A128S-P129S-S130T-S224A-N243V, and A88T-N109Q-A116TA128S-S224A-N243V, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to the BPN', BPN'-v3, and BPN'-v36, and a greater PI value than that of BPN', BPN'-v3 and BPN'-v36 in this assay.

[0412] The following BPN'-v36 variant was determined to have a PI value equal to or greater than 0.5 and equal to or less than 1.0 relative to BPN'-v36 in an egg BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising amino acid substitutions selected from the group consisting of substitutions S33T-N76D-A128S-N218S, N76D-N109G-A128S-S224A and S063G-N76D, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

[0413] The following BPN'-v36 variants were determined to have a PI value greater than 1.0, at least 1.1, at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2, from greater than 1.0 to about 10, from greater than 1.0 to about 8, or from greater than 1.0 to about 5 relative to BPN'-v36 in an AAPF proteolytic assay: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of G24S-G53S-N78S-G97A-N101S-A128S, I31L-S33T-S63G-N109G-A128S-G169A-N218S-N243V, A1G-131L-S33T-T55P-N61P-S63G-A88T-N109G-A116T-A128S-G131H-G169A-S224A-N243V-S249Q, S33T-N61G-S63G-N109G-A128S-G131H-G169A-N218S-N243V, S33T-S63G-N109G-A128S-G169A-N218S-N243V, S33T-T55P-N61P-S63G-A88T-N109G-A116T-A128S-G131H-S224A-N243V, S33T-T55P-N61P-S63G-A88T-N109G-A116T-A128S-G131H-S224A-N243V-S249Q, S33T-N61G-S63G-N109G-A128S-N218S-N243V, S33T-S63G-N109G-A128S-N218S-N243V, S33T-T55P-N61P-S63G-

N109Q-A128S-G131H-S224A-N243V, N61P-S63G-N109Q-A128S-G131H-G169A-S224A-N243V-S249Q, S33T-N61G-A88T-N109G-A116T-A128S-N218S-N243V, S33T-N109G-A128S-N218S-N243V, S33T-N76D-N109G-A128S-N218S-N243V, S33T-N76D-N109G-A128S-N218S-N243V-S248N-K256R, S33T-N61G-N109G-A128S-N218S-N243V, S33T-N76D-A128S-N218S, S33T-A128S-N218S, A1G-N61P-S63G-N109Q-A128S-G131H-S224A-N243V, N61P-S63G-N109Q-A128S-G131H-S224A-N243V-S249Q, N61P-S63G-N109Q-A128S-G131H-S224A-N243V, S63G-N109Q-A128S-G131H-S224A-N243V, N61P-S63G-N109Q-A128S-S224A-N243V, A88T-N109G-A114S-A116T-A128S-N243V, A88T-N109G-A114S-A116T-A128S-S183L-S224A-N243V, N109G-A114S-A128S, N109G-A114S-A128S-S183L-S224A, N109G-A114S-A128S-S224A, N109G-A114S-A128S-S224A-N243V, A88T-N109G-A116T-A128S-S224A-N243V, N61G-A88T-N109G-A116T-A128S-S224A-N243V, N109G-A128S-S183V, N109G-A114S-A128S-N243V, N109G-A128S-N243V-S248A, N109G-A128S-S224A-N243V, N109G-A128S-N243V-K256R, N109G-A128S-S224A, N109G-A128S-S183L-S224A, N61G-N109G-A128S-S224A, N76D-N109G-A128S-S224A, N109M-A128S-S224A, N109G-A128S-S183L, S33T-N76D, A88T-N109S-A116T-A128S-S224A-N243V, N109Q-A128S-S224A-N243V, N109S-A128S-S224A, A88T-N109M-A116T-A128S-S224A-N243V, N101Q-N109Q-A128S-P129S-S130T-S224A-N243V, S63G-N109Q-A128S-S224A-N243V, N109M-A128S-S224A-N243V, S63G-A128S, N109S-A128S-S224AN243V, A88T-N109G-A116T-N243V, N61S-N109G-N243V, N101Q-N109Q-A128S-S224A-N243V, N109G-A116T-N243V-K256R, A88T-N109G-A116T-T158S-N243V-K256R, N109G-A116T, S63G-N109G, A88T-N109G, N109G-K256R, N61G-N109G-N243V, S33T-N61P-S63G-N109G-A128S-G131H-G169A-N218S-N243V, S33T-N109G-A128S-G169A-N218S-N243V, S33T-N109G-A128S-N218S-S224A-N243V, N109G-A128S-P129S-S130T-S224A-N243V, and A88T-N109Q-A116T-A128S-S224A-N243V, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Such variants have enhanced proteolytic activity compared to the BPN', BPN'-v3, and BPN'-v36, and a greater PI value than that of BPN', BPN'-v3 and BPN'-v36 in this assay.

