(54) Title: VARIANT ENZYMES

(57) Abstract: Disclosed are glycosyl hydrolase enzyme variants, particularly variants of certain oxidoreductases of glycosyl hydrolase family 61. Nucleic acids encoding the glycosyl hydrolyase variants, compositions including the glycosyl hydrolase variants, methods of producing the variants, and methods of using the variants are also described.

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SEQ ID NO:1 (Top); SEQ ID NO:2 (Bottom: signal sequence underlined)

1     atgatccacaagctttccaacctccttgtccaccgcactggcggtggctactggcgttgtc
1     M  I  Q  K  L  S  N  L  L  V  T  A  L  A  V  A  T  G  V  V

61    ggacatggacatattaatgacattgtcatcaacggggtgtggtatcaggcctatgatcct
21    G  H  G  H  I  N  D  I  V  I  N  G  V  W  Y  Q  A  Y  D  P

121   acaacgtttccatacgagtcaaacccccccatagtagtgggcggacggctgccgacctt
41    T  T  F  P  Y  E  S  N  P  P  I  V  V  G  W  T  A  A  D  L

181   gacaacggcttcgtttcacccgacgcataccaaaacctgacatcatctgccacaagaat
61    D  N  G  F  V  S  P  D  A  Y  Q  N  P  D  I  I  C  H  K  N

241   gctacgaatgccaagggcacgcgtctgtcaaggccggagacactattctcttccagtgg
81    A  T  N  A  K  G  H  A  S  V  K  A  G  D  T  I  L  F  Q  W

301   gtgccagttccatggccgcaccctggtcccattgtcgactacctggccaactgcaatggt
101   V  P  V  P  W  P  H  P  G  P  I  V  D  Y  L  A  N  C  N  G

361   gactgcgacaccgttgacaagacgacgcttgagttcttcaagatcgatggcgttgctctc
121   D  C  E  T  V  D  K  T  T  L  E  F  F  K  I  D  G  V  G  L

421   ctcagcggcggggatccgggcacctgggcctcagacgtgctgatctccaacaacaacacc
141   L  S  G  G  D  P  G  T  W  A  S  D  V  L  I  S  N  N  N  T

481   tgggtcgtcaagatccccgacaatcttgcgccaggcaattacgtgctccgccacaagatc
161   W  V  V  K  I  P  D  N  L  A  P  G  N  Y  V  L  R  H  E  I

541   atcgcgttacacagcgccgggcaggcaaacggcgctcagaaccaccccccagtgcttcaac
181   I  A  L  H  S  A  G  Q  A  N  G  A  Q  N  Y  P  Q  C  F  N

601   attgccgtctcaggctcgggttctctgcagcccagcggcgttctagggaccgacctctat
201   I  A  V  S  G  S  G  S  L  Q  P  S  G  V  L  G  T  D  L  Y

661   cacgcgaccgaccctggtgttctcatcaacatctacaccagcccgctcaactacatcatc
221   H  A  T  D  P  G  V  L  I  N  I  Y  T  S  P  L  N  Y  I  I

721   cctggacctaccgtggtatcaggcctgccaacgagtgttgcccaggggagctccgccgcg
241   P  G  P  T  V  V  S  G  L  P  T  S  V  A  Q  G  S  S  A  A

781   acggccaccgccagcgccactgttcctggaggcggtagcggcccgaccagcagaaccacg
261   T  A  T  A  S  A  T  V  P  G  G  G  S  G  P  T  S  R  T  T

841   acaacggccaggacgacgcaggcctcaagcaggcccagctctacgcctccgcaaccacg
281   T  T  A  R  T  T  Q  A  S  S  R  P  S  S  T  P  P  A  T  T

901   tcggcacctgctggcggcccaacccagactctgtacggccagtgtggtggcagcgcttac
301   S  A  P  A  G  G  P  T  Q  T  L  Y  G  Q  C  G  G  S  G  Y

961   agcgggcctactcgatgcgcgccgccagccacttgctctacctgaaccctactacgcc
321   S  G  P  T  R  C  A  P  P  A  T  C  S  T  L  N  P  Y  Y  A

1021  cagtgccttaac
341   Q  C  L  N
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FIG. 1

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VARIANT ENZYMES

CROSS REFERENCE TO RELATED APPLICATIONS

[01]   This application claims benefit of priority from United States Provisional Patent
Applications Serial Nos. 61/859,630; 61/859,666; 61/859,680; 61/859,704; 61/859,712;
61/859,721, and 61/859,735, all filed on 29 July 2013, the contents of all of which are
incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[02]   The present disclosure generally relates to glycosyl hydrolase enzyme variants,
particularly variants of certain oxidoreductases of glycosyl hydrolase family 61. Nucleic acids
encoding the glycosyl hydrolyase variants, compositions including the glycosyl hydrolase
variants, methods of producing the variants, and methods of using the variants are also
described.

GOVERNMENT RIGHTS

BACKGROUND OF THE INVENTION

[04]   Cellulose and hemicellulose are the most abundant plant materials produced by
photosynthesis. They can be degraded and used as an energy source by numerous
microorganisms, including bacteria, yeast and fungi, that produce extracellular enzymes
capable of hydrolysis of the polymeric substrates to monomeric sugars (Aro et al., 2001). As
the limits of non-renewable resources approach, the potential of cellulose to become a major
renewable energy resource is enormous (Krishna et al., 2001). The effective utilization of
cellulose through biological processes is one approach to overcoming the shortage of foods,
feeds, and fuels (Ohmiya et al., 1997).

[05]   Cellulases are enzymes that hydrolyze cellulose (beta-1,4-glucan or beta D-glucosidic
linkages) resulting in the formation of glucose, cellobiose, cellooligosaccharides, and the like.
Cellulases have been traditionally divided into three major classes: endoglucanases (EC
3.2.1.4) ("EG"), exoglucanases or cellobiohydrolases (EC 3.2.1.91) ("CBH"), and beta-
glucosidases (β -D-glucoside glucohydrolase; EC 3.2.1.21) ("BG"). (Knowles et al., 1987;
Shulein, 1988). Endoglucanases act mainly on the amorphous parts of the cellulose fiber,
whereas cellobiohydrolases are also able to degrade crystalline cellulose (Nevalainen and

Penttila, 1995). Beta-glucosidase acts to liberate D-glucose units from cellobiose, cello-oligosaccharides, and other glucosides (Freer, 1993).

[06]   Cellulases are known to be produced by a large number of bacteria, yeast and fungi. Certain fungi produce a complete cellulase system capable of degrading crystalline forms of cellulose, such that the cellulases are readily produced in large quantities via fermentation. Filamentous fungi play a special role since many yeast, such as *Saccharomyces cerevisiae*, lack the ability to hydrolyze cellulose. (See, *e.g.*, Aro *et al.*, 2001; Aubert *et al.*, 1988; Wood *et al.*, 1988, and Coughlan, *et al.*)

[07]   The fungal cellulase classifications of CBH, EG and BG can be further expanded to include multiple components within each classification. For example, multiple CBHs, EGs and BGs have been isolated from a variety of fungal sources including *Trichoderma reesei* which contains known genes for 2 CBHs, *e.g.,* CBH I (also known as Cel7A or glycosyl hydrolase family (GH)7A) and CBH II (also known as Cel6A or GH6A), a number of EGs, e.g., EG I (also known as Cel7B or GH7B), EG II (also known as Cel5A or GH5A), EG III (also known as Cel12A or GH12A), EGV (also known as Cel45A or GH45A), EGVI (also known as Cel74A or GH74A), EGVII (also known as Cel61B or GH61b) and EGVIII, and a series of BGs, e.g., BG1, BG3, and BG5.

[08]   In order to efficiently convert crystalline cellulose to glucose, a complete cellulase system comprising components or enzymatic activities from each of the CBH, EG and BG classifications is typically required, with isolated components less effective in hydrolyzing crystalline cellulose (Filho *et al.*, 1996). A synergistic relationship has been observed amongst cellulase components from different classifications. In particular, the EG-type cellulases and CBH- type cellulases synergistically interact to more efficiently degrade cellulose. (See, *e.g.,* Wood, 1985.).

[09]   Cellulases are known in the art to be useful in the treatment of textiles for the purposes of enhancing the cleaning ability of detergent compositions, for use as a softening agent, for improving the feel and appearance of cotton fabrics, and the like (Kumar *et al.*, 1997).

[10]   Cellulase-containing detergent compositions with improved cleaning performance (US Pat. No. 4,435,307; GB App. Nos. 2,095,275 and 2,094,826) and for use in the treatment of fabric to improve the feel and appearance of the textile (US Pat. Nos. 5,648,263, 5,691,178, and 5,776,757; GB App. No. 1,358,599; The Shizuoka Prefectural Hammamatsu Textile Industrial Research Institute Report, Vol. 24, pp. 54-61, 1986), have been described.

[11]   Cellulases are further known in the art to be useful in the conversion of cellulosic feedstocks into ethanol. This process has a number of advantages, including the ready availability of large amounts of feedstock that is otherwise discarded (e.g., burning or land filling the feedstock). Other materials that consist primarily of cellulose, hemicellulose, and lignin, e.g., wood, herbaceous crops, and agricultural or municipal waste, have been considered for use as

feedstock in ethanol production. In recent years, new classes of glycosyl hydrolases have been identified that provide further auxiliary effects that enhance or augment the enzymatic hydrolysis of cellulosic materials, although the mechanisms of action of many of these new auxiliary enzymes have not been fully elucidated. One such family of glycosyl hydrolases,

5   which had earlier been annotated as GH61 family (see, e.g., Harris et al. "Stimulation of Lignocellulosic Biomass Hydrolysis by Proteins of Glycoside Hydrolase Family 61: Structure and Function of a Large, Enigmatic Family" Biochemistry 2010, vol. 49, pp. 3305–3316), had been repeatedly re-annotated, most recently to Auxiliary Activity (AA) Family 9 after the discovery that some family members are lytic polysaccharide monooxygenases (Levasseur A.

10   et al, "Expansion of the enzymatic repertoire of the CAZy database to integrate auxiliary redox enzymes" Biotechnol Biofuels 2013, vol 6, issue 1, pp. 41). At least two GH61 enzymes are present in the *T. reesei* (Saloheimo M., "cDNA cloning of a *Trichoderma reesei* cellulase and demonstration of endoglucanase activity by expression in yeast" Eur J Biochem. 1997 vol. 249, issue 2: pp. 584-91; Karlsson et al., Homologous expression and characterization of Cel61A

15   (EG IV) of *Trichoderma reesei*" Eur. J. Biochem. 2001 vol. 268, pp. 6498-6507; Karkehabadi et al., "The first structure of a glycoside hydrolase family 61 member, Cel61B from Hypocrea jecorina, at 1.6 A resolution" J Mol Biol. 2008, vol. 383 issue 1: pp 144-154; Martinez et al., "Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*)" Nature Biotechnology 2008, vol. 26, pp. 553-560). In the very recent past,

20   it was reported that up to four more of these glycosyl hydrolases have been identified in the *Trichoderma reesei* genome (Häkkinen M. et al, "Re-annotation of the CAZy genes of Trichoderma reesei and transcription in the presence of lignocellulosic substrates" 2012, Microb Cell Fact. Vol 4, issue 11, pp. 134).

[12]   It would be an advantage in the art to provide a set of GH61 enzyme variants with

25   improved capacity, when combined with one or more cellulases, and optionally also one or more hemicellulases, to augment the efficacy and efficiency of hydrolyzing lignocellulosic biomass substrates to monosaccharides, disaccharides, and polysaccharides. Improved properties of the variant GH61 polypeptide include, but are not limited to: altered temperature-dependent activity profiles, thermostability, pH activity, pH stability, substrate specificity,

30   product specificity, and chemical stability.

BRIEF SUMMARY OF THE INVENTION

[13]   The present disclosure describes isolated variant polypeptides having glycosyl hydrolyase family 61 (GH61) activity, nucleic acids encoding such enzymes, host cells containing GH61-encoding polynucleotides (e.g., host cells that express the GH61

35   polypeptides), compositions containing the GH61 polypeptides, and methods for producing and using the same.

[14]   As such, aspects of the present invention provide variants of a parent GH61 enzyme, where the variant has cellulase augmenting activity, has at least 80% sequence identity to SEQ ID NO:3, and has at least one improved property or performance over the parent GH61 enzyme selected from: (a) expression (yield), (b) Thermostability and/or Tm, (c) Whole Hydrolysate Dilute Acid Pretreated Corn Stover (whPCS) Hydrolysis Assay activity, and (d) Dilute Ammonia Pretreated Corn Stover (daCS) Hydrolysis Assay activity. In certain embodiment, the GH61 enzyme is a GH61A enzyme.

[15]   In certain embodiments, a GH61 variant has a few mutations, where by "a few" is meant from 1 to 10 mutations (e.g., from 1 to 10 amino acid substitutions as compared to a parent GH61 enzyme).

[16]   In certain embodiments, a GH61 variant has at least two, at least three, or all four improved properties selected from the list above over the parent GH61.

[17]   In certain embodiments, a GH61 variant has one or more highly combinable substitutions that fall into Group A as defined herein (see Examples section below).

[18]   In certain embodiments, a GH61 variant has one or more highly combinable substitutions that fall into Group B as defined herein, where the variant further may have one or more amino acid substitutions that fall into Group A.

[19]   In certain embodiments, a GH61 variant has one or more highly combinable substitutions that fall into Group C as defined herein, where the variant further may have one or more amino acid substitutions that fall into Group A or Group B.

[20]   In certain embodiments, the GH61 variant has an amino acid substitution at one or more sites that each has a productivity score of 4 as defined herein (see Examples section below and Table 4).

[21]   In certain embodiments, the GH61 variant has an amino acid substitution at one or more sites that each has a productivity score of 3, where the variant further may have at least one additional substitution at a site that has a productivity score of 4.

[22]   In certain embodiments, the GH61 variant has an amino acid substitution at one or more sites that each has a productivity score of 2, where the variant further may have at least one additional substitution at a site that has a productivity score of 3 or 4.

[23]   In certain embodiments, the GH61 variant has an amino acid substitution at one or more sites that each has a productivity score of 1, where the variant further may have at least one additional substitution at a site that has a productivity score of 2 or 3 or 4.

[24]   In certain embodiments, the GH61 variant has at least one amino acid substitution that has a variant suitability score of +++++ as defined herein (see Examples section below and Table 4).

[25] In certain embodiments, the GH61 variant has at least one amino acid substitution that has a variant suitability score of ++++, where the variant further may have at least one additional substitution that has a variant suitability score of +++++.

[26] In certain embodiments, the GH61 variant has at least one amino acid substitution that has a variant suitability score of +++, where the variant further may have at least one additional substitution that has a variant suitability score of ++++ or +++++.

[27] In certain embodiments, the GH61 variant has at least one amino acid substitution that has a variant suitability score of ++, where the variant further may have at least one additional substitution that has a variant suitability score of +++ or ++++ or +++++.

[28] In certain embodiments, the GH61 variant has at least one amino acid substitution that has a variant suitability score of +, where the variant further may have at least one additional substitution that has a variant suitability score of ++ or +++ or ++++ or +++++.

[29] In certain embodiments, the GH61 variant has at least one amino acid substitution that falls into one of the specific productivity score/variant suitability score categories A to T as set forth in Table 1 below:

Table 1: GH61 Variant Categories

		Variant Suitability Score				
		+++++	++++	+++	++	+
Productivity Score	4	A	B	C	D	E
	3	F	G	H	I	J
	2	K	L	M	N	O
	1	P	Q	R	S	T

[30] The specific amino acid positions and substitutions for each category can be readily identified in Table 4 of Example 3 herein. For example, a GH61 variant can have a substitution selected from the following, each of which falls into category A: W13G, W13L, W13N, W13R, W13T, or W13V.

[31] Examples of GH61 variants include, but are not limited to the following:

[32] 1. An GH61 variant having cellulase augmenting activity, at least 80% sequence identity to SEQ ID NO:3, and having at least one improved property over a parent GH61 enzyme selected from: (a) expression, (b) thermostability and/or Tm, (c) performance in the presence of at least one cellulase, in a Whole Hydrolysate Dilute Acid Pretreated Corn Stover (whPCS) hydrolysis assay, (f) performance in the presence of at least one cellulase, in a Dilute Ammonia Pretreated Corn Stover (daCS) hydrolysis assay. In certain embodiments, the GH61 variant is in an isolated form.

[33]   2. The GH61 variant of 1, where the variant has at least one amino acid substitution at a position selected from the group consisting of: W13, L288, P19, T20, P29, A37, L39, D47, A48, S68, T107, S121, G122, T127, S164, G166, Q167, A168, N216, I218, I219, V224, T287, T289, Y291, S297, T303, R304, A306, P308, S312, T313, N315, Y317, Y318, N5, I7, V12, Q15, T21, F22, S26, P28, V32, G33, T35, A36, N41, Q50, N51, K58, N59, V80, P89, T108, G116, L120, G123, V132, S135, N147, A161, A181, S183, S185, Q189, G195, T196, H200, A201, T202, I208, P222, T223, S226, S231, G235, L290, G295, G296, G298, Y299, S300, G301, P302, P307, A309, T310, P316, A319, L322, I4, I9, N10, P23, Y24, E25, N27, D38, F43, V44, Y49, P52, I54, T61, N62, K64, V69, K70, A71, I90, V91, N96, N98, D100, T103, E110, V117, L119, D124, P125, G126, V141, D146, A149, I160, H163, A171, N173, P175, I180, S187, L188, S191, L194, D197, P204, V206, L207, N209, Y211, S213, P214, Y217, T230, V232, A233, G292, Q293, Q320, and N323, where the position of each amino acid substitution corresponds to that of SEQ ID NO:3.

[34]   3. The GH61 variant of 1 or 2, where the variant has at least one amino acid substitution selected from Table 4 as set forth herein.

[35]   4. Any preceding GH61 variant, where the variant has an amino acid substitution at position W13 selected from the group consisting of: G, L, N, R, T, V, F, Q, Y, A, E, I, K, and M.

[36]   5. Any preceding GH61 variant, where the variant has an amino acid substitution at position L288 selected from the group consisting of: E, F, G, I, K, M, N, R, S, T, A, C, D, V, and W.

[37]   6. Any preceding GH61 variant, where the variant has an amino acid substitution at position P19 selected from the group consisting of: F, M, E, H, Q, T, K, and W.

[38]   7. Any preceding GH61 variant, where the variant has an amino acid substitution at position T20 selected from the group consisting of: K, P, G, M, R, Y, A, and N.

[39]   8. Any preceding GH61 variant, where the variant has an amino acid substitution at position P29 selected from the group consisting of: C, E, F, K, T, Y D, G, M, R, and S.

[40]   9. Any preceding GH61 variant, where the variant has an amino acid substitution at position A37 selected from the group consisting of: D, S, E, L, W, F, K, and Q.

[41]   10. Any preceding GH61 variant, where the variant has an amino acid substitution at position L39 selected from the group consisting of: A, D, E, M, T, Y, F, I, N, P, Q, S, and V.

[42]   11. Any preceding GH61 variant, where the variant has an amino acid substitution at position D47 selected from the group consisting of: A, K, T, H, R, P, and W.

[43]   12. Any preceding GH61 variant, where the variant has an amino acid substitution at position A48 selected from the group consisting of: C, E, G, K, L, N, T, Y, Q, W, H, I, and S.

[44]   13. Any preceding GH61 variant, where the variant has an amino acid substitution at position S68 selected from the group consisting of: E, I, L, M, N, T, V, and Y.

[45] 14. Any preceding GH61 variant, where the variant has an amino acid substitution at position T107 selected from the group consisting of: A, D, E, K, M, R, S, and G.

[46] 15. Any preceding GH61 variant, where the variant has an amino acid substitution at position S121 selected from the group consisting of: E, F, N, T, C, L, V, D, G, K, M, and Y.

[47] 16. Any preceding GH61 variant, where the variant has an amino acid substitution at position G122 selected from the group consisting of: E, N, S, V, A, M, T, F, and L.

[48] 17. Any preceding GH61 variant, where the variant has an amino acid substitution at position T127 selected from the group consisting of: A, E, F, I, L, M, V, W, and Y.

[49] 18. Any preceding GH61 variant, where the variant has an amino acid substitution at position S164 selected from the group consisting of: A, D, E, G, K, L, M, Q, R, V, and Y.

[50] 19. Any preceding GH61 variant, where the variant has an amino acid substitution at position G166 selected from the group consisting of: A, D, E, F, H, K, M, N, Q, and S.

[51] 20. Any preceding GH61 variant, where the variant has an amino acid substitution at position Q167 selected from the group consisting of: E, A, D, F, G, K, L, N, R, V, and Y.

[52] 21. Any preceding GH61 variant, where the variant has an amino acid substitution at position A168 selected from the group consisting of: E, M, D, F, I, L, N, P, Q, R, T, and Y.

[53] 22. Any preceding GH61 variant, where the variant has an amino acid substitution at position N216 selected from the group consisting of: D, Q, T, K, and P.

[54] 23. Any preceding GH61 variant, where the variant has an amino acid substitution at position I218 selected from the group consisting of: T, A, D, E, K, M, N, P, S, and V.

[55] 24. Any preceding GH61 variant, where the variant has an amino acid substitution at position I219 selected from the group consisting of: M, A, D, L, N, P, and Q.

[56] 25. Any preceding GH61 variant, where the variant has an amino acid substitution at position V224 selected from the group consisting of: Q, I, A, E, N, P, S, and T.

[57] 26. Any preceding GH61 variant, where the variant has an amino acid substitution at position T287 selected from the group consisting of: H, A, F, G, S, and I.

[58] 27. Any preceding GH61 variant, where the variant has an amino acid substitution at position T289 selected from the group consisting of: A, D, K, L, M, R, and S.

[59] 28. Any preceding GH61 variant, where the variant has an amino acid substitution at position Y291 selected from the group consisting of: A, F, G, I, K, L, M, N, R, S, V, W, D, and E.

[60] 29. Any preceding GH61 variant, where the variant has an amino acid substitution at position S297 selected from the group consisting of: D, E, K, N, Q, R, and T.

[61] 30. Any preceding GH61 variant, where the variant has an amino acid substitution at position T303 selected from the group consisting of: K, M, P, R, S, V, and Y.

[62] 31. Any preceding GH61 variant, where the variant has an amino acid substitution at position R304 selected from the group consisting of: A, C, D, E, N, Q, T, and V.

[63]   32. Any preceding GH61 variant, where the variant has an amino acid substitution at position A306 selected from the group consisting of: E, F, G, I, K, L, M, Q, T, and Y.

[64]   33. Any preceding GH61 variant, where the variant has an amino acid substitution at position P308 selected from the group consisting of: A, G, H, I, K, L, Q, R, S, T, V, W, and Y.

[65]   34. Any preceding GH61 variant, where the variant has an amino acid substitution at position S312 selected from the group consisting of: Q, F, G, I, K, L, M, N, T, V, D, R, and Y.

[66]   35. Any preceding GH61 variant, where the variant has an amino acid substitution at position T313 selected from the group consisting of: S, D, G, L, P, V, E, F, I, K, M, and Y.

[67]   36. Any preceding GH61 variant, where the variant has an amino acid substitution at position N315 selected from the group consisting of: S, H, L, Q, R, V, Y, E, K, and M.

[68]   37. Any preceding GH61 variant, where the variant has an amino acid substitution at position Y317 selected from the group consisting of: Q, S, V, R, E, G, L, M, P, and T.

[69]   38. Any preceding GH61 variant, where the variant has a Y318T amino acid substitution.

[70]   39. Any preceding GH61 variant, where the variant has an amino acid substitution at position N5 selected from the group consisting of: A, D, G, and S.

[71]   40. Any preceding GH61 variant, where the variant has an amino acid substitution at position I7 selected from the group consisting of: L, M, and V.

[72]   41. Any preceding GH61 variant, where the variant has an amino acid substitution at position V12 selected from the group consisting of: M and T.

[73]   42. Any preceding GH61 variant, where the variant has an amino acid substitution at position Q15 selected from the group consisting of: H, K, and D.

[74]   43. Any preceding GH61 variant, where the variant has an amino acid substitution at position T21 selected from the group consisting of: I, K, M, and S.

[75]   44. Any preceding GH61 variant, where the variant has an amino acid substitution at position F22 selected from the group consisting of: K, I, L, and R.

[76]   45. Any preceding GH61 variant, where the variant has an amino acid substitution at position S26 selected from the group consisting of: M, P, and T.

[77]   46. Any preceding GH61 variant, where the variant has an amino acid substitution at position P28 selected from the group consisting of: M, S, and V.

[78]   47. Any preceding GH61 variant, where the variant has an amino acid substitution at position V32 selected from the group consisting of: A and P.

[79]   48. Any preceding GH61 variant, where the variant has an amino acid substitution at position G33 selected from the group consisting of: Q, A, and S.

[80]   49. Any preceding GH61 variant, where the variant has an amino acid substitution at position T35 selected from the group consisting of: A, D, E, K, N, and S.

[81]   50. Any preceding GH61 variant, where the variant has an amino acid substitution at position A36 selected from the group consisting of: T, I, N, S, and Y.

[82]   51. Any preceding GH61 variant, where the variant has an amino acid substitution at position N41 selected from the group consisting of: D, E, L, and M.

[83]   52. Any preceding GH61 variant, where the variant has an amino acid substitution at position Q50 selected from the group consisting of: A, T, Y, D, E, and K.

[84]   53. Any preceding GH61 variant, where the variant has an amino acid substitution at position N51 selected from the group consisting of: S, E, H, K, L, and T.

[85]   54. Any preceding GH61 variant, where the variant has an amino acid substitution at position K58 selected from the group consisting of: I, R, and V.

[86]   55. Any preceding GH61 variant, where the variant has an amino acid substitution at position N59 selected from the group consisting of: G, E, Q, and S.

[87]   56. Any preceding GH61 variant, where the variant has an amino acid substitution at position V80 selected from the group consisting of: A, E, L, and M.

[88]   57. Any preceding GH61 variant, where the variant has an amino acid substitution at position P89 selected from the group consisting of: T, S, V, and L.

[89]   58. Any preceding GH61 variant, where the variant has an amino acid substitution at position T108 selected from the group consisting of: A, Q, R, and S.

[90]   59. Any preceding GH61 variant, where the variant has an amino acid substitution at position G116 selected from the group consisting of: H, Q, and S.

[91]   60. Any preceding GH61 variant, where the variant has an amino acid substitution at position L120 selected from the group consisting of: V, T, I, and S.

[92]   61. Any preceding GH61 variant, where the variant has an amino acid substitution at position G123 selected from the group consisting of: E, Q, and R.

[93]   62. Any preceding GH61 variant, where the variant has an amino acid substitution at position V132 selected from the group consisting of: D, E, M, and R.

[94]   63. Any preceding GH61 variant, where the variant has an amino acid substitution at position S135 selected from the group consisting of: A, E, G, H, M, and N.

[95]   64. Any preceding GH61 variant, where the variant has an amino acid substitution at position N147 selected from the group consisting of: E, M, S, and T.

[96]   65. Any preceding GH61 variant, where the variant has an amino acid substitution at position A161 selected from the group consisting of: E, G, L, and S.

[97]   66. Any preceding GH61 variant, where the variant has an amino acid substitution at position A181 selected from the group consisting of: K, N, and R.

[98]   67. Any preceding GH61 variant, where the variant has an amino acid substitution at position S183 selected from the group consisting of: E, K, N, and T.

[99]   68. Any preceding GH61 variant, where the variant has an amino acid substitution at position S185 selected from the group consisting of: D, G, and T.

[100]   69. Any preceding GH61 variant, where the variant has an amino acid substitution at position Q189 selected from the group consisting of: E, L, M, R, G, and T.

[101]   70. Any preceding GH61 variant, where the variant has an amino acid substitution at position G195 selected from the group consisting of: A, E, and S.

[102]   71. Any preceding GH61 variant, where the variant has an amino acid substitution at position T196 selected from the group consisting of: H, Y, and R.

[103]   72. Any preceding GH61 variant, where the variant has an amino acid substitution at position H200 selected from the group consisting of: R, A, Q, and S.

[104]   73. Any preceding GH61 variant, where the variant has an amino acid substitution at position A201 selected from the group consisting of: P, K, and Q.

[105]   74. Any preceding GH61 variant, where the variant has an amino acid substitution at position T202 selected from the group consisting of: S, E, and Q.

[106]   75. Any preceding GH61 variant, where the variant has an amino acid substitution at position I208 selected from the group consisting of: F, L, and V.

[107]   76. Any preceding GH61 variant, where the variant has an amino acid substitution at position P222 selected from the group consisting of: D and G.

[108]   77. Any preceding GH61 variant, where the variant has an amino acid substitution at position T223 selected from the group consisting of: S, E, and Q.

[109]   78. Any preceding GH61 variant, where the variant has an amino acid substitution at position S226 selected from the group consisting of: D, G, K, M, P, and Q.

[110]   79. Any preceding GH61 variant, where the variant has an amino acid substitution at position S231 selected from the group consisting of: C and I.

[111]   Any preceding GH61 variant, where the variant has an amino acid substitution at position G235 selected from the group consisting of: A, F, I, M, N, Q, V.

[112]   80. Any preceding GH61 variant, where the variant has an amino acid substitution at position L290 selected from the group consisting of: K, M, and T.

[113]   81. Any preceding GH61 variant, where the variant has an amino acid substitution at position G295 selected from the group consisting of: A, F, H, Q, and R.

[114]   82. Any preceding GH61 variant, where the variant has an amino acid substitution at position G296 selected from the group consisting of: A and M.

[115]   83. Any preceding GH61 variant, where the variant has an amino acid substitution at position G298 selected from the group consisting of: K, A, I, N, and Q.

[116]   84. Any preceding GH61 variant, where the variant has an amino acid substitution at position Y299 selected from the group consisting of: N, S, F, and P.

[117]   85. Any preceding GH61 variant, where the variant has an amino acid substitution at position S300 selected from the group consisting of: F, L, N, T, and Q.

[118]   86. Any preceding GH61 variant, where the variant has an amino acid substitution at position G301 selected from the group consisting of: D, S, and Y.

[119]   87. Any preceding GH61 variant, where the variant has an amino acid substitution at position P302 selected from the group consisting of: G, L, T, V, Y, and F.

[120]   88. Any preceding GH61 variant, where the variant has an amino acid substitution at position P307 selected from the group consisting of: A, F, H, L, and V.

[121]   89. Any preceding GH61 variant, where the variant has an amino acid substitution at position A309 selected from the group consisting of: I, K, R, T, V, and Y.

[122]   90. Any preceding GH61 variant, where the variant has an amino acid substitution at position T310 selected from the group consisting of: L, M, Q, S, and W.

[123]   91. Any preceding GH61 variant, where the variant has an amino acid substitution at position P316 selected from the group consisting of: D, N, R, and T.

[124]   92. Any preceding GH61 variant, where the variant has an amino acid substitution at position A319 selected from the group consisting of: Q, W, D, F, and S.

[125]   93. Any preceding GH61 variant, where the variant has an amino acid substitution at position L322 selected from the group consisting of: A, E, S, T, and V.

[126]   94. Any preceding GH61 variant, where the variant has an amino acid substitution at position I4 selected from the group consisting of: V and T.

[127]   95. Any preceding GH61 variant, where the variant has an amino acid substitution at position I9 selected from the group consisting of: V and A.

[128]   96. Any preceding GH61 variant, where the variant has an N10D amino acid substitution.

[129]   97. Any preceding GH61 variant, where the variant has a P23G amino acid substitution.

[130]   98. Any preceding GH61 variant, where the variant has a Y24T amino acid substitution.

[131]   99. Any preceding GH61 variant, where the variant has an amino acid substitution at position E25 selected from the group consisting of: N and Q.

[132]   100. Any preceding GH61 variant, where the variant has an N27E amino acid substitution.

[133]   101. Any preceding GH61 variant, where the variant has an amino acid substitution at position D38 selected from the group consisting of: E and N.

[134]   102. Any preceding GH61 variant, where the variant has an F43Y amino acid substitution.

[135]   103. Any preceding GH61 variant, where the variant has a V44I amino acid substitution.

[136]   104. Any preceding GH61 variant, where the variant has a Y49W amino acid substitution.

[137]   105. Any preceding GH61 variant, where the variant has an amino acid substitution at position P52 selected from the group consisting of: A and N.

[138]   106. Any preceding GH61 variant, where the variant has an amino acid substitution at position I54 selected from the group consisting of: Q and E.

[139]   107. Any preceding GH61 variant, where the variant has an amino acid substitution at position T61 selected from the group consisting of: Q and S.

[140]   108. Any preceding GH61 variant, where the variant has an N62P amino acid substitution.

[141]   109. Any preceding GH61 variant, where the variant has an amino acid substitution at position K64 selected from the group consisting of: A and Q.

[142]   110. Any preceding GH61 variant, where the variant has a V69Y amino acid substitution.

[143]   111. Any preceding GH61 variant, where the variant has a K70R amino acid substitution.

[144]   112. Any preceding GH61 variant, where the variant has an amino acid substitution at position A71 selected from the group consisting of: Y and P.

[145]   113. Any preceding GH61 variant, where the variant has an I90V amino acid substitution.

[146]   114. Any preceding GH61 variant, where the variant has an amino acid substitution at position V91 selected from the group consisting of: L and I.

[147]   115. Any preceding GH61 variant, where the variant has an amino acid substitution at position N96 selected from the group consisting of: A and S.

[148]   116. Any preceding GH61 variant, where the variant has an N98H amino acid substitution.

[149]   117. Any preceding GH61 variant, where the variant has an amino acid substitution at position D100 selected from the group consisting of: P and S.

[150]   118. Any preceding GH61 variant, where the variant has a T103A amino acid substitution.

[151]   119. Any preceding GH61 variant, where the variant has an E110K amino acid substitution.

[152]   120. Any preceding GH61 variant, where the variant has a V117A amino acid substitution.

[153]   121. Any preceding GH61 variant, where the variant has an amino acid substitution at position L119 selected from the group consisting of: N and T.

[154]   122. Any preceding GH61 variant, where the variant has a D124N amino acid substitution.

[155]   123. Any preceding GH61 variant, where the variant has a P125D amino acid substitution.

[156]   124. Any preceding GH61 variant, where the variant has an amino acid substitution at position G126 selected from the group consisting of: M and Q.

[157]   125. Any preceding GH61 variant, where the variant has a V141T amino acid substitution.

[158]   126. Any preceding GH61 variant, where the variant has a D146E amino acid substitution.

[159]   127. Any preceding GH61 variant, where the variant has an A149E amino acid substitution.

[160]   128. Any preceding GH61 variant, where the variant has an amino acid substitution at position I160 selected from the group consisting of: F and M.

[161]   129. Any preceding GH61 variant, where the variant has an H163A amino acid substitution.

[162]   130. Any preceding GH61 variant, where the variant has an A171T amino acid substitution.

[163]   131. Any preceding GH61 variant, where the variant has an N173H amino acid substitution.

[164]   132. Any preceding GH61 variant, where the variant has a P175F amino acid substitution.

[165]   133. Any preceding GH61 variant, where the variant has an I180L amino acid substitution.

[166]   134. Any preceding GH61 variant, where the variant has an amino acid substitution at position S187 selected from the group consisting of: A and D.

[167]   135. Any preceding GH61 variant, where the variant has an amino acid substitution at position L188 selected from the group consisting of: A and D.

[168]   136. Any preceding GH61 variant, where the variant has an S191D amino acid substitution.

[169]   137. Any preceding GH61 variant, where the variant has an amino acid substitution at position L194 selected from the group consisting of: D and M.

[170]   138. Any preceding GH61 variant, where the variant has an amino acid substitution at position D197 selected from the group consisting of: A and V.

[171]   139. Any preceding GH61 variant, where the variant has a P204A amino acid substitution.

[172]   140. Any preceding GH61 variant, where the variant has a V206I amino acid substitution.

[173]   141. Any preceding GH61 variant, where the variant has an L207F amino acid substitution.

[174]   142. Any preceding GH61 variant, where the variant has an N209D amino acid substitution.

[175]   143. Any preceding GH61 variant, where the variant has a Y211A amino acid substitution.

[176]   144. Any preceding GH61 variant, where the variant has an S213T amino acid substitution.

[177]   145. Any preceding GH61 variant, where the variant has a P214S amino acid substitution.

[178]   146. Any preceding GH61 variant, where the variant has a Y217H amino acid substitution.

[179]   147. Any preceding GH61 variant, where the variant has a T230S amino acid substitution.

[180]   148. Any preceding GH61 variant, where the variant has an amino acid substitution at position V232 selected from the group consisting of: F and Y.

[181]   149. Any preceding GH61 variant, where the variant has an amino acid substitution at position A233 selected from the group consisting of: F and S.

[182]   150. Any preceding GH61 variant, where the variant has an amino acid substitution at position G292 selected from the group consisting of: D and K.

[183]   151. Any preceding GH61 variant, where the variant has an amino acid substitution at position Q293 selected from the group consisting of: K and P.

[184]   152. Any preceding GH61 variant, where the variant has a Q320R amino acid substitution.

[185]   153. Any preceding GH61 variant, where the variant has an N323V amino acid substitution.

[186]   154. The GH61 variant of 1, where the variant has a first amino acid substitution, where the first amino acid substitution is in SEL Cohort 1.

[187]   155. The GH61 variant of 1, where the variant has a first amino acid substitution, where the first amino acid substitution is in SEL Cohort 1 and SEL Cohort 2.

[188]   156. The GH61 variant of 1, where the variant has a first amino acid substitution, where the first amino acid substitution is in SEL Cohort 1, SEL Cohort 2, and SEL Cohort 3.

[189]   157. The GH61 variant of 1, where the variant has a first amino acid substitution, where the first amino acid substitution is in SEL Cohort 2.

[190]   158. The GH61 variant of 1, where the variant has a first amino acid substitution, where the first amino acid substitution is in SEL Cohort 2 and SEL Cohort 3.

[191]   159. The GH61 variant of 1, where the variant has a first amino acid substitution, where the first amino acid substitution is in SEL Cohort 3.

[192]   160. The GH61 variant of any one of 154 to 159, where the variant has a second amino acid substitution, where the second amino acid substitution is in SEL Cohort 1, SEL Cohort 2, SEL Cohort 3 or the Wild-Type SEL Cohort.

[193]   161. The GH61 variant of 160, where the variant has from 3 to 20 amino acid substitutions, where the amino acid substitutions are in SEL Cohort 1, SEL Cohort 2, SEL Cohort 3 and/or the Wild-Type SEL Cohort.

[194]   162. The GH61 variant of 1, where the variant is a combinatorial variant in one or more of Combinatorial Cohorts 1 to 11.

[195]   163. The GH61 variant of 1, where the variant has a first amino acid substitution, where the first amino acid substitution is in Deconvoluted Cohort 1.

[196]   164. The GH61 variant of 1, where the variant has a first amino acid substitution, where the first amino acid substitution is in Deconvoluted Cohort 1 and Deconvoluted Cohort 2.

[197]   165. The GH61 variant of 1, where the variant has a first amino acid substitution, where the first amino acid substitution is in Deconvoluted Cohort 1, Deconvoluted Cohort 2, and Deconvoluted Cohort 3.

[198]   166. The GH61 variant of 1, where the variant has a first amino acid substitution, where the first amino acid substitution is in Deconvoluted Cohort 2.

[199]   167. The GH61 variant of 1, where the variant has a first amino acid substitution, where the first amino acid substitution is in Deconvoluted Cohort 2 and Deconvoluted Cohort 3.

[200]   168. The GH61 variant of any one of 163 to 167, where the variant has a second amino acid substitution, where the second amino acid substitution is in Deconvoluted Cohort 1, Deconvoluted Cohort 2, and/or Deconvoluted Cohort 3.

[201]   169. Any preceding GH61 variant, where the parent GH61 polypeptide is a fungal glycosyl hydrolase 61a (GH61A).

[202]   170. The GH61 variant of 169, where the fungal GH61A is from *Hypocrea jecorina, Hypocrea rufa, Hypocrea orientalis, Hypocrea atroviridis, Hypocrea virens, Emericella nidulans, Aspergillus terreus, Aspergillus oryzae, Aspergillus niger, Aspergillus kawachii, Aspergillus flavus, Aspergillus clavatus, Gaeumannomyces graminis, Trichoderma saturnisporum, Neurospora tetrasperma, Neurospora crassa, Neosartorya fumigate, Neosartorya fumigate, Neosartorya fischeri, Thielavia terrestris, and Thielavia heterothallica*.

[203]   171. Any preceding GH61 variant, where the variant has at least 90% sequence identity to SEQ ID NO:3.

[204]   172. Any preceding GH61 variant, where the variant has at least 95% sequence identity to SEQ ID NO:3.

[205]   In certain embodiments, the parent GH61 is a fungal glycosyl hydrolase 61 (GH61), e.g., GH61A from *Hypocrea jecorina, Hypocrea atroviridis, Hypocrea virens, Thielavia terrestris,* or *Thielavia heterothallica* (or their respective anamorph, teleomorph or holomorph counterpart forms), e.g., GH61A selected from any one of SEQ ID NOs: 3, 72, 73, 74, and 77.

[206]   Aspects of the invention include variants in the catalytic and/or the carbohydrate binding domain of enzymes having homology to the catalytic domain of *H. jecorina* GH61A (SEQ ID NO:34) and/or the carbohydrate binding domain of *H. jecorina* GH61A (SEQ ID NO:51). Thus, any one or any combination of the variants described above that fall within the catalytic domain of *H. jecorina* GH61A can be applied to a catalytic domain that is homologous to the catalytic domain of the *H. jecorina* GH61A enzyme. Likewise, any one or any combination of the variants described above that fall within the carbohydrate binding domain of *H. jecorina* GH61A can be applied to a carbohydrate binding domain that is homologous to the carbohydrate binding domain of the *H. jecorina* GH61A enzyme. As described above, these catalytic domain and/or carbohydrate binding domain variants have at least one improved property over their respective parent enzyme. Examples of catalytic domains homologous to *H. jecorina* GH61A (SEQ ID NO:34) are shown in Figures 2A to 2C.   Examples of carbohydrate binding domains homologous to *H. jecorina* GH61A (SEQ ID NO:51) are shown in Figure 3. Moreover, chimeric enzymes comprising either (1) a catalytic domain from a GH61 variant and a carbohydrate binding domain of a second enzyme or (2) a carbohydrate binding domain from a GH61 variant and a catalytic domain of a second enzyme are contemplated, where the GH61 domain of the chimeric enzyme contains one or more variant amino acids as described herein.

[207]   Aspects of the subject invention include an isolated polynucleotide comprising a polynucleotide sequence encoding a variant of a parent GH61 as described herein. The isolated polynucleotide may be present in a vector, e.g., an expression vector or a vector for propagation of the polynucleotide. The vector may be present in a host cell to propagate the vector and/or that expresses the encoded GH61 variant as described herein. The host cell can be any cell that finds use in propagation of the GH61 variant polynucleotide and/or expression of the encoded GH61 variant, e.g., a bacterial cell, a fungal cell, etc. Examples of suitable fungal cell types that can be employed include filamentous fungal cells, e.g., cells of *Trichoderma reesei, Trichoderma longibrachiatum, Trichoderma viride, Trichoderma koningii, Trichoderma harzianum, Penicillium, Humicola, Humicola insolens, Humicola grisea, Chrysosporium, Chrysosporium lucknowense, Myceliophthora thermophila, Gliocladium, Aspergillus, Fusarium, Neurospora, Hypocrea, Emericella, Aspergillus niger, Aspergillus awamori, Aspergillus aculeatus,* and *Aspergillus nidulans.* Alternatively, the fungal host cell can be a yeast cell, e.g., *Saccharomyces cervisiae, Schizzosaccharomyces pombe, Schwanniomyces occidentalis, Kluveromyces lactus, Candida utilis, Candida albicans, Pichia*

stipitis, Pichia pastoris, Yarrowia lipolytica, Hansenula polymorpha, Phaffia rhodozyma, Arxula adeninivorans, Debaryomyces hansenii, or *Debaryomyces polymorphus.*

[208]   Aspects of the present invention include methods of producing a variant GH61 that includes culturing a host cell that contains a polynucleotide encoding the GH61 variant in a suitable culture medium under suitable conditions to express (or produce) the GH61 variant from the polynucleotide, e.g., where the polynucleotide encoding the GH61 variant is present in an expression vector (i.e., where the GH61 variant-encoding polynucleotide is operably linked to a promoter that drives expression of the GH61 variant in the host cell). In certain embodiments, the method further includes isolating the produced GH61 variant.

[209]   Aspects of the present invention also include compositions containing a GH61 variant as described herein. Examples of suitable compositions include, but are not limited to detergent compositions, feed additives, and compositions for treating (or hydrolyzing) a cellulosic substrate (e.g., a cellulose containing textile, e.g., denim; a cellulose containing biomass material, e.g., a mixture of lignocellulosic biomass material which has optionally been subject to pre-treatment of pre-hydrolysis processing, etc.). Compositions that include a GH61 variant as described herein and a cellulosic substrate represent further aspects of the present invention. GH61 variant-containing detergent compositions include laundry detergents and dish detergents, where such detergents may further include additional components, e.g., surfactants. Examples of suitable cellulosic substrates include, but are not limited to: grass, switch grass, cord grass, rye grass, reed canary grass, miscanthus, sugar-processing residues, sugarcane bagasse, agricultural wastes, rice straw, rice hulls, barley straw, corn cobs, cereal straw, wheat straw, canola straw, oat straw, oat hulls, corn fiber, stover, soybean stover, corn stover, forestry wastes, wood pulp, recycled wood pulp fiber, paper sludge, sawdust, hardwood, softwood, and combinations thereof.

[210]   Aspects of the present invention include methods for hydrolyzing a cellulosic substrate comprising contacting the substrate with a variant GH61 as described herein. In certain embodiments, the GH61 variant is provided as a cell-free composition, whereas in other embodiments, the GH61 variant is provided as a host cell composition in which the host cell expresses the GH61 variant. Thus, certain embodiments of the methods for hydrolyzing a cellulosic substrate contacting the substrate with a host cell containing a GH61 variant expression vector. In certain embodiments, the method is for converting a lignocellulosic biomass to glucose, where in some of these embodiments, the lignocellulosic biomass is selected, without limitation, from: grass, switch grass, cord grass, rye grass, reed canary grass, miscanthus, sugar-processing residues, sugarcane bagasse, agricultural wastes, rice straw, rice hulls, barley straw, corn cobs, cereal straw, wheat straw, canola straw, oat straw, oat hulls, corn fiber, stover, soybean stover, corn stover, forestry wastes, wood pulp, recycled wood pulp fiber, paper sludge, sawdust, hardwood, softwood, and combinations thereof. In certain other

embodiments, the cellulosic substrate is a cellulosic-containing textile, e.g., denim, where in some of these embodiments the method is for treating indigo dyed denim (e.g., in a stonewashing process).

[211]   Aspects of the present invention include cell culture supernatant compositions that contain a GH61 variant as described herein. For example, a cell culture supernatant obtained by culturing a host cell that contains a polynucleotide encoding the GH61 variant in a suitable culture medium under suitable conditions to express the GH61 variant from the polynucleotide and secrete the GH61 variant into the cell culture supernatant. Such a cell culture supernatant can include other proteins and/or enzymes produced by the host cell, including endogenously-and/or exogenously-expressed proteins and/or enzymes. Such supernatant of the culture medium can be used as is, with minimum or no post-production processing, which may typically include filtration to remove cell debris, cell-kill procedures, and/or ultrafiltration or other steps to enrich or concentrate the enzymes therein. Such supernatants are referred to herein as "whole broths" or "whole cellulase broths".

[212]   The GH61 variants can be produced by co-expression with one or more cellulases, and/or one or more hemicellulases. Alternatively, the GH61 variants can be produced without cellulases or hemicellulases. In the latter case, the GH61 variant optionally can be physically mixed with one or more cellulases and/or one or more hemicellulases to form an enzyme composition that is useful for a particular application, e.g., in hydrolyzing lignocellulosic biomass substrates. In a further embodiment the GH61 variants can be further co-expressed or physically mixed with one or more accessory enzymes. Known accessory enzymes include, for example, certain mannanases, which can sometimes be characterized as hemicellulases but more often are deemed accessory enzymes, galactanases, arabinases, ligninases, amylases, glucuronidases, proteases, esterases (e.g., ferulic acid esterases, acetyl xylan esterases, coumaric acid esterases, pectin methyl esterases), lipases, certain other GH61 family enzymes, xyloglucanases, CIP1, CIP1-like proteins, CIP2, CIP2-like proteins, swollenin, expansions, cellobiose hydrogenases, manganese peroxidases, and cellulose disrupting proteins, which may be, for example, cellulose binding modules.

[213]   Other compositions containing a desired variant GH61 enzyme, as well as methods for using such compositions, are also contemplated.

BRIEF DESCRIPTION OF THE DRAWINGS

[214]   Figure 1 shows the nucleic acid sequence (top line) (SEQ ID NO:1) and the amino acid sequence (bottom line) (SEQ ID NO:2) of the wild type GH61A (GH61A) from *H. jecorina*. The signal sequence in SEQ ID NO:2 is underlined.

[215]   Figure 2A, 2B and 2C show an amino acid sequence alignment (Uniprot) of the catalytic domains of glycosyl hydrolase homologous to *H. jecorina* GH61A. The catalytic domains of the following enzymes are aligned (SEQ ID NOs represent the sequence of the catalytic domains of

each enzyme): *Hypocrea jecorina* GH61A (SEQ ID NO:34), *Hypocrea rufa* EGIV (SEQ ID

NO:35), *Trichoderma saturnisporum* EGIV (SEQ ID NO:36), *Hypocrea orientalis* EGIV (SEQ ID

NO:37), *Trichoderma sp.* EGIV (SEQ ID NO:38), *Hypocrea atroviridis* GH61 (SEQ ID NO:39),

Hypocrea virens GH61 (SEQ ID NO:40), *Thielavia terrestris* GH61 (SEQ ID NO:41),

5   *Neurospora tetrasperma* EGIV (SEQ ID NO:42), *Neurospora tetrasperma* putative protein (SEQ

ID NO:43), *Thielavia heterothallica* GH61 (SEQ ID NO:44), *Neurospora crassa* EGIV (SEQ ID

NO:45), *Sordaria macrospora* putative protein (SEQ ID NO:46), *Gaeumannomyces graminis*

EGIV (SEQ ID NO:47), *Nectria haematococca* putative protein (SEQ ID NO:48), *Fusarium*

pseudograminearum putative protein (SEQ ID NO:49), and *Gibberella zeae* putative protein

10   (SEQ ID NO:50).

[216]   Figure 3 shows an amino acid sequence alignment (Uniprot) of the following

carbohydrate binding domains of the indicated glycosyl hydrolase enzymes: GH61A from

Hypocrea jecorina (SEQ ID NO:51), GH61 enzyme from *Hypocrea virens* (SEQ ID NO:52),

Glycosyl hydrolase family 28 enzyme from *Thielavia terrestris* (SEQ ID NO:53), Glycosyl

15   hydrolase family 45 enzyme from *Hypocrea atroviridis* (SEQ ID NO:54), putative

Endoglucanase from *Neosartorya fumigata* (SEQ ID NO:55), putative enzyme from *Aspergillus*

terreus (SEQ ID NO:56), Cip1 from *Hypocrea jecorina* (SEQ ID NO:57), Exoglucanase 1 from

Hypocrea rufa (SEQ ID NO:58), Glycosyl hydrolase family 7 enzyme from *Hypocrea virens*

(SEQ ID NO:59), Glycosyl hydrolase family 5 enzyme from *Hypocrea atroviridis* (SEQ ID

20   NO:60), Glycosyl hydrolase family 45 enzyme from *Neosartorya fischeri* (SEQ ID NO:61),

Exoglucanase 1 from *Trichoderma koningii* (SEQ ID NO:62), Glycosyl hydrolase family 61 from

Colletotrichum graminicola (SEQ ID NO:63), Glycosyl hydrolase family 61 from *Colletotrichum*

graminicola (SEQ ID NO:64), putative enzyme from *Arthrobotrys oligospora* (SEQ ID NO:65),

Cellobiohydrolase from *Trichoderma harzianum* (SEQ ID NO:66), and Endoglucanase from

25   *Penicillium sp.* (SEQ ID NO:67).

DETAILED DESCRIPTION

[217]   The invention will now be described in detail by way of reference only using the

following definitions and examples. All patents and publications, including all sequences

30   disclosed within such patents and publications, referred to herein are expressly incorporated by

reference.

[218]   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 belongs. Singleton, *et al.,* DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY,

35   3RD ED., John Wiley and Sons, Ltd., New York (2007), and Hale & Marham, THE HARPER

COLLINS DICTIONARY OF BIOLOGY, Harper Perennial, NY (1991) provide one of skill with a

general dictionary of many of the terms used in this invention. Although any methods and

materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. 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

5 to carboxy orientation. Practitioners are particularly directed to Green and Sambrook *Molecular Cloning: A Laboratory Manual* (Fourth Edition), *Cold Spring Harbor Laboratory Press* 2012, and Ausubel FM *et al.,* 1993, for definitions and terms of the art. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary.

10 **[219]**   The headings provided herein are not limitations of the various aspects or embodiments 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.

[220]   All publications cited herein are expressly incorporated herein by reference for the

15 purpose of describing and disclosing compositions and methodologies which might be used in connection with the invention.

I.   DEFINITIONS

[221]   The term "amino acid sequence" is synonymous with the terms "polypeptide," "protein," and "peptide," and are used interchangeably. Where such amino acid sequences exhibit

20 activity, they may be referred to as an "enzyme." The conventional one-letter or three-letter codes for amino acid residues are used, with amino acid sequences being presented in the standard amino-to-carboxy terminal orientation (*i.e.*, N→C).

[222]   The term "nucleic acid" encompasses DNA, RNA, heteroduplexes, and synthetic molecules capable of encoding a polypeptide. Nucleic acids may be single stranded or double

25 stranded, and may have chemical modifications. The terms "nucleic acid" and "polynucleotide" are used interchangeably. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and the present compositions and methods encompass nucleotide sequences that encode a particular amino acid sequence. As such, the present invention contemplates every possible variant nucleotide sequence encoding GH61 or

30 a variant thereof, all of which are possible given the degeneracy of the genetic code. Unless otherwise indicated, nucleic acid sequences are presented in 5'-to-3' orientation.

[223]   "Cellulase" or "cellulase enzyme" means bacterial or fungal exoglucanases or exocellobiohydrolases, and/or endoglucanases, and/or β-glucosidases. These three different types of cellulase enzymes are known to act synergistically to convert cellulose and its

35 derivatives to glucose.

[224]   "Endoglucanase" or "EG" or "EG enzyme" or "EG polypeptide," as used herein is defined as an endo-1,4-β-D-glucanase which catalyzes the endohydrolysis of 1,4 β-D-

glucosidic linkages in cellulose, lichenin and cereal β-D-glucans. In cellulose hydrolysis, this activity generates new chain ends that are substrates for CBH action. EGs will also hydrolyze 1,4-linkages in β-D-glucans that also contain 1,3-linkages. Certain EGs have been shown to act "processively" on crystalline cellulose [see, e.g., Wilson, D.B. *Three microbial strategies for plant cell wall degradation*. Ann. N. Y. Acad. Sci. 2008, 1125, 289–297; and Li, Y, et al. *Increased crystalline cellulose activity via combinations of amino acid changes in the family 9 catalytic domain and family 3c cellulose binding module of Thermobifida fusca Cel9A*. Appl. Environ. Microbiol. 2010, 76, 2582–2588].

[225] By "GH61" or "GH61 enzyme" and the like is meant an enzyme that belongs to the glycosyl hydrolase 61 family, e.g., the glycosyl hydrolase 61a (GH61A) family. The GH61 enzyme can be from a fungal cell, including filamentous fungus of the subdivision Eumycota or Oomycota. The filamentous fungi are characterized by vegetative mycelium having a cell wall composed of chitin, glucan, chitosan, mannan, and other complex polysaccharides, with vegetative growth by hyphal elongation and carbon catabolism that is obligately aerobic. A filamentous fungal parent cell may be a cell of a species of, but not limited to, *Trichoderma, e.g., Trichoderma longibrachiatum, Trichoderma viride, Trichoderma koningii, Trichoderma harzianum; Penicillium sp.; Humicola sp.,* including *Humicola insolens* and *Humicola grisea; Chrysosporium sp.,* including *C. lucknowense; Myceliophthora sp.; Gliocladium sp.; Aspergillus sp.; Fusarium sp., Neurospora sp., Hypocrea sp., e.g., Hypocrea jecorina, and Emericella sp.* As used herein, the term "*Trichoderma*" or "*Trichoderma sp.*" refers to any fungal strains which have previously been classified as *Trichoderma* or are currently classified as *Trichoderma.* In certain embodiments, a GH61 enzyme can be from a non-filamentous fungal cell. Examples of GH61A enzymes include those found in *Hypocrea jecorina (Trichoderma reesei), Hypocrea rufa, Hypocrea orientalis, Hypocrea atroviridis, Hypocrea virens, Emericella nidulans, Aspergillus terreus, Aspergillus oryzae, Aspergillus niger, Aspergillus kawachii, Aspergillus flavus, Aspergillus clavatus, Gaeumannomyces graminis, Trichoderma saturnisporum, Neurospora tetrasperma, Neurospora crassa, Neosartorya fumigate, Neosartorya fumigate, Neosartorya fischeri, Thielavia terrestris,* and *Thielavia heterothallica.* In certain aspects, a GH61 enzyme comprises the amino acid sequence of any one of the mature GH61 enzyme sequences shown in SEQ ID NOs: 3, 72, 73, 74, 77, an amino acid sequence having at least 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereto, an allelic variant thereof, or a fragment thereof that has cellulase augmenting activity. In certain embodiments, a GH61A enzyme has cellulase augmenting activity and contains an amino acid sequence that is at least 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO:3, or a fragment or derivative thereof having cellulase augmenting activity.

[226] By "GH61 activity" or "GH61A activity" or "activity" when in reference to a GH61 enzyme is meant the cellulase augmenting activity that is characteristic of a GH61 family member. Specifically, GH61 enzymes demonstrate an improved capacity, when combined with certain cellulases, to augment the efficacy and efficiency of hydrolyzing lignocellulosic biomass

5   substrates, e.g., to generate monosaccharides, disaccharides, and polysaccharides.

[227] A "variant" of an enzyme, protein, polypeptide, nucleic acid, or polynucleotide as used herein means that the variant is derived from a parent polypeptide or parent nucleic acid (*e.g.,* native, wildtype or other defined parent polypeptide or nucleic acid) that includes at least one modification or alteration as compared to that parent, where such modification or alteration is

10   produced by human intervention. Thus, a variant may have a few mutations as compared to a parent, where by "a few" is meant from 1 to 10 mutations. For example, a variant having from 1 to 10 amino acid substitutions as compared to SEQ ID NO:3 can be referred to as a GH61 variant having a few substitutions. Alterations/modifications can include a substitution of an amino acid/nucleic acid residue in the parent for a different amino acid/nucleic acid residue at

15   one or more sites, deletion of an amino acid/nucleic acid residue (or a series of amino acid/nucleic acid residues) in the parent at one or more sites, insertion of an amino acid/nucleic acid residue (or a series of amino acid/nucleic acid residues) in the parent at one or more sites, truncation of amino- and/or carboxy-terminal amino acid sequences or 5' and or 3' nucleic acid sequences, and any combination thereof. A variant GH61 enzyme (sometimes referred to as a

20   "GH61 variant" or a "GH61A variant") according to aspects of the invention retains cellulase augmenting activity but may have an altered property in some specific aspect, e.g., an improved property. For example, a variant GH61 enzyme may have an altered pH optimum, improved thermostability or oxidative stability, or a combination thereof, but will retain its characteristic cellulase augmenting activity. In certain embodiments, the variant GH61 enzyme

25   is a variant of a GH61A enzyme as defined above and which has cellulase augmenting activity. In some aspects of the invention, a variant GH61A enzyme contains an amino acid sequence that is at least 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO:3, or an enzymatically active fragment thereof.

30   [228] "Combinatorial variants" are variants comprising two or more mutations, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more, substitutions, deletions, and/or insertions.

[229] A "parent" or "parental" polynucleotide, polypeptide, or enzyme sequence (e.g., a "parent GH61 enzyme"), or equivalents thereto, as used herein refers to a polynucleotide,

35   polypeptide, or enzyme sequence that was used as a starting point or template for designing a variant polynucleotide, polypeptide, or enzyme. In certain embodiments, the parent enzyme is

a GH61A enzyme as described above (e.g., SEQ ID NO:3). It is further noted that the words "parent" and "parental" are used interchangeably in this context.

[230]   The term "wild-type" refers to a naturally-occurring polypeptide or polynucleotide sequence, i.e., one that does not include a man-made variation. In some cases, a wild-type sequence is used as the parental sequence.

[231]   The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not normally found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences, e.g., from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. Similarly, a heterologous polypeptide will often refer to two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion polypeptide).

[232]   The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, polypeptide, or vector, indicates that the cell, nucleic acid, polypeptide or vector, has been modified by the introduction of a heterologous nucleic acid or polypeptide or the alteration of a native nucleic acid or polypeptide, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[233]   The terms "isolated" or "purified" as used herein refer to a component that is removed from the environment in which it is naturally produced. In general, in an isolated or purified nucleic acid or polypeptide sample, the nucleic acid(s) or polypeptide(s) of interest are present at an increased absolute or relative concentration as compared to the environment in which they are naturally produced.

[234]   The term "enriched" when describing a component or material in a composition (e.g., a polypeptide or polynucleotide) means that the component or material is present at a relatively increased concentration in that composition as compared to the starting composition from which the enriched composition was generated. For example, an enriched GH61 composition (or sample) is one in which the relative or absolute concentration of GH61 is increased as compared to the initial fermentation product from the host organism.

[235]   As used herein, the terms "promoter" refers to a nucleic acid sequence that functions to direct transcription of a downstream gene. The promoter will generally be appropriate to the host cell in which the target gene is being expressed. The promoter, together with other transcriptional and translational regulatory nucleic acid sequences (also termed "control sequences"), are necessary to express a given gene. In general, the transcriptional and translational regulatory sequences include, but are not limited to, promoter sequences,

ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation. An example of an

5   inducible promoter useful in the present invention is the *T. reesei* (*H. jecorina*) *cbh1* promoter which is deposited in GenBank under Accession Number D86235. In another aspect the promoter is a *cbh II* or xylanase promoter from *H. jecorina*. Examples of suitable promoters include the promoter from the *A. awamori* or *A. niger* glucoamylase genes (Nunberg, J. H. et al. (1984) Mol. Cell. Biol. 4, 2306-2315; Boel, E. et al. (1984) EMBO J. 3, 1581-1585), the *Mucor*

10   *miehei* carboxyl protease gene, the *H. jecorina* cellobiohydrolase I gene (Shoemaker, S. P. et al. (1984) European Patent Application No. EP0137280A1), the *A. nidulans* trpC gene (Yelton, M. et al. (1984) Proc. Natl. Acad. Sci. USA 81, 1470-1474; Mullaney, E. J. et al. (1985) Mol. Gen. Genet. 199, 37-45) the *A. nidulans* alcA gene (Lockington, R. A. et al. (1986) Gene 33, 137-149), the *A. nidulans* tpiA gene (McKnight, G. L. et al. (1986) Cell 46, 143-147), the *A.*

15   *nidulans* amdS gene (Hynes, M. J. et al. (1983) Mol. Cell Biol. 3, 1430-1439), the *H. jecorina* xln1 gene, the *H. jecorina* cbh2 gene, the *H. jecorina* eg1 gene, the *H. jecorina* eg2 gene, the *H. jecorina* eg3 gene, and higher eukaryotic promoters such as the SV40 early promoter (Barclay, S. L. and E. Meller (1983) Molecular and Cellular Biology 3, 2117-2130).

[236]   A nucleic acid is "operably linked" when it is placed into a functional relationship with

20   another nucleic acid sequence. For example, DNA encoding a secretory leader, i.e., a signal peptide, is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally,

25   "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice. Thus, the term "operably linked" refers to a functional linkage between a

30   nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

[237]   The term "signal sequence", "signal peptide", "secretory sequence", "secretory peptide", "secretory signal sequence", "secretory signal peptide" and the like denotes a peptide

35   sequence that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized, as well as nucleic acids encoding such peptides. In general, the larger polypeptide (or protein) is commonly cleaved to remove the

secretory/signal peptide during transit through the secretory pathway, where the cleaved form of the polypeptide (i.e., the form without the signal/secretory peptide) is often referred to herein as the "mature form" of the polypeptide. For example, SEQ ID NO:2 provides the amino acid sequence of GH61A from *H. jecorina* with the signal peptide while SEQ ID NO:3 provides the

5   amino acid sequence of the mature form of GH61A from *H. jecorina*, i.e., without the signal peptide.

[238]   As used herein, the term "vector" refers to a nucleic acid construct designed for transfer between different host cells. An "expression vector" refers to a vector that has the ability to incorporate and express heterologous DNA fragments in a foreign cell. Many prokaryotic and

10   eukaryotic expression vectors are commercially available. Selection of appropriate expression vectors is within the knowledge of those having skill in the art.

[239]   Accordingly, an "expression cassette" or "expression vector" is a nucleic acid construct generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a target cell. The recombinant expression

15   cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid sequence to be transcribed and a promoter.

[240]   As used herein, the term "plasmid" refers to a circular double-stranded (ds) DNA

20   construct that forms an extrachromosomal self-replicating genetic element when present in many bacteria and some eukaryotes. Plasmids may be employed for any of a number of different purposes, e.g., as cloning vectors, propagation vectors, expression vectors, etc.

[241]   As used herein, the term "selectable marker" refers to a nucleotide sequence or polypeptide encoded thereby which is capable of expression in cells and where expression of

25   the selectable marker in cells confers the ability to be differentiated from cells that do not express the selectable marker. In certain embodiments, a selectable marker allows a cell expressing it to grow in the presence of a corresponding selective agent, or under corresponding selective growth conditions. In other embodiments, a selectable marker allows a cell expressing it to be identified and/or isolated from cells that do not express it by virtue of a

30   physical characteristic, e.g., by differences in fluorescence, immuno-reactivity, etc.

[242]   In general, nucleic acid molecules which encode the variant GH61A will hybridize, under moderate to high stringency conditions to the wild type sequence (or its complement) provided herein as SEQ ID NO:1 (native *H. jecorina* GH61A). However, in some cases a GH61A-encoding nucleotide sequence is employed that possesses a substantially different codon

35   usage, while the enzyme encoded by the GH61A-encoding nucleotide sequence has the same or substantially the same amino acid sequence as the native enzyme. For example, the coding sequence may be modified to facilitate faster expression of GH61A in a particular prokaryotic or

eukaryotic expression system, in accordance with the frequency with which a particular codon is utilized by the host (commonly referred to as "codon optimization"). Te'o, *et al.* (FEMS Microbiology Letters 190:13-19, 2000), for example, describes the optimization of genes for expression in filamentous fungi. Such nucleic acid sequences are sometimes referred to as

5   "degenerate" or "degenerated sequences".

[243]   A nucleic acid sequence is considered to be "selectively hybridizable" to a reference nucleic acid sequence if the two sequences specifically hybridize to one another under moderate to high stringency hybridization and wash conditions. Hybridization conditions are based on the melting temperature (Tm) of the nucleic acid binding complex or probe. For

10   example, "maximum stringency" typically occurs at about Tm-5°C (5° below the Tm of the probe); "high stringency" at about 5-10° below the Tm; "moderate" or "intermediate stringency" at about 10-20° below the Tm of the probe; and "low stringency" at about 20-25° below the Tm. Functionally, maximum stringency conditions may be used to identify sequences having strict identity or near-strict identity with the hybridization probe; while high stringency conditions are

15   used to identify sequences having about 80% or more sequence identity with the probe.

[244]   Moderate and high stringency hybridization conditions are well known in the art (see, for example, Sambrook, *et al,* 1989, Chapters 9 and 11, and in Ausubel, F.M., *et al.,* 1993, expressly incorporated by reference herein). An example of high stringency conditions includes hybridization at about 42°C in 50% formamide, 5X SSC, 5X Denhardt's solution, 0.5% SDS and

20   100 µg/ml denatured carrier DNA followed by washing two times in 2X SSC and 0.5% SDS at room temperature and two additional times in 0.1X SSC and 0.5% SDS at 42°C.

[245]   As used herein, the terms "transformed", "stably transformed" or "transgenic" with reference to a cell means the cell has a non-native (heterologous) nucleic acid sequence integrated into its genome or as an episomal plasmid that is maintained through multiple

25   generations.

[246]   As used herein, the term "expression" refers to the process by which a polypeptide is produced based on the nucleic acid sequence of a gene. The process generally includes both transcription and translation.

[247]   The term "introduced" in the context of inserting a nucleic acid sequence into a cell,

30   means "transfection", or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid sequence into a eukaryotic or prokaryotic cell where the nucleic acid sequence may be incorporated into the genome of the cell (for example, chromosome, plasmid, plastid, or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (for example, transfected mRNA).

35   [248]   It follows that the term "desired glycosyl hydrolase expression" or equivalents refers to transcription and translation of the desired glycosyl hydrolase gene, the products of which include precursor RNA, mRNA, polypeptide, post-translationally processed polypeptides. By

way of example, assays for GH61A expression include Western blot for GH61A enzyme, Northern blot analysis and reverse transcriptase polymerase chain reaction (RT-PCR) assays for GH61A mRNA, and cellulase augmenting activity assays, for example augmentation of assays as described in Shoemaker S.P. and Brown R.D.Jr. (Biochim. Biophys. Acta, 1978, 5   523:133-146) and Schulein (1988).

[249] By the term "host cell" is meant a cell that contains a vector and supports the replication, and/or transcription and/or transcription and translation (expression) of the expression construct. Host cells for use in the present invention can be prokaryotic cells, such as *E. coli*, or eukaryotic cells such as yeast, plant, insect, amphibian, or mammalian cells. In certain 10   embodiments, host cells are filamentous fungi.