[0414] The following BPN'-v36 variant was determined to have a PI value equal to or greater than 0.5 and equal to or less than 1.0 relative to BPN'-v36 in an AAPF proteolytic assay: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising amino acid substitutions G24S-G53S-N78S-G97A-N101S or S063G-N76D, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

EXAMPLE 21

Cleaning Performance of Polypeptide BPN'-v36 Polypeptides Variants from Combinatorial Libraries Based on BPN'-v36 Polypeptide

[0415] Two separate combinatorial libraries (AJ1 and AJ2) were synthesized by DNA2.0 (Menlo Park, CA) and were delivered as individual ligation reactions. The pHPLT-BPN'-v36 plasmid (Figure 4) containing the BPN' expression cassette served as template DNA (parent plasmid) for library construction. A list of the possible amino acid positions and substitutions for each library is shown in Table 21-1. The ligation reactions for each library were used to transform *B. subtilis*, and the library variants were grown up for protein expression as described in Example 11. The variants were tested for performance in the BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C as described in Example 1.

Table 21-1: Possible Substitutions for Combinatorial Libraries AJ1 and AJ2

AJ1		AJ2	
Position	**Possible Substitutions**	**Position**	**Possible Substitutions**
S33	G, S	E54	E,Q
D60	D,G	D99	D,N
N62	N,L,S	D120	D,N
S63	S,R,L,N,G	D140	D,N
S125	S,A	E156	E,Q
Q217	Q,R,E,L,G	D197	D,N
M222	M,L,S	K12	K,T
		K27	K,S
		K43	K,T
		K141	K,Y
		K213	K,Q

Table 21-1: Possible Substitutions for Combinatorial Libraries AJ1 and AJ2			
AJ1		AJ2	
Position	Possible Substitutions	Position	Possible Substitutions
		K237	K,A
		K256	K,Q

[0416] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.5 and less or equal to 1.0 relative to BPN'-v36 in the BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of G024S-G053S-N078S-G097A-N101S (i.e., BPN'-v3), G024S-G053S-N078S-G097AN101S-A128S (i.e., BPN'-v12), N062L, N062L-S063G, N062S, N062S-S063G-Q217L, N062S-S063L-Q217L, N062S-S063N, N062S-S063R, Q217E, S063G, S063G-Q217L, S063G-Q217L-M222S, S063L-Q217L, S063N, S063N-Q217L, D099N-K141Y-K213Q, D099N-K141Y-K256Q, K043T, K043T-K141Y-E156Q, N062L-Q217E, N062L-Q217L, N062L-S063G-Q217E, N062L-S063L, N062L-S063N-Q217L, N062S-Q217L, N062S-S063G, N062S-S063L, N062S-S063N-Q217L, N062S-S063R-Q217E, Q217L, S063G-Q217E, S063N-Q217E, S063R, S063R-Q217E, S063R-Q217L,), D099N-K141Y-K213Q, D099N-K141Y-K256Q, K043T, K043T-K141Y-E156Q, N062L-Q217E, N062L-Q217L, N062L-S063G-Q217E, N062L-S063L, N062S-Q217L, N062S-S063G, N062S-S063L, N062S-S063N-Q217L, Q217L, S063G-Q217E, S063N-Q217E, S063R, S063R-Q217E, and S063R-Q217L, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2. Note that a protease variant which is BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising the amino acid substitutions G024S-G053S-N078S-G097A-N101S is BPN'-v3 (SEQ ID NO:4), because the G at position 24, G at position 53, N at position 78, and N at position 101 of SEQ ID NO:6 have been substituted with S at each of positions 24, 53, 78, and 101 of SEQ ID NO:6. In addition, G at position 97 of SEQ ID NO:6 has been substituted with A. Thus, the resultant sequence is SEQ ID NO:4.

[0417] The following BPN'-v36 variants were determined to have a PI value equal to or greater than 0.5 and less than 0.9 relative to BPN'-v36 in the BMI microswatch cleaning assay in Detergent Composition 4 at pH 8 and 16°C: BPN'-S024G-S053G-S078N-S101N-G128A-Y217Q amino acid sequence (SEQ ID NO:6) comprising at least one set of amino acid substitutions selected from the group consisting of D099N, K141Y-E156Q, N062L-S063L-Q217L, N062L-S063N, N062L-SO63N-Q217E, N062L-S063R, N062L-S063R-Q217L, N062S-Q217E, N062S-S063G-Q217E, N062S-S063G-Q217R, N062S-S063N-Q217R, S063G-S125A, D060G-Q217L, D120N-K141Y-K213Q, K043T-D099N-D120N-K141Y, K043T-D099N-K141Y-K256Q, K043T-K237A, N062L-S063G-Q217R, N062L-S063G-S125A, N062L-S063L-Q217E, N062L-S063N-S125A-Q217L, N062S-Q217R, N062S-S063L-Q217E, N062S-S063R-Q217L, S063G-M222S, S063G-Q217R, D120N-E156Q-K256Q, K141Y-D197N, N062L-Q217R, N062L-S063G-Q217L-M222S, N062L-S063L-Q217R, N062L-S063N-Q217R, N062S-Q217G, N062S-S063G-Q217G, N062S-S063G-Q217L-M222L, N062S-S063G-S125A-Q217L, N062S-S063N-Q217E, Q217G, S033G-N062S-S063G, S063G-Q217G, S063G-Q217L-M222L, S063G-S125A-Q217R, S063L-Q217R, S063N-M222S, S063N-Q217R, S063N-S125A-Q217L, S063R-Q217R, S063R-S125A-Q217L, D099N-E156Q-K256Q, E156Q, K012T-D099N-K213Q, K012T-K256Q, K043T-D099N-K141Y-K213Q, IC043T-E156Q, K141Y-K213Q, N062L-Q217G, N062L-Q217L-M222L, N062L-Q217L-M222S, N062L-S063G-M222S, N062L-S063G-Q217L-M222L, N062L-S063G-Q217R-M222S, N062L-S063N-Q217L-M222S, N062L-S063N-S125A, N062L-S063R-S125A, N062L-S125A, N062S-S063G-M222S, N062S-S063G-Q217G-M222S, N062S-S063G-S125A, N062S-S063N-Q217L-M222L, N062S-S063N-S125A-Q217L, N062S-S063R-Q217G, N062S-S063R-Q217L-M222S, Q217G-M222S, Q217L-M222S, Q217R, S033G-S063G-Q217R, S063G-Q217E-M222S, S063G-S125A-Q217G, S063L-Q217E, S063N-Q217G, S063N-Q217G-M222S, S063N-Q217L-M222S, S063R-Q217L-M222S, and S063R-S125A, wherein amino acid positions of the variant are numbered by correspondence with the sequence of SEQ ID NO:2.

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- WO9529979A [0004]
- US20040209343A [0005]
- WO9630010A [0006]
- WO9934011A [0025] [0078]
- US6376450B [0025] [0202] [0203] [0203] [0204] [0207] [0207]
- US5352604A [0028]
- US4683195A [0044]
- US4683202A [0044]
- US4965188A [0044]
- US6582914B [0071]
- US6605458B [0078] [0163] [0198] [0202] [0207]
- US5264366A [0129]
- US4760025A [0129]
- USRE34606E [0129] [0185] [0185]
- US4450235A [0130]
- US4302544A [0130]
- EP0134048A [0130]
- US20050202535A [0131]
- US6610642B [0163] [0198] [0202] [0202] [0203] [0204] [0207]
- US5705464A [0163] [0198]
- US5710115A [0163] [0198]
- US5698504A [0163] [0198]
- US5695679A [0163] [0198]
- US5686014A [0163] [0198]
- US5646101A [0163] [0198]
- WO9711151A [0170]
- EP0922499A [0170]
- US4977252A [0170]
- US5354559A [0170]
- US5935826A [0170]
- US5955340A [0185] [0185]
- US5700676A [0185] [0185]
- US6312936B [0185] [0185]
- US6482628B [0185] [0185]
- WO8906270A [0185]
- WO9523221A [0185]
- WO9221760A [0185]
- US20080090747A [0185]
- US5801039A [0185]
- US5340735A [0185]
- US5500364A [0185]
- US5855625A [0185]
- WO07044993A [0185] [0236]
- EP258068A [0186]
- EP305216A [0186]
- EP238023A [0196]
- EP214761A [0186]
- EP218272A [0186]
- EP331376A [0186]
- GB1372034A [0186]
- JP64744992B [0186]
- WO9116422A [0186]
- WO8809367A [0186]
- WO9009446A [0188]
- GB1296839A [0191]
- US4436307A [0193]
- EP0495257A [0193]
- US5874276A [0193]