[250] As used herein, the term "detergent composition" refers to a mixture which is intended for use in a wash medium for the laundering of soiled cellulose containing fabrics. In the context of the present invention, such compositions may include, in addition to cellulases and surfactants, additional hydrolytic enzymes, builders, bleaching agents, bleach activators, bluing 15   agents and fluorescent dyes, caking inhibitors, masking agents, cellulase activators, antioxidants, and solubilizers.

[251] As used herein, the term "surfactant" refers to any compound generally recognized in the art as having surface active qualities. Thus, for example, surfactants comprise anionic, cationic and nonionic surfactants such as those commonly found in detergents. Anionic 20   surfactants include linear or branched alkylbenzenesulfonates; alkyl or alkenyl ether sulfates having linear or branched alkyl groups or alkenyl groups; alkyl or alkenyl sulfates; olefinsulfonates; and alkanesulfonates. Ampholytic surfactants include quaternary ammonium salt sulfonates, and betaine-type ampholytic surfactants. Such ampholytic surfactants have both the positive and negative charged groups in the same molecule. Nonionic surfactants may 25   comprise polyoxyalkylene ethers, as well as higher fatty acid alkanolamides or alkylene oxide adduct thereof, fatty acid glycerine monoesters, and the like.

[252] As used herein, the term "cellulose containing fabric" refers to any sewn or unsewn fabrics, yarns or fibers made of cotton or non-cotton containing cellulose or cotton or non-cotton containing cellulose blends including natural cellulosics and manmade cellulosics (such as jute, 30   flax, ramie, rayon, and lyocell).

[253] As used herein, the term "cotton-containing fabric" refers to sewn or unsewn fabrics, yarns or fibers made of pure cotton or cotton blends including cotton woven fabrics, cotton knits, cotton denims, cotton yarns, raw cotton and the like.

[254] As used herein, the term "stonewashing composition" refers to a formulation for use in 35   stonewashing cellulose containing fabrics. Stonewashing compositions are used to modify cellulose containing fabrics prior to sale, *i.e.*, during the manufacturing process. In contrast,

detergent compositions are intended for the cleaning of soiled garments and are not used during the manufacturing process.

[255]   When an amino acid position (or residue) in a first polypeptide is noted as being "equivalent" to an amino acid position in a second, related polypeptide, it means that the amino acid position of the first polypeptide corresponds to the position noted in the second, related polypeptide by one or more of (i) primary sequence alignment (see description of sequence alignment and sequence identity below); (ii) structural sequence homology; or (iii) analogous functional property. Thus, an amino acid position in a first GH61 enzyme (or a variant thereof) can be identified as "equivalent" (or "homologous") to an amino acid position in a second GH61 enzyme (or even multiple different GH61 enzymes).

[256]   *Primary sequence alignment:* Equivalent amino acid positions can be determined using primary amino acid sequence alignment methodologies, many of which are known in the art. For example, by aligning the primary amino acid sequences of two or more different GH61 enzymes, it is possible to designate an amino acid position number from one GH61 enzyme as equivalent to the position number of another one of the aligned GH61 enzymes. In this manner, the numbering system originating from the amino acid sequence of one GH61 enzyme (e.g., the GH61A enzyme denoted in SEQ ID NO: 3) can be used to identify equivalent (or homologous) amino acid residues in other GH61 enzymes. See, e.g., the alignments shown in Figures 2 and 3.

[257]   *Structural sequence homology:* In addition to determining "equivalent" amino acid positions using primary sequence alignment methodologies, "equivalent" amino acid positions may also be defined by determining homology at the level of secondary and/or tertiary structure. For example, for a glycosyl hydrolase whose tertiary structure has been determined by x-ray crystallography, equivalent residues can be defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of the glycosyl hydrolase are within 0.13 nm and preferably 0.1 nm after alignment with *H. jecorina* GH61A (N on N, CA on CA, C on C, and O on O). Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the glycosyl hydrolase in question to *H. jecorina* GH61A. The best model is the crystallographic model that gives the highest resolution available. Where two or more different models have equal resolution, the model with the lowest R factor for experimental diffraction data, using the equation below, is used.

$$R\, factor \;=\; \frac{\Sigma_h |Fo(h)| - |Fc(h)|}{\Sigma_h |Fo(h)|}$$

[258]   *Analogous functional property:* Equivalent amino acid residues in a first polypeptide which are functionally analogous to a specific residue of a second related polypeptide (e.g., a

first glycosyl hydrolase and *H. jecorina* GH61A) are defined as those amino acids in the first polypeptide that adopt a conformation such that they alter, modify, or contribute to polypeptide structure, substrate binding, or catalysis in a manner defined and attributed to a specific residue of the second related polypeptide (e.g., *H. jecorina* GH61A). When a tertiary structure has

5   been obtained by x-ray crystallography for the first polypeptide,   amino acid residues of the first polypeptide that are functionally analogous to the second polypeptide occupy an analogous position to the extent that, although the main chain atoms of the given residue may not satisfy the criteria of equivalence on the basis of occupying a homologous position, the atomic coordinates of at least two of the side chain atoms of the residue lie within 0.13 nm of the

10   corresponding side chain atoms of the second polypeptide (e.g., *H. jecorina* GH61A).

[259]   The term "improved" or "improved property" or "improved performance" and the like with respect to a variant enzyme (e.g., a GH61 variant) is defined herein as a characteristic or activity associated with a variant enzyme which is improved as compared to its respective parent enzyme. Improved properties include, but are not limited to, improved production from or

15   expression in a host cell (sometimes referred to as yield), improved thermostability or altered temperature-dependent activity profile, improved activity or stability at a desired pH or pH range, improved substrate specificity, improved product specificity, and improved stability in the presence of a chemical or other component in a cellulosic hydrolysis process step, etc. Improved performance may be determined using a particular assay(s) including, but not limited

20   to: (a) expression (Protein Content Determination, or yield), (b) Thermostability and/or melting temperature (Tm), (c) Whole Hydrolysate Dilute Acid Pretreated Corn Stover (whPCS) Hydrolysis Assay, and (d) Dilute Ammonia Pretreated Corn Stover (daCS) Hydrolysis Assay.

[260]   The term "improved thermostability" with respect to a variant polypeptide (e.g., a GH61 variant) is defined herein as a variant enzyme displaying retention of enzymatic activity (or in

25   the specific case of a GH61 enzyme, the retention of an enzyme's capability to augment cellulase activities) after a period of incubation at an elevated temperature relative to the parent enzyme. Such a variant may or may not display an altered thermal activity profile relative to the parent. For example, a variant may have an improved ability to refold following incubation at elevated temperature relative to the parent.

30   [261]   By "improved product specificity" is meant a variant enzyme displaying an altered product profile as compared to the parent enzyme, where the altered product profile of the variant is improved in a given application as compared to the parent. A "product profile" is defined herein as the chemical composition of the reaction products produced by the enzyme of interest.

35   [262]   By "improved chemical stability" is meant that a variant enzyme displays retention of enzymatic activity after a period of incubation in the presence of a chemical or chemicals that reduce the enzymatic activity of the parent enzyme under the same conditions. Variants with

improved chemical stability are better able to catalyze a reaction in the presence of such chemicals as compared to the parent enzyme.

[263] A "pH range," with reference to an enzyme, refers to the range of pH values under which the enzyme exhibits catalytic activity.

5   [264] The terms "pH stable" and "pH stability," with reference to an enzyme, relate to the ability of the enzyme to retain activity over a wide range of pH values for a predetermined period of time (*e.g.*, 15 min., 30 min., 1 hr.).

[265] "Percent sequence identity" or grammatical equivalents means that a particular sequence has at least a certain percentage of amino acid residues identical to those in a

10   specified reference sequence using an alignment algorithm. An example of an algorithm that is suitable for determining sequence similarity is the BLAST algorithm, which is described in 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 (<www.ncbi.nlm.nih.gov>). This algorithm involves first identifying high scoring sequence pairs

15   (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.

20   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 word length (W) of 11, the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA*

25   **89**:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M'5, N'-4, and a comparison of both strands.

[266] The BLAST algorithm then performs a statistical analysis of the similarity between two sequences (*see, e.g.,* Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* **90**:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability

30   (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, an amino acid sequence is considered similar to a protease if the smallest sum probability in a comparison of the test amino acid sequence to a protease amino acid sequence is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

35   [267] When questions of percent sequence identity arise, alignment using the CLUSTAL W algorithm with default parameters will govern. *See* Thompson *et al.* (1994) *Nucleic Acids Res.* 22:4673-4680. Default parameters for the CLUSTAL W algorithm are:

Gap opening penalty:	10.0
Gap extension penalty:	0.05
Protein weight matrix:	BLOSUM series
DNA weight matrix:	IUB
Delay divergent sequences %:	40
Gap separation distance:	8
DNA transitions weight:	0.50
List hydrophilic residues:	GPSNDQEKR
Use negative matrix:	OFF
Toggle Residue specific penalties:	ON
Toggle hydrophilic penalties:	ON
Toggle end gap separation penalty	OFF.

II.   MOLECULAR BIOLOGY

[268]   Embodiments of the subject invention provide for the expression of a desired glycosyl hydrolase enzyme (or combination of glycosyl hydrolase enzymes) from glycosyl hydrolase-encoding nucleic acids under control of a promoter functional in a host cell of interest, e.g., a filamentous fungus. Therefore, this invention relies on a number of routine techniques in the field of recombinant genetics. Basic texts disclosing examples of suitable recombinant genetics methods are noted above.

[269]   Any method known in the art that can introduce mutations into a parent nucleic acid/polypeptide is contemplated by the present invention.

[270]   The present invention relates to the expression, purification and/or isolation and use of variant GH61 enzymes, e.g., GH61A enzymes. These enzymes may be prepared by recombinant methods utilizing any of a number of gh61 genes encoding the GH61 enzymes known in the art, including the GH61A/GH61 enzymes in SEQ ID NOs:2 to 11, 13, 14, and 16, e.g., GH61A from H. jecorina. Any convenient method for introducing mutations may be employed, including site directed mutagenesis. As indicated above, mutations (or variations) include substitutions, additions, deletions or truncations that will correspond to one or more amino acid changes in the expressed GH61 variant. Again, site directed mutagenesis and other methods of incorporating amino acid changes in expressed proteins at the DNA level can be found in numerous references, e.g., Green and Sambrook, et al. 2012 and Ausubel, et al.

[271]   DNA encoding an amino acid sequence variant of a parent GH61 is prepared by a variety of methods known in the art. These methods include, but are not limited to, preparation by site-directed (or oligonucleotide-mediated) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared DNA encoding the parent GH61A enzyme.

[272] Site-directed mutagenesis is one method that can be employed in preparing substitution variants. This technique is well known in the art (see, e.g.,Carter et al. Nucleic Acids Res. 13:4431-4443 (1985) and Kunkel et al., Proc. Natl. Acad.Sci.USA 82:488 (1987)). Briefly, in carrying out site-directed mutagenesis of DNA, the starting DNA is altered by first hybridizing an oligonucleotide encoding the desired mutation to a single strand of such starting DNA. After hybridization, a DNA polymerase is used to synthesize an entire second strand, using the hybridized oligonucleotide as a primer, and using the single strand of the starting DNA as a template. Thus, the oligonucleotide encoding the desired mutation is incorporated in the resulting double-stranded DNA.

[273] PCR mutagenesis is also suitable for making amino acid sequence variants of the parent GH61. See Higuchi, in PCR Protocols, pp.177-183 (Academic Press, 1990); and Vallette et al., Nuc. Acids Res. 17:723-733 (1989). Briefly, when small amounts of template DNA are used as starting material in a PCR, primers that differ slightly in sequence from the corresponding region in a template DNA can be used to generate relatively large quantities of a specific DNA fragment that differs from the template sequence only at the positions where the primers differ from the template.

[274] Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells et al., Gene 34:315-323 (1985). The starting material is the plasmid (or other vector) comprising the starting polypeptide DNA to be mutated. The codon(s) in the starting DNA to be mutated are identified. There must be a unique restriction endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at appropriate locations in the starting polypeptide DNA. The plasmid DNA is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures, wherein the two strands of the oligonucleotide are synthesized separately and then hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 5' and 3' ends that are compatible with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated DNA sequence.

[275] Alternatively, or additionally, the desired amino acid sequence of a desired GH61 variant can be determined, and a nucleic acid sequence encoding such GH61 variant can be generated synthetically.

[276] The desired GH61 so prepared may be subjected to further modifications, oftentimes depending on the intended use. Such modifications may involve further alteration of the amino acid sequence, fusion to heterologous polypeptide(s) and/or covalent modifications.

III. VARIANT GH61 POLYPEPTIDES AND NUCLEIC ACIDS ENCODING SAME

[277]   In one aspect, variant GH61 enzymes are provided. In certain embodiments, variant GH61 enzymes have one or more mutations, as set forth herein, with respect to a parent GH61 enzyme and further have at least 60% (i.e., 60% or greater but less than 100%) amino acid sequence identity to the mature form of *H. jecorina* GH61A (SEQ ID NO:3), including at least 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, up to and including 99.6% amino acid sequence identity to SEQ ID NO:3. In certain embodiments, the parent GH61 is a fungal GH61A (as defined above). Further, the variant GH61 enzyme has cellulase augmenting activity, where in certain embodiments, the variant GH61 has an improved property as compared to the parent GH61 (as detailed herein). The amino acid sequence for the wild type, full-length form of *H. jecorina* GH61A is shown in Figure 1 (SEQ ID NO:2). Sequence alignments of the catalytic domains and carbohydrate binding domains of *H. jecorina* GH61A with numerous other glycosyl hydrolases are shown in Figures 2 and 3, respectively.

[278]   In certain embodiments, a variant GH61 enzyme comprises an amino acid mutation at one or more amino acid positions in the mature form of GH61A from *H. jecorina* (SEQ ID NO:3). Because certain parent GH61 enzymes according to aspects of the invention may not have the same amino acid as wild type GH61A from *H. jecorina,* amino acid positions corresponding to the residues noted above (e.g., amino acid position W13) may also be designated either by the position number alone (e.g., amino acid position 13, as denoted in Table 4) or with an "X" prefix (e.g., amino acid position X13).   It is noted here that all three ways of designating the amino acid positions corresponding to a specific amino acid residue in GH61A from *H. jecorina* are interchangeable. In some instances, the word "position" may be left our (e.g., amino acid 13, amino acid W13, or amino acid X13).

[279]   The amino acid sequence of the GH61 variant differs from the parent GH61 amino acid sequence by the substitution, deletion or insertion of one or more amino acids of the parent amino acid sequence. A residue (amino acid) of a GH61 variant is equivalent to a residue of *H. jecorina* GH61A if it is either homologous (*i.e.*, corresponding in position in either primary or tertiary structure) or is functionally analogous to a specific residue or portion of that residue in *H. jecorina* GH61A (*i.e.,* having the same or similar functional capacity to combine, react, or interact chemically or structurally). As used herein, numbering is intended to correspond to that of the mature GH61A amino acid sequence as illustrated in Figure 1.

[280]   Alignment of amino acid sequences to determine homology can be determined by using a "sequence comparison algorithm." Optimal alignment of sequences for comparison can be conducted, *e.g.,* by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* **2**:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* **48**:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l Acad. Sci. USA*

85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), by visual inspection or MOE by Chemical Computing Group, Montreal Canada. See also the description of "percent sequence identity" provided in the Definitions section above.

[281] In certain embodiments, the mutation(s) in a variant GH61 enzyme is an amino acid substitution shown in Table 4 (see Example 3) and/or that are present in any of the SEL Cohorts (1, 2, 3, and Wild-type), Combinatorial Cohorts (1 to 11), or Deconvoluted Cohorts (1 to 4), where the sites of the substitutions correspond to the mature form of GH61A from *H. jecorina* (SEQ ID NO:3). All possible combinations of the substitutions shown in Table 4 and/or the Cohorts noted above are contemplated embodiments of the invention, including but not limited to the following:

[282] 1.   GH61 variant having at least one amino acid substitution at a position selected from the group consisting of: D1, H2, S3, I4, N5, D6, I7, V8, I9, N10, V12, W13, Q15, A16, P19, T20, T21, F22, P23, Y24, E25, S26, N27, P28, P29, V32, G33, T35, A36, A37, D38, L39, N41, F43, V44, D47, A48, Y49, Q50, N51, P52, I54, K58, N59, A60, T61, N62, K64, S68, V69, K70, A71, L76, V80, P83, P89, I90, V91, N96, N98, T103, V104, D105, K106, T107, T108, E110, F112, K113, I114, G116, V117, L119, L120, S121, G122, G123, D124, P125, G126, T127, A129, V132, S135, V141, I144, P145, D146, N147, A149, G151, Y153, V154, E158, I160, A161, H163, S164, G166, C167, A168, A171, N173, P175, I180, A181, S183, S185, S187, L188, Q189, S191, L194, G195, T196, D197, A201, T202, P204, V206, L207, I208, N209, Y211, T212, S213, P214, N216, Y217, I218, I219, P222, T223, V224, S226, T230, S231, V232, A233, G235, T287, L288, T289, L290, Y291, G292, Q293, C294, G295, G296, S297, G298, Y299, G301, P302, T303, R304, C305, A306, P307, P308, A309, T310, C311, S312, T313, N315, P316, Y317, Y318, A319, Q320, C321, L322, and N323.

[283] 2.   GH61 variant of 1 above having a D1L substitution.

[284] 3.   GH61 variant of 1 above having a D1P substitution.

[285] 4.   GH61 variant of 1 above having a D1S substitution.

[286] 5.   GH61 variant of any one of 1 to 4 above having an H2A substitution.

[287] 6.   GH61 variant of any one of 1 to 4 above having an H2D substitution.

[288] 7.   GH61 variant of any one of 1 to 4 above having an H2Q substitution.

[289] 8.   GH61 variant of any one of 1 to 4 above having an H2R substitution.

[290] 9.   GH61 variant of any one of 1 to 4 above having an H2S substitution.

[291] 10.   GH61 variant of any one of 1 to 9 above having an S3F substitution.

[292] 11.   GH61 variant of any one of 1 to 9 above having an S3L substitution.

[293] 12.   GH61 variant of any one of 1 to 9 above having an S3N substitution.

[294] 13.   GH61 variant of any one of 1 to 9 above having an S3Q substitution.

[295]   14.   GH61 variant of any one of 1 to 9 above having an S3T substitution.

[296]   15.   GH61 variant of any one of 1 to 14 above having an I4T substitution.

[297]   16.   GH61 variant of any one of 1 to 14 above having an I4V substitution.

[298]   17.   GH61 variant of any one of 1 to 16 above having an N5A substitution.

[299]   18.   GH61 variant of any one of 1 to 16 above having an N5D substitution.

[300]   19.   GH61 variant of any one of 1 to 16 above having an N5E substitution.

[301]   20.   GH61 variant of any one of 1 to 16 above having an N5G substitution.

[302]   21.   GH61 variant of any one of 1 to 16 above having an N5R substitution.

[303]   22.   GH61 variant of any one of 1 to 16 above having an N5S substitution.

[304]   23.   GH61 variant of any one of 1 to 22 above having a D6S substitution.

[305]   24.   GH61 variant of any one of 1 to 23 above having an I7L substitution.

[306]   25.   GH61 variant of any one of 1 to 23 above having an I7M substitution.

[307]   26.   GH61 variant of any one of 1 to 23 above having an I7V substitution.

[308]   27.   GH61 variant of any one of 1 to 26 above having an V8I substitution.

[309]   28.   GH61 variant of any one of 1 to 27 above having an I9A substitution.

[310]   29.   GH61 variant of any one of 1 to 27 above having an I9V substitution.

[311]   30.   GH61 variant of any one of 1 to 29 above having an N10D substitution.

[312]   31.   GH61 variant of any one of 1 to 29 above having an N10R substitution.

[313]   32.   GH61 variant of any one of 1 to 31 above having a V12M substitution.

[314]   33.   GH61 variant of any one of 1 to 31 above having a V12T substitution.

[315]   34.   GH61 variant of any one of 1 to 33 above having a W13A substitution.

[316]   35.   GH61 variant of any one of 1 to 33 above having a W13E substitution.

[317]   36.   GH61 variant of any one of 1 to 33 above having a W13F substitution.

[318]   37.   GH61 variant of any one of 1 to 33 above having a W13G substitution.

[319]   38.   GH61 variant of any one of 1 to 33 above having a W13I substitution.

[320]   39.   GH61 variant of any one of 1 to 33 above having a W13K substitution.

[321]   40.   GH61 variant of any one of 1 to 33 above having a W13L substitution.

[322]   41.   GH61 variant of any one of 1 to 33 above having a W13M substitution.

[323]   42.   GH61 variant of any one of 1 to 33 above having a W13N substitution.

[324]   43.   GH61 variant of any one of 1 to 33 above having a W13Q substitution.

[325]   44.   GH61 variant of any one of 1 to 33 above having a W13R substitution.

[326]   45.   GH61 variant of any one of 1 to 33 above having a W13T substitution.

[327]   46.   GH61 variant of any one of 1 to 33 above having a W13V substitution.

[328]   47.   GH61 variant of any one of 1 to 33 above having a W13Y substitution.

[329]   48.   GH61 variant of any one of 1 to 47 above having a Q15D substitution.

[330]   49.   GH61 variant of any one of 1 to 47 above having a Q15H substitution.

[331]   50.   GH61 variant of any one of 1 to 47 above having a Q15K substitution.

[332]   51.   GH61 variant of any one of 1 to 50 above having an A16N substitution.

[333]   52.   GH61 variant of any one of 1 to 51 above having a P19E substitution.

[334]   53.   GH61 variant of any one of 1 to 51 above having a P19F substitution.

[335]   54.   GH61 variant of any one of 1 to 51 above having a P19H substitution.

5   [336]   55.   GH61 variant of any one of 1 to 51 above having a P19K substitution.

[337]   56.   GH61 variant of any one of 1 to 51 above having a P19M substitution.

[338]   57.   GH61 variant of any one of 1 to 51 above having a P19Q substitution.

[339]   58.   GH61 variant of any one of 1 to 51 above having a P19T substitution.

[340]   59.   GH61 variant of any one of 1 to 51 above having a P19W substitution.

10   [341]   60.   GH61 variant of any one of 1 to 59 above having a T20A substitution.

[342]   61.   GH61 variant of any one of 1 to 59 above having a T20G substitution.

[343]   62.   GH61 variant of any one of 1 to 59 above having a T20K substitution.

[344]   63.   GH61 variant of any one of 1 to 59 above having a T20M substitution.

[345]   64.   GH61 variant of any one of 1 to 59 above having a T20N substitution.

15   [346]   65.   GH61 variant of any one of 1 to 59 above having a T20P substitution.

[347]   66.   GH61 variant of any one of 1 to 59 above having a T20R substitution.

[348]   67.   GH61 variant of any one of 1 to 59 above having a T20Y substitution.

[349]   68.   GH61 variant of any one of 1 to 67 above having a T21I substitution.

[350]   69.   GH61 variant of any one of 1 to 67 above having a T21K substitution.

20   [351]   70.   GH61 variant of any one of 1 to 67 above having a T21M substitution.

[352]   71.   GH61 variant of any one of 1 to 67 above having a T21S substitution.

[353]   72.   GH61 variant of any one of 1 to 71 above having an F22A substitution.

[354]   73.   GH61 variant of any one of 1 to 71 above having an F22D substitution.

[355]   74.   GH61 variant of any one of 1 to 71 above having an F22G substitution.

25   [356]   75.   GH61 variant of any one of 1 to 71 above having an F22I substitution.

[357]   76.   GH61 variant of any one of 1 to 71 above having an F22K substitution.

[358]   77.   GH61 variant of any one of 1 to 71 above having an F22L substitution.

[359]   78.   GH61 variant of any one of 1 to 71 above having an F22R substitution.

[360]   79.   GH61 variant of any one of 1 to 78 above having a P23G substitution.

30   [361]   80.   GH61 variant of any one of 1 to 79 above having a Y24A substitution.

[362]   81.   GH61 variant of any one of 1 to 79 above having a Y24S substitution.

[363]   82.   GH61 variant of any one of 1 to 79 above having a Y24T substitution.

[364]   83.   GH61 variant of any one of 1 to 82 above having an E25L substitution.

[365]   84.   GH61 variant of any one of 1 to 82 above having an E25M substitution.

35   [366]   85.   GH61 variant of any one of 1 to 82 above having an E25N substitution.

[367]   86.   GH61 variant of any one of 1 to 82 above having an E25Q substitution.

[368]   87.   GH61 variant of any one of 1 to 86 above having an S26M substitution.

[369]   88.   GH61 variant of any one of 1 to 86 above having an S26P substitution.

[370]   89.   GH61 variant of any one of 1 to 86 above having an S26T substitution.

[371]   90.   GH61 variant of any one of 1 to 89 above having an N27E substitution.

[372]   91.   GH61 variant of any one of 1 to 89 above having an N27E substitution.

5   [373]   92.   GH61 variant of any one of 1 to 89 above having an N27H substitution.

[374]   93.   GH61 variant of any one of 1 to 92 above having a P28A substitution.

[375]   94.   GH61 variant of any one of 1 to 92 above having a P28K substitution.

[376]   95.   GH61 variant of any one of 1 to 92 above having a P28M substitution.

[377]   96.   GH61 variant of any one of 1 to 92 above having a P28S substitution.

10   [378]   97.   GH61 variant of any one of 1 to 92 above having a P28V substitution.

[379]   98.   GH61 variant of any one of 1 to 97 above having a P29C substitution.

[380]   99.   GH61 variant of any one of 1 to 97 above having a P29D substitution.

[381]   100.   GH61 variant of any one of 1 to 97 above having a P29E substitution.

[382]   101.   GH61 variant of any one of 1 to 97 above having a P29F substitution.

15   [383]   102.   GH61 variant of any one of 1 to 97 above having a P29G substitution.

[384]   103.   GH61 variant of any one of 1 to 97 above having a P29K substitution.

[385]   104.   GH61 variant of any one of 1 to 97 above having a P29M substitution.

[386]   105.   GH61 variant of any one of 1 to 97 above having a P29R substitution.

[387]   106.   GH61 variant of any one of 1 to 97 above having a P29S substitution.

20   [388]   107.   GH61 variant of any one of 1 to 97 above having a P29T substitution.

[389]   108.   GH61 variant of any one of 1 to 97 above having a P29Y substitution.

[390]   109.   GH61 variant of any one of 1 to 108 above having a V32A substitution.

[391]   110.   GH61 variant of any one of 1 to 108 above having a V32P substitution.

[392]   111.   GH61 variant of any one of 1 to 110 above having a G33A substitution.

25   [393]   112.   GH61 variant of any one of 1 to 110 above having a G33Q substitution.

[394]   113.   GH61 variant of any one of 1 to 110 above having a G33S substitution.

[395]   114.   GH61 variant of any one of 1 to 113 above having a T35A substitution.

[396]   115.   GH61 variant of any one of 1 to 113 above having a T35D substitution.

[397]   116.   GH61 variant of any one of 1 to 113 above having a T35E substitution.

30   [398]   117.   GH61 variant of any one of 1 to 113 above having a T35K substitution.

[399]   118.   GH61 variant of any one of 1 to 113 above having a T35N substitution.

[400]   119.   GH61 variant of any one of 1 to 113 above having a T35S substitution.

[401]   120.   GH61 variant of any one of 1 to 119 above having an A36I substitution.

[402]   121.   GH61 variant of any one of 1 to 119 above having an A36N substitution.

35   [403]   122.   GH61 variant of any one of 1 to 119 above having an A36S substitution.

[404]   123.   GH61 variant of any one of 1 to 119 above having an A36T substitution.

[405]   124.   GH61 variant of any one of 1 to 119 above having an A36Y substitution.

[406] 125. GH61 variant of any one of 1 to 124 above having an A37D substitution.

[407] 126. GH61 variant of any one of 1 to 124 above having an A37E substitution.

[408] 127. GH61 variant of any one of 1 to 124 above having an A37F substitution.

[409] 128. GH61 variant of any one of 1 to 124 above having an A37K substitution.

5 [410] 129. GH61 variant of any one of 1 to 124 above having an A37L substitution.

[411] 130. GH61 variant of any one of 1 to 124 above having an A37Q substitution.

[412] 131. GH61 variant of any one of 1 to 124 above having an A37S substitution.

[413] 132. GH61 variant of any one of 1 to 124 above having an A37W substitution.

[414] 133. GH61 variant of any one of 1 to 132 above having a D38E substitution.

10 [415] 134. GH61 variant of any one of 1 to 132 above having a D38N substitution.

[416] 135. GH61 variant of any one of 1 to 134 above having an L39A substitution.

[417] 136. GH61 variant of any one of 1 to 134 above having an L39D substitution.

[418] 137. GH61 variant of any one of 1 to 134 above having an L39E substitution.

[419] 138. GH61 variant of any one of 1 to 134 above having an L39F substitution.

15 [420] 139. GH61 variant of any one of 1 to 134 above having an L39I substitution.

[421] 140. GH61 variant of any one of 1 to 134 above having an L39M substitution.

[422] 141. GH61 variant of any one of 1 to 134 above having an L39N substitution.

[423] 142. GH61 variant of any one of 1 to 134 above having an L39P substitution.

[424] 143. GH61 variant of any one of 1 to 134 above having an L39Q substitution.

20 [425] 144. GH61 variant of any one of 1 to 134 above having an L39S substitution.

[426] 145. GH61 variant of any one of 1 to 134 above having an L39T substitution.

[427] 146. GH61 variant of any one of 1 to 134 above having an L39V substitution.

[428] 147. GH61 variant of any one of 1 to 134 above having an L39Y substitution.

[429] 148. GH61 variant of any one of 1 to 147 above having an N41D substitution.

25 [430] 149. GH61 variant of any one of 1 to 147 above having an N41E substitution.

[431] 150. GH61 variant of any one of 1 to 147 above having an N41L substitution.

[432] 151. GH61 variant of any one of 1 to 147 above having an N41M substitution.

[433] 152. GH61 variant of any one of 1 to 151 above having an F43Y substitution.

[434] 153. GH61 variant of any one of 1 to 152 above having a V44I substitution.

30 [435] 154. GH61 variant of any one of 1 to 153 above having a D47A substitution.

[436] 155. GH61 variant of any one of 1 to 153 above having a D47H substitution.

[437] 156. GH61 variant of any one of 1 to 153 above having a D47K substitution.

[438] 157. GH61 variant of any one of 1 to 153 above having a D47P substitution.

[439] 158. GH61 variant of any one of 1 to 153 above having a D47R substitution.

35 [440] 159. GH61 variant of any one of 1 to 153 above having a D47T substitution.

[441] 160. GH61 variant of any one of 1 to 153 above having a D47W substitution.

[442] 161. GH61 variant of any one of 1 to 160 above having an A48C substitution.

[443] 162. GH61 variant of any one of 1 to 160 above having an A48E substitution.

[444] 163. GH61 variant of any one of 1 to 160 above having an A48G substitution.

[445] 164. GH61 variant of any one of 1 to 160 above having an A48H substitution.

[446] 165. GH61 variant of any one of 1 to 160 above having an A48I substitution.

5   [447] 166. GH61 variant of any one of 1 to 160 above having an A48K substitution.

[448] 167. GH61 variant of any one of 1 to 160 above having an A48L substitution.

[449] 168. GH61 variant of any one of 1 to 160 above having an A48N substitution.

[450] 169. GH61 variant of any one of 1 to 160 above having an A48Q substitution.

[451] 170. GH61 variant of any one of 1 to 160 above having an A48S substitution.

10   [452] 171. GH61 variant of any one of 1 to 160 above having an A48T substitution.

[453] 172. GH61 variant of any one of 1 to 160 above having an A48W substitution.

[454] 173. GH61 variant of any one of 1 to 160 above having an A48Y substitution.

[455] 174. GH61 variant of any one of 1 to 173 above having a Y49W substitution.

[456] 175. GH61 variant of any one of 1 to 174 above having a Q50A substitution.

15   [457] 176. GH61 variant of any one of 1 to 174 above having a Q50D substitution.

[458] 177. GH61 variant of any one of 1 to 174 above having a Q50E substitution.

[459] 178. GH61 variant of any one of 1 to 174 above having a Q50G substitution.

[460] 179. GH61 variant of any one of 1 to 174 above having a Q50K substitution.

[461] 180. GH61 variant of any one of 1 to 174 above having a Q50T substitution.

20   [462] 181. GH61 variant of any one of 1 to 174 above having a Q50Y substitution.

[463] 182. GH61 variant of any one of 1 to 181 above having an N51E substitution.

[464] 183. GH61 variant of any one of 1 to 181 above having an N51H substitution.

[465] 184. GH61 variant of any one of 1 to 181 above having an N51K substitution.

[466] 185. GH61 variant of any one of 1 to 181 above having an N51L substitution.

25   [467] 186. GH61 variant of any one of 1 to 181 above having an N51S substitution.

[468] 187. GH61 variant of any one of 1 to 181 above having an N51T substitution.

[469] 188. GH61 variant of any one of 1 to 187 above having a P52A substitution.

[470] 189. GH61 variant of any one of 1 to 187 above having a P52F substitution.

[471] 190. GH61 variant of any one of 1 to 187 above having a P52H substitution.

30   [472] 191. GH61 variant of any one of 1 to 187 above having a P52N substitution.

[473] 192. GH61 variant of any one of 1 to 191 above having an I54E substitution.

[474] 193. GH61 variant of any one of 1 to 191 above having an I54Q substitution.

[475] 194. GH61 variant of any one of 1 to 193 above having a K58I substitution.

[476] 195. GH61 variant of any one of 1 to 193 above having a K58R substitution.

35   [477] 196. GH61 variant of any one of 1 to 193 above having a K58V substitution.

[478] 197. GH61 variant of any one of 1 to 196 above having an N59E substitution.

[479] 198. GH61 variant of any one of 1 to 196 above having an N59G substitution.

[480]　199.　GH61 variant of any one of 1 to 196 above having an N59Q substitution.

[481]　200.　GH61 variant of any one of 1 to 196 above having an N59S substitution.

[482]　201.　GH61 variant of any one of 1 to 200 above having an A60G substitution.

[483]　202.　GH61 variant of any one of 1 to 201 above having a T61A substitution.

5　　[484]　203.　GH61 variant of any one of 1 to 201 above having a T61D substitution.

[485]　204.　GH61 variant of any one of 1 to 201 above having a T61Q substitution.

[486]　205.　GH61 variant of any one of 1 to 201 above having a T61R substitution.

[487]　206.　GH61 variant of any one of 1 to 201 above having a T61S substitution.

[488]　207.　GH61 variant of any one of 1 to 206 above having an N62C substitution.

10　　[489]　208.　GH61 variant of any one of 1 to 206 above having an N62H substitution.

[490]　209.　GH61 variant of any one of 1 to 206 above having an N62P substitution.

[491]　210.　GH61 variant of any one of 1 to 209 above having a K64A substitution.

[492]　211.　GH61 variant of any one of 1 to 209 above having a K64L substitution.

[493]　212.　GH61 variant of any one of 1 to 209 above having a K64Q substitution.

15　　[494]　213.　GH61 variant of any one of 1 to 212 above having an S68E substitution.

[495]　214.　GH61 variant of any one of 1 to 212 above having an S68I substitution.

[496]　215.　GH61 variant of any one of 1 to 212 above having an S68L substitution.