- US6566114B [0194]
- US6602842B [0194]
- US6440991B [0194]
- WO9412621A [0195]
- WO9501426A [0195]
- WO05056782A [0196]
- US5879584A [0208] [0228] [0230]
- US5691297A [0208] [0228] [0230]
- US5574005A [0208] [0228] [0230]
- US5569645A [0208] [0228] [0230]
- US5565422A [0208] [0230]
- US5516448A [0208] [0228] [0230]
- US5489392A [0208] [0228] [0230]
- US5486303A [0208] [0228] [0230]
- US5576282A [0209] [0223]
- US6306812B [0209]
- US6326348B [0209]
- EP2100949A [0212] [0215] [0220] [0221] [0222] [0227] [0231]
- WO07145964A [0219]
- US4246612A [0222]
- US5227084A [0222]
- US4810410A [0222]
- WO9906521A [0222]
- US4430243A [0223]
- US5597936A [0223]
- US5595967A [0223]
- WO200032601A [0225]
- US6225464B [0225]
- WO9426860A [0227]
- WO9426859A [0227]
- US4515705A [0230]
- US4537706A [0230]
- US4515707A [0230]
- US4550862A [0230]
- US4561998A [0230]
- US4597898A [0230]
- US4968451A [0230]
- US5565145A [0230]
- US5929022A [0230]
- US6294514B [0230]
- US6376445B [0230]
- WO02102955A [0231]
- US4765916A [0231]
- US4972017A [0231]
- WO04111178A [0231]
- EP2100947A [0231]
- WO9418314A [0236]
- WO9605295A [0236]
- US6566112B [0262]

Non-patent literature cited in the description

- **BRODE et al.**describe the use of site-directed and random mutagenesis to produce variants of subtilisin BPN' (bacillus amyloliquefaciens) protease with variable surface adsorption propertiesbiochemistry, 1996, vol. 35, 3162-3169 [0003]

- **DEL MAR et al.**Anal. Biochem., 1979, vol. 99, 316-320 [0025]
- **RAWLINGSBARRETT**Biochem. J., 1993, vol. 290, 205-218 [0026]
- Genetics**FERRARI et al.**BacillusPlenum Publishing Corp.1989000057-72 [0037] [0133]
- **SMITHWATERMAN**Adv. Appl. Math., 1981, vol. 2, 482- [0058]
- **NEEDLEMANWUNSCH**J. Mol. Biol., 1970, vol. 48, 443- [0058]
- **PEARSONLIPMAN**Proc. Natl. Acad. Sci. USA, 1988, vol. 85, 2444- [0058]
- **DEVEREUX et al.**Nucl. Acid Res., 1984, vol. 12, 387-395 [0058]
- **FENGDOOLITTLE**J. Mol. Evol., 1987, vol. 35, 351-360 [0058]
- **HIGGINSSHARP**CABIOS, 1989, vol. 5, 151-153 [0058]
- **ALTSCHUL et al.**J. Mol. Biol., 1990, vol. 215, 403-410 [0058] [0061]
- **KARLINALTSCHUL**Proc. Natl. Acad. Sci. USA, 1993, vol. 90, 5873-5787 [0058]
- **ALTSCHUL et al.**Meth. Enzymol., 1996, vol. 266, 460-480 [0058]
- **HENIKOFFHENIKOFF**Proc. Natl. Acad. Sci. USA, 1992, vol. 89, 10915- [0061]
- **ALTSCHUL et al.**Nucleic Acids Res., 1997, vol. 25, 173389-3402 [0065]
- **R SCOPES**Protein PurificationSpringer-Verlag19820000 [0069]
- Guide to Protein Purification**DEUTSCHER**Methods in Enzymol.Academic Press, Inc.19900000vol. 182, [0069]
- **BEAUCAGE et al.**Tetrahedron Letters, 1981, vol. 22, 1859-69 [0117]
- **MATTHES et al.**EMBO J., 1984, vol. 3, 801-805 [0117]
- **ITAKURA et al.**Ann. Rev. Biochem., 1984, vol. 53, 323- [0117]
- **ITAKURA et al.**Science, 1984, vol. 198, 1056- [0117]
- Overview of principles of hybridization and the strategy of nucleic acid probe assays**P. TIJSSEN** Laboratory Techniques in Biochemistry and Molecular Biology - Hybridization with Nucleic Acid ProbesElsevier19930000vol. 24, [0119]
- **HAMESHIGGINS**Gene Probes 1 and 2, 1995, [0119]
- **SAMBROOK et al.**Molecular Cloning: A Laboratory ManualCold Spring Harbor Press19890000 [0122]
- **STEMMER WP**Proc. Natl. Acad. Sci. USA, 1994, vol. 91, 2210747-51 [0123]
- **OSTERMEIER et al.**Bioorg. Med. Chem., 1999, vol. 7, 2139-44 [0123]
- **LUTZ et al.**Proc. Natl. Acad. Sci. USA, 2001, vol. 98, 11248-53 [0123]
- **SIEBER et al.**Nat. Biotechnol., 2001, vol. 19, 456-60 [0123]
- **BITTKER et al.**Nat. Biotechnol., 2001, vol. 20, 1024-9 [0123]
- **BITTKER et al.**Proc Natl. Acad. Sci. USA, 2004, vol. 101, 7011-6 [0123]
- **NESS et al.**Nat. Biotechnol., 2002, vol. 20, 1251-5 [0123]
- **COCO et al.**Nat. Biotechnol., 2002, vol. 20, 1246-50 [0123]
- **ZHA et al.**Chembiochem, 2003, vol. 4, 34-9 [0123]
- **GLASER et al.**J. Immunol., 1992, vol. 149, 3903-13 [0123]
- **ARKINYOUVAN**Biotechnology, 1992, vol. 10, 297-300 [0123]
- **REIDHAAR-OLSON et al.**Methods Enzymol., 1991, vol. 208, 564-86 [0123]
- **PISARCHIK et al.**Prot. Eng. Des. Select., 2007, vol. 20, 257-265 [0124]
- Molecular Biological Methods for BacillusJohn Wiley & Sons19900000 [0127]
- **PEREGO, M.**Integrational Vectors for Genetic Manipulations in Bacillus subtilis, 1993, 615-624 [0127]
- Bacillus subtilis and other Gram-positive bacteria: biochemistry, physiology and molecular geneticsAmerican Society for Microbiology [0127]
- **HOCH et al.**Genetics, 1973, vol. 73, 215-228 [0130]
- **PALVA et al.**Gene, 1982, vol. 19, 81-87 [0130]
- **FAHNESTOCKFISCHER**J. Bacteriol., 1986, vol. 165, 796-804 [0130]
- **WANG et al.**Gene, 1988, vol. 69, 39-47 [0130]
- **MSADEK et al.**J. Bacteriol., 1990, vol. 172, 824-834 [0131]
- **OLMOS et al.**Mol. Gen. Genet., 1997, vol. 253, 562-567 [0131]
- **CALDWELL et al.**J. Bacteriol., 2001, vol. 183, 7329-7340 [0131]
- **ARIGONI et al.**Mol. Microbiol., 1999, vol. 31, 1407-1415 [0131]
- **PEREGO et al.**Mol. Microbiol., 1991, vol. 5, 173-185 [0131]
- **SAUNDERS et al.**J. Bacteriol., 1984, vol. 157, 718-726 [0133]
- **HOCH et al.**J. Bacteriol., 1967, vol. 93, 1925-1937 [0133]
- **MANN et al.**Current Microbiol., 1986, vol. 13, 131-135 [0133]
- **HOLUBOVA**Folia Microbiol., 1985, vol. 30, 97- [0133]
- **CHANG et al.**Mol. Gen. Genet., 1979, vol. 168, 11-115 [0133]
- **VOROBJEVA et al.**FEMS Microbiol. Lett., 1980, vol. 7, 261-263 [0133]
- **SMITH et al.**Appl. Env. Microbiol., 1986, vol. 51, 634- [0133]
- **FISHER et al.**Arch. Microbiol., 1981, vol. 139, 213-217 [0133]

- **MCDONALD**J. Gen. Microbiol., 1984, vol. 130, 203- [0133]
- **CONTENTE et al.**Plasmid, 1979, vol. 2, 555-571 [0133]
- **HAIMA et al.**Mol. Gen. Genet., 1990, vol. 223, 185-191 [0133]
- **WEINRAUCH et al.**J. Bacteriol., 1983, vol. 154, 1077-1087 [0133]
- **WEINRAUCH et al.**J. Bacteriol., 1987, vol. 169, 1205-1211 [0133]
- **STAHL et al.**J. Bacteriol., 1984, vol. 158, 411-418 [0134]
- **PALMEROS et al.**Gene, 2000, vol. 247, 255-264 [0134]
- **HOPWOOD**Practical Streptomyces GeneticsJohn Innes Foundation20000000 [0135]
- **HARDWOOD et al.**Molecular Biological Methods for BacillusJohn Wiley and from the American Type Culture Collection (ATCC)19900000 [0135]
- **KROLL, D.J. et al.**DNA Cell Biol., 1993, vol. 12, 441-53 [0137]
- **PORATH J.**Protein Expr. Purif., 1992, vol. 3, 263-281 [0137]
- Methods of Enzymatic Analysis**BERGMEYER et al.**Peptidases, Proteinases and their InhibitorsVerlag Chemie19840000vol. 5, [0138]
- **WARD**ProteinasesMicrobial Enzymes and Biotechnology, Applied Science, 1983, 251-317 [0138]
- **WELLS et al.**Nucleic Acids Res., 1983, vol. 11, 7911-7925 [0138]
- **CHRISTIANSON et al.**Anal. Biochem., 1994, vol. 223, 119-129 [0138]
- **HSIA et al.**Anal Biochem., 1999, vol. 242, 221-227 [0138]
- **MADDOX et al.**J. Exp. Med., 1983, vol. 158, 1211- [0139]
- **STEWART et al.**Solid-Phase Peptide SynthesisW.H. Freeman Co19690000 [0142]
- **MERRIFIELD**J. Am. Chem. Soc, 1963, vol. 85, 2149-2154 [0142]
- **DARTOIS et al.**Biochem. Biophys. Acta, 1993, vol. 1131, 253-260 [0186]
- **YAMAGUCHI et al.**Gene, 1991, vol. 103, 61-67 [0187]
- **SCHIMADA et al.**J. Biochem., 1989, vol. 106, 383-388 [0187]
- **HASS et al.**Gene, 1991, vol. 109, 117-113 [0187]
- **KUGIMIYA et al.**Biosci. Biotech. Biochem., 1992, vol. 56, 716-719 [0187]
- **MCKENZIE et al.**Plasmid, 1986, vol. 15, 293-103 [0262]
- **NEIDHARDT et al.**J. Bacteriol., 1974, vol. 119, 736-747 [0264]