[497]　216.　GH61 variant of any one of 1 to 212 above having an S68M substitution.

[498]　217.　GH61 variant of any one of 1 to 212 above having an S68N substitution.

20　　[499]　218.　GH61 variant of any one of 1 to 212 above having an S68T substitution.

[500]　219.　GH61 variant of any one of 1 to 212 above having an S68V substitution.

[501]　220.　GH61 variant of any one of 1 to 212 above having an S68Y substitution.

[502]　221.　GH61 variant of any one of 1 to 220 above having a V69Y substitution.

[503]　222.　GH61 variant of any one of 1 to 221 above having a K70A substitution.

25　　[504]　223.　GH61 variant of any one of 1 to 221 above having a K70E substitution.

[505]　224.　GH61 variant of any one of 1 to 221 above having a K70L substitution.

[506]　225.　GH61 variant of any one of 1 to 221 above having a K70N substitution.

[507]　226.　GH61 variant of any one of 1 to 221 above having a K70R substitution.

[508]　227.　GH61 variant of any one of 1 to 221 above having a K70S substitution.

30　　[509]　228.　GH61 variant of any one of 1 to 227 above having an A71P substitution.

[510]　229.　GH61 variant of any one of 1 to 227 above having an A71Y substitution.

[511]　230.　GH61 variant of any one of 1 to 229 above having an L76D substitution.

[512]　231.　GH61 variant of any one of 1 to 229 above having an L76E substitution.

[513]　232.　GH61 variant of any one of 1 to 231 above having a V80A substitution.

35　　[514]　233.　GH61 variant of any one of 1 to 231 above having a V80E substitution.

[515]　234.　GH61 variant of any one of 1 to 231 above having a V80L substitution.

[516]　235.　GH61 variant of any one of 1 to 231 above having a V80M substitution.

[517]   236.   GH61 variant of any one of 1 to 231 above having a V80T substitution.

[518]   237.   GH61 variant of any one of 1 to 236 above having a P83K substitution.

[519]   238.   GH61 variant of any one of 1 to 237 above having a P89L substitution.

[520]   239.   GH61 variant of any one of 1 to 237 above having a P89S substitution.

5   [521]   240.   GH61 variant of any one of 1 to 237 above having a P89T substitution.

[522]   241.   GH61 variant of any one of 1 to 237 above having a P89V substitution.

[523]   242.   GH61 variant of any one of 1 to 241 above having an I90V substitution.

[524]   243.   GH61 variant of any one of 1 to 242 above having a V91I substitution.

[525]   244.   GH61 variant of any one of 1 to 242 above having a V91L substitution.

10   [526]   245.   GH61 variant of any one of 1 to 244 above having an N96A substitution.

[527]   246.   GH61 variant of any one of 1 to 244 above having an N96D substitution.

[528]   247.   GH61 variant of any one of 1 to 244 above having an N96H substitution.

[529]   248.   GH61 variant of any one of 1 to 244 above having an N96S substitution.

[530]   249.   GH61 variant of any one of 1 to 244 above having an N98H substitution.

15   [531]   250.   GH61 variant of any one of 1 to 249 above having a T103A substitution.

[532]   251.   GH61 variant of any one of 1 to 249 above having a T103E substitution.

[533]   252.   GH61 variant of any one of 1 to 251 above having a V104A substitution.

[534]   253.   GH61 variant of any one of 1 to 251 above having a V104K substitution.

[535]   254.   GH61 variant of any one of 1 to 253 above having a D105S substitution.

20   [536]   255.   GH61 variant of any one of 1 to 254 above having a K106C substitution.

[537]   256.   GH61 variant of any one of 1 to 254 above having a K106E substitution.

[538]   257.   GH61 variant of any one of 1 to 254 above having a K106F substitution.

[539]   258.   GH61 variant of any one of 1 to 254 above having a K106L substitution.

[540]   259.   GH61 variant of any one of 1 to 254 above having a K106R substitution.

25   [541]   260.   GH61 variant of any one of 1 to 259 above having a T107A substitution.

[542]   261.   GH61 variant of any one of 1 to 259 above having a T107C substitution.

[543]   262.   GH61 variant of any one of 1 to 259 above having a T107D substitution.

[544]   263.   GH61 variant of any one of 1 to 259 above having a T107E substitution.

[545]   264.   GH61 variant of any one of 1 to 259 above having a T107F substitution.

30   [546]   265.   GH61 variant of any one of 1 to 259 above having a T107G substitution.

[547]   266.   GH61 variant of any one of 1 to 259 above having a T107K substitution.

[548]   267.   GH61 variant of any one of 1 to 259 above having a T107M substitution.

[549]   268.   GH61 variant of any one of 1 to 259 above having a T107N substitution.

[550]   269.   GH61 variant of any one of 1 to 259 above having a T107Q substitution.

35   [551]   270.   GH61 variant of any one of 1 to 259 above having a T107R substitution.

[552]   271.   GH61 variant of any one of 1 to 259 above having a T107S substitution.

[553]   272.   GH61 variant of any one of 1 to 271 above having a T108A substitution.

[554]   273.   GH61 variant of any one of 1 to 271 above having a T108C substitution.

[555]   274.   GH61 variant of any one of 1 to 271 above having a T108D substitution.

[556]   275.   GH61 variant of any one of 1 to 271 above having a T108E substitution.

[557]   276.   GH61 variant of any one of 1 to 271 above having a T108K substitution.

[558]   277.   GH61 variant of any one of 1 to 271 above having a T108L substitution.

[559]   278.   GH61 variant of any one of 1 to 271 above having a T108Q substitution.

[560]   279.   GH61 variant of any one of 1 to 271 above having a T108R substitution.

[561]   280.   GH61 variant of any one of 1 to 271 above having a T108S substitution.

[562]   281.   GH61 variant of any one of 1 to 280 above having an E110K substitution.

[563]   282.   GH61 variant of any one of 1 to 280 above having an E110L substitution.

[564]   283.   GH61 variant of any one of 1 to 280 above having an E110M substitution.

[565]   284.   GH61 variant of any one of 1 to 283 above having an F112W substitution.

[566]   285.   GH61 variant of any one of 1 to 284 above having a K113P substitution.

[567]   286.   GH61 variant of any one of 1 to 285 above having an I114F substitution.

[568]   287.   GH61 variant of any one of 1 to 286 above having a G116H substitution.

[569]   288.   GH61 variant of any one of 1 to 286 above having a G116Q substitution.

[570]   289.   GH61 variant of any one of 1 to 286 above having a G116S substitution.

[571]   290.   GH61 variant of any one of 1 to 289 above having a V117A substitution.

[572]   291.   GH61 variant of any one of 1 to 289 above having a V117I substitution.

[573]   292.   GH61 variant of any one of 1 to 291 above having an L119N substitution.

[574]   293.   GH61 variant of any one of 1 to 291 above having an L119T substitution.

[575]   294.   GH61 variant of any one of 1 to 293 above having an L120I substitution.

[576]   295.   GH61 variant of any one of 1 to 293 above having an L120S substitution.

[577]   296.   GH61 variant of any one of 1 to 293 above having an L120T substitution.

[578]   297.   GH61 variant of any one of 1 to 293 above having an L120V substitution.

[579]   298.   GH61 variant of any one of 1 to 297 above having an S121C substitution.

[580]   299.   GH61 variant of any one of 1 to 297 above having an S121D substitution.

[581]   300.   GH61 variant of any one of 1 to 297 above having an S121E substitution.

[582]   301.   GH61 variant of any one of 1 to 297 above having an S121F substitution.

[583]   302.   GH61 variant of any one of 1 to 297 above having an S121G substitution.

[584]   303.   GH61 variant of any one of 1 to 297 above having an S121K substitution.

[585]   304.   GH61 variant of any one of 1 to 297 above having an S121L substitution.

[586]   305.   GH61 variant of any one of 1 to 297 above having an S121M substitution.

[587]   306.   GH61 variant of any one of 1 to 297 above having a S121N substitution.

[588]   307.   GH61 variant of any one of 1 to 297 above having an S121T substitution.

[589]   308.   GH61 variant of any one of 1 to 297 above having an S121V substitution.

[590]   309.   GH61 variant of any one of 1 to 297 above having an S121Y substitution.

[591]   310.   GH61 variant of any one of 1 to 309 above having an G122A substitution.

[592]   311.   GH61 variant of any one of 1 to 309 above having an G122E substitution.

[593]   312.   GH61 variant of any one of 1 to 309 above having an G122F substitution.

[594]   313.   GH61 variant of any one of 1 to 309 above having an G122L substitution.

5   [595]   314.   GH61 variant of any one of 1 to 309 above having a G122M substitution.

[596]   315.   GH61 variant of any one of 1 to 309 above having a G122N substitution.

[597]   316.   GH61 variant of any one of 1 to 309 above having a G122S substitution.

[598]   317.   GH61 variant of any one of 1 to 309 above having a G122T substitution.

[599]   318.   GH61 variant of any one of 1 to 309 above having a G122V substitution.

10   [600]   319.   GH61 variant of any one of 1 to 318 above having a G123E substitution.

[601]   320.   GH61 variant of any one of 1 to 318 above having a G123Q substitution.

[602]   321.   GH61 variant of any one of 1 to 318 above having a G123R substitution.

[603]   322.   GH61 variant of any one of 1 to 321 above having a D124N substitution.

[604]   323.   GH61 variant of any one of 1 to 321 above having a D124S substitution.

15   [605]   324.   GH61 variant of any one of 1 to 323 above having a P125D substitution.

[606]   325.   GH61 variant of any one of 1 to 324 above having a G126M substitution.

[607]   326.   GH61 variant of any one of 1 to 324 above having a G126Q substitution.

[608]   327.   GH61 variant of any one of 1 to 326 above having a T127A substitution.

[609]   328.   GH61 variant of any one of 1 to 326 above having a T127E substitution.

20   [610]   329.   GH61 variant of any one of 1 to 326 above having a T127F substitution.

[611]   330.   GH61 variant of any one of 1 to 326 above having a T127I substitution.

[612]   331.   GH61 variant of any one of 1 to 326 above having a T127L substitution.

[613]   332.   GH61 variant of any one of 1 to 326 above having a T127M substitution.

[614]   333.   GH61 variant of any one of 1 to 326 above having a T127V substitution.

25   [615]   334.   GH61 variant of any one of 1 to 326 above having a T127W substitution.

[616]   335.   GH61 variant of any one of 1 to 326 above having a T127Y substitution.

[617]   336.   GH61 variant of any one of 1 to 335 above having an A129N substitution.

[618]   337.   GH61 variant of any one of 1 to 336 above having a V132D substitution.

[619]   338.   GH61 variant of any one of 1 to 336 above having a V132E substitution.

30   [620]   339.   GH61 variant of any one of 1 to 336 above having a V132L substitution.

[621]   340.   GH61 variant of any one of 1 to 336 above having a V132M substitution.

[622]   341.   GH61 variant of any one of 1 to 336 above having a V132R substitution.

[623]   342.   GH61 variant of any one of 1 to 341 above having an S135A substitution.

[624]   343.   GH61 variant of any one of 1 to 341 above having an S135E substitution.

35   [625]   344.   GH61 variant of any one of 1 to 341 above having an S135G substitution.

[626]   345.   GH61 variant of any one of 1 to 341 above having an S135H substitution.

[627]   346.   GH61 variant of any one of 1 to 341 above having an S135M substitution.

[628]   347.   GH61 variant of any one of 1 to 341 above having an S135N substitution.

[629]   348.   GH61 variant of any one of 1 to 347 above having a V141R substitution.

[630]   349.   GH61 variant of any one of 1 to 347 above having a V141T substitution.

[631]   350.   GH61 variant of any one of 1 to 349 above having a V142S substitution.

5   [632]   351.   GH61 variant of any one of 1 to 350 above having an I144Y substitution.

[633]   352.   GH61 variant of any one of 1 to 351 above having a P145D substitution.

[634]   353.   GH61 variant of any one of 1 to 351 above having a P145F substitution.

[635]   354.   GH61 variant of any one of 1 to 353 above having a D146E substitution.

[636]   355.   GH61 variant of any one of 1 to 354 above having an N147E substitution.

10   [637]   356.   GH61 variant of any one of 1 to 354 above having an N147M substitution.

[638]   357.   GH61 variant of any one of 1 to 354 above having an N147S substitution.

[639]   358.   GH61 variant of any one of 1 to 354 above having an N147T substitution.

[640]   359.   GH61 variant of any one of 1 to 358 above having an A149E substitution.

[641]   360.   GH61 variant of any one of 1 to 359 above having a G151D substitution.

15   [642]   361.   GH61 variant of any one of 1 to 359 above having a G151P substitution.

[643]   362.   GH61 variant of any one of 1 to 361 above having a Y153A substitution.

[644]   363.   GH61 variant of any one of 1 to 362 above having a V154D substitution.

[645]   364.   GH61 variant of any one of 1 to 362 above having a V154K substitution.

[646]   365.   GH61 variant of any one of 1 to 364 above having an E158G substitution.

20   [647]   366.   GH61 variant of any one of 1 to 364 above having an E158W substitution.

[648]   367.   GH61 variant of any one of 1 to 366 above having an I160F substitution.

[649]   368.   GH61 variant of any one of 1 to 366 above having an I160M substitution.

[650]   369.   GH61 variant of any one of 1 to 368 above having an A161E substitution.

[651]   370.   GH61 variant of any one of 1 to 368 above having an A161G substitution.

25   [652]   371.   GH61 variant of any one of 1 to 368 above having an A161L substitution.

[653]   372.   GH61 variant of any one of 1 to 368 above having an A161S substitution.

[654]   373.   GH61 variant of any one of 1 to 372 above having an H163A substitution.

[655]   374.   GH61 variant of any one of 1 to 372 above having an H163I substitution.

[656]   375.   GH61 variant of any one of 1 to 372 above having an H163Q substitution.

30   [657]   376.   GH61 variant of any one of 1 to 372 above having an H163Y substitution.

[658]   377.   GH61 variant of any one of 1 to 376 above having an S164A substitution.

[659]   378.   GH61 variant of any one of 1 to 376 above having an S164D substitution.

[660]   379.   GH61 variant of any one of 1 to 376 above having an S164E substitution.

[661]   380.   GH61 variant of any one of 1 to 376 above having an S164G substitution.

35   [662]   381.   GH61 variant of any one of 1 to 376 above having an S164K substitution.

[663]   382.   GH61 variant of any one of 1 to 376 above having an S164L substitution.

[664]   383.   GH61 variant of any one of 1 to 376 above having an S164M substitution.

[665]   384.   GH61 variant of any one of 1 to 376 above having an S164Q substitution.

[666]   385.   GH61 variant of any one of 1 to 376 above having an S164R substitution.

[667]   386.   GH61 variant of any one of 1 to 376 above having an S164V substitution.

[668]   387.   GH61 variant of any one of 1 to 376 above having an S164Y substitution.

5 [669]   388.   GH61 variant of any one of 1 to 387 above having a G166A substitution.

[670]   389.   GH61 variant of any one of 1 to 387 above having a G166D substitution.

[671]   390.   GH61 variant of any one of 1 to 387 above having a G166E substitution.

[672]   391.   GH61 variant of any one of 1 to 387 above having a G166F substitution.

[673]   392.   GH61 variant of any one of 1 to 387 above having a G166H substitution.

10 [674]   393.   GH61 variant of any one of 1 to 387 above having a G166K substitution.

[675]   394.   GH61 variant of any one of 1 to 387 above having a G166M substitution.

[676]   395.   GH61 variant of any one of 1 to 387 above having a G166N substitution.

[677]   396.   GH61 variant of any one of 1 to 387 above having a G166Q substitution.

[678]   397.   GH61 variant of any one of 1 to 387 above having a G166S substitution.

15 [679]   398.   GH61 variant of any one of 1 to 387 above having a G166W substitution.

[680]   399.   GH61 variant of any one of 1 to 398 above having a C167R substitution.

[681]   400.   GH61 variant of any one of 1 to 398 above having a Q167A substitution.

[682]   401.   GH61 variant of any one of 1 to 398 above having a Q167D substitution.

[683]   402.   GH61 variant of any one of 1 to 398 above having a Q167E substitution.

20 [684]   403.   GH61 variant of any one of 1 to 398 above having a Q167F substitution.

[685]   404.   GH61 variant of any one of 1 to 398 above having a Q167G substitution.

[686]   405.   GH61 variant of any one of 1 to 398 above having a Q167K substitution.

[687]   406.   GH61 variant of any one of 1 to 398 above having a Q167L substitution.

[688]   407.   GH61 variant of any one of 1 to 398 above having a Q167N substitution.

25 [689]   408.   GH61 variant of any one of 1 to 398 above having a Q167V substitution.

[690]   409.   GH61 variant of any one of 1 to 398 above having a Q167Y substitution.

[691]   410.   GH61 variant of any one of 1 to 409 above having an A168D substitution.

[692]   411.   GH61 variant of any one of 1 to 409 above having an A168E substitution.

[693]   412.   GH61 variant of any one of 1 to 409 above having an A168F substitution.

30 [694]   413.   GH61 variant of any one of 1 to 409 above having an A168I substitution.

[695]   414.   GH61 variant of any one of 1 to 409 above having an A168K substitution.

[696]   415.   GH61 variant of any one of 1 to 409 above having an A168L substitution.

[697]   416.   GH61 variant of any one of 1 to 409 above having an A168M substitution.

[698]   417.   GH61 variant of any one of 1 to 409 above having an A168N substitution.

35 [699]   418.   GH61 variant of any one of 1 to 409 above having an A168P substitution.

[700]   419.   GH61 variant of any one of 1 to 409 above having an A168Q substitution.

[701]   420.   GH61 variant of any one of 1 to 409 above having an A168R substitution.

[702] 421. GH61 variant of any one of 1 to 409 above having an A168T substitution.

[703] 422. GH61 variant of any one of 1 to 409 above having an A168Y substitution.

[704] 423. GH61 variant of any one of 1 to 422 above having an A171E substitution.

[705] 424. GH61 variant of any one of 1 to 422 above having an A171T substitution.

5   [706] 425. GH61 variant of any one of 1 to 424 above having an N173H substitution.

[707] 426. GH61 variant of any one of 1 to 424 above having an N173S substitution.

[708] 427. GH61 variant of any one of 1 to 426 above having a P175F substitution.

[709] 428. GH61 variant of any one of 1 to 427 above having an I180L substitution.

[710] 429. GH61 variant of any one of 1 to 428 above having an A181K substitution.

10 [711] 430. GH61 variant of any one of 1 to 428 above having an A181N substitution.

[712] 431. GH61 variant of any one of 1 to 428 above having an A181R substitution.

[713] 432. GH61 variant of any one of 1 to 431 above having an S183E substitution.

[714] 433. GH61 variant of any one of 1 to 431 above having an S183K substitution.

[715] 434. GH61 variant of any one of 1 to 431 above having an S183N substitution.

15 [716] 435. GH61 variant of any one of 1 to 431 above having an S183R substitution.

[717] 436. GH61 variant of any one of 1 to 431 above having an S183T substitution.

[718] 437. GH61 variant of any one of 1 to 436 above having an S185D substitution.

[719] 438. GH61 variant of any one of 1 to 436 above having an S185G substitution.

[720] 439. GH61 variant of any one of 1 to 436 above having an S185H substitution.

20 [721] 440. GH61 variant of any one of 1 to 436 above having an S185K substitution.

[722] 441. GH61 variant of any one of 1 to 436 above having an S185T substitution.

[723] 442. GH61 variant of any one of 1 to 441 above having an S187A substitution.

[724] 443. GH61 variant of any one of 1 to 441 above having an S187D substitution.

[725] 444. GH61 variant of any one of 1 to 441 above having an S187E substitution.

25 [726] 445. GH61 variant of any one of 1 to 441 above having an S187H substitution.

[727] 446. GH61 variant of any one of 1 to 441 above having an S187K substitution.

[728] 447. GH61 variant of any one of 1 to 441 above having an S187P substitution.

[729] 448. GH61 variant of any one of 1 to 447 above having an L188A substitution.

[730] 449. GH61 variant of any one of 1 to 447 above having an L188D substitution.

30 [731] 450. GH61 variant of any one of 1 to 449 above having a Q189D substitution.

[732] 451. GH61 variant of any one of 1 to 449 above having a Q189E substitution.

[733] 452. GH61 variant of any one of 1 to 449 above having a Q189G substitution.

[734] 453. GH61 variant of any one of 1 to 449 above having a Q189L substitution.

[735] 454. GH61 variant of any one of 1 to 449 above having a Q189M substitution.

35 [736] 455. GH61 variant of any one of 1 to 449 above having a Q189R substitution.

[737] 456. GH61 variant of any one of 1 to 449 above having a Q189T substitution.

[738] 457. GH61 variant of any one of 1 to 456 above having an S191D substitution.

[739] 458. GH61 variant of any one of 1 to 456 above having an S191R substitution.

[740] 459. GH61 variant of any one of 1 to 458 above having an L194D substitution.

[741] 460. GH61 variant of any one of 1 to 458 above having an L194M substitution.

[742] 461. GH61 variant of any one of 1 to 460 above having a G195A substitution.

[743] 462. GH61 variant of any one of 1 to 460 above having a G195E substitution.

[744] 463. GH61 variant of any one of 1 to 460 above having a G195S substitution.

[745] 464. GH61 variant of any one of 1 to 463 above having a T196H substitution.

[746] 465. GH61 variant of any one of 1 to 463 above having a T196R substitution.

[747] 466. GH61 variant of any one of 1 to 463 above having a T196Y substitution.

[748] 467. GH61 variant of any one of 1 to 466 above having a D197A substitution.

[749] 468. GH61 variant of any one of 1 to 466 above having a D197E substitution.

[750] 469. GH61 variant of any one of 1 to 466 above having a D197L substitution.

[751] 470. GH61 variant of any one of 1 to 466 above having a D197M substitution.

[752] 471. GH61 variant of any one of 1 to 466 above having a D197T substitution.

[753] 472. GH61 variant of any one of 1 to 466 above having a D197V substitution.

[754] 473. GH61 variant of any one of 1 to 472 above having an A201D substitution.

[755] 474. GH61 variant of any one of 1 to 472 above having an A201E substitution.

[756] 475. GH61 variant of any one of 1 to 472 above having an A201F substitution.

[757] 476. GH61 variant of any one of 1 to 472 above having an A201K substitution.

[758] 477. GH61 variant of any one of 1 to 472 above having an A201P substitution.

[759] 478. GH61 variant of any one of 1 to 472 above having an A201Q substitution.

[760] 479. GH61 variant of any one of 1 to 478 above having a T202A substitution.

[761] 480. GH61 variant of any one of 1 to 478 above having a T202E substitution.

[762] 481. GH61 variant of any one of 1 to 478 above having a T202Q substitution.

[763] 482. GH61 variant of any one of 1 to 478 above having a T202S substitution.

[764] 483. GH61 variant of any one of 1 to 482 above having a P204A substitution.

[765] 484. GH61 variant of any one of 1 to 483 above having a V206I substitution.

[766] 485. GH61 variant of any one of 1 to 484 above having an L207F substitution.

[767] 486. GH61 variant of any one of 1 to 485 above having an I208F substitution.

[768] 487. GH61 variant of any one of 1 to 485 above having an I208L substitution.

[769] 488. GH61 variant of any one of 1 to 485 above having an I208V substitution.

[770] 489. GH61 variant of any one of 1 to 488 above having an N209D substitution.

[771] 490. GH61 variant of any one of 1 to 489 above having a Y211A substitution.

[772] 491. GH61 variant of any one of 1 to 490 above having a T212D substitution.

[773] 492. GH61 variant of any one of 1 to 490 above having a T212K substitution.

[774] 493. GH61 variant of any one of 1 to 490 above having a T212R substitution.

[775] 494. GH61 variant of any one of 1 to 493 above having an S213E substitution.

[776] 495. GH61 variant of any one of 1 to 493 above having an S213R substitution.

[777] 496. GH61 variant of any one of 1 to 493 above having an S213T substitution.

[778] 497. GH61 variant of any one of 1 to 496 above having a P214S substitution.

[779] 498. GH61 variant of any one of 1 to 497 above having an N216D substitution.

5 [780] 499. GH61 variant of any one of 1 to 497 above having an N216I substitution.

[781] 500. GH61 variant of any one of 1 to 497 above having an N216K substitution.

[782] 501. GH61 variant of any one of 1 to 497 above having an N216P substitution.

[783] 502. GH61 variant of any one of 1 to 497 above having an N216Q substitution.

[784] 503. GH61 variant of any one of 1 to 497 above having an N216T substitution.

10 [785] 504. GH61 variant of any one of 1 to 503 above having a Y217H substitution.

[786] 505. GH61 variant of any one of 1 to 504 above having an I218A substitution.

[787] 506. GH61 variant of any one of 1 to 504 above having an I218D substitution.

[788] 507. GH61 variant of any one of 1 to 504 above having an I218E substitution.

[789] 508. GH61 variant of any one of 1 to 504 above having an I218K substitution.

15 [790] 509. GH61 variant of any one of 1 to 504 above having an I218M substitution.

[791] 510. GH61 variant of any one of 1 to 504 above having an I218N substitution.

[792] 511. GH61 variant of any one of 1 to 504 above having an I218P substitution.

[793] 512. GH61 variant of any one of 1 to 504 above having an I218R substitution.

[794] 513. GH61 variant of any one of 1 to 504 above having an I218S substitution.

20 [795] 514. GH61 variant of any one of 1 to 504 above having an I218T substitution.

[796] 515. GH61 variant of any one of 1 to 504 above having an I218V substitution.

[797] 516. GH61 variant of any one of 1 to 515 above having an I219A substitution.

[798] 517. GH61 variant of any one of 1 to 515 above having an I219D substitution.

[799] 518. GH61 variant of any one of 1 to 515 above having an I219L substitution.

25 [800] 519. GH61 variant of any one of 1 to 515 above having an I219M substitution.

[801] 520. GH61 variant of any one of 1 to 515 above having an I219N substitution.

[802] 521. GH61 variant of any one of 1 to 515 above having an I219P substitution.

[803] 522. GH61 variant of any one of 1 to 515 above having an I219Q substitution.

[804] 523. GH61 variant of any one of 1 to 522 above having a P222D substitution.

30 [805] 524. GH61 variant of any one of 1 to 522 above having a P222G substitution.

[806] 525. GH61 variant of any one of 1 to 524 above having a T223E substitution.

[807] 526. GH61 variant of any one of 1 to 524 above having a T223Q substitution.

[808] 527. GH61 variant of any one of 1 to 524 above having a T223S substitution.

[809] 528. GH61 variant of any one of 1 to 527 above having a V224A substitution.

35 [810] 529. GH61 variant of any one of 1 to 527 above having a V224E substitution.

[811] 530. GH61 variant of any one of 1 to 527 above having a V224I substitution.

[812] 531. GH61 variant of any one of 1 to 527 above having a V224N substitution.

[813]   532.   GH61 variant of any one of 1 to 527 above having a V224P substitution.

[814]   533.   GH61 variant of any one of 1 to 527 above having a V224Q substitution.

[815]   534.   GH61 variant of any one of 1 to 527 above having a V224S substitution.

[816]   535.   GH61 variant of any one of 1 to 527 above having a V224T substitution.

5   [817]   536.   GH61 variant of any one of 1 to 535 above having an S226D substitution.

[818]   537.   GH61 variant of any one of 1 to 535 above having an S226G substitution.

[819]   538.   GH61 variant of any one of 1 to 535 above having an S226K substitution.

[820]   539.   GH61 variant of any one of 1 to 535 above having an S226M substitution.

[821]   540.   GH61 variant of any one of 1 to 535 above having an S226P substitution.

10   [822]   541.   GH61 variant of any one of 1 to 535 above having an S226Q substitution.

[823]   542.   GH61 variant of any one of 1 to 541 above having a T230S substitution.

[824]   543.   GH61 variant of any one of 1 to 542 above having an S231C substitution.

[825]   544.   GH61 variant of any one of 1 to 542 above having an S231I substitution.

[826]   545.   GH61 variant of any one of 1 to 544 above having a V232F substitution.

15   [827]   546.   GH61 variant of any one of 1 to 544 above having a V232Y substitution.

[828]   547.   GH61 variant of any one of 1 to 544 above having an A233C substitution.

[829]   548.   GH61 variant of any one of 1 to 544 above having an A233F substitution.

[830]   549.   GH61 variant of any one of 1 to 544 above having an A233S substitution.

[831]   550.   GH61 variant of any one of 1 to 549 above having a G235A substitution.

20   [832]   551.   GH61 variant of any one of 1 to 549 above having a G235D substitution.

[833]   552.   GH61 variant of any one of 1 to 549 above having a G235F substitution.

[834]   553.   GH61 variant of any one of 1 to 549 above having a G235I substitution.

[835]   554.   GH61 variant of any one of 1 to 549 above having a G235K substitution.

[836]   555.   GH61 variant of any one of 1 to 549 above having a G235L substitution.

25   [837]   556.   GH61 variant of any one of 1 to 549 above having a G235M substitution.

[838]   557.   GH61 variant of any one of 1 to 549 above having a G235N substitution.

[839]   558.   GH61 variant of any one of 1 to 549 above having a G235Q substitution.

[840]   559.   GH61 variant of any one of 1 to 549 above having a G235S substitution.

[841]   560.   GH61 variant of any one of 1 to 549 above having a G235V substitution.

30   [842]   561.   GH61 variant of any one of 1 to 560 above having a T287A substitution.

[843]   562.   GH61 variant of any one of 1 to 560 above having a T287D substitution.

[844]   563.   GH61 variant of any one of 1 to 560 above having a T287F substitution.

[845]   564.   GH61 variant of any one of 1 to 560 above having a T287G substitution.

[846]   565.   GH61 variant of any one of 1 to 560 above having a T287H substitution.

35   [847]   566.   GH61 variant of any one of 1 to 560 above having a T287I substitution.

[848]   567.   GH61 variant of any one of 1 to 560 above having a T287S substitution.

[849]   568.   GH61 variant of any one of 1 to 567 above having an L288A substitution.

[850]   569.   GH61 variant of any one of 1 to 567 above having an L288C substitution.

[851]   570.   GH61 variant of any one of 1 to 567 above having an L288D substitution.

[852]   571.   GH61 variant of any one of 1 to 567 above having an L288E substitution.

[853]   572.   GH61 variant of any one of 1 to 567 above having an L288F substitution.

5   [854]   573.   GH61 variant of any one of 1 to 567 above having an L288G substitution.

[855]   574.   GH61 variant of any one of 1 to 567 above having an L288I substitution.

[856]   575.   GH61 variant of any one of 1 to 567 above having an L288K substitution.

[857]   576.   GH61 variant of any one of 1 to 567 above having an L288M substitution.

[858]   577.   GH61 variant of any one of 1 to 567 above having an L288N substitution.

10   [859]   578.   GH61 variant of any one of 1 to 567 above having an L288R substitution.

[860]   579.   GH61 variant of any one of 1 to 567 above having an L288S substitution.

[861]   580.   GH61 variant of any one of 1 to 567 above having an L288T substitution.

[862]   581.   GH61 variant of any one of 1 to 567 above having an L288V substitution.

[863]   582.   GH61 variant of any one of 1 to 567 above having an L288W substitution.

15   [864]   583.   GH61 variant of any one of 1 to 582 above having a T289A substitution.

[865]   584.   GH61 variant of any one of 1 to 582 above having a T289D substitution.

[866]   585.   GH61 variant of any one of 1 to 582 above having a T289K substitution.

[867]   586.   GH61 variant of any one of 1 to 582 above having a T289L substitution.

[868]   587.   GH61 variant of any one of 1 to 582 above having a T289M substitution.

20   [869]   588.   GH61 variant of any one of 1 to 582 above having a T289R substitution.

[870]   589.   GH61 variant of any one of 1 to 582 above having a T289S substitution.

[871]   590.   GH61 variant of any one of 1 to 589 above having an L290A substitution.

[872]   591.   GH61 variant of any one of 1 to 589 above having an L290K substitution.

[873]   592.   GH61 variant of any one of 1 to 589 above having an L290M substitution.

25   [874]   593.   GH61 variant of any one of 1 to 589 above having an L290T substitution.

[875]   594.   GH61 variant of any one of 1 to 593 above having a Y291A substitution.

[876]   595.   GH61 variant of any one of 1 to 593 above having a Y291D substitution.

[877]   596.   GH61 variant of any one of 1 to 593 above having a Y291E substitution.

[878]   597.   GH61 variant of any one of 1 to 593 above having a Y291F substitution.

30   [879]   598.   GH61 variant of any one of 1 to 593 above having a Y291G substitution.

[880]   599.   GH61 variant of any one of 1 to 593 above having a Y291I substitution.

[881]   600.   GH61 variant of any one of 1 to 593 above having a Y291K substitution.

[882]   601.   GH61 variant of any one of 1 to 593 above having a Y291L substitution.

[883]   602.   GH61 variant of any one of 1 to 593 above having a Y291M substitution.

35   [884]   603.   GH61 variant of any one of 1 to 593 above having a Y291N substitution.

[885]   604.   GH61 variant of any one of 1 to 593 above having a Y291R substitution.

[886]   605.   GH61 variant of any one of 1 to 593 above having a Y291S substitution.

[887] 606. GH61 variant of any one of 1 to 593 above having a Y291V substitution.

[888] 607. GH61 variant of any one of 1 to 593 above having a Y291W substitution.

[889] 608. GH61 variant of any one of 1 to 607 above having a G292D substitution.

[890] 609. GH61 variant of any one of 1 to 607 above having a G292K substitution.

5   [891] 610. GH61 variant of any one of 1 to 609 above having a Q293K substitution.

[892] 611. GH61 variant of any one of 1 to 609 above having a Q293P substitution.

[893] 612. GH61 variant of any one of 1 to 611 above having a C294S substitution.

[894] 613. GH61 variant of any one of 1 to 612 above having a G295A substitution.

[895] 614. GH61 variant of any one of 1 to 612 above having a G295C substitution.

10 [896] 615. GH61 variant of any one of 1 to 612 above having a G295D substitution.

[897] 616. GH61 variant of any one of 1 to 612 above having a G295F substitution.

[898] 617. GH61 variant of any one of 1 to 612 above having a G295H substitution.

[899] 618. GH61 variant of any one of 1 to 612 above having a G295Q substitution.

[900] 619. GH61 variant of any one of 1 to 612 above having a G295R substitution.

15 [901] 620. GH61 variant of any one of 1 to 612 above having a G295T substitution.

[902] 621. GH61 variant of any one of 1 to 612 above having a G295W substitution.

[903] 622. GH61 variant of any one of 1 to 621 above having a G296A substitution.

[904] 623. GH61 variant of any one of 1 to 621 above having a G296M substitution.

[905] 624. GH61 variant of any one of 1 to 623 above having an S297C substitution.

20 [906] 625. GH61 variant of any one of 1 to 623 above having an S297D substitution.

[907] 626. GH61 variant of any one of 1 to 623 above having an S297E substitution.

[908] 627. GH61 variant of any one of 1 to 623 above having an S297E substitution.

[909] 628. GH61 variant of any one of 1 to 623 above having an S297K substitution.

[910] 629. GH61 variant of any one of 1 to 623 above having an S297N substitution.

25 [911] 630. GH61 variant of any one of 1 to 623 above having an S297Q substitution.

[912] 631. GH61 variant of any one of 1 to 623 above having an S297R substitution.

[913] 632. GH61 variant of any one of 1 to 623 above having an S297T substitution.

[914] 633. GH61 variant of any one of 1 to 632 above having a G298A substitution.

[915] 634. GH61 variant of any one of 1 to 632 above having a G298C substitution.

30 [916] 635. GH61 variant of any one of 1 to 632 above having a G298H substitution.

[917] 636. GH61 variant of any one of 1 to 632 above having a G298I substitution.

[918] 637. GH61 variant of any one of 1 to 632 above having a G298K substitution.

[919] 638. GH61 variant of any one of 1 to 632 above having a G298N substitution.

[920] 639. GH61 variant of any one of 1 to 632 above having a G298Q substitution.

35 [921] 640. GH61 variant of any one of 1 to 639 above having a Y299F substitution.

[922] 641. GH61 variant of any one of 1 to 639 above having a Y299N substitution.

[923] 642. GH61 variant of any one of 1 to 639 above having a Y299P substitution.

[924]   643.   GH61 variant of any one of 1 to 639 above having a Y299S substitution.

[925]   644.   GH61 variant of any one of 1 to 643 above having a G301D substitution.

[926]   645.   GH61 variant of any one of 1 to 643 above having a G301P substitution.

[927]   646.   GH61 variant of any one of 1 to 643 above having a G301S substitution.

5   [928]   647.   GH61 variant of any one of 1 to 643 above having a G301Y substitution.

[929]   648.   GH61 variant of any one of 1 to 647 above having a P302F substitution.

[930]   649.   GH61 variant of any one of 1 to 647 above having a P302G substitution.

[931]   650.   GH61 variant of any one of 1 to 647 above having a P302L substitution.

[932]   651.   GH61 variant of any one of 1 to 647 above having a P302T substitution.

10   [933]   652.   GH61 variant of any one of 1 to 647 above having a P302V substitution.

[934]   653.   GH61 variant of any one of 1 to 647 above having a P302Y substitution.

[935]   654.   GH61 variant of any one of 1 to 653 above having a T303G substitution.

[936]   655.   GH61 variant of any one of 1 to 653 above having a T303K substitution.

[937]   656.   GH61 variant of any one of 1 to 653 above having a T303M substitution.

15   [938]   657.   GH61 variant of any one of 1 to 653 above having a T303P substitution.

[939]   658.   GH61 variant of any one of 1 to 653 above having a T303R substitution.

[940]   659.   GH61 variant of any one of 1 to 653 above having a T303S substitution.

[941]   660.   GH61 variant of any one of 1 to 653 above having a T303V substitution.

[942]   661.   GH61 variant of any one of 1 to 653 above having a T303Y substitution.

20   [943]   662.   GH61 variant of any one of 1 to 661 above having an R304A substitution.

[944]   663.   GH61 variant of any one of 1 to 661 above having an R304C substitution.

[945]   664.   GH61 variant of any one of 1 to 661 above having an R304D substitution.

[946]   665.   GH61 variant of any one of 1 to 661 above having an R304E substitution.

[947]   666.   GH61 variant of any one of 1 to 661 above having an R304N substitution.

25   [948]   667.   GH61 variant of any one of 1 to 661 above having an R304P substitution.

[949]   668.   GH61 variant of any one of 1 to 661 above having an R304Q substitution.

[950]   669.   GH61 variant of any one of 1 to 661 above having an R304T substitution.

[951]   670.   GH61 variant of any one of 1 to 661 above having an R304V substitution.

[952]   671.   GH61 variant of any one of 1 to 670 above having a C305E substitution.

30   [953]   672.   GH61 variant of any one of 1 to 670 above having a C305G substitution.

[954]   673.   GH61 variant of any one of 1 to 670 above having a C305K substitution.

[955]   674.   GH61 variant of any one of 1 to 673 above having an A306E substitution.

[956]   675.   GH61 variant of any one of 1 to 673 above having an A306F substitution.

[957]   676.   GH61 variant of any one of 1 to 673 above having an A306G substitution.

35   [958]   677.   GH61 variant of any one of 1 to 673 above having an A306I substitution.

[959]   678.   GH61 variant of any one of 1 to 673 above having an A306K substitution.

[960]   679.   GH61 variant of any one of 1 to 673 above having an A306L substitution.

[961]   680.   GH61 variant of any one of 1 to 673 above having an A306M substitution.

[962]   681.   GH61 variant of any one of 1 to 673 above having an A306Q substitution.

[963]   682.   GH61 variant of any one of 1 to 673 above having an A306T substitution.

[964]   683.   GH61 variant of any one of 1 to 673 above having an A306Y substitution.

5   [965]   684.   GH61 variant of any one of 1 to 683 above having a P307A substitution.

[966]   685.   GH61 variant of any one of 1 to 683 above having a P307F substitution.

[967]   686.   GH61 variant of any one of 1 to 683 above having a P307H substitution.

[968]   687.   GH61 variant of any one of 1 to 683 above having a P307L substitution.

[969]   688.   GH61 variant of any one of 1 to 683 above having a P307V substitution.

10   [970]   689.   GH61 variant of any one of 1 to 688 above having a P308A substitution.

[971]   690.   GH61 variant of any one of 1 to 688 above having a P308G substitution.

[972]   691.   GH61 variant of any one of 1 to 688 above having a P308H substitution.

[973]   692.   GH61 variant of any one of 1 to 688 above having a P308I substitution.

[974]   693.   GH61 variant of any one of 1 to 688 above having a P308K substitution.

15   [975]   694.   GH61 variant of any one of 1 to 688 above having a P308L substitution.

[976]   695.   GH61 variant of any one of 1 to 688 above having a P308Q substitution.

[977]   696.   GH61 variant of any one of 1 to 688 above having a P308R substitution.

[978]   697.   GH61 variant of any one of 1 to 688 above having a P308S substitution.

[979]   698.   GH61 variant of any one of 1 to 688 above having a P308T substitution.

20   [980]   699.   GH61 variant of any one of 1 to 688 above having a P308V substitution.

[981]   700.   GH61 variant of any one of 1 to 688 above having a P308W substitution.

[982]   701.   GH61 variant of any one of 1 to 688 above having a P308Y substitution.

[983]   702.   GH61 variant of any one of 1 to 701 above having an A309I substitution.

[984]   703.   GH61 variant of any one of 1 to 701 above having an A309K substitution.

25   [985]   704.   GH61 variant of any one of 1 to 701 above having an A309R substitution.

[986]   705.   GH61 variant of any one of 1 to 701 above having an A309T substitution.

[987]   706.   GH61 variant of any one of 1 to 701 above having an A309V substitution.

[988]   707.   GH61 variant of any one of 1 to 701 above having an A309Y substitution.

[989]   708.   GH61 variant of any one of 1 to 707 above having a T310L substitution.

30   [990]   709.   GH61 variant of any one of 1 to 707 above having a T310M substitution.

[991]   710.   GH61 variant of any one of 1 to 707 above having a T310Q substitution.

[992]   711.   GH61 variant of any one of 1 to 707 above having a T310S substitution.

[993]   712.   GH61 variant of any one of 1 to 707 above having a T310W substitution.

[994]   713.   GH61 variant of any one of 1 to 712 above having a C311S substitution.

35   [995]   714.   GH61 variant of any one of 1 to 713 above having an S312D substitution.

[996]   715.   GH61 variant of any one of 1 to 713 above having an S312F substitution.

[997]   716.   GH61 variant of any one of 1 to 713 above having an S312G substitution.

[998]   717.   GH61 variant of any one of 1 to 713 above having an S312I substitution.

[999]   718.   GH61 variant of any one of 1 to 713 above having an S312K substitution.

[1000] 719.   GH61 variant of any one of 1 to 713 above having an S312L substitution.

[1001] 720.   GH61 variant of any one of 1 to 713 above having an S312M substitution.

5   [1002] 721.   GH61 variant of any one of 1 to 713 above having an S312N substitution.

[1003] 722.   GH61 variant of any one of 1 to 713 above having an S312Q substitution.

[1004] 723.   GH61 variant of any one of 1 to 713 above having an S312R substitution.

[1005] 724.   GH61 variant of any one of 1 to 713 above having an S312T substitution.

[1006] 725.   GH61 variant of any one of 1 to 713 above having an S312V substitution.

10   [1007] 726.   GH61 variant of any one of 1 to 713 above having an S312Y substitution.

[1008] 727.   GH61 variant of any one of 1 to 726 above having a T313D substitution.

[1009] 728.   GH61 variant of any one of 1 to 726 above having a T313E substitution.

[1010] 729.   GH61 variant of any one of 1 to 726 above having a T313F substitution.

[1011] 730.   GH61 variant of any one of 1 to 726 above having a T313G substitution.

15   [1012] 731.   GH61 variant of any one of 1 to 726 above having a T313I substitution.

[1013] 732.   GH61 variant of any one of 1 to 726 above having a T313K substitution.

[1014] 733.   GH61 variant of any one of 1 to 726 above having a T313L substitution.

[1015] 734.   GH61 variant of any one of 1 to 726 above having a T313M substitution.

[1016] 735.   GH61 variant of any one of 1 to 726 above having a T313P substitution.

20   [1017] 736.   GH61 variant of any one of 1 to 726 above having a T313S substitution.

[1018] 737.   GH61 variant of any one of 1 to 726 above having a T313V substitution.

[1019] 738.   GH61 variant of any one of 1 to 726 above having a T313Y substitution.

[1020] 739.   GH61 variant of any one of 1 to 738 above having an N315E substitution.

[1021] 740.   GH61 variant of any one of 1 to 738 above having an N315H substitution.

25   [1022] 741.   GH61 variant of any one of 1 to 738 above having an N315K substitution.

[1023] 742.   GH61 variant of any one of 1 to 738 above having an N315L substitution.

[1024] 743.   GH61 variant of any one of 1 to 738 above having an N315M substitution.

[1025] 744.   GH61 variant of any one of 1 to 738 above having an N315Q substitution.

[1026] 745.   GH61 variant of any one of 1 to 738 above having an N315R substitution.

30   [1027] 746.   GH61 variant of any one of 1 to 738 above having an N315S substitution.

[1028] 747.   GH61 variant of any one of 1 to 738 above having an N315V substitution.

[1029] 748.   GH61 variant of any one of 1 to 738 above having an N315W substitution.

[1030] 749.   GH61 variant of any one of 1 to 738 above having an N315Y substitution.

[1031] 750.   GH61 variant of any one of 1 to 749 above having a P316A substitution.

35   [1032] 751.   GH61 variant of any one of 1 to 749 above having a P316C substitution.

[1033] 752.   GH61 variant of any one of 1 to 749 above having a P316D substitution.

[1034] 753.   GH61 variant of any one of 1 to 749 above having a P316F substitution.

[1035] 754.   GH61 variant of any one of 1 to 749 above having a P316N substitution.

[1036] 755.   GH61 variant of any one of 1 to 749 above having a P316R substitution.

[1037] 756.   GH61 variant of any one of 1 to 749 above having a P316T substitution.

[1038] 757.   GH61 variant of any one of 1 to 756 above having a Y317E substitution.

5   [1039] 758.   GH61 variant of any one of 1 to 756 above having a Y317G substitution.

[1040] 759.   GH61 variant of any one of 1 to 756 above having a Y317L substitution.

[1041] 760.   GH61 variant of any one of 1 to 756 above having a Y317M substitution.

[1042] 761.   GH61 variant of any one of 1 to 756 above having a Y317P substitution.

[1043] 762.   GH61 variant of any one of 1 to 756 above having a Y317Q substitution.

10   [1044] 763.   GH61 variant of any one of 1 to 756 above having a Y317R substitution.

[1045] 764.   GH61 variant of any one of 1 to 756 above having a Y317S substitution.

[1046] 765.   GH61 variant of any one of 1 to 756 above having a Y317T substitution.

[1047] 766.   GH61 variant of any one of 1 to 756 above having a Y317V substitution.

[1048] 767.   GH61 variant of any one of 1 to 756 above having a Y317W substitution.

15   [1049] 768.   GH61 variant of any one of 1 to 767 above having a Y318T substitution.

[1050] 769.   GH61 variant of any one of 1 to 767 above having a Y318W substitution.

[1051] 770.   GH61 variant of any one of 1 to 769 above having an A319D substitution.

[1052] 771.   GH61 variant of any one of 1 to 769 above having an A319F substitution.

[1053] 772.   GH61 variant of any one of 1 to 769 above having an A319Q substitution.

20   [1054] 773.   GH61 variant of any one of 1 to 769 above having an A319S substitution.

[1055] 774.   GH61 variant of any one of 1 to 769 above having an A319W substitution.

[1056] 775.   GH61 variant of any one of 1 to 774 above having a Q320A substitution.

[1057] 776.   GH61 variant of any one of 1 to 774 above having a Q320P substitution.

[1058] 777.   GH61 variant of any one of 1 to 774 above having a Q320R substitution.

25   [1059] 778.   GH61 variant of any one of 1 to 774 above having a Q320S substitution.

[1060] 779.   GH61 variant of any one of 1 to 778 above having a C321E substitution.

[1061] 780.   GH61 variant of any one of 1 to 778 above having a C321G substitution.

[1062] 781.   GH61 variant of any one of 1 to 778 above having a C321R substitution.

[1063] 782.   GH61 variant of any one of 1 to 781 above having an L322A substitution.

30   [1064] 783.   GH61 variant of any one of 1 to 781 above having an L322E substitution.

[1065] 784.   GH61 variant of any one of 1 to 781 above having an L322S substitution.

[1066] 785.   GH61 variant of any one of 1 to 781 above having an L322T substitution.

[1067] 786.   GH61 variant of any one of 1 to 781 above having an L322V substitution.

[1068] 787.   GH61 variant of any one of 1 to 786 above having an N323V substitution.

35   [1069] In another aspect, nucleic acids encoding a variant GH61 enzyme having one or more mutations with respect to a parent GH61 enzyme (e.g., as described above) are provided. In certain embodiments, the parent GH61 enzyme encoded by the nucleic acid has at least 80%

(i.e., 80% or greater but less than 100%) amino acid sequence identity to *H. jecorina* GH61A (SEQ ID NO:3). In certain embodiments, the nucleic acid encoding a variant GH61 enzyme is at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%,

5   at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or even at least 99.9% homology/identity to SEQ ID NO: 1 (excluding the portion of the nucleic acid that encodes the signal sequence). It will be appreciated that due to the degeneracy of the genetic code, a plurality of nucleic acids may

10   encode the same variant GH61 enzyme. Moreover, nucleic acids encoding a variant GH61 enzyme as described herein may be engineered to be codon optimized, e.g., to improve expression in a host cell of interest. Certain codon optimization techniques are known in the art.

[1070] In certain embodiments, the variant GH61 enzyme-encoding nucleic acid hybridizes

15   under stringent conditions to a nucleic acid encoding (or complementary to a nucleic acid encoding) a GH61 having at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%,

20   at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.9% homology/identity to SEQ ID NO:1 (excluding the portion of the nucleic acid that encodes the signal sequence).

[1071] Nucleic acids may encode a "full-length" ("fl" or "FL") variant GH61 enzyme, which includes a signal sequence, only the mature form of a variant GH61 enzyme, which lacks the signal sequence, or a truncated form of a variant GH61 enzyme, which lacks portions of the N

25   and/or C-terminus of the mature form.

[1072] A nucleic acid that encodes a variant GH61 enzyme can be operably linked to various promoters and regulators in a vector suitable for expressing the variant GH61 enzyme in a host cell(s) of interest, as described below.

30   **IV. EXPRESSION OF RECOMBINANT GH61 VARIANTS**

[1073] Aspects of the subject invention include methods and compositions related to the generation nucleic acids encoding GH61 variants, host cells containing such nucleic acids, the production of GH61 variants by such host cells, and the isolation, purification and/or use of the GH61 variants.

35   [1074] As such, embodiments of the invention provide host cells that have been transduced, transformed or transfected with an expression vector comprising a desired GH61 variant-encoding nucleic acid sequence. For example, a filamentous fungal cell or yeast cell is

transfected with an expression vector having a promoter or biologically active promoter fragment or one or more (*e.g.*, a series) of enhancers which functions in the host cell line, operably linked to a DNA segment encoding a desired GH61 variant, such that desired GH61 variant is expressed in the cell line.

5   **A. Nucleic Acid Constructs/Expression Vectors.**

[1075] Natural or synthetic polynucleotide fragments encoding a desired GH61 variant may be incorporated into heterologous nucleic acid constructs or vectors, capable of introduction into, and replication in, a host cell of interest (e.g., a filamentous fungal or yeast cell). The vectors and methods disclosed herein are suitable for use in host cells for the expression of a desired

10   GH61 variant. Any vector may be used as long as it meets the desired replication/expression characteristics in the host cell(s) into which it is introduced (such characteristics generally being defined by the user). Large numbers of suitable vectors and promoters are known to those of skill in the art, some of which are commercially available. Cloning and expression vectors are also described in Sambrook *et al.,* 1989, Ausubel FM *et al.,* 1989, and Strathern *et al.,* 1981,

15   each of which is expressly incorporated by reference herein. Appropriate expression vectors for fungi are described in van den Hondel, C.A.M.J.J. et al. (1991) In: Bennett, J.W. and Lasure, L.L. (eds.) More Gene Manipulations in Fungi. Academic Press, pp. 396-428. The appropriate DNA sequence may be inserted into a plasmid or vector (collectively referred to herein as "vectors") by a variety of procedures. In general, the DNA sequence is inserted into an

20   appropriate restriction endonuclease site(s) by standard procedures. Such procedures and related sub-cloning procedures are deemed to be within the scope of knowledge of those skilled in the art.

[1076] Recombinant host cells comprising the coding sequence for a desired GH61 variant may be produced by introducing a heterologous nucleic acid construct comprising the desired

25   GH61 variant coding sequence into the desired host cells (e.g., as described in further detail below). For example, a desired GH61 variant coding sequence may be inserted into a suitable vector according to well-known recombinant techniques and used to transform a filamentous fungus capable of GH61 expression. As has been noted above, due to the inherent degeneracy of the genetic code, other nucleic acid sequences which encode substantially the

30   same or a functionally equivalent amino acid sequence may be used to clone and express a desired GH61 variant. Therefore it is appreciated that such substitutions in the coding region fall within the sequence variants covered by the present invention.

[1077] The present invention also includes recombinant nucleic acid constructs comprising one or more of the desired GH61 variant-encoding nucleic acid sequences as described above.

35   The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation.

[1078] Heterologous nucleic acid constructs may include the coding sequence for a desired GH61 variant: (i) in isolation; (ii) in combination with additional coding sequences; such as fusion polypeptide or signal peptide coding sequences, where the desired GH61 variant coding sequence is the dominant coding sequence; (iii) in combination with non-coding sequences,

5   such as introns and control elements, such as promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host; and/or (iv) in a vector or host environment in which the desired GH61 variant coding sequence is a heterologous gene.

[1079] In one aspect of the present invention, a heterologous nucleic acid construct is

10   employed to transfer a desired GH61 variant-encoding nucleic acid sequence into a host cell *in vitro*, e.g., into established filamentous fungal and yeast lines. Long-term production of a desired GH61 variant can be achieved by generating a host cell that has stable expression of the GH61 variant. Thus, it follows that any method effective to generate stable transformants may be used in practicing the invention.

15   [1080] Appropriate vectors are typically equipped with a selectable marker-encoding nucleic acid sequence, insertion sites, and suitable control elements, such as promoter and termination sequences. The vector may comprise regulatory sequences, including, for example, non-coding sequences, such as introns and control elements, *i.e.*, promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in

20   host cells (and/or in a vector or host cell environment in which a modified soluble protein antigen coding sequence is not normally expressed), operably linked to the coding sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, many of which are commercially available and/or are described in Sambrook, *et al.*, (*supra*).

[1081] Examples of suitable promoters include both constitutive promoters and inducible

25   promoters, examples of which include a CMV promoter, an SV40 early promoter, an RSV promoter, an EF-1α promoter, a promoter containing the *tet* responsive element (TRE) in the tet-on or tet-off system as described (ClonTech and BASF), the beta actin promoter and the metallothionine promoter that can upregulated by addition of certain metal salts. A promoter sequence is a DNA sequence which is recognized by the particular host cell for expression

30   purposes. It is operably linked to DNA sequence encoding a variant GH61A polypeptide. Such linkage comprises positioning of the promoter with respect to the initiation codon of the DNA sequence encoding the variant GH61A polypeptide in the expression vector such that the promoter can drive transcription/translation of the GH61 variant-encoding sequence. The promoter sequence contains transcription and translation control sequence which mediate the

35   expression of the variant GH61A polypeptide. Examples include the promoters from the *Aspergillus niger*, *A awamori* or *A. oryzae* glucoamylase, alpha-amylase, or alpha-glucosidase encoding genes; the *A. nidulans gpdA* or *trpC* Genes; the *Neurospora crassa cbh1* or *trp1*

genes; the *A. niger* or *Rhizomucor miehei* aspartic proteinase encoding genes; the *H. jecorina* *cbh1*, *cbh2*, *egl1*, *egl2*, or other cellulase encoding genes.

[1082] The choice of the proper selectable marker will depend on the host cell, and appropriate markers for different hosts are well known in the art. Typical selectable marker genes include argB from *A. nidulans* or *H. jecorina*, amdS from *A. nidulans*, pyr4 from *Neurospora crassa* or *H. jecorina*, pyrG from *Aspergillus niger or A. nidulans*. Additional examples of suitable selectable markers include, but are not limited to trpc, trp1, oliC31, niaD or leu2, which are included in heterologous nucleic acid constructs used to transform a mutant strain such as trp-, pyr-, leu- and the like.

[1083] Such selectable markers confer to transformants the ability to utilize a metabolite that is usually not metabolized by the filamentous fungi. For example, the amdS gene from *H. jecorina* which encodes the enzyme acetamidase that allows transformant cells to grow on acetamide as a nitrogen source. The selectable marker (e.g. pyrG) may restore the ability of an auxotrophic mutant strain to grow on a selective minimal medium or the selectable marker (e.g. olic31) may confer to transformants the ability to grow in the presence of an inhibitory drug or antibiotic.

[1084] The selectable marker coding sequence is cloned into any suitable plasmid using methods generally employed in the art. Examples of suitable plasmids include pUC18, pBR322, pRAX and pUC100. The pRAX plasmid contains AMA1 sequences from *A. nidulans*, which make it possible to replicate in *A. niger*.

[1085] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Sambrook *et al.*, 1989; Freshney, 1987; Ausubel, *et al.*, 1993; and Coligan *et al.*, 1991.

B. Host Cells and Culture Conditions For GH61 and Variant GH61 Enzyme Production

[1086] After DNA sequences that encode the GH61A variantGH61A variants have been cloned into DNA constructs, the DNA is used to transform microorganisms. The microorganism to be transformed for the purpose of expressing a variant GH61A according to the present invention can be chosen from a wide variety of host cells. The sections below are provided as examples of host cells/microorganisms and are not meant to limit the scope of host cells that can be employed in practicing aspects of the present invention.

(i) Filamentous Fungi

[1087] Aspect of the present invention include filamentous fungi which have been modified, selected and cultured in a manner effective to result in desired GH61 variant production or expression relative to the corresponding non-transformed parental filamentous fungi.

[1088] Examples of species of parental filamentous fungi that may be treated and/or modified
5   for desired glycosyl hydrolase expression include, but are not limited to *Trichoderma*, *Penicillium sp., Humicola sp.*, including *Humicola insolens; Aspergillus sp.*, including *Aspergillus niger, Chrysosporium sp., Myceliophthora sp., Fusarium sp., Hypocrea* sp., *Talaromyces sp., Sporotricum sp,* and *Emericella* sp.

[1089] Cells expressing a desired GH61 variant are cultured under conditions typically
10   employed to culture the parental fungal line. Generally, cells are cultured in a standard medium containing physiological salts and nutrients, such as described in Pourquie, J. et al., Biochemistry and Genetics of Cellulose Degradation, eds. Aubert, J. P. et al., Academic Press, pp. 71-86, 1988 and Ilmen, M. et al., Appl. Environ. Microbiol. 63:1298-1306, 1997. Standard culture conditions are known in the art, *e.g.*, cultures are incubated at 28°C in shaker cultures
15   or fermenters until desired levels of desired GH61 variant expression are achieved.

[1090] Culture conditions for a given filamentous fungus can be found, for example, in the scientific literature and/or from the source of the fungi such as the American Type Culture Collection (ATCC). After fungal growth has been established, the cells are exposed to conditions effective to cause or permit the expression of a desired GH61 variant.

20   [1091] In cases where a desired GH61 variant coding sequence is under the control of an inducible promoter, the inducing agent, *e.g.*, a sugar, metal salt or antibiotic, is added to the medium at a concentration effective to induce expression of the desired GH61 variant.

[1092] In one embodiment, the strain is an *Aspergillus niger* strain, which is a useful strain for obtaining overexpressed protein. For example *A. niger var awamori dgr246* is known to secrete
25   elevated amounts of secreted cellulases (Goedegebuur et al, Curr. Genet (2002) 41: 89-98). Other strains of *Aspergillus niger var awamori* such as GCDAP3, GCDAP4 and GAP3-4 are known Ward et al (Ward, M, Wilson, L.J. and Kodama, K.H., 1993, Appl. Microbiol. Biotechnol. 39:738-743).

[1093] In another embodiment, the strain is a *Trichoderma reesei* strain, which is a useful strain
30   for obtaining overexpressed protein. For example, RL-P37, described by Sheir-Neiss, *et al., Appl. Microbiol. Biotechnol.* 20:46-53 (1984) is known to secrete elevated amounts of cellulase enzymes. Functional equivalents of RL-P37 include *Trichoderma reesei* strain RUT-C30 (ATCC No. 56765) and strain QM9414 (ATCC No. 26921). It is contemplated that these strains would also be useful in overexpressing variant GH61.

35   [1094] Where it is desired to obtain a GH61 variant in the absence of potentially detrimental native glycosyl hydrolase or cellulase activity, it is useful to obtain a host cell strain which has had one or more glycosyl hydrolase genes (e.g., the *gh61a* gene) and/or cellulase genes

deleted prior to introduction of a DNA construct or plasmid containing the DNA fragment encoding the desired GH61 variant. Such strains may be prepared in any convenient manner, for example by the method disclosed in U.S. Patent No. 5,246,853 and WO 92/06209, which disclosures are hereby incorporated by reference. By expressing a desired GH61 variant in a
5   host microorganism that is missing one or more glycosyl hydrolase genes (e.g., the endogenous *gh61a* gene of a host cell), identification and subsequent purification procedures, where desired, are simplified.

[1095] Gene deletion may be accomplished by inserting a form of the desired gene to be deleted or disrupted into a plasmid by methods known in the art. The deletion plasmid is then
10   cut at an appropriate restriction enzyme site(s), internal to the desired gene coding region, and the gene coding sequence or part thereof replaced with a selectable marker. Flanking DNA sequences from the locus of the gene to be deleted or disrupted, for example from about 0.5 to about 2.0 kb may remain on either side of the selectable marker gene. An appropriate deletion plasmid will generally have unique restriction enzyme sites present therein to enable the
15   fragment containing the deleted gene, including flanking DNA sequences, and the selectable marker gene to be removed as a single linear piece.

[1096] In certain embodiments, more than one copy of DNA encoding a desired GH61 variant may be present in a host strain to facilitate overexpression of the GH61 variant. For example, a host cell may have multiple copies of a desired GH61 variant integrated into the genome or,
20   alternatively, include a plasmid vector that is capable of replicating autonomously in the host organism.

(ii) Yeast

[1097] The present invention also contemplates the use of yeast as a host cell for desired GH61 production. Several other genes encoding hydrolytic enzymes have been expressed in
25   various strains of the yeast *S. cerevisiae*. These include sequences encoding for two endoglucanases (Penttila *et al.*, 1987), two cellobiohydrolases (Penttila *et al.*, 1988) and one beta-glucosidase from *Trichoderma reesei* (Cummings and Fowler, 1996), a xylanase from *Aureobasidlium pullulans* (Li and Ljungdahl, 1996), an alpha-amylase from wheat (Rothstein *et al.*, 1987), etc. In addition, a cellulase gene cassette encoding the *Butyrivibrio fibrisolvens*
30   endo- [beta] -1,4-glucanase (END1), *Phanerochaete chrysosporium* cellobiohydrolase (CBH1), the *Ruminococcus flavefaciens* cellodextrinase (CEL1) and the *Endomyces fibrilizer* cellobiase (Bgl1) was successfully expressed in a laboratory strain of *S. cerevisiae* (Van Rensburg *et al.*, 1998).

(iii) Other

[1098] It is further contemplated that in some embodiments, expression systems in host cells other than filamentous fungal cells or yeast cells may be employed, including insect cell or bacterial cell expression systems. Certain of the bacterial host cells can, for example, be one that is also an ethanologen, such as an engineered *Zymomonas mobilis,* which is not only capable of expressing the enzyme(s)/variant(s) of interest but also capable of metabolizing certain monomeric and other fermentable sugars, turning them into ethanol. The selection of a host cell may be determined by the desires of the user of the GH61 variants described herein, and thus no limitation in that regard is intended.

C. Introduction of a Desired GH61-Encoding Nucleic Acid Sequence into Host Cells.

[1099] The invention further provides cells and cell compositions which have been genetically modified to comprise an exogenously provided desired GH61 variant-encoding nucleic acid sequence. A parental cell or cell line may be genetically modified (*e.g.,* transduced, transformed or transfected) with a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc., as further described above.

[1100] The methods of transformation of the present invention may result in the stable integration of all or part of the transformation vector into the genome of the host cell. However, transformation resulting in the maintenance of a self-replicating extra-chromosomal transformation vector is also contemplated.

[1101] Any of the well-known procedures for introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, polybrene, infection, protoplast fusion, electroporation, biolistics, liposomes, microinjection, plasma vectors, viral vectors and any of the other well known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell (*see, e.g.,* Sambrook *et al., supra*). In essence, the particular genetic engineering procedure used should be capable of successfully introducing a polynucleotide (e.g., an expression vector) into the host cell that is capable of expressing the desired GH61 variant.

[1102] Many standard transfection methods can be used to produce *Trichoderma reesei* cell lines that express large quantities of the heterologous polypeptide. Some of the published methods for the introduction of DNA constructs into cellulase-producing strains of *Trichoderma* include Lorito, Hayes, DiPietro and Harman, 1993, Curr. Genet. 24: 349-356; Goldman, VanMontagu and Herrera-Estrella, 1990, Curr. Genet. 17:169-174; Penttila, Nevalainen, Ratto, Salminen and Knowles, 1987, Gene 6: 155-164, for *Aspergillus* Yelton, Hamer and Timberlake, 1984, Proc. Natl. Acad. Sci. USA 81: 1470-1474, for *Fusarium* Bajar, Podila and Kolattukudy,

1991, Proc. Natl. Acad. Sci. USA 88: 8202-8212, for *Streptomyces* Hopwood et al., 1985, The John Innes Foundation, Norwich, UK and for *Bacillus* Brigidi, DeRossi, Bertarini, Riccardi and Matteuzzi, 1990, FEMS Microbiol. Lett. 55: 135-138). An example of a suitable transformation process for *Aspergillus sp.* can be found in Campbell *et al.* Improved transformation efficiency

5   of *A.niger* using homologous *nia*D gene for nitrate reductase. Curr. Genet. 16:53-56; 1989.
[1103] The invention further includes novel and useful transformants of host cells, e.g., filamentous fungi such as *H. jecorina* and *A. niger*, for use in producing fungal cellulase and glycosyl hydrolase compositions. Thus, aspects of the subject invention include transformants of filamentous fungi comprising the desired GH61 variant coding sequence, sometimes also

10   including a deletion or an inactivating mutation of one or more endogenous glycosyl hydrolase coding sequence (e.g., deletion of *gh61a* coding sequence; host cells with deleted glycosyl hydrolyase and/or cellulase genes are also describe in the Examples).
[1104] In addition, heterologous nucleic acid constructs comprising a desired glycosyl hydrolase-encoding nucleic acid sequence can be transcribed *in vitro*, and the resulting RNA

15   introduced into the host cell by well-known methods, *e.g.*, by injection.

D. Analysis For GH61 Nucleic Acid Coding Sequences and/or Protein Expression.

[1105] In order to evaluate the expression of a desired GH61 variant by a cell line that has been transformed with a desired GH61 variant-encoding nucleic acid construct, assays can be

20   carried out at the protein level, the RNA level or by use of functional bioassays particular to GH61 activity and/or production.
[1106] In general, assays employed to analyze the expression of a desired GH61 variant include, but are not limited to, Northern blotting, dot blotting (DNA or RNA analysis), RT-PCR (reverse transcriptase polymerase chain reaction), or *in situ* hybridization, using an

25   appropriately labeled probe (based on the nucleic acid coding sequence) and conventional Southern blotting and autoradiography.
[1107] In addition, the production and/or expression of modified GH61 may be measured in a sample directly, for example, by assays for GH61 activity (cellulase augmenting activity), expression and/or production. Assays in which GH61 cellulase augmenting activity may be

30   assessed are described, for example, in Shoemaker, S. P. and Brown, R. D. Jr. (Biochim. Biophys. Acta, 1978, 523:133 146), Schulein (1988), and U.S. Pat. Nos. 5,246,853 and 5,475,101 each of which is expressly incorporated by reference herein. The ability of modified GH61 to augment the hydrolysis of isolated soluble and insoluble substrates can be measured using assays described in Suurnakki et al. (2000) and Ortega et al. (2001). Substrates useful

35   for assaying augmentation by GH61 on cellobiohydrolase, endoglucanase or β-glucosidase activities include crystalline cellulose, filter paper, phosphoric acid swollen cellulose, hydroxyethyl cellulose, carboxymethyl cellulose, cellooligosaccharides, methylumbelliferyl

lactoside, methylumbelliferyl cellobioside, orthonitrophenyl lactoside, paranitrophenyl lactoside, orthonitrophenyl cellobioside, paranitrophenyl cellobioside, orthonitrophenyl glucoside, paranitrophenyl glucoside, methylumbelliferyl glycoside.

[1108] In addition, protein expression may be evaluated by immunological methods, such as ELISA, competitive immunoassays, radioimmunoassays, Western blot, indirect immunofluorescent assays, and the like. Certain of these assays can be performed using commercially available reagents and/or kits designed for detecting GH61 enzymes. Such immunoassays can be used to qualitatively and/or quantitatively evaluate expression of a desired GH61 variant. The details of such methods are known to those of skill in the art and many reagents for practicing such methods are commercially available. In certain embodiments, an immunological reagent that is specific for a desired variant GH61 enzyme but not its parent GH61 may be employed, e.g., an antibody that is specific for a GH61 substitution or a fusion partner of the GH61 variant (e.g., an N or C terminal tag sequence, e.g., a hexa-Histidine tag or a FLAG tag). Thus, aspects of the present invention include using a purified form of a desired GH61 variant to produce either monoclonal or polyclonal antibodies specific to the expressed polypeptide for use in various immunoassays. (See, e.g., Hu et al., 1991).