SAMMENSÆTNINGER OG FREMGANGSMÅDER OMFATTENDE PROTEASEVARIANTER

PATENTKRAV

1. Isoleret proteasevariant, hvilken variant omfatter en aminosyresekvens med mindst 80 % sekvensidentitet med aminosyresekvensen ifølge SEQ ID NO: 2 og omfatter aminosyresubstitutionerne X024G/R, X053G, X078N, X101N, X128A/S og X217Q/L, hvor varianten har forbedret proteolytisk aktivitet og/eller rensningsaktivitet sammenlignet med den proteolytiske aktivitet og/eller rensningsaktivitet for BPN'-proteasen med sekvensen ifølge SEQ ID NO: 2, og hver aminosyreposition af varianten er nummereret i forhold til en aminosyreposition i aminosyresekvensen ifølge SEQ ID NO: 2 som bestemt ved alignment af aminosyresekvensen for varianten med SEQ ID NO: 2.

2. Variant ifølge krav 1, hvor aminosyresekvensvarianten endvidere omfatter aminosyresubstitutionen X097A.

3. Variant ifølge krav 1, hvor aminosyresekvensvarianten omfatter aminosyresubstitutionerne S024G+S053G+S078N+S101N+G128S+Y217Q eller S024G+S053G+S078N+S101N+G128A+Y217Q.

4. Variant ifølge krav 3, hvor aminosyresekvensvarianten endvidere omfatter

(i) en substitution udvalgt fra gruppen bestående af N109G, N076D, S033T, N243V, S248A, A088T og S063G; eller

(ii) et sæt af aminosyresubstitutioner udvalgt fra gruppen bestående af:

A088T+N109G+A116T+G131H+N243V+L257G,

S033T+N076D,

S009T+N109G+K141R+N243V,

S162G+K256R,

N109G+A116T,

N109G+L257G,

S162G+L257G,

N061G+N109G+N243V,

N 109G+N243V+S248A,

S033T+N076D+N109G+N218S+N243V+S248N+K256R,

N109G+A116T+N243V+K256R,

A088T+N109G+A116T+G131H+N243V,

A088T+N109G,

N109G+N243V,

T158S+L257G,

N061S+N109G+N243V,

P040A+N109G+N243V+S248N+K256R,

S009T+S018T+Y021N+N109G+K141R,

A088T+N109G+A116T+T158S+N243V+K256R,

A088T+N109G+A116T+T158S+N218S+L257G,

N109G+K256R,

N109G+N243V+K256R,

S063G+K256R,

S063G+N109G,

S063G,

S063G+N076D,

S033T+N076D+N218S og

N076D+N218S

5. Variant ifølge et hvilket som helst af de foregående krav, hvor aminosyresekvensvarianten har mindst 85 % sekvensidentitet med aminosyresekvensen ifølge SEQ ID NO: 2 eller SEQ ID NO: 6.