V. METHODS FOR ENRICHMENT, ISOLATION AND/OR PURIFICATION OF GH61 VARIANT POLYPEPTIDE

[1109] In general, a desired GH61 variant polypeptide produced in a host cell culture is secreted into the medium (producing a culture supernatant containing the GH61 variant) and may be enriched, purified or isolated, e.g., by removing unwanted components from the cell culture medium. However, in some cases, a desired GH61 variant polypeptide may be produced in a cellular form necessitating recovery from a cell lysate. The desired GH61 variant polypeptide is harvested from the cells or cell supernatants in which it was produced using techniques routinely employed by those of skill in the art. Examples include, but are not limited to, filtration (e.g., ultra- or micro-filtration), centrifugation, density gradient fractionation (e.g., density gradient ultracentrifugation), affinity chromatography (Tilbeurgh et al., 1984), ion-exchange chromatographic methods (Goyal et al., 1991; Fliess et al., 1983; Bhikhabhai et al., 1984; Ellouz et al., 1987), including ion-exchange using materials with high resolution power (Medve et al., 1998), hydrophobic interaction chromatography (Tomaz and Queiroz, 1999), and two-phase partitioning (Brumbauer, et al., 1999).

[1110] While enriched, isolated, or purified GH61 variant polypeptide is sometimes desired, in other embodiments a host cell expressing a GH61 variant polypeptide is employed directly in an assay that requires GH61-mediated cellulase augmenting activity. Thus, enrichment, isolation or purification of the desired GH61 variant polypeptide is not always required to obtain a GH61 variant polypeptide composition that finds use in a cellulosic biomass hydrolysis assay or process. For example, a cellulase-and glycosyl hydrolase-comprising system according to

aspects of the present invention might be designed to allow a host cell that expresses a variant GH61A as described herein to be used directly in a cellulosic hydrolysis process, i.e., without isolation of the GH61A away from the host cell prior to its use in an assay of interest.

VI. UTILITY OF GH61 VARIANTS

5   **[1111]** It can be appreciated that the desired GH61 variant-encoding nucleic acids, the desired GH61 variant polypeptide and compositions comprising the same find utility in a wide variety applications, some of which are described below. The improved property or properties of the GH61 variants described herein can be exploited in many ways. For example, GH61 variants with improved performance under conditions of thermal stress can be used to increase

10   cellulase augmenting activity in assays carried out at high temperatures (e.g., temperatures at which the parent GH61 would perform poorly), allowing a user to reduce the total amount of GH61 employed (as compared to using the parent GH61). Other improved properties of GH61 variant polypeptides can be exploited in assays suitable for determining cellulosic hydrolysis activities of cellulase compositions, including GH61 variants having altered pH optima,

15   increased stability or activity in the presence of surfactants, increased specific activity for a substrate, altered substrate cleavage pattern, and/or high level expression in a host cell of interest.

[1112] GH61 variants as described herein can be used for augmenting the treatment of virtually any cellulosic material, for example for augmenting processes in the textile industry (e.g. in

20   biofinishing or biostoning), in detergents, in animal feed, in the pulp and paper industry and/or bioethanol production.

[1113] Thus, GH61 variant polypeptides as describe herein find use in detergent compositions that exhibit enhanced cleaning ability, function as a softening agent and/or improve the feel of cotton fabrics (*e.g.*, "stone washing" or "biopolishing"), in compositions for degrading wood pulp

25   into sugars (*e.g.*, for bio-ethanol production), and/or in feed compositions. The isolation and characterization of GH61 variants provides the ability to control characteristics and activity of such compositions.

[1114] An enzyme mixture composition containing a desired GH61 variant as described herein finds use in ethanol production. Ethanol from this process can be further used as an octane

30   enhancer or directly as a fuel in lieu of gasoline which is advantageous because ethanol as a fuel source is more environmentally friendly than petroleum derived products. It is known that the use of ethanol will improve air quality and possibly reduce local ozone levels and smog. Moreover, utilization of ethanol in lieu of gasoline can be of strategic importance in buffering the impact of sudden shifts in non-renewable energy and petro-chemical supplies.

35   **[1115]** Separate saccharification and fermentation is a process whereby cellulose present in biomass, e.g., corn stover, is converted to glucose and subsequently yeast strains convert the glucose into ethanol. Simultaneous saccharification and fermentation is a process whereby

cellulose present in biomass is converted to glucose and, at the same time and in the same reactor, yeast strains convert glucose into ethanol. Thus, the GH61 variants of the invention find use in the both of these processes for the degradation of biomass to ethanol. Ethanol production from readily available sources of cellulose provides a stable, renewable fuel source.

5   It is further noted that in some processes, biomass is not fully broken down to glucose (containing, e.g., disaccharides), as such products find uses apart from ethanol production.

[1116] Cellulose-based feedstocks can take a variety of forms and can contain agricultural wastes, grasses and woods and other low-value biomass such as municipal waste (e.g., recycled paper, yard clippings, etc.). Ethanol may be produced from the fermentation of any of

10   these cellulosic feedstocks. As such, a large variety of feedstocks may be used with the inventive desired glycosyl hydrolase(s) and the one selected for use may depend on the region where the conversion is being done. For example, in the Midwestern United States agricultural wastes such as wheat straw, corn stover and bagasse may predominate while in California rice straw may predominate. However, it should be understood that any available cellulosic

15   biomass may be used in any region.

[1117] In another embodiment the cellulosic feedstock may be pretreated. Pretreatment may be by elevated temperature and the addition of dilute acid, concentrated acid or dilute alkali solution. The pretreatment solution is added for a time sufficient to at least partially hydrolyze the hemicellulose components and then neutralized.

20   [1118] In addition to biomass conversion, GH61 variant polypeptides as described herein can be present in detergent compositions which can include any one or more detergent components, e.g., a surfactant (including anionic, non-ionic and ampholytic surfactants), a hydrolase, building agents, bleaching agents, bluing agents and fluorescent dyes, caking inhibitors, solubilizers, cationic surfactants and the like. All of these components are known in

25   the detergent art. The GH61 variant polypeptide-containing detergent composition can be in any convenient form, including liquid, granule, emulsion, gel, paste, and the like. In certain forms (e.g., granules) the detergent composition can be formulated so as to contain a cellulase protecting agent. (see, e.g., WO1997020025 entitled "Enzymatic detergent compositions", incorporated herein by reference). In certain embodiments, the GH61 variant polypeptide is

30   present in the detergent compositions from 0.00005 weight percent to 5 weight percent relative to the total detergent composition, e.g., from about 0.0002 weight percent to about 2 weight percent relative to the total detergent composition.

[1119] As seen from above, GH61 variant polypeptides (and the nucleic acids encoding them) with improved properties as compared to their parent GH61 enzymes find use in improving any

35   of a number of assays and processes that employ glycosyl hydrolases, typically in the presence of at least one cellulase.

EXAMPLES

[1120] The present invention is described in further detain in the following examples, which are not in any way intended to limit the scope of the invention as claimed. The attached Figures are meant to be considered as integral parts of the specification and description of the disclosure. All references cited are herein specifically incorporated by reference for all that is described therein.

[1121] In the experimental disclosure which follows, the following abbreviations apply: M (molar); mM (millimolar); µM (micromolar); nM (nanomolar); mol (moles); mmol (millimoles); µmol (micromoles); nmol (nanomoles); g and 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); NA (not applicable); rpm (revolutions per minute); 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); g (gravity); OD (optical density); ABTS (2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt; HPLC (high pressure liquid chromatography); PAGE (polyacrylamide gel electrophoresis); PCR (polymerase chain reaction); whPCS (whole hydrolystae dilute acid-pretreated corn stover); daCS (diluta ammonia pre-treated corn stover); Pi or PI (performance index); RT-PCR (reverse transcription PCR); TFA (Trifluoroacetic acid); FAB (a certain hybrid beta-glucosidase, described in PCT Publication WO2012/125951); SEC (size-exclusion chromatography); RPC (reverse phase chromatography); and SEL (site evaluation library).

EXAMPLE 1

ASSAYS

[1122] The following assays were used in the examples described below. Any deviations from the protocols provided below are indicated in the examples. In these experiments, a spectrophotometer was used to measure the absorbance of the products formed after the completion of the reactions.

I.   Measurement of Glucose

A. Hexokinase Assay for Measurement of Residual Glucose

[1123] Glucose produced from whPCS was measured using a hexokinase assay. Ten (10) µL of 10X diluted supernatant was added to 190 µL of a glucose hexokinase assay mixture

(Instrumentation Laboratory, Breda, Netherlands) in a 96-well microtiter plate (Costar Flat Bottom PS). The plates were incubated at room temperature for 15 min. Following incubation, absorbance of the supernatant was measured at 340 nm. Supernatants of cultures containing residual glucose were excluded from pooling for further studies.

B. ABTS Assay for Measurement of Glucose

[1124] Monomeric glucose generated in the GH61A Avicel activity assays was detected using the ABTS assay. The assay buffer contained 5.48 g/L 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS, Sigma, catalog no. A1888), 0.2 U/mL horseradish peroxidase Type VI-A (Sigma, catalog no. P8375), and 2 U/mL food grade glucose oxidase (GENENCOR® 5989 U/mL) in 50 mM sodium acetate buffer pH 5.0. Fifty (50) μL GH61A activity assay mix (from the Avicel assay described in VIII below) was added to 50 μL ABTS assay solution. After adding the activity assay mix, the reaction was followed kinetically for 5 min at OD_{420}, at ambient temperature of 22 °C. An appropriate calibration curve of glucose for each assay condition was always included.

C. HPLC assay for glucose concentration determination

[1125] Glucose concentrations were determined using an Agilent 1200 (Agilent Technologies) HPLC Equipped with an REZEX RFQ-Fast Fruit H+ (8%) 100 x 7.8 mm (Phenomenex), The column was operating at 80 °C and a flow rate of 0.9 ml/min with 0.01 N H_2SO_4 as eluent. Thirty (30) μL of sample was mixed with 90 μL of milliQ and filtered under vacuum over a 0.22 μm Millipore Multiscreen HTS 96 well filtration system. Ten (10 μL) of 4 x diluted sample was injected. Appropriate glucose calibration sets were used to determine exact glucose concentrations.

II.   Protein purification and dialysis

[1126] Supernatants from *H. jecorina* (Δ*eg1*, Δ*eg2*, Δ*eg3*, Δ*eg5*, Δ*eg6*, *Δgh61a*, Δ*cbh1*, Δ*cbh2*, Δ*man1*) expressing wild type or variant GH61A were diluted 4X in 1M HEPES pH 8.0 to yield a final volume of 500 μL. The mixture was incubated for 30 minutes, with intermediate mixing (5 times), with 200 μL Biokal Workbead 40 IDA High, charged with 100 mM $CuSO_4$. Purified protein sample, present in the flow through, was obtained by centrifuging for 2 min at 1,000 rpm. Purified samples were dialyzed (40 x) overnight at 4°C to 50 mM sodium acetate, pH 5.0 using "Harvard apparatus 96 well dispo dialyzer" 10kD MWCO (#74-0903) plates.

III.   Protein Determination by Bradford, normalization and endoH treatment

Protein concentrations were determined using the BioRad Bradford assay with BSA as standards. For selected samples the protein concentrations obtained by Bradford were

compared with protein data obtained by SEC HPLC and/or RP HPLC with purified GH61A as a reference. Normalization of the GH61A samples to 100 ppm by diluting appropriately with 50 mM NaAC pH 5.0 was done by taking (if needed) a correction factor between the Bradford and HPLC measurement into account, were the HPLC values were directive. Protein samples were treated with 10 ppm endoH glycosidase from *S. plicatus* (*e.g.*, NEB P0702L).and incubated for 4-20 h at 37 °C and 800 rpm.

IV.   HPLC Assay For Protein Content Determination after normalisation
A. Size Exclusion Chromatography (SEC) for protein determination

[1127] The concentration of endoH treated and normalized GH61A variant was determined by an Agilent 1200 (Agilent Technologies) HPLC equipped with a Waters Acquity BEH125 SEC 1.7 μm (4.6x150 mm) column. Twenty five (25) μL of sample was mixed with 75 μL of milliQ. Ten (10) μL of the sample was injected on the column. Compounds were eluted using NaH_2PO_4 pH 6.75 running isocratic for 4.5 min at a flow of 0.35 mL/min. Proteins were detected at a wavelength of 220 nm. Protein concentrations of GH61A variants were determined from a calibration curve generated using purified wild-type GH61A (3.125, 6.25, 12.5, 25, 50, 100, 200, 400 μg/mL). To calculate performance index (PI), the concentration of a GH61A variant was divided by that of the average wild-type GH61A (e.g., a reference enzyme) in the same plate.

B. Reversed Phase Chromatography (RPC) for protein determination

[1128] The concentration of GH61A variant proteins from purified culture supernatants was determined by an Agilent 1200 (Agilent Technologies) HPLC equipped with a Phenomenex Aeris Widepore 3.6 u XB-C8 (50 x 2.1mm) column. Ninety (90) μL of sample was mixed with 10 μL of 50% acetonitrile. Ten (10) μL of the sample was injected on the column. Compounds were eluted using the following gradient: Eluent A (0 min, 90%); (1.5 min 70%); (3.5 min 55%); (3.6 min 5%); (4.1 min 5%); (4.2 min 90%); (4.5 min 90%). Eluent A was MilliQ + 0.1% TFA and eluent B was acetonitrile + 0.07% TFA. Proteins were detected at a wavelength of 220 nm. Protein concentrations of GH61A variants were determined from a calibration curve generated using purified wild-type GH61A (15.625, 31.25, 62.5, 125, 250, 500 μg/mL). To calculate performance index (PI), the concentration of a GH61A variant was divided by that of the average wild-type GH61A (e.g., a reference enzyme) in the same plate.

V.   Thermostability Assays
A. Avicel assay to measure thermostability (also called "Avicel After")

[1129] Residual activity of GH61A polypeptides (including wild type and variants) after heat incubation was determined using the Avicel assay. Twenty five (25) μL aliquots were incubated

in quadruplicate in a 96-well PCR plate in a PCR machine at 66 ºC for 1 hr. After incubation the residual specific activity of GH61A polypeptides was determined as described below (in section VIII). The relative residual activity of the variants to that of the wild-type enzyme was determined by comparing the averaged specific activity after incubation and the averaged

5   specific activity before incubation.

B. Protein thermal shift assay using SYPRO® Orange and RT-PCR machine (also called "Tm")
[1130] Unfolding of GH61A polypeptide (including wild type and variants) was measured as follows. Twenty five (25) µL GH61A wild type and variant protein sample (non EndoH treated)

10   and 8 µL (1000 X diluted in 50 mM NaAC pH 5.0) SYPRO® orange were mixed in a 96 well Hard Shell Plate (HSP9645 BioRad). The sample was incubated in a BioRad CFX connect RT-PCR machine. Samples were incubated for 1 min at 30 ˚C followed by a gradient from 30 ˚C to 90 ˚C incrementing every 5 seconds with 0.2 ˚C. Every 5 seconds fluorescence data was collected, data was analyzed using the BioRad CFX manager software. The apparent melting

15   temperature Tm of each GH61A variant was determined and compared to the average (median) Tm of the wild type GH61A as per the method described above. The extent of improvements seen in Tm was recorded.

VI.   Whole Hydrolysate Dilute Acid Pretreated Corn Stover (whPCS) Hydrolysis Assay

20   **[1131]** Corn stover was pretreated with 2% w/w H_2SO_4 as described (*see,* Schell et al., *J. Appl. Biochem. Biotechnol.,* 105:69-86, 2003) and titrated to *a* pH of 5.0 with 3M ammonium hydroxide, a final concentration 0.01 % of sodium azide was added for preservation. A sodium acetate buffer (pH 5.0) was then added to get a final concentration of 10% solids. The cellulose concentration in the reaction mixture was about 3% Seventy (70) µL of this cellulose

25   suspension was added per well in a 96-well microtiter plate (Corning Flat bottom non binding PS). Two different methods were employed to measure performance on whPCS: **A)** Fourty seven (47) µL of a 2 g/L enzyme background mixture was added to the whPCS. This enzyme mixture provided CBH:BG:EG enzymes at approximately an 8:1:1 ratio, respectively. Then 22, 11, 5 and 3 µL of a 100 ug/mL purified supernatants from *H. jecorina* cells expressing either

30   wild-type GH61A or a GH61A variant were added to the whPCS /background enzyme mixture. Compensating volumes of sodium acetate buffer were added to make up for the differences in total volume. **B)** Twenty five (25) µL of a 0.225 g/L enzyme background mixture was added to the whPCS. The enzyme background mixture provided CBH:BG:EG enzymes at approximately a 4:1.5:1 ratio, respectively, as well as accessory xylanase and hemicellulase enzymes

35   (representing approximately 5% and 20% of the enzymes in the mixture). Then 25 µL of a 50 ug/mL purified supernatant from *H. jecorina* cells expressing either wild-type GH61A or a

GH61A variant were added to the whPCS /background enzyme mixture. Compensating volumes of sodium acetate buffer were added to make up for the differences in total volume. **[1132]** After sealing, the plates were placed in a thermostatted incubator at 50 ºC with continuous shaking at 900 rpm. After 72 hr the plates were put on ice for 5 min and the

5   hydrolysis reaction was stopped by the addition of 100 µL of 100 mM glycine buffer, pH 10, to each well. The plates were sealed and centrifuged at 3,000 rpm at room temperature for 2 min. The glucose released hydrolysis reaction products in the supernatant were analyzed by Hexokinase and/or the HPLC glucose concentration determination method. A dose response curve was generated for wild-type GH61A enzyme. To calculate performance index (PI), the

10   (average) total sugar produced by a variant GH61A was divided by the (average) total sugar produced by the wild-type GH61A (e.g., a reference enzyme) at the same dose.

VII.   Dilute Ammonia Pretreated Corn Stover (daCS) Hydrolysis Assay

[1133] Corn stover was ground to pass a 0.9 mm screen then pretreated with dilute ammonia in

15   accordance with the description of PCT Publication WO06110901, or the published US Patent Applications 20070031918, 20070031919, 2007-0031953, or 20070037259, and titrated to a pH of 5.0 with 1M H_2SO_4, a final concentration 0.01 % of sodium azide was added for preservation. A sodium acetate buffer (pH 5.0) was then added to get a final concentration of 10% solids. The cellulose concentration in the reaction mixture was about 3%. Seventy (70)

20   µL of this cellulose suspension was added per well in a 96-well microtiter plate (Corning, Flat bottom, non-binding). Twenty five (25) µL of a 0.225 g/L enzyme background mixture was added to the daCS. The enzyme background mixture provided CBH:BG:EG enzymes (at approximately 4:1.5:1 ratio, respectively)as well as accessory xylanase and hemicellulase enzymes (representing approximately 5% and 20% of the enzymes in the mixture).Then 25 µL

25   of a 25 ug/mL purified supernatants from *H. jecorina* cells expressing either wild-type GH61A or a GH61A variant were added to the daCS /background enzyme mixture. Compensating volumes of sodium acetate buffer were added to make up for the differences in total volume. After sealing, the plates were placed in a thermostatted incubator at 50 ºC with continuous shaking at 900 rpm. After 72 hr the plates were put on ice for 5 min and the hydrolysis reaction

30   was stopped by the addition of 100 µL of 100 mM glycine buffer, pH 10, to each well. The plates were sealed and centrifuged at 3,000 rpm at room temperature for 2 min. The glucose released hydrolysis reaction products in the supernatant were analyzed by the HPLC glucose concentration determination method. A dose response curve was generated for wild-type GH61A enzyme. To calculate performance index (PI), the (average) total sugar produced by a

35   variant GH61A was divided by the (average) total sugar produced by the wild-type GH61A (e.g., a reference enzyme) at the same dose.

VIII.   Avicel Activity Assay

[1134] Avicel was diluted with sodium acetate 50 mM (pH 5.0) to achieve a 3.33 % w/v mixture. Seventy five (75) μL of a this suspension was dispensed into a 96-well microtiterplate (Corning Flat bottom non binding PS). Subsequently, 15 μL of 10 mM ascorbic acid, 15 μL 1mM CuCl$_2$

5   and thirty five (35) μL of a 714 μg/mL culture supernatant from a (Δeg1, Δeg2, Δeg3, Δeg5, Δeg6, Δgh61a, , Δcbh1, Δcbh2, ΔMan1) strain expressing FAB (see, PCT publication WO2012/125951) was added to the Avicel solution. Then 10 μL of a 100 μg/mL purified GH61A wild-type or GH61A variant were added to the Avicel/FAB mixture. Each wild type and variant was assayed in quadruplicate. The microtiter plate was sealed and incubated in a

10   thermostatted incubator at 50℃ with continuous shaking at 900 rpm. After 20 hr, the hydrolysis reaction was stopped by the addition of 100 μL 100 mM glycine buffer, pH 10 to each well. The plates were sealed and centrifuged at 3,000 rpm at room temperature for 2 min. The hydrolysis reaction products in the supernatant (also called the GH61A activity assay mix in I.B above) were analyzed by the ABTS assay (see I.B, above). A dose response curve was generated for

15   the wild-type GH61A. To calculate performance index (PI), the (average) total sugar produced by a variant GH61A was divided by the (average) total sugar produced by the wild-type GH61A (e.g., a reference enzyme) at the same dose.

EXAMPLE 2

20   **I.   Generation of *Hypocrea jecorina* GH61A Site Evaluation Libraries ("SELs")**

[1135] *H. jecorina* GH61A enzyme-encoding sequence (SEQ ID NO:1) was cloned into the pTTTpyr2 vector to produce the pTTTpyr2-*GH61A* plasmid (the pTTTpyr2 vector is similar to the pTTTpyrG vector described in PCT publication WO 2011/063308, incorporated herein by reference, except that the pyrG gene is replaced with the pyr2 gene). The amino acid

25   sequence of the full length GH61A enzyme is shown in SEQ ID NO: 2. Using the pTTTpyr2-*GH61A* plasmid or PTTTpyrG, Site Evaluation Libraries (SELs) were generated at all of the sites in the GH61A mature enzyme (SEQ ID NO: 3) except for amino acid positions 236 to 286 and amino acid residue 314 of SEQ ID NO:3. Wild type GH61A enzymes were produced where an identical amino acid was encoded at a site (e.g., H1H, G2G, etc.).

30   [1136] SEQ ID NO:1 below sets forth the reference *H. jecorina GH61A* coding DNA sequence: ATGATCCAGAAGCTTTCCAACCTCCTTGTCACCGCACTGGCGGTGGCTACTGGCGTTGTC GGACATGGACATATTAATGACATTGTCATCAACGGGGTGTGGTATCAGGCCTATGATCCTA CAACGTTTCCATACGAGTCAAACCCCCCCATAGTAGTGGGCTGGACGGCTGCCGACCTTG ACAACGGCTTCGTTTCACCCGACGCATACCAAAACCCTGACATCATCTGCCACAAGAATGC

35   TACGAATGCCAAGGGGCACGCGTCTGTCAAGGCCGGAGACACTATTCTCTTCCAGTGGGT GCCAGTTCCATGGCCGCACCCTGGTCCCATTGTCGACTACCTGGCCAACTGCAATGGTGA CTGCGAGACCGTTGACAAGACGACGCTTGAGTTCTTCAAGATCGATGGCGTTGGTCTCCT

CAGCGGCGGGGATCCGGGCACCTGGGCCTCAGACGTGCTGATCTCCAACAACAACACCT
GGGTCGTCAAGATCCCCGACAATCTTGCGCCAGGCAATTACGTGCTCCGCCACGAGATCA
TCGCGTTACACAGCGCCGGGCAGGCAAACGGCGCTCAGAACTACCCCCAGTGCTTCAAC
ATTGCCGTCTCAGGCTCGGGTTCTCTGCAGCCCAGCGGCGTTCTAGGGACCGACCTCTAT

5   CACGCGACGGACCCTGGTGTTCTCATCAACATCTACACCAGCCCGCTCAACTACATCATC
CCTGGACCTACCGTGGTATCAGGCCTGCCAACGAGTGTTGCCCAGGGGAGCTCCGCCGC
GACGGCCACCGCCAGCGCCACTGTTCCTGGAGGCGGTAGCGGCCCGACCAGCAGAACC
ACGACAACGGCGAGGACGACGCAGGCCTCAAGCAGGCCCAGCTCTACGCCTCCCGCAAC
CACGTCGGCACCTGCTGGCGGCCCAACCCAGACTCTGTACGGCCAGTGTGGTGGCAGCG

10   GTTACAGCGGGCCTACTCGATGCGCGCCGCCAGCCACTTGCTCTACCTTGAACCCCTACT
ACGCCCAGTGCCTTAAC

[1137] SEQ ID NO:2 below sets forth the sequence of the *H. jecorina* GH61A full length enzyme:

MIQKLSNLLVTALAVATGVVGHGHINDIVINGVWYQAYDPTTFPYESNPPIVVGWTAADLDNGF

15   VSPDAYQNPDIICHKNATNAKGHASVKAGDTILFQWVPVPWPHPGPIVDYLANCNGDCETVDK
TTLEFFKIDGVGLLSGGDPGTWASDVLISNNNTWVVKIPDNLAPGNYVLRHEIIALHSAGQANG
AQNYPQCFNIAVSGSGSLQPSGVLGTDLYHATDPGVLINIYTSPLNYIIPGPTVVSGLPTSVAQG
SSAATATASATVPGGGSGPTSRTTTTARTTQASSRPSSTPPATTSAPAGGPTQTLYGQCGGS
GYSGPTRCAPPATCSTLNPYYAQCLN

20   [1138] SEQ ID NO:3 below sets forth the sequence of the *H. jecorina* GH61A mature enzyme:
HGHINDIVINGVWYQAYDPTTFPYESNPPIVVGWTAADLDNGFVSPDAYQNPDIICHKNATNAK
GHASVKAGDTILFQWVPVPWPHPGPIVDYLANCNGDCETVDKTTLEFFKIDGVGLLSGGDPGT
WASDVLISNNNTWVVKIPDNLAPGNYVLRHEIIALHSAGQANGAQNYPQCFNIAVSGSGSLQP
SGVLGTDLYHATDPGVLINIYTSPLNYIIPGPTVVSGLPTSVAQGSSAATATASATVPGGGSGPT

25   SRTTTTARTTQASSRPSSTPPATTSAPAGGPTQTLYGQCGGSGYSGPTRCAPPATCSTLNPY
YAQCLN

[1139] For each of the 178 sites selected, typically 14-16 substitution variants were obtained. The SEL variants were received as individually purified plasmids each encoding a GH61A variant sequence substituted at the indicated position.

30

II.   Production of GH61A Variants

[1140] Protoplasts of *H. jecorina* strain (Δeg1, Δeg2, Δeg3, Δeg5, Δeg6, Δgh61a, Δcbh1, Δcbh2, Δman1) were transformed with the individual pTTTpyr2-*GH61A* or pTTTpyrG-*GH61A*

35   SEL constructs (a single GH61A variant per transformation) and grown on selective agar containing acetamide at 28 ºC for 7 d as previously described in, for example, PCT Patent Application Publication WO 2009/048488 (incorporated herein by reference). Protoplasts of *H.*

jecorina were generated, harvested, replated on acetamide agar, and incubated at 28 °C for 7

d. Spores were harvested in 15% glycerol and stored at -20 °C. For GH61A variant production,

a volume of 10 μL spore suspension was added to 200 μL of a glycine minimal medium

supplemented with 2% glucose/sophorose mixture in a PVDF filter plate. Each GH61A variant

5   was grown in quadruplicate. After sealing the plate with an oxygen permeable membrane, the

plates were incubated at 28 °C for 6 d, with shaking at 220 rpm. Supernatants were harvested

by transferring the culture medium to a microtiter plate under low pressure. Residual glucose

was measured using the hexokinase assay as described in Example 1, section I.A.

10   EXAMPLE 3

Expression, Activity and Performance of GH61A Variants

[1141] *H. jecorina* GH61A SEL variant enzymes were tested for various properties of interest.

In particular, the GH61A variants were tested for protein expression as set forth in Example 1,

section IV.A or B, thermostability as set forth in Example 1, section V.B (Tm), hydrolysis of

15   whPCS as set forth in Example 1, section VI.A, using the Hexokinase Assay for Measurement

of Residual Glucose (whPCS HK), and hydrolysis of whPCS as set forth in Example 1, section

VI.B, using the HPLC assay for glucose concentration determination (whPCS HPLC).

[1142] The performance indices (PI) for each of the GH61A variants tested were determined for

both of the whPCS assays noted above, and at above a certain level of protein production. PI

20   is the ratio of performance of the GH61A variant tested to a reference GH61A (i.e., a GH61A

having the wild type amino acid at that site). PIs that were less than or equal to 0.05 were

generally fixed to 0.05. However, HPLC protein expression values of 0.0 were fixed to 0.04. PI

values for GH61A enzymes with wild type residues were set at 1.00. Improvements in Tm were

demonstrated by comparison of the Tm measurements of the variants with the average Tm

25   measurement of the wild type parent GH61A.

[1143] Below is a listing of GH61A substitution variants having an improved property over wild

type GH61A (for example, a PI > 1.0 for WhPCS HPLC or WhPCS HK, or higher Tm) in at least

one of the assays. The GH61A variants fall into the following SEL Cohorts.

[1144] <u>SEL Cohort 1</u> - GH61A variants having improved PI in whPCS (HPLC): G2C, G2F, G2I,

30   G2N, G2R, G2S, G2T, G2Y, H3F, H3P, H3Q, I4K, N5H, N5I, D6C, D6F, D6H, D6I, D6P, D6R,

D6S, D6V, V8M, N10H, A16D, A16E, A16I, A16N, A16V, A16Y, Y17M, Y17S, Y17V, D18L,

F22A, F22C, F22E, F22N, P23C, P23E, P23Q, P23S, P23T, P23V, P23W, P23Y, Y24C, Y24N,

E25C, E25D, E25V, P28C, P28D, P28E, P28F, P28Q, V32F, V32W, A36C, A36P, A37H,

A37N, A37Y, D38H, D38Q, N41K, D47F, D47I, D47V, A48D, A48R, Y49S, Q50G, Q50N,

35   D53A, D53C, D53L, D53Q, D53R, D53S, D53T, D53V, D53W, D53Y, H57F, H57I, H57K,

H57N, H57P, H57Q, H57S, H57T, H57V, H57W, H57Y, A60C, A60E, A60F, A60L, A60Q,

A60W, T61A, T61D, T61E, T61G, T61M, T61N, T61W, T61Y, N62A, N62C, N62D, N62F,

N62S, N62T, A63D, A63G, A63H, A63R, K64C, K64D, K64G, K64M, K64P, K64S, K64T, G65D, G65F, G65H, G65K, G65L, G65T, G65W, H66C, H66I, H66N, H66Q, H66S, H66V, H66W, A67F, A67G, S68A, K70D, T74V, V80F, P83N, W84F, W84I, W84K, W84Q, W84R, W84S, W84T, W84V, P85A, P85D, P85L, P85M, P85Q, P85S, P85V, P85W, G88P, D92Q,

5   N96I, C97D, C97E, N98Q, G99H, V104A, V104C, V104D, V104E, V104F, V104G, V104H, V104I, V104K, V104L, V104M, V104N, V104P, V104Q, D105Y, K106A, K106C, K106D, K106E, K106F, K106G, K106H, K106L, K106M, K106N, K106Q, K106R, K106Y, T107A, T107C, T107D, T107E, T107F, T107G, T107H, T107I, T107K, T107L, T107M, T107N, T107P, T107Q, T107R, T108A, T108C, T108D, T108E, T108F, T108G, T108H, T108I, T108K, T108L,

10   E110D, G116K, G116M, L119C, L119D, L119E, L119F, L119G, L119N, L119Q, L119W, L120A, L120C, L120D, L120E, L120F, L120G, L120I, S121C, S121D, S121E, S121F, S121G, S121L, S121P, S121Q, G122A, G122C, G122E, G122F, G122L, G122P, G122Y, L133H, V141I, V141P, V142D, V142W, V142Y, K143A, K143D, K143H, P145G, D146K, D146Q, D146Y, N147F, Y153K, V154A, V154C, V154D, V154E, V154G, V154H, V154M, V154P,

15   V154Q, V154T, R156A, R156C, R156D, R156E, R156F, R156H, R156I, R156K, R156L, R156M, R156P, R156Q, E158A, E158C, E158D, E158F, E158H, E158I, E158M, E158N, E158P, E158Q, E158T, H163A, H163C, H163C, H163D, H163D, H163E, H163F, H163F, H163G, H163I, H163K, H163M, H163P, H163T, H163W, S164F, S164H, G166P, A168C, Q172G, Q172I, Q172M, Y174E, Y174H, Y174I, Y174K, Y174L, Y174M, C177M, C177Y,

20   F178C, F178Q, S185C, S185M, G186D, S187C, S187G, S191E, S191H, G192C, G192D, G192E, G192F, G192H, G192I, G192K, G192L, G192M, G192N, G192Q, G192R, G192T, G192V, V193D, L194N, G195D, G195M, T196G, D197C, D197D, D197F, D197G, Y199A, Y199C, Y199D, Y199F, Y199G, Y199H, Y199K, Y199N, Y199P, Y199Q, Y199T, Y199W, H200A, H200D, H200E, H200M, H200N, A201C, A201E, A201F, A201M, T202A, T202D,

25   T202E, T202G, T202L, T202N, T202W, Y211C, Y211I, Y211M, Y211Q, N216A, N216C, N216E, N216H, N216L, N216W, N216Y, I218W, I219E, I219H, I219K, G221A, G221D, G221I, G221S, G221V, P222F, P222H, P222L, P222M, P222S, P222V, T223C, T223F, T223I, T223M, V225A, V225C, V225F, V225H, S226E, S226T, G227C, G227N, G227T, L228G, L228N, L228T, P229C, P229I, P229T, T230D, T230V, V232C, V232H, V232L, A233C, A233D, A233E,

30   A233W, G235A, G235D, G235E, G235F, G235H, G235K, G235L, G235P, G235S, G235Y, Q288Y, L290A, C294R, G295C, G295D, G295E, G295K, G295M, G295N, G295S, G295T, G295V, G295W, G295Y, S297C, S297D, S297E, S297F, S297G, S297H, S297I, S297K, S297L, S297M, S297P, S297R, S297W, S297Y, G298A, G298C, G298E, G298F, G298H, G298I, G298P, G298W, G298Y, Y299C, C305A, C305E, C305G, C305K, C305L, C305M,

35   C305N, C305P, C305Q, C305S, C305T, C305V, C305W, C305Y, S312P, S312W, T313W, N315P, P316W, Q320I, Q320L, Q320M, C321G, C321I, C321M, C321P, C321Q, C321R, and C321V.

[1145] <u>Cohort 2</u> - GH61A variants having improved PI in whPCS (HK): H1D, H1K, G2C, G2D, G2F, G2I, G2L, G2M, G2N, G2Q, G2R, G2S, G2T, G2Y, H3F, H3I, H3K, H3L, H3P, H3Q, H3V, I4D, I4F, I4K, I4M, I4N, I4Q, N5C, N5D, N5F, N5H, N5I, N5K, N5M, N5P, N5R, N5S, N5T, N5V, D6C, D6F, D6G, D6H, D6I, D6K, D6L, D6M, D6P, D6Q, D6S, D6T, D6V, D6Y, V8I, V8L, N10H, N10M, G11D, G11W, V12I, W13A, W13D, W13E, W13H, W13K, W13L, W13P, Y14F, Y14M, Y14Q, Q15H, Q15R, Q15Y, A16C, A16D, A16I, A16M, A16N, A16Q, A16S, A16T, Y17G, Y17H, Y17I, Y17L, Y17M, Y17N, Y17P, Y17R, Y17S, Y17V, D18A, D18C, D18F, D18I, D18K, D18L, D18M, D18N, D18R, D18Y, P19E, P19H, P19W, T20F, T20G, T20L, T21C, T21F, T21H, T21I, T21K, T21L, T21N, T21Q, T21Y, F22A, F22C, F22D, F22E, F22I, P23C, P23D, E25C, E25D, P28A, P28C, P28D, P28F, P28G, P28K, P28W, I30K, V32A, V32C, V32D, V32L, V32W, G33D, W34A, W34D, W34H, W34K, W34N, T35D, T35G, T35K, A37I, A37R, D38A, D40I, N41H, G42C, G42K, F43A, F43D, F43Y, D47C, D47F, D47V, A48D, A48R, A48T, Y49D, Y49G, Y49P, Q50S, I55G, I55M, I55Y, K58A, K58D, K58E, K58F, K58R, K58V, K58W, N59F, N59K, N59P, N59V, A60C, A60D, A60L, A60Q, T61D, T61E, T61G, T61M, N62A, N62C, N62D, N62F, N62L, A63C, A63D, A63E, A63F, A63G, A63H, A63I, A63L, A63M, A63R, K64A, K64G, K64I, K64M, G65D, G65H, G65K, G65M, G65Y, H66M, H66N, H66S, A67H, S68A, S68D, S68H, S68N, S68V, V69D, V69E, V69G, V69W, K70A, K70C, K70D, K70E, K70I, K70L, K70M, K70N, K70Q, K70T, K70W, A71E, G72A, G72D, G72E, G72F, G72H, G72I, G72L, G72M, G72N, G72P, G72Q, G72T, G72W, D73A, D73C, D73H, D73L, D73Q, D73T, D73Y, I75K, L76A, L76C, L76C, L76D, L76E, L76E, L76F, L76F, L76G, L76H, L76I, L76N, F77A, F77E, F77H, Q78D, Q78F, Q78H, W79E, P83N, H86D, H86N, G88C, G88N, G88R, P89D, D92A, D92C, D92F, D92T, N96C, N96I, N96K, C97A, C97D, C97E, C97G, C97H, C97I, C97K, C97L, C97M, N98A, N98C, N98D, N98E, N98G, N98I, N98K, N98L, N98P, N98Q, N98S, G99A, G99C, G99D, G99E, G99H, G99I, G99K, G99L, G99N, D100A, D100F, D100G, D100N, D100P, D100Q, D100S, D100W, C101A, C101F, C101W, E102A, E102H, E102K, E102L, E102N, E102P, E102S, V104A, V104C, V104E, V104G, V104H, V104I, V104K, V104L, V104N, D105A, D105C, D105E, D105G, D105H, D105I, D105K, D105L, D105M, D105N, D105Q, D105S, D105W, D105Y, K106C, K106D, K106E, K106F, K106G, K106R, K106Y, T107A, T107C, T107F, T107H, T107I, T107L, T107N, T108A, T108C, T108E, T108K, E110F, E110H, E110I, E110L, E110P, F112R, F112W, K113H, K113P, K113Q, I114Q, G116E, G116I, G116K, G116L, G116M, L119C, L119D, L119H, L119K, L119M, L119P, L119Q, L119Q, L119W, L120C, L120E, L120F, L120H, L120I, L120K, L120P, L120Q, L120R, L120T, L120W, S121A, S121D, S121E, S121F, S121Q, S121Q, S121R, G122C, G123H, W128S, A129H, A129N, A129R, A129V, A129W, S130C, S130E, S130F, S130M, S130R, V132D, L133Y, S135F, S135H, N136A, N136E, N136Q, N136R, N137C, T139A, T139G, T139V, W140A, W140H, V141G, V141N, V142F, V142H, V142L, K143A, K143F, K143Q, K143V, I144K, I144M, I144R, I144S, P145G, P145H, P145I, P145K, P145R, P145S, P145V, D146A, D146C, D146E, D146F,

D146K, D146M, D146T, N147F, N147M, L148K, L148V, A149C, A149D, A149F, A149G,

A149I, A149N, A149V, P150A, P150C, P150D, P150E, P150F, P150G, P150H, P150I, P150K,

P150L, P150Q, G151E, G151F, G151H, G151I, G151K, G151M, G151P, G151Q, G151S,

G151V, G151W, G151Y, N152E, N152F, N152G, N152H, N152K, N152P, Y153F, Y153K,

5 Y153L, Y153M, Y153P, Y153Q, Y153R, Y153S, Y153V, V154A, V154I, V154K, V154N,

V154R, L155C, L155F, L155K, L155M, L155N, R156C, R156D, R156H, R156I, R156K,

R156Q, H157D, E158A, E158D, E158F, E158L, E158N, E158Q, E158S, E158W, A161C,

L162I, L162N, H163C, H163D, H163F, H163P, H163R, H163T, H163V, H163W, H163Y,

S164C, S164G, S164M, S164N, A168K, A168N, N169A, N169D, N169H, N169K, N169Q,

10 N169T, G170F, G170H, G170I, G170W, A171C, A171I, A171K, A171L, A171R, A171W,

Q172G, Q172I, Q172M, Q172N, Q172P, Q172R, Q172S, Q172T, Q172V, Q172W, Q172Y,

N173I, N173M, Y174A, Y174D, Y174E, Y174F, Y174G, Y174H, Y174I, Y174K, Y174L, Y174M,

Y174N, Y174P, Y174Q, Y174R, Y174T, P175F, Q176N, C177D, C177M, C177R, F178A,

F178C, F178L, F178N, F178Q, N179A, N179H, I180F, I180H, I180N, I180S, S183D, S183R,

15 S185A, S185C, S185D, S185E, S185H, S185I, S185K, S185L, S185M, S185N, S185Q,

S185R, S185W, S185Y, G186A, G186C, G186D, G186E, G186F, G186H, G186I, G186K,

G186L, G186N, G186P, G186Q, G186R, G186T, G186W, G186Y, S187A, S187C, S187D,

S187E, S187G, S187I, S187M, S187N, S187P, S187Q, L188I, L188P, Q189A, Q189G, S191E,

S191H, G192A, G192C, G192D, G192E, G192F, G192H, G192I, G192K, G192L, G192M,

20 G192N, G192Q, G192R, G192T, G192V, G192W, G192Y, V193C, V193D, V193E, V193T,

V193W, L194A, L194D, G195A, G195D, D197C, D197F, D197G, L198E, L198K, Y199A,

Y199C, Y199D, Y199E, Y199F, Y199G, Y199H, Y199I, Y199K, Y199L, Y199N, Y199P, Y199T,

Y199W, H200C, H200I, H200K, H200M, H200N, H200P, A201C, A201H, A201I, A201L,

A201V, T202D, T202N, T202W, D203G, D203N, P204F, P204H, G205M, I208F, I208G, I208H,

25 I208M, N209A, N209D, N209F, N209G, N209I, N209K, N209L, N209S, N209T, N209V, I210A,

I210E, I210F, I210H, I210L, I210M, Y211C, Y211F, Y211H, Y211I, Y211L, Y211M, Y211P,

Y211Q, Y211T, Y211W, T212K, T212R, S213N, P214C, P214I, P214N, L215C, L215I, L215M,

L215P, N216C, N216W, I219E, I219K, I219V, P220K, P220W, G221C, P222F, P222I, P222V,

T223A, V225F, S226V, G227L, L228G, P229C, T230A, S231A, S231C, S231D, S231G,

30 S231H, S231K, S231L, S231M, S231N, S231R, V232H, V232I, V232Q, V232W, A233E,

A233G, A233W, G235D, G235E, G235F, G235H, G235K, G235L, G235N, G235P, G235T,

G235Y, T287A, T287C, T287E, T287I, T287K, T287L, T287M, T287P, T287Q, T287R, T287V,

Q288L, Q288Y, L290A, L290C, L290D, L290F, L290H, L290K, L290M, L290N, L290P, L290S,

L290V, G292I, Q293G, Q293M, Q293W, C294L, G295A, G295E, G295H, G295I, G295K,

35 G295M, G295N, G295S, G295V, G295W, G295Y, G296C, G296K, S297C, S297F, S297G,

S297H, S297I, S297L, S297M, S297N, S297P, S297Q, S297T, S297W, S297Y, G298C,

G298D, G298E, G298F, G298L, G298P, G298S, G298W, G298Y, Y299A, S300C, S300H,

S300K, P302W, R304A, R304D, R304S, C305A, C305G, C305K, C305M, C305N, C305P,

C305Q, C305R, C305S, C305V, C305W, C305Y, A306M, P307H, P307I, S312N, S312P,

T313N, T313Q, T313R, N315C, N315G, N315I, N315L, N315P, N315T, N315V, P316A,

P316E, P316G, P316N, P316Q, P316W, Y317D, Y317H, Y317I, Y318T, A319A, A319G,

5   A319H, A319I, A319N, A319R, Q320N, Q320V, C321H, C321M, C321P, C321V, L322F,

L322R, N323L, and N323T.

[1146] <u>Cohort 3</u> - GH61A variants having improved Tm: H1G, H1P, H1T, G2A, G2E, G2I, G2P,

G2Q, G2V, G2W, H3C, H3D, H3E, H3K, H3N, H3R, H3S, H3T, I4A, I4C, I4G, I4P, I4T, I4T,

I4V, I4V, I4Y, N5C, N5D, D6G, D6V, D6W, D6Y, I7L, I9C, I9I, I9V, N10L, N10N, N10Q, N10S,

10   N10V, V12F, V12I, V12M, V12T, W13C, W13I, W13K, W13V, Y14R, A16N, P19I, T20I, T20L,

T20M, T21D, T21I, T21K, F22L, F22R, P23G, P23R, Y24K, E25D, E25K, E25N, S26A, S26D,

S26E, S26I, S26K, S26L, S26M, S26N, S26P, S26Q, S26R, S26T, N27E, N27L, P28L, P29A,

P29C, P29D, P29E, P29F, P29G, P29I, P29K, P29L, P29M, P29N, P29Q, P29R, P29S, P29T,

P29V, P29W, P29Y, V32P, T35M, A36D, A36E, A36F, A36G, A36N, A36Y, A37D, A37L,

15   A37W, D38N, D38P, L39F, L39I, L39V, L39Y, D40G, D40I, N41D, N41E, N41F, N41Q, N41T,

N41V, F43Y, V44K, V44N, V44Q, V44Y, S45H, S45R, S45Y, P46H, P46I, P46M, P46Q, D47N,

D47P, D47R, A48H, A48R, Y49Y, Q50T, N51C, N51E, N51F, N51G, N51H, N51K, N51L,

N51M, N51Q, N51R, N51S, N51T, N51Y, P52N, D53H, D53V, I54E, I54Q, I55I, I55M, I55V,

C56G, C56R, K58M, K58V, N59E, N59Q, N59S, A60H, A60L, A60N, A60R, T61A, T61Q,

20   T61S, N62C, N62I, N62K, N62P, N62S, A63C, A63K, K64L, K64Q, H66W, S68F, S68G, S68L,

S68Q, S68T, S68Y, K70H, K70I, K70N, K70Q, K70R, K70S, K70T, K70V, K70Y, A71Y, G72A,

G72K, D73A, D73E, D73N, D73Q, D73S, F77P, Q78H, Q78L, Q78M, W79A, W79D, V80I,

V80K, V82I, V82L, V82Y, P83Y, W84D, P85K, P85R, P85T, H86Y, P87E, G88D, G88N, P89A,

P89C, I90V, V91G, V91L, V91P, V91R, V91T, D92G, D92H, D92M, D92P, D92V, Y93D, Y93H,

25   Y93N, Y93P, Y93S, L94T, A95W, N96A, N98H, N98P, N98W, G99E, G99Y, D100F, D100I,

D100L, D100N, D100P, D100Q, D100Y, C101M, E102E, T103A, T103C, T103D, T103F,

T103M, T103N, T103Q, T103S, T103V, T103Y, D105H, D105I, D105V, K106I, K106R, K106V,

L109Q, L109T, L109V, E110E, E110K, E110M, E110Q, E110R, E110Y, F111A, F112F, K113L,

K113T, D115C, D115E, D115I, D115L, V117R, L119N, L120P, L120R, L120V, S121Y, G123A,

30   G123P, G123Q, G123S, G123T, D124N, D124Q, D124S, D124W, G126M, G126Q, G126Y,

T127F, T127I, T127K, T127L, T127M, T127N, T127R, T127V, T127W, T127Y, W128H,

W128L, W128Q, A129C, A129E, A129F, A129N, A129S, A129V, S130C, S130E, S130H,

S130I, S130L, S130N, S130P, S130Q, S130T, D131K, D131R, I134K, I134M, I134V, S135A,

S135E, S135H, S135M, N137I, N137P, N138S, T139M, T139N, T139Q, T139V, W140C,

35   W140F, V142I, V142T, V142Y, K143C, K143M, K143W, I144G, I144V, D146F, L148P, A149P,

G151Q, G151V, N152F, N152F, N152G, N152Q, N152S, N152Y, V154V, L155L, L155M,

R156S, H157H, H157W, E158W, E158Y, I159Q, I160D, I160F, A161E, A161L, A161Y, L162A,

L162F, H163L, H163Q, H163R, H163Y, S164A, S164H, S164K, S164L, S164M, S164N, S164Q, S164R, S164V, S164W, S164Y, A165M, A165N, A165P, A165Y, G166A, G166F, G166H, G166K, G166L, G166M, G166N, G166Q, G166R, G166S, G166T, G166V, G166W, Q167A, Q167F, Q167I, Q167K, Q167L, Q167M, Q167N, Q167R, Q167S, Q167V, Q167W,

5   Q167Y, A168E, A168F, A168H, A168I, A168K, A168L, A168M, A168N, A168P, A168Q, A168R, A168S, A168T, A168V, A168Y, N169N, G170P, A171H, Q172H, N173N, P175F, P175H, Q176C, Q176Q, C177S, C177V, N179C, N179M, I180L, I180M, I180T, A181E, A181H, A181K, A181L, A181N, A181R, A181S, V182G, V182N, V182R, V182T, V182Y, S183A, S183D, S183E, S183H, S183I, S183K, S183N, S183P, S183R, S183T, S183V, S183Y,

10   G184H, G184N, G184R, S185D, S185F, S185G, S185H, S185I, S185K, S185L, S185N, S185P, S185Q, S185T, S185V, G186L, G186P, S187A, S187D, S187E, S187H, S187K, S187L, S187M, S187N, S187Q, S187R, S187T, S187V, S187W, L188M, Q189D, Q189E, Q189R, P190R, S191M, S191P, S191Q, G192S, G192Y, V193F, L194D, L194M, L194Q, G195I, G195K, G195L, G195N, G195R, G195S, G195V, T196L, T196N, T196Q, T196V, D197I,

15   D197L, D197M, D197P, D197Q, D197T, D197V, L198M, L198Y, H200C, H200I, H200R, A201G, A201Q, T202A, T202E, T202H, T202Q, T202S, D203K, D203M, G205H, V206I, V206T, L207F, L207M, L207W, L207Y, I208V, N209D, Y211G, T212L, S213K, S213R, S213T, S213W, S213Y, N216D, N216I, N216K, N216M, N216Q, N216T, Y217D, Y217G, Y217H, Y217M, Y217Q, Y217T, I218Q, I218T, I218V, I219M, I219T, T223Q, T223S, S226D, S226L,

20   S226M, S226N, S226P, S226Q, T230W, S231W, S231Y, A233F, A233Q, A233R, A233S, G235L, T287A, T287C, T287F, T287G, T287H, T287I, T287S, Q288A, Q288C, Q288D, Q288E, Q288F, Q288G, Q288I, Q288K, Q288L, Q288M, Q288N, Q288R, Q288S, Q288T, Q288V, Q288W, Q288Y, T289A, T289C, T289D, T289F, T289I, T289K, T289L, T289M, T289N, T289R, T289S, T289V, T289Y, L290A, L290I, L290K, L290M, L290S, L290T, Y291A, Y291C,

25   Y291H, Y291I, Y291M, Y291N, Y291P, Y291S, Y291T, Y291V, Y291W, G292L, G292M, G292P, G292S, G292T, G292W, Q293T, Q293W, Q293Y, G295E, G296A, G296C, G296F, G296I, G296L, G296M, G296P, G296R, G296S, G296T, G296V, G296Y, S297W, G298I, G298P, Y299C, Y299E, Y299F, Y299G, Y299H, Y299I, Y299K, Y299L, Y299N, Y299P, Y299S, S300C, S300E, S300F, S300L, S300Q, S300Q, S300T, G301A, G301D, G301K, G301M,

30   G301P, G301T, G301V, G301Y, P302M, P302R, P302T, P302Y, T303A, T303G, T303H, T303I, T303K, T303M, T303N, T303P, T303Q, T303R, T303S, T303V, T303Y, R304A, R304C, R304D, R304E, R304G, R304H, R304I, R304L, R304M, R304N, R304Q, R304S, R304T, R304V, R304Y, C305E, C305L, C305M, C305N, C305P, C305Q, C305R, C305S, C305T, C305V, C305W, C305Y, A306D, A306E, A306F, A306G, A306H, A306I, A306K, A306L,

35   A306M, A306N, A306P, A306Q, A306R, A306S, A306T, A306V, A306W, A306Y, P307A, P307C, P307F, P307G, P307H, P307L, P307M, P307N, P307Q, P307R, P307S, P307T, P307V, P307Y, P308C, P308D, P308G, P308H, P308K, P308L, P308M, P308Q, P308R,

P308S, P308T, P308V, P308W, P308Y, A309L, A309P, A309S, A309V, T310F, T310I, T310L, T310M, T310P, T310R, T310S, T310V, T310W, T310Y, S312C, S312F, S312I, S312L, S312M, S312N, S312P, S312Q, S312R, S312T, S312V, S312W, S312Y, T313F, T313M, T313P, T313T, T313V, T313W, T313Y, N315M, N315S, P316A, P316C, P316D, P316F, P316H,

5   P316I, P316L, P316N, P316Q, P316R, P316S, P316T, P316V, P316W, Y317A, Y317E, Y317P, Y317Q, Y317S, Y317T, Y318Q, Y318T, A319D, A319E, A319F, A319I, A319L, A319M, A319Q, A319R, A319S, A319T, A319V, A319W, Q320A, Q320C, Q320H, Q320I, Q320L, Q320M, Q320P, Q320R, Q320S, Q320T, Q320Y, C321E, C321L, C321M, C321P, C321Q, C321V, C321Y, L322S, L322T, L322V, L322W, N323M, N323P, N323S, N323T, and N323V.

10   [1147] Any one of the GH61A variants from cohorts 1, 2 or 3 find use in the subject invention. Certain GH61A variants are present in more than one SEL Cohort, and are of particular interest as GH61A variants according to aspects of the subject invention, e.g., GH61A variants that are present in SEL Cohorts 1 and 2, SEL Cohorts 1 and 3, SEL Cohorts 2 and 3, or SEL Cohorts 1, 2 and 3. Further, any combination of substitutions from the GH61A variants in SEL Cohorts 1,

15   2, and/or 3 can be used to generate a combinatorial GH61A variant (i.e., a GH61A variant having more than one substitution). Combinatorial GH61A variants are described in further detail below.

[1148] In addition to the GH61A variants in SEL Cohorts 1, 2 and 3 above, GH61A substitution variants were identified that, while not having an improved property as compared to wild-type

20   GH61A, displayed wild-type levels of activity in either the whPCS (HPLC) assay, the whPCS (HK) assay, or both. These GH61A variants are as follows, forming what is referred to herein as the **Wild-Type SEL Cohort**: H1A, H1C, H1E, H1F, H1I, H1L, H1M, H1N, H1Q, H1R, H1S, H1V, H1W, H1Y, G2H, G2K, H3A, H3G, H3M, H3W, H3Y, I4A, I4C, I4D, I4E, I4F, I4G, I4H, I4I, I4K, I4L, I4M, I4N, I4P, I4Q, I4R, I4S, I4W, N5A, N5E, N5G, N5L, N5Q, N5W, N5Y, D6A, D6E,

25   D6N, I7A, I7C, I7D, I7F, I7G, I7H, I7K, I7M, I7N, I7P, I7Q, I7R, I7S, I7T, I7V, I7W, I7Y, V8A, V8C, V8D, V8E, V8F, V8G, V8H, V8K, V8N, V8P, V8Q, V8R, V8S, V8T, V8W, V8Y, I9A, I9D, I9E, I9F, I9G, I9H, I9K, I9L, I9M, I9N, I9P, I9Q, I9R, I9S, I9T, I9W, I9Y, N10A, N10C, N10D, N10E, N10F, N10G, N10I, N10K, N10P, N10R, N10T, N10W, N10Y, G11A, G11C, G11E, G11F, G11H, G11I, G11K, G11L, G11M, G11N, G11P, G11Q, G11R, G11S, G11T, G11V,

30   G11Y, V12A, V12C, V12D, V12E, V12G, V12H, V12K, V12L, V12N, V12P, V12Q, V12R, V12S, V12W, V12Y, W13F, W13G, W13M, W13N, W13Q, W13R, W13S, W13T, W13Y, Y14C, Y14E, Y14G, Y14H, Y14I, Y14K, Y14L, Y14N, Y14P, Y14S, Y14T, Y14V, Y14W, Q15A, Q15C, Q15D, Q15E, Q15F, Q15G, Q15I, Q15K, Q15L, Q15M, Q15N, Q15P, Q15S, Q15T, Q15V, Q15W, A16F, A16G, A16H, A16K, A16L, A16P, A16R, A16W, Y17A, Y17C, Y17D, Y17E, Y17F, Y17K,

35   Y17Q, Y17T, Y17W, D18E, D18G, D18H, D18P, D18Q, D18S, D18T, D18V, D18W, P19A, P19C, P19D, P19F, P19G, P19K, P19L, P19M, P19N, P19Q, P19R, P19S, P19T, P19V, P19Y, T20A, T20C, T20D, T20E, T20H, T20K, T20N, T20P, T20R, T20S, T20V, T20W, T20Y, T21A,

T21E, T21G, T21M, T21P, T21R, T21S, T21V, T21W, F22G, F22H, F22K, F22M, F22P, F22Q,

F22S, F22T, F22V, F22W, F22Y, P23A, P23F, P23H, P23I, P23K, P23L, P23M, P23N, P23T,

Y24D, Y24E, Y24F, Y24G, Y24H, Y24I, Y24L, Y24M, Y24P, Y24Q, Y24R, Y24S, Y24T, Y24V,

Y24W, E25A, E25F, E25G, E25H, E25I, E25L, E25M, E25P, E25Q, E25R, E25S, E25T, E25W,

5 E25Y, S26C, S26F, S26G, S26H, S26V, S26W, S26Y, N27A, N27C, N27D, N27F, N27G,

N27H, N27I, N27K, N27M, N27P, N27Q, N27R, N27S, N27T, N27V, N27W, N27Y, P28H, P28I,

P28M, P28N, P28R, P28S, P28T, P28V, P28Y, P29H, I30A, I30C, I30D, I30E, I30F, I30G,

I30H, I30L, I30M, I30N, I30P, I30Q, I30R, I30S, I30T, I30V, I30W, I30Y, V31A, V31C, V31D,

V31E, V31F, V31G, V31H, V31I, V31K, V31L, V31M, V31N, V31P, V31Q, V31R, V31S, V31T,

10 V31W, V31Y, V32E, V32G, V32H, V32I, V32K, V32M, V32N, V32Q, V32R, V32S, V32T, V32V,

V32Y, G33A, G33C, G33E, G33F, G33H, G33I, G33K, G33L, G33M, G33N, G33P, G33Q,

G33R, G33S, G33T, G33V, G33W, G33Y, W34C, W34E, W34F, W34G, W34I, W34L, W34M,

W34P, W34Q, W34R, W34S, W34T, W34V, W34Y, T35A, T35C, T35E, T35F, T35H, T35I,

T35L, T35N, T35P, T35Q, T35R, T35S, T35V, T35W, T35Y, A36H, A36I, A36K, A36L, A36M,

15 A36Q, A36R, A36S, A36T, A36V, A37A, A37C, A37E, A37F, A37G, A37K, A37M, A37P, A37Q,

A37S, A37T, A37V, D38C, D38E, D38F, D38G, D38I, D38K, D38L, D38M, D38R, D38S, D38T,

D38V, D38W, D38Y, L39A, L39C, L39D, L39E, L39G, L39H, L39K, L39M, L39N, L39P, L39Q,

L39R, L39S, L39T, L39W, D40A, D40C, D40E, D40F, D40H, D40K, D40L, D40M, D40N, D40P,

D40Q, D40R, D40S, D40T, D40V, D40W, D40Y, N41A, N41C, N41G, N41I, N41L, N41M,

20 N41P, N41R, N41S, N41W, N41Y, G42A, G42D, G42E, G42F, G42H, G42I, G42L, G42M,

G42N, G42P, G42Q, G42R, G42S, G42T, G42V, G42W, G42Y, F43C, F43E, F43G, F43H,

F43I, F43K, F43L, F43M, F43N, F43P, F43Q, F43R, F43S, F43T, F43V, F43W, V44A, V44C,

V44D, V44E, V44F, V44G, V44H, V44I, V44L, V44M, V44P, V44R, V44S, V44T, V44W, S45A,

S45C, S45D, S45E, S45F, S45G, S45I, S45K, S45L, S45M, S45N, S45P, S45Q, S45T, S45V,

25 S45W, P46A, P46C, P46D, P46E, P46F, P46G, P46K, P46L, P46N, P46R, P46S, P46T, P46V,

P46W, P46Y, D47A, D47E, D47G, D47H, D47K, D47L, D47M, D47Q, D47S, D47T, D47W,

D47Y, A48C, A48E, A48F, A48G, A48I, A48K, A48L, A48M, A48N, A48P, A48Q, A48S, A48V,

A48W, A48Y, Y49A, Y49C, Y49E, Y49F, Y49H, Y49I, Y49K, Y49L, Y49M, Y49N, Y49Q, Y49R,

Y49T, Y49V, Y49W, Q50A, Q50C, Q50D, Q50E, Q50F, Q50H, Q50I, Q50K, Q50L, Q50M,

30 Q50P, Q50R, Q50V, Q50W, Q50Y, N51A, N51D, N51I, N51P, N51V, N51W, P52A, P52C,

P52D, P52E, P52F, P52G, P52H, P52I, P52K, P52L, P52M, P52Q, P52R, P52S, P52T, P52V,

P52W, P52Y, D53I, D53P, I54A, I54C, I54D, I54F, I54G, I54H, I54K, I54L, I54M, I54N, I54P,

I54R, I54S, I54T, I54V, I54W, I54Y, I55A, I55C, I55D, I55E, I55F, I55H, I55K, I55L, I55N, I55P,

I55Q, I55R, I55S, I55T, I55W, C56A, C56D, C56E, C56F, C56H, C56I, C56K, C56L, C56M,

35 C56N, C56P, C56Q, C56S, C56T, C56V, C56W, C56Y, H57A, H57C, H57D, H57G, H57L,

H57M, H57R, K58C, K58G, K58H, K58I, K58L, K58N, K58P, K58Q, K58S, K58T, K58Y, N59A,

N59C, N59D, N59G, N59H, N59I, N59L, N59M, N59R, N59T, N59W, N59Y, A60G, A60I, A60K,

A60M, A60P, A60S, A60T, A60V, A60Y, T61C, T61F, T61H, T61I, T61K, T61L, T61P, T61R,
T61V, N62E, N62G, N62H, N62M, N62Q, N62R, N62V, N62W, N62Y, A63P, A63Q, A63S,
A63T, A63V, A63W, A63Y, K64E, K64F, K64H, K64N, K64R, K64V, K64W, K64Y, G65A,
G65C, G65E, G65I, G65N, G65P, G65Q, G65R, G65S, G65V, H66A, H66D, H66E, H66F,

5   H66G, H66K, H66L, H66P, H66R, H66T, H66Y, A67C, A67D, A67E, A67I, A67K, A67L, A67M,
A67N, A67P, A67Q, A67R, A67S, A67T, A67V, A67W, A67Y, S68C, S68E, S68I, S68K, S68M,
S68P, S68R, S68W, V69A, V69C, V69F, V69H, V69I, V69K, V69L, V69M, V69N, V69P, V69Q,
V69R, V69S, V69T, V69Y, K70F, K70G, K70P, A71C, A71D, A71F, A71G, A71H, A71I, A71K,
A71L, A71M, A71N, A71P, A71Q, A71R, A71S, A71T, A71V, A71W, G72C, G72R, G72S,

10   G72V, G72Y, D73F, D73G, D73I, D73K, D73M, D73P, D73R, D73V, D73W, T74A, T74C,
T74D, T74E, T74F, T74G, T74H, T74I, T74K, T74L, T74M, T74N, T74P, T74Q, T74R, T74S,
T74W, T74Y, I75A, I75C, I75D, I75E, I75F, I75G, I75H, I75L, I75M, I75N, I75P, I75Q, I75R,
I75S, I75T, I75V, I75W, I75Y, L76A, L76D, L76G, L76H, L76I, L76K, L76K, L76M, L76N, L76P,
L76Q, L76R, L76S, L76T, L76V, L76W, L76Y, F77C, F77D, F77G, F77I, F77K, F77L, F77M,

15   F77N, F77Q, F77R, F77S, F77T, F77V, F77W, F77Y, Q78A, Q78C, Q78E, Q78G, Q78I, Q78K,
Q78N, Q78P, Q78R, Q78S, Q78T, Q78V, Q78W, Q78Y, W79C, W79F, W79G, W79H, W79K,
W79L, W79M, W79N, W79P, W79R, W79T, W79Y, V80A, V80C, V80D, V80E, V80G, V80H,
V80L, V80M, V80N, V80P, V80Q, V80R, V80S, V80T, V80W, V80Y, P81A, P81C, P81D,
P81E, P81F, P81G, P81H, P81I, P81K, P81L, P81M, P81N, P81Q, P81R, P81S, P81T, P81W,

20   P81Y, V82A, V82C, V82D, V82E, V82F, V82G, V82H, V82K, V82M, V82N, V82P, V82Q,
V82R, V82S, V82T, V82W, P83A, P83C, P83D, P83E, P83F, P83G, P83H, P83I, P83K, P83L,
P83M, P83Q, P83R, P83S, P83T, P83V, P83W, W84A, W84C, W84E, W84G, W84H, W84L,
W84M, W84N, W84P, W84Y, P85C, P85E, P85F, P85G, P85H, P85I, P85N, P85Y, H86A,
H86C, H86E, H86F, H86G, H86I, H86K, H86L, H86M, H86P, H86Q, H86R, H86S, H86V,

25   P87A, P87C, P87F, P87H, P87I, P87K, P87L, P87M, P87N, P87Q, P87R, P87S, P87V, P87W,
P87Y, G88A, G88E, G88F, G88H, G88I, G88K, G88L, G88M, G88Q, G88S, G88T, G88V,
G88Y, P89A, P89E, P89F, P89G, P89H, P89I, P89K, P89L, P89M, P89N, P89Q, P89R, P89S,
P89T, P89V, P89W, P89Y, I90A, I90C, I90D, I90E, I90F, I90G, I90H, I90K, I90L, I90M, I90N,
I90P, I90Q, I90R, I90S, I90T, I90W, I90Y, V91A, V91C, V91D, V91E, V91F, V91H, V91I, V91K,

30   V91M, V91N, V91Q, V91S, V91W, V91Y, D92E, D92I, D92K, D92L, D92N, D92R, D92S,
D92W, D92Y, Y93A, Y93C, Y93E, Y93F, Y93G, Y93I, Y93K, Y93L, Y93M, Y93Q, Y93R, Y93T,
Y93V, Y93W, L94A, L94C, L94D, L94E, L94F, L94G, L94H, L94I, L94K, L94M, L94N, L94P,
L94Q, L94R, L94S, L94V, L94W, L94Y, A95C, A95D, A95E, A95F, A95G, A95H, A95I, A95K,
A95L, A95M, A95N, A95P, A95Q, A95R, A95S, A95T, A95V, A95Y, N96D, N96E, N96F, N96G,

35   N96H, N96L, N96M, N96P, N96Q, N96R, N96S, N96T, N96V, N96W, N96Y, C97F, C97N,
C97P, C97Q, C97R, C97S, C97T, C97V, C97W, C97Y, N98F, N98M, N98R, N98T, N98V,
N98Y, G99F, G99M, G99P, G99Q, G99R, G99S, G99T, G99V, G99W, D100C, D100E, D100H,

D100K, D100M, D100R, D100T, D100V, C101D, C101E, C101G, C101H, C101I, C101K, C101L, C101N, C101P, C101Q, C101R, C101S, C101T, C101V, C101Y, E102C, E102D, E102F, E102G, E102I, E102M, E102Q, E102R, E102T, E102V, E102W, E102Y, T103E, T103G, T103H, T103I, T103K, T103L, T103P, T103R, T103W, V104R, V104S, V104T, V104W,

5 V104Y, D105F, D105P, D105R, D105T, K106P, K106S, K106T, K106W, T107S, T107V, T107W, T107Y, T108M, T108N, T108P, T108Q, T108R, T108S, T108T, T108V, T108W, T108Y, L109A, L109C, L109D, L109E, L109F, L109G, L109H, L109I, L109K, L109M, L109N, L109P, L109R, L109S, L109W, L109Y, E110A, E110C, E110G, E110N, E110S, E110T, E110V, E110W, F111C, F111D, F111E, F111G, F111H, F111I, F111K, F111L, F111M, F111N,

10 F111P, F111Q, F111R, F111S, F111T, F111V, F111W, F111Y, F112A, F112C, F112D, F112E, F112G, F112H, F112I, F112K, F112L, F112M, F112N, F112P, F112Q, F112S, F112T, F112V, F112Y, K113A, K113C, K113D, K113E, K113F, K113G, K113I, K113M, K113N, K113R, K113S, K113V, K113W, K113Y, I114A, I114C, I114D, I114E, I114F, I114G, I114H, I114K, I114L, I114M, I114N, I114P, I114R, I114S, I114T, I114V, I114W, I114Y, D115A, D115F,

15 D115G, D115H, D115K, D115M, D115N, D115P, D115Q, D115R, D115S, D115T, D115V, D115W, D115Y, G116A, G116C, G116D, G116F, G116H, G116N, G116P, G116Q, G116R, G116S, G116T, G116V, G116W, G116Y, V117A, V117C, V117D, V117E, V117F, V117G, V117H, V117I, V117K, V117L, V117M, V117N, V117P, V117Q, V117S, V117T, V117W, V117Y, G118A, G118C, G118D, G118E, G118F, G118H, G118I, G118K, G118L, G118M,