6. Variant ifølge krav 1, hvor varianten har aminosyresekvensen ifølge SEQ ID NO: 6 eller har sekvensen:

ai) BPN' S024G-S053G-S078N-S101N-G128S-Y217Q;

a) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+A088T+N109G+A116T+G131H+N243V+L257G;

b) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+S033T+N076D;

c) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+S009T+N109G+ K141R+N243V;

d) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+S162G+K256R;

e) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N109G+A116T;

f) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N109G+L257G;

g) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+S162G+L257G;

h) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N061G+N109G+N243V;

i) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+N109G+N243V+S248A;

j) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+S033T+N076D+N109G+N218S+N243V+S248N+K256R;

k) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N109G+A116T+N243V+ K256R;

l) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+A088T+N109G+A116T+G131H+N243V;

m) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+A088T+N109G;

n) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N109G+N243V;

o) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+T158S+L257G;

p) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N061S+N109G+N243V;

q) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+P040A+N109G+N243V+S248N+K256R;

r) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+S009T+S018T+Y021N+N109G+K141R;

s) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+A088T+N109G+A116T+T158S+N243V+K256R;

t) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+A088T+N109G+A116T+T158S+N218S+L257G;

u) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N109G+K256R;

v) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+N109G+N243V+K256R;

w) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+S063G+K256R;

x) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+S063G+N109G;

y) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+S063G;

z) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+S063G+N076D;

aa) BPN'-S024G+S053G+S078N+S101N+G128S+Y217Q+S033T+N076D+N218S;

bb) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q+N076D+N218S eller

cc) BPN'-S024G+S053G+S078N+S101N+G128A+Y217Q.

7. Variant ifølge krav 4, hvor varianten har forbedret proteolytisk aktivitet sammenlignet med den proteolytiske aktivitet for proteasen med sekvensen ifølge SEQ ID NO: 6.

8. Isoleret nukleinsyre, der omfatter en polynukleotidsekvens, der koder for varianten ifølge et hvilket som helst af kravene 1 til 7, der f.eks. har mindst 80 % sekvensidentitet med polynukleotidsekvensen ifølge SEQ ID NO: 3 eller SEQ ID NO: 5, eller en komplementær polynukleotidsekvens deraf.

9. Ekspressionsvektor, der omfatter mindst én nukleinsyre ifølge krav 8, eventuelt hvor den mindst ene nukleinsyre er operativt bundet til en promoter.

10. Rekombinant værtscelle, der omfatter: (a) en nukleinsyre ifølge krav 8, eller (b) en ekspressionsvektor ifølge krav 9; hvor værtscellen eventuelt er en bakteriecelle, f.eks. en *Bacillus*-celle, såsom en *Bacillus subtilis*-celle.

11. Fremgangsmåde til fremstilling af en proteasevariant, hvilken fremgangsmåde omfatter dyrkning af en rekombinant værtscelle ifølge krav 10 under forhold, der bidrager til fremstilling af varianten, og eventuelt indvinding af varianten fra cellekulturen.

12. Sammensætning omfattende varianten ifølge et hvilket som helst af kravene 1 til 7, hvilken sammensætning ikke er et stof- eller rengøringsprodukt; f.eks. hvor sammensætningen er en rensemiddelsammensætning til rensning af en kontaktlinse eller en rensemiddelsammensætning, der kan anvendes til personlig pleje.

13. Sammensætning ifølge krav 12, hvor sammensætningen omfatter et supplerende enzym, f.eks. hvor det supplerende enzym er udvalgt fra gruppen bestående af en hemicellulase, cellulase, amylase, peroxidase, protease, xylanase, lipase, phospholipase, esterase, cutinase, pectinase, pectatlyase, mannanase, keratinase, reductase, oxidase, phenoloxidase, lipoxygenase, ligninase, pullulanase, tannase, pentosanase, malanase, ß-glucanase, arabinosidase, hyaluronidase, chondroitinase og laccase.

14. Sammensætning ifølge krav 12 eller krav 13, hvilken sammensætning endvidere omfatter mindst én adjuvans og/eller mindst ét overfladeaktivt middel.

15. Fremgangsmåde til rensning af et emne eller en overflade, der har behov for at blive renset, hvilken fremgangsmåde omfatter etablering af kontakt mellem emnet og overfladen med en variant ifølge et hvilket som helst af kravene 1 til 7, eller en sammensætning ifølge krav 12 til 14, og eventuelt skylning af emnet eller overfladen med vand.

DRAWINGS

FIGURE 1

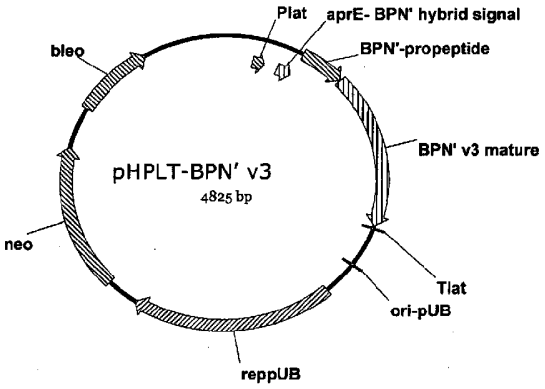

FIGURE 2

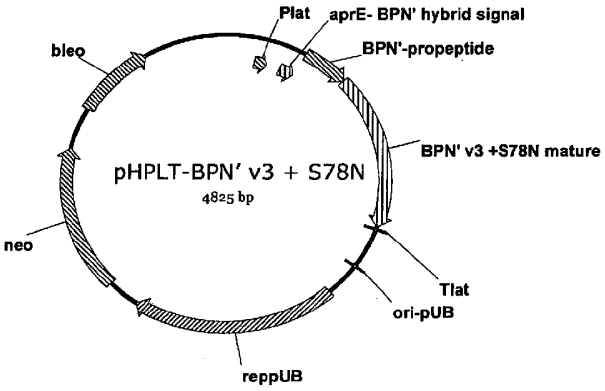
Plat
aprE- BPN' hybrid signal
BPN'-propeptide
bleo
BPN' v3 +S78N mature
pHPLT-BPN' v3 + S78N
4825 bp
neo
Tlat
ori-pUB
reppUB

FIGURE 3

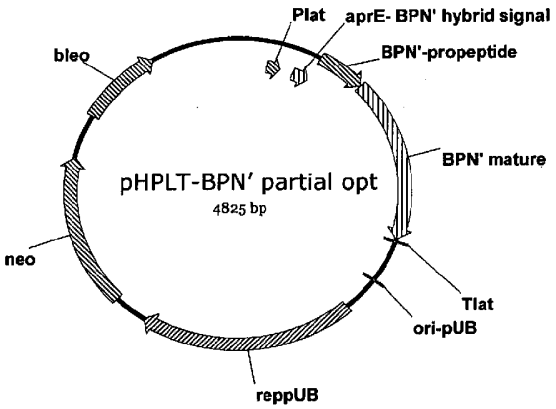
Plat
aprE- BPN' hybrid signal
BPN'-propeptide
bleo
BPN' mature
pHPLT-BPN' partial opt
4825 bp
neo
Tlat
ori-pUB
reppUB

FIGURE 4

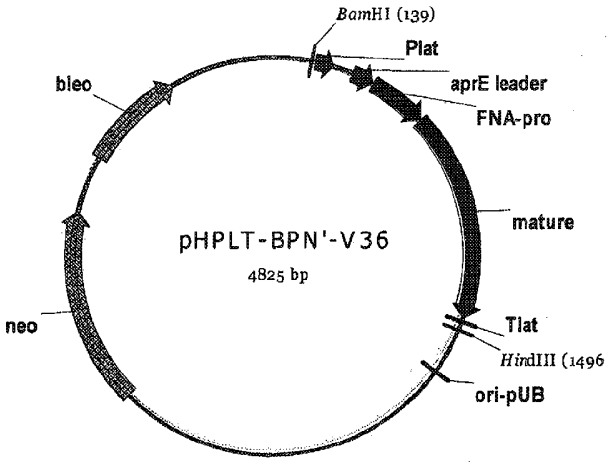
BamHI (139)
Plat
aprE leader
FNA-pro
mature
Tlat
HindIII (1496
ori-pUB
neo
bleo
pHPLT-BPN'-V36
4825 bp

FIGURE 5

```
BPN'          1 AQSVPYGVSQ IKAPALHSQG YTGSNVKVAV IDSGIDSSHP DLKVAGGASM VPSETNPFQD
GG36          1 AQSVPWGISR VQAPAAHNRG LTGSGVKVAV LDTGIS-THP DLNIRGGASF VPGEPST-QD

BPN'         61 NNSHGTHVAG TVAALNNSIG VLGVAPSASL YAVKVLGADG SGQYSWIING IEWAIANNMD
GG36         59 GNGHGTHVAG TIAALNNSIG VLGVAPSAEL YAVKVLGASG SGSVSSIAQG LEWAGNNGMH

                                                          161
BPN'        121 VINMSLGGPS GSAALKAAVD KAVASGVVVV AAAGNEGTSG SSSTVGYPGK YPSVIAVGAV
GG36        119 VANLSLGSPS PSATLEQAVN SATSRGVLVV AASGNSGAGS ----ISYPAR YANAMAVGAT

BPN'        181 DSSNQRASFS QVGPELDVMA PGVSIQSTLP GNKYGAYNGT SMASPHVAGA AALILSKHPN
GG36        175 DQNNNRASFS QYGAGLDIVA PGVNVQSTYP GSTYASLNGT SMATPHVAGA AALVKQKNPS

BPN'        241 WTNTQVRSSL ENTTTKLGDS FYYGKGLINV QAAAQ
GG36        235 WSNVQIRNHL KNTATSLGST NLYGSGLVNA EAATR
```