20 G118N, G118P, G118Q, G118R, G118S, G118T, G118V, G118W, G118Y, L119A, L119C, L119D, L119E, L119F, L119G, L119H, L119I, L119I, L119K, L119L, L119M, L119N, L119P, L119Q, L119R, , L119S, L119T, L119V, L119W, L119Y, L120A, L120C, L120D, L120E, L120F, L120G, L120H, L120I, L120K, L120M, L120M, L120N, L120N, L120P, L120Q, L120S, L120S, L120T, L120V, L120W, L120Y, L120Y, S121A, S121C, S121D, S121E, S121F, S121G, S121H,

25 S121I, S121K, S121L, S121M, S121N, S121P, S121R, S121S, S121T, S121V, S121W, S121Y, G122A, G122C, G122D, G122E, G122F, G122H, G122I, G122K, G122L, G122M, G122N, G122P, G122Q, G122R, G122S, G122T, G122V, G122W, G122Y, G123C, G123D, G123E, G123F, G123I, G123K, G123L, G123M, G123N, G123R, G123V, G123W, G123Y, D124A, D124C, D124E, D124F, D124G, D124H, D124I, D124K, D124L, D124M, D124P,

30 D124R, D124T, D124V, D124Y, P125A, P125C, P125D, P125E, P125F, P125G, P125H, P125I, P125K, P125L, P125M, P125N, P125Q, P125R, P125S, P125T, P125V, P125W, P125Y, G126A, G126C, G126D, G126E, G126F, G126H, G126I, G126K, G126L, G126N, G126P, G126R, G126S, G126T, G126V, G126W, T127A, T127C, T127D, T127E, T127G, T127H, T127P, T127Q, T127S, W128A, W128C, W128D, W128E, W128F, W128G, W128I,

35 W128K, W128M, W128N, W128P, W128R, W128T, W128V, W128Y, A129D, A129G, A129I, A129K, A129L, A129M, A129P, A129Q, A129T, A129Y, S130A, S130D, S130G, S130K, S130V, S130W, S130Y, D131A, D131C, D131E, D131H, D131I, D131L, D131M, D131P,

D131Q, D131S, D131T, D131W, D131Y, V132A, V132C, V132E, V132F, V132G, V132H,
V132I, V132K, V132L, V132M, V132N, V132P, V132Q, V132R, V132S, V132T, V132W,
V132Y, L133A, L133C, L133D, L133E, L133F, L133G, L133I, L133K, L133M, L133N, L133P,
L133Q, L133R, L133S, L133T, L133V, L133W, I134A, I134D, I134E, I134F, I134G, I134L,

5   I134N, I134P, I134Q, I134R, I134S, I134T, I134W, I134Y, S135C, S135D, S135G, S135I,
S135K, S135L, S135N, S135P, S135Q, S135R, S135T, S135V, S135W, S135Y, N136C,
N136D, N136F, N136G, N136H, N136I, N136K, N136L, N136M, N136P, N136S, N136T,
N136V, N136W, N136Y, N137A, N137D, N137E, N137F, N137G, N137H, N137K, N137L,
N137M, N137Q, N137R, N137S, N137T, N137V, N137W, N137Y, N138A, N138C, N138D,

10   N138E, N138G, N138H, N138I, N138K, N138L, N138M, N138P, N138Q, N138R, N138T,
N138V, N138Y, T139C, T139D, T139E, T139F, T139H, T139I, T139K, T139L, T139P, T139R,
T139S, T139Y, W140D, W140E, W140G, W140I, W140K, W140L, W140M, W140N, W140P,
W140Q, W140R, W140S, W140T, W140V, W140Y, V141A, V141C, V141D, V141E, V141F,
V141H, V141K, V141L, V141M, V141Q, V141R, V141S, V141T, V141W, V141Y, V142A,

15   V142C, V142E, V142G, V142K, V142M, V142N, V142P, V142Q, V142R, V142S, K143E,
K143G, K143I, K143L, K143N, K143P, K143R, K143S, K143T, K143Y, I144A, I144C, I144D,
I144E, I144F, I144H, I144L, I144N, I144P, I144Q, I144T, I144W, I144Y, P145A, P145C,
P145D, P145E, P145F, P145L, P145M, P145N, P145Q, P145T, P145W, P145Y, D146G,
D146H, D146I, D146L, D146N, D146P, D146R, D146S, D146V, D146W, N147A, N147C,

20   N147D, N147E, N147G, N147H, N147I, N147K, N147L, N147P, N147Q, N147R, N147S,
N147T, N147V, N147W, N147Y, L148A, L148C, L148D, L148E, L148F, L148G, L148H, L148I,
L148M, L148N, L148P, L148Q, L148R, L148S, L148T, L148W, L148Y, A149E, A149H, A149H,
A149K, A149L, A149M, A149Q, A149R, A149S, A149T, A149W, A149Y, P150M, P150N,
P150R, P150S, P150T, P150V, P150W, P150Y, G151A, G151C, G151D, G151L, G151N,

25   G151R, G151T, N152A, N152C, N152D, N152E, N152H, N152I, N152K, N152L, N152M,
N152P, N152Q, N152R, N152T, N152V, N152W, N152Y, Y153A, Y153C, Y153D, Y153E,
Y153G, Y153H, Y153I, Y153N, Y153T, Y153W, V154F, V154L, V154S, V154W, V154Y,
L155A, L155D, L155E, L155G, L155H, L155I, L155P, L155Q, L155R, L155S, L155T, L155V,
L155W, L155Y, R156G, R156N, R156T, R156V, R156W, R156Y, H157A, H157C, H157E,

30   H157F, H157G, H157I, H157K, H157L, H157M, H157N, H157P, H157Q, H157R, H157S,
H157T, H157V, H157Y, E158G, E158K, E158R, E158V, I159A, I159C, I159D, I159E, I159F,
I159G, I159H, I159K, I159L, I159M, I159N, I159P, I159R, I159S, I159T, I159V, I159W, I159Y,
I160A, I160C, I160E, I160G, I160H, I160K, I160L, I160M, I160N, I160P, I160Q, I160R, I160S,
I160T, I160V, I160W, I160Y, A161D, A161F, A161G, A161H, A161I, A161K, A161M, A161N,

35   A161P, A161Q, A161R, A161S, A161T, A161V, A161W, L162C, L162D, L162E, L162G,
L162H, L162K, L162M, L162P, L162Q, L162R, L162S, L162T, L162V, L162W, L162Y, H163A,
H163G, H163I, H163K, H163M, H163N, H163N, H163Q, H163R, H163S, H163S, S164D,

S164E, S164I, S164P, S164T, A165C, A165D, A165E, A165F, A165G, A165H, A165I, A165K, A165L, A165Q, A165R, A165S, A165T, A165V, A165W, G166C, G166D, G166E, G166I, G166Y, Q167C, Q167D, Q167E, Q167G, Q167H, Q167P, Q167T, A168D, A168G, A168W, N169C, N169E, N169F, N169G, N169I, N169L, N169M, N169P, N169R, N169S, N169V,

5   N169W, N169Y, G170A, G170C, G170D, G170E, G170K, G170L, G170M, G170N, G170Q, G170R, G170S, G170T, G170V, G170Y, A171D, A171E, A171F, A171G, A171M, A171N, A171P, A171Q, A171S, A171T, A171V, A171Y, Q172A, Q172C, Q172D, Q172E, Q172F, Q172K, Q172L, N173A, N173C, N173D, N173E, N173F, N173G, N173H, N173K, N173L, N173P, N173Q, N173R, N173S, N173T, N173V, N173W, N173Y, Y174C, Y174S, Y174V,

10   P175A, P175C, P175D, P175E, P175G, P175I, P175K, P175L, P175M, P175N, P175Q, P175R, P175S, P175T, P175V, P175W, P175Y, Q176A, Q176D, Q176E, Q176F, Q176G, Q176H, Q176I, Q176K, Q176L, Q176M, Q176P, Q176R, Q176S, Q176T, Q176V, Q176W, Q176Y, C177A, C177E, C177F, C177G, C177H, C177I, C177K, C177L, C177N, C177P, C177Q, C177T, C177W, F178D, F178E, F178G, F178H, F178I, F178K, F178M, F178P,

15   F178R, F178S, F178T, F178V, F178W, F178Y, N179D, N179E, N179F, N179G, N179I, N179K, N179L, N179P, N179Q, N179R, N179S, N179T, N179V, N179W, N179Y, I180A, I180C, I180D, I180E, I180G, I180K, I180P, I180Q, I180R, I180V, I180W, I180Y, A181C, A181D, A181F, A181G, A181M, A181P, A181Q, A181T, A181V, A181W, A181Y, V182A, V182C, V182D, V182E, V182F, V182H, V182I, V182K, V182L, V182M, V182P, V182Q, V182S,

20   V182W, S183C, S183F, S183G, S183L, S183M, S183Q, S183W, G184C, G184D, G184E, G184F, G184I, G184K, G184L, G184M, G184P, G184Q, G184S, G184T, G184Y, G186M, G186S, G186V, S187F, S187S, S187Y, L188A, L188C, L188D, L188E, L188F, L188G, L188H, L188K, L188N, L188Q, L188R, L188S, L188T, L188V, L188W, L188Y, Q189C, Q189F, Q189H, Q189I, Q189K, Q189L, Q189M, Q189N, Q189P, Q189S, Q189T, Q189V, Q189W, Q189Y,

25   P190A, P190C, P190D, P190E, P190F, P190G, P190H, P190I, P190K, P190L, P190M, P190N, P190Q, P190S, P190T, P190V, P190W, P190Y, S191A, S191C, S191D, S191F, S191G, S191I, S191K, S191L, S191N, S191R, S191T, S191V, S191W, S191Y, G192P, V193A, V193G, V193H, V193I, V193K, V193L, V193M, V193N, V193P, V193Q, V193R, V193S, V193Y, L194C, L194E, L194F, L194G, L194H, L194I, L194K, L194P, L194R, L194S,

30   L194T, L194V, L194W, L194Y, G195C, G195E, G195F, G195H, G195P, G195Q, G195T, G195W, G195Y, T196A, T196C, T196D, T196E, T196F, T196H, T196I, T196M, T196P, T196S, T196W, T196Y, D197A, D197E, D197H, D197K, D197N, D197R, D197S, D197W, D197Y, L198A, L198C, L198D, L198F, L198G, L198H, L198I, L198N, L198P, L198Q, L198R, L198S, L198T, L198V, L198W, Y199M, Y199R, Y199S, Y199V, H200F, H200G, H200L, H200Q,

35   H200S, H200T, H200V, H200W, H200Y, A201D, A201K, A201N, A201P, A201R, A201S, A201T, A201W, A201Y, T202C, T202F, T202I, T202K, T202M, T202P, T202R, T202T, T202V, T202Y, D203A, D203C, D203F, D203I, D203L, D203P, D203Q, D203R, D203S, D203T,

D203V, D203W, D203Y, P204A, P204C, P204D, P204E, P204G, P204I, P204K, P204L,

P204M, P204N, P204Q, P204R, P204S, P204T, P204V, P204W, P204Y, G205A, G205C,

G205D, G205E, G205F, G205I, G205K, G205L, G205N, G205P, G205Q, G205R, G205S,

G205T, G205V, G205W, G205Y, V206A, V206C, V206D, V206E, V206F, V206G, V206H,

5   V206K, V206L, V206M, V206N, V206P, V206Q, V206S, V206W, V206Y, L207A, L207C,

L207D, L207E, L207G, L207H, L207I, L207K, L207N, L207P, L207Q, L207R, L207S, L207T,

L207V, I208A, I208C, I208D, I208E, I208K, I208L, I208N, I208P, I208Q, I208R, I208S, I208T,

I208W, I208Y, N209C, N209E, N209H, N209M, N209P, N209Q, N209R, N209W, N209Y,

I210C, I210D, I210G, I210K, I210P, I210Q, I210R, I210S, I210T, I210V, I210W, I210Y, Y211A,

10   Y211D, Y211E, Y211K, Y211N, Y211S, Y211V, T212A, T212C, T212D, T212E, T212F,

T212G, T212H, T212I, T212M, T212N, T212P, T212Q, T212S, T212V, T212W, T212Y, S213A,

S213C, S213D, S213E, S213F, S213G, S213H, S213I, S213L, S213M, S213P, S213Q,

S213V, P214A, P214D, P214E, P214F, P214G, P214H, P214K, P214L, P214M, P214Q,

P214R, P214S, P214T, P214V, P214W, P214Y, L215A, L215D, L215E, L215F, L215G, L215H,

15   L215K, L215N, L215Q, L215R, L215S, L215T, L215V, L215W, L215Y, N216F, N216G, N216P,

N216R, N216S, N216V, Y217A, Y217C, Y217E, Y217F, Y217I, Y217K, Y217L, Y217N, Y217P,

Y217R, Y217S, Y217V, Y217W, I218A, I218C, I218D, I218E, I218F, I218G, I218H, I218K,

I218L, I218M, I218N, I218P, I218R, I218S, I218Y, I219A, I219C, I219D, I219F, I219G, I219L,

I219N, I219P, I219Q, I219R, I219S, I219W, I219Y, P220A, P220C, P220D, P220E, P220F,

20   P220G, P220H, P220I, P220L, P220M, P220N, P220Q, P220R, P220S, P220T, P220V,

P220Y, G221E, G221F, G221H, G221K, G221L, G221M, G221N, G221P, G221Q, G221T,

G221W, G221Y, P222A, P222C, P222D, P222E, P222G, P222K, P222N, P222Q, P222R,

P222T, P222W, P222Y, T223D, T223E, T223G, T223H, T223K, T223L, T223N, T223P,

T223R, T223V, T223W, T223Y, V224A, V224C, V224D, V224E, V224F, V224G, V224H,

25   V224I, V224K, V224L, V224N, V224P, V224Q, V224R, V224S, V224T, V224W, V224Y,

V225D, V225E, V225G, V225I, V225K, V225L, V225M, V225N, V225P, V225Q, V225R,

V225S, V225T, V225W, V225Y, S226A, S226C, S226F, S226G, S226H, S226I, S226K,

S226R, S226W, S226Y, G227A, G227D, G227E, G227F, G227H, G227I, G227K, G227M,

G227P, G227Q, G227R, G227S, G227V, G227W, G227Y, L228A, L228C, L228D, L228E,

30   L228F, L228H, L228I, L228K, L228M, L228P, L228Q, L228R, L228S, L228V, L228W, L228Y,

P229A, P229D, P229E, P229F, P229G, P229H, P229K, P229L, P229M, P229N, P229Q,

P229R, P229S, P229V, P229W, P229Y, T230C, T230E, T230F, T230G, T230H, T230I, T230K,

T230L, T230M, T230N, T230P, T230Q, T230R, T230S, T230Y, S231E, S231F, S231I, S231P,

S231Q, S231T, S231V, V232A, V232D, V232E, V232F, V232G, V232K, V232M, V232N,

35   V232P, V232R, V232S, V232T, V232Y, A233H, A233I, A233K, A233L, A233M, A233N, A233N,

A233P, A233T, A233V, A233Y, Q234A, Q234C, Q234D, Q234E, Q234F, Q234G, Q234H,

Q234I, Q234K, Q234L, Q234M, Q234N, Q234P, Q234R, Q234S, Q234T, Q234V, Q234W,

Q234Y, G235C, G235I, G235M, G235Q, G235R, G235V, G235W, T287D, T287N, T287W,
T287Y, Q288H, Q288P, T289E, T289G, T289H, T289P, T289Q, T289W, L290E, L290G,
L290Q, L290R, L290W, L290Y, Y291D, Y291E, Y291F, Y291G, Y291K, Y291L, Y291Q,
Y291R, G292A, G292C, G292D, G292E, G292F, G292H, G292K, G292N, G292Q, G292R,
G292V, G292Y, Q293A, Q293D, Q293E, Q293F, Q293H, Q293I, Q293K, Q293L, Q293N,
Q293P, Q293R, Q293S, Q293V, C294D, C294E, C294F, C294G, C294H, C294I, C294K,
C294M, C294N, C294P, C294Q, C294S, C294T, C294V, C294W, C294Y, G295F, G295L,
G295P, G295Q, G295R, G296D, G296E, G296H, G296N, G296Q, G296W, S297A, S297V,
G298K, G298M, G298N, G298Q, G298R, G298T, G298V, Y299D, Y299M, Y299Q, Y299R,
Y299T, Y299V, Y299W, S300A, S300D, S300G, S300I, S300M, S300N, S300P, S300R,
S300W, S300Y, G301A, G301C, G301E, G301F, G301H, G301I, G301L, G301N, G301Q,
G301R, G301S, G301W, P302A, P302C, P302D, P302E, P302F, P302G, P302H, P302I,
P302K, P302L, P302N, P302Q, P302S, P302V, T303C, T303D, T303E, T303F, T303L,
T303W, R304F, R304K, R304P, R304R, R304W, C305D, C305F, C305H, C305I, A306C,
P307D, P307E, P307K, P307W, P308A, P308E, P308F, P308I, P308N, A309A, A309C,
A309D, A309E, A309F, A309G, A309H, A309I, A309K, A309M, A309N, A309Q, A309R,
A309T, A309W, A309Y, T310A, T310C, T310D, T310E, T310G, T310H, T310K, T310N,
T310Q, C311A, C311D, C311F, C311G, C311H, C311I, C311K, C311L, C311M, C311N,
C311P, C311Q, C311R, C311S, C311T, C311V, C311W, C311Y, S312A, S312D, S312E,
S312G, S312H, S312K, T313A, T313C, T313D, T313E, T313G, T313H, T313I, T313K, T313L,
T313S, N315A, N315D, N315E, N315F, N315H, N315K, N315Q, N315R, N315W, N315Y,
P316K, P316M, P316Y, Y317C, Y317F, Y317K, Y317L, Y317M, Y317N, Y317R, Y317V,
Y317W, Y318A, Y318C, Y318D, Y318E, Y318F, Y318G, Y318H, Y318I, Y318K, Y318L,
Y318M, Y318N, Y318P, Y318R, Y318S, Y318V, Y318W, A319C, A319K, A319P, A319Y,
Q320D, Q320E, Q320F, Q320G, Q320K, Q320W, C321A, C321D, C321F, C321K, C321N,
C321S, C321T, C321W, L322A, L322C, L322D, L322E, L322G, L322H, L322I, L322K, L322M,
L322N, L322P, L322Q, L322Y, N323A, N323C, N323D, N323E, N323F, N323G, N323H,
N323I, N323K, N323Q, N323R, N323W, and N323Y.

[1149] In certain embodiments of the subject invention, one or a combination of substitutions in
this Wild-Type SEL Cohort find use in generating combinatorial GH61A variants with one or
more of the substitutions identified in SEL Cohorts 1, 2 and/or 3 above.

[1150] Using the SEL data described above, Productive positions in GH61A were identified.
Productive positions are defined herein as those positions within a molecule that are most
useful for making combinatorial variants exhibiting an improved characteristic, where the
position itself allows for at least one combinable mutation. Highly combinable mutations are
defined herein as mutations at any amino acid position that can be used to make combinatorial
variants. Highly combinable mutations improve at least one desired property of the molecule,

while not significantly decreasing expression, activity, or stability. Highly combinable mutations can be grouped as follows:

[1151] <u>Group A</u>: A mutation that produces a variant wherein (1)(a) the minimum performance indices (PI) relative to a defined parent polypeptide in whPCS Hydrolysis Assays as described in section VI in Example 1, are greater than or equal to 0.9; and (b) Tm value is no less than 2°C below the median Tm of the wild type polypeptide as measured in accordance with section V.B. of Example 1; and (2) (a) the minimum performance indices PI relative to a defined parent polypeptide in whPCS Hydrolysis Assays as described in section VI of Example 1, are greater than or equal to 1.0; or (b) Tm value greater than that of the defined parent polypeptide, as measured in accordance with section V. B of Example 1.

[1152] <u>Group B</u>: A mutation that produces a variant wherein (1)(a) the minimum performance indices (PI) relative to a defined parent polypeptide for whPCS Hydrolysis Assays as described in section VI in Example 1, are greater than or equal to 0.8; and (b) Tm value is no less than 3°C below the median Tm of the wild type polypeptide as measured in accordance with section V.B. of Example 1; and (2) (a) the minimum performance indices PI relative to a defined parent polypeptide in whPCS Hydrolysis Assays as described in section VI of Example 1, are greater than or equal to 1.2; or (b) Tm value that is at least 1°C greater than that of the defined parent polypeptide, as measured in accordance with Section V.B. of Example 1.

[1153] <u>Group C</u>: A mutation that produces a variant wherein (1)(a) the minimum performance indices (PI) relative to a defined parent polypeptide for whPCS Hydrolysis Assays as described in section VI in Example 1, are greater than or equal to 0.5; and (b) Tm value is no less than 4°C below the median Tm of the wild type polypeptide as measured in accordance with section V.B. of Example 1; and (2) (a) the minimum performance indices PI relative to a defined parent polypeptide in whPCS Hydrolysis Assays as described in section VI of Example 1, are greater than or equal to 1.5; or (b) Tm value that is at least 3°C greater than that of the defined parent polypeptide, as measured in accordance with Section V.B. of Example 1.

[1154] Preferred combinable mutations are at "productive positions," as described, below. In the case of the present GH61, "activity" refers to GH61-mediated cellulase augmenting activity, which can be measured as described herein.

[1155] Productive positions are amino acid positions that are tolerant to substitution with different amino acid residues, wherein the resulting variants meet a set of performance criteria for combinability, as set forth above. Productive positions can be assigned a Productivity Score as follows:

- Positions where less than 15% of the substitutions at a given position fall within groups A, B, or C are given a **Productivity Score of "1".**
- Positions where less than 40%, but greater than, or equal to 15% of the substitutions at a given position fall within groups A, B, or C are given a **Productivity Score of "2".**

- Positions where less than 75%, but greater than, or equal to 40% of the substitutions at a given position fall within groups A, B, or C are given a **Productivity Score of "3"**.

- Positions where 75% or more of the substitutions at a given position fall within groups A, B, or C are given a **Productivity Score of "4"**.

[1156] Preferred productive positions are highly combinable mutations.

[1157] Suitability score refers to the ability of one or more highly combinable mutations to be used to make combinatorial variants, based on the performance criteria for combinability, (i.e., A, B, and C, as set forth, above) in which each of the mutations fall. A higher suitability score indicates a mutation or mutations that are more suitable for use in making combinatorial variants. Suitability scores are described in Table 2 below.

Table 2: Definitions of suitability scores

Substitutions Occur in Group(s)	Suitability Score
A, B and C	+++++
A and B	++++
A or (B and C)	+++
B	++
C	+

[1158] Table 3 shows the Productivity Score (4, 3, 2, or 1) calculated for each position in the GH61A polypeptide. For each GH61A position, variants are listed according to the suitability score they received (+, ++, +++, ++++, or +++++). Position numbering is based on the mature GH61A polypeptide listed in SEQ ID NO: 3. Any individual or any combination of GH61A substitutions in Table 3 below find use as GH61A variants according to aspects of the subject invention.

Table 3: Productivity and Suitability Scores for GH61A Variant Polypeptides

POS	Productivity score	Variant Suitability Score				
		+++++	++++	+++	++	+
13	4	G,L,N,R,T,V	F,Q,Y	A,E,I,K,M		
288	4		E,F,G,I,K,M,N,R,S,T	A,C,D,V,W		
19	3	F,M		E,H,Q,T	K,W	
20	3	K	P	G,M,R,Y	A,N	
29	3	C,E,F,K,T,Y		D,G,M,R,S		

37	3	D,S	E,L,W	F,K,Q		
39	3	A,D,E,M,T,Y	F,I,N,P,Q,S,V			
47	3	A,K,T		H,R	P	W
48	3	C,E,G,K,L,N,T,Y	Q,W			H,I,S
68	3			E,I,L,M,N,T,V,Y		
107	3		A,D,E	K,M,R,S	G	
121	3	E,F,N,T	C,L,V	D,G,K,M,Y		
122	3	E,N,S,V	A,M,T	F,L		
127	3			A,E,F,I,L,M,V,W,Y		
164	3		A,D,E	G,K,L,M,Q,R,V,Y		
166	3			A,D,E,F,H,K,M,N,Q,S		
167	3		E	A,D,F,G,K,L,N,R,V,Y		
168	3		E,M	D,F,I,L,N,P,Q,R,T,Y		
216	3		D,Q,T	K,P		
218	3		T	A,D,E,K,M,N,P,S,V		
219	3		M	A,D,L,N,P,Q		
224	3	Q	I	A,E,N,P,S,T		
287	3		H	A,F,G,S	I	
289	3			A,D,K,L,M,R,S		
291	3		A	F,G,I,K,L,M,N,R,S,V,W	D,E	
297	3			D,E,K,N,Q,R,T		
303	3			K,M,P,R,S,V,Y		
304	3			A,C,D,E,N,Q,T,V		
306	3			E,F,G,I,K,L,M,Q,T,Y		
308	3			A,G,H,I,K,L,Q,R,S,T,V,W,Y		
312	3		Q	F,G,I,K,L,M,N,T,V	D,R,Y	
313	3	S	D,G,L,P,V	E,F,I,K,M,Y		
315	3		S	H,L,Q,R,V,Y	E,K,M	
317	3		Q,S,V	R	E,G,L,M,P,T	

318	3			T		
5	2			A,D,G,S		
7	2		L	M,V		
12	2			M,T		
15	2		H	K	D	
21	2		I	K,M,S		
22	2		K	I,L,R		
26	2			M,P,T		
28	2			M,S,V		
32	2		A	P		
33	2		Q	A,S		
35	2			A,D,E,K,N,S		
36	2		T	I,N,S	Y	
41	2			D,E,L,M		
50	2	A,T,Y	D,E,K			
51	2		S	E,H,K,L,T		
58	2			I,R,V		
59	2		G	E,Q,S		
80	2			A,E,L,M		
89	2	T	S,V		L	
108	2		A,Q,R	S		
116	2			H,Q,S		
120	2	V	T	I,S		
123	2			E,Q,R		
132	2			D,E,M,R		
135	2		A,E	G,H,M,N		
147	2	E		M,S,T		
161	2		E,G	L,S		
181	2		K	N,R		
183	2		E	K,N,T		
185	2		D	G,T		
189	2		E,L,M,R	G,T		
195	2			A,E,S		
196	2			H,Y	R	
200	2		R	A,Q,S		
201	2		P	K,Q		
202	2		S	E,Q		
208	2			F,L,V		
222	2		D	G		
223	2		S	E,Q		
226	2			D,G,K,M,P,Q		
231	2		C		I	
235	2			A,F,I,M,N,Q,V		
290	2			K,M,T		

295	2		A	F,H,Q,R		
296	2			A,M		
298	2		K	A,I,N,Q		
299	2		N,S	F,P		
300	2			F,L,N,T	Q	
301	2		D	S,Y		
302	2		G	L,T,V,Y	F	
307	2		A	F,H,L,V		
309	2		I	K,R,T,V,Y		
310	2			L,M,Q,S,W		
316	2			D,N,R,T		
319	2	Q,W	D,F,S			
322	2			A,E,S,T,V		
4	1		V	T		
9	1		V	A		
10	1		D			
23	1		G			
24	1		T			
25	1			N,Q		
27	1			E		
38	1		E	N		
43	1		Y			
44	1			I		
49	1	W				
52	1			A,N		
54	1	Q		E		
61	1			Q,S		
62	1			P		
64	1			A,Q		
69	1			Y		
70	1			R		
71	1	Y	P			
90	1	V				
91	1		L	I		
96	1			A,S		
98	1			H		
100	1			P,S		
103	1			A		
110	1			K		
117	1			A		
119	1			N,T		
124	1			N		
125	1			D		
126	1			M,Q		
141	1			T		

146	1			E		
149	1			E		
160	1			F,M		
163	1					A
171	1			T		
173	1			H		
175	1			F		
180	1		L			
187	1			A,D		
188	1			A,D		
191	1			D		
194	1			D,M		
197	1			A,V		
204	1			A		
206	1	I				
207	1			F		
209	1		D			
211	1			A		
213	1			T		
214	1			S		
217	1		H			
230	1			S		
232	1			F,Y		
233	1			F,S		
292	1			D,K		
293	1			K,P		
320	1			R		
323	1			V		

EXAMPLE 4

GH61A Combinatorial Library Variants and the Identification of Highly Combinable and Productive Substitutions

[1159] Table 4 below lists substitutions in GH61A that were selected from the SELs in Example 3, above, for inclusion in the construction of GH61A combinatorial variant libraries. Some of these variants are present in Table 3 above while others are not. Each individual member of the GH61A combinatorial variant libraries produced based on the substitutions in Table 4 has at least two different amino acid substitutions. The amino acid residue numbers in Table 4 were assigned in reference to the mature amino acid sequence of wild type GH61A, i.e., SEQ ID NO:3. The combinatorial GH61A variant libraries were constructed. It is emphasized here that GH61A substitutions in the SELs above (e.g., SELs in Cohorts 1, 2, 3 and/or the Wild-Type Cohort) that were not selected for combinatorial library construction in this example still find use

in combinatorial GH61A variants having improved properties according to aspects of the present invention. The substitutions in Table 4 were selected merely as representative substitutions having improved properties of interest.

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Table 4: Substitutions selected for generating an

H. jecorina GH61A combinatorial library.

H. jecorina GH61A Position (SEQ ID NO:3)	Wild-type AA	Variant AA
002	G	V
002	G	W
010	N	D
016	A	N
028	P	K
030	I	E
048	A	R
050	Q	G
051	N	T
051	N	E
051	N	H
052	P	C
052	P	I
052	P	F
058	K	V
059	N	K
060	A	L
061	T	K
061	T	R
061	T	D
062	N	S
062	N	C
064	K	L
070	K	A
070	K	N
070	K	S
070	K	R
070	K	E
070	K	L
073	D	E
073	D	A
080	V	T
082	V	W
087	P	H
096	N	D

096	N	P
099	G	E
100	D	Q
102	E	S
104	V	A
104	V	K
104	V	I
104	V	R
106	K	C
106	K	H
106	K	R
106	K	F
106	K	E
107	T	A
107	T	F
107	T	C
107	T	G
107	T	H
107	T	D
107	T	N
107	T	Q
107	T	I
107	T	M
107	T	E
107	T	K
107	T	L
107	T	R
108	T	A
108	T	C
108	T	K
108	T	I
108	T	E
108	T	L
108	T	H
108	T	Q
108	T	D
108	T	M
110	E	Q
117	V	I
124	D	N
127	T	I
129	A	N
130	S	L
132	V	N
135	S	E

163	H	Y
164	S	M
165	A	Y
165	A	G
167	Q	A
167	Q	I
167	Q	K
168	A	E
168	A	K
182	V	R
182	V	N
185	S	D
185	S	H
187	S	D
192	G	A
193	V	T
194	L	D
194	L	G
196	A	C
197	E	M
197	E	L
197	D	E
197	D	A
200	H	A
201	A	D
205	G	H
212	T	D
212	T	K
231	S	A
231	S	C
233	A	C
235	G	A
235	G	F
235	G	K
235	G	L
235	G	S
287	T	A
287	T	H
290	L	A
290	L	D
290	L	K
291	Y	W
291	Y	F
295	G	C
295	G	T

297	S	C
297	S	K
297	S	E
297	S	D
297	S	R
298	G	C
300	S	K
300	S	T
301	G	A
301	G	P
301	G	S
302	P	G
303	T	P
303	T	G
304	R	P
304	R	A
304	R	D
313	T	K
315	N	W
316	P	F
316	P	C
316	P	A
317	Y	W
318	Y	W
320	Q	A
320	Q	P
320	Q	S

[1160] GH61A combinatorial variant-encoding polynucleotides were made using the pTTTpyr2-GH61A vector as a template, transformed into *E. coli,* and plated onto 2 x TY agar plates (16 g/L Bacto Tryptone (Difco, USA), 10 g/L Bacto Yeast Extract (Difco, USA), 5 g/L NaCl, 16 g/L Bacto Agar (Difco, USA)) with 100 µg/mL ampicillin. After overnight incubation at 37 °C, ampicillin resistant *E. coli* colonies were picked from the 2 x TYagar plates containing 100 µg/mL ampicillin and grown for 24 hr at 37 °C in a microtiterplate containing 1 mL of a 2 x TYmedium with 100 µg/mL ampicillin and 50 µg/mL kanamycin. These bacterial cultures were used for purification of plasmid DNA.

[1161] Purified pTTTpyr2-GH61A derived plasmids encoding GH61A combinatorial variants were used at concentrations of 150-300 ng/µL. Five (5) µL of plasmid DNA was used for fungal transformation as described in, for example, U.S. Patent Application Publication US2006/0094080 A1. Protoplasts of *H. jecorina* strain (Δ*eg1*, Δ*eg2*, Δ*eg3*, Δ*eg5*, Δ*eg6*, Δ*gh61a*, , Δ*cbh1*, Δ*cbh2*, Δ*man1*) were transformed with individual pTTTpyr2-*GH61A* constructs

(i.e., including a single GH61A variant per transformation) and grown in 24-well microtiter plates on selective medium containing acetamide at 28 ºC for 7 d.

[1162] Spores from the initial population of *H. jecorina* transformants of individual variants were harvested and reselected on acetamide agar plates to increase the number of plasmid-borne spores. Spores were harvested using saline physiological solution, re-arrayed in 96-well microtiter plates, and used for inoculation of a number of production media to generate GH61A variant samples. For this purpose, 96-well filter plates (Corning, Art. No. 3505) containing in each well 250 µL of a glycine production medium, containing 4.7 g/L $(NH_4)_2SO_4$; 33 g/L 1,4-piperazinebis(propanesulfonic acid) pH 5.5; 6.0 g/L glycine; 5.0 g/L KH_2PO_4; 1.0 g/L $CaCl_2$ x $2H_2O$; 1.0 g/L $MgSO_4$ x $7H_2O$; 2.5 mL/L of 400X *T. reesei* trace elements, containing 5 g/L $FeSO_4$ x $7H_2O$, 1.4 g/L $ZnSO_4$ x $7H_2O$, 1.6 g/L $MnSO_4$ x H_2O, 3.7 g/L $CoCl_2$ x $6H_2O$; 20 g/L Glucose; and 6.5 g/L Sophorose, were inoculated in quadruplicate with spore suspensions of *H. jecorina* transformants. Plates were incubated at 28 °C and 80% humidity for 6 to 8 d. Culture supernatants were harvested by vacuum filtration.

[1163] GH61A combinatorial variants were isolated from the culture supernatants and tested for various activities. Two methods of quantitation were used for each GH61A combinatorial variant: SEC as described in section IV.A in Example 1, and RPC as described section IV.B in Example 1. GH61A variants that have increased amounts of enzyme present in the culture supernatant as compared to wild-type GH61A are said to have improved production (or yield). Improved thermostability in variants were determined, for example, using the Thermostability Assay as described in section V.B of Example 1 (Tm). Performance index (PI) values were determined for the purified GH61A combinatorial variants (both SEC and RPC) in one or more of the following assays: (i) whPCS Hydrolysis Assay as described in section VI in Example 1 (whPCS); (ii) daCS Hydrolysis Assay as described in section VII in Example 1 (daCS); and (iii) Avicel Activity as described in section VIII in Example 1 above (Avicel).

[1164] Below is a listing of combinatorial GH61A variants having an improved property (e.g., improved production, improved thermostability) / improved PI over wild type GH61A (i.e., a PI>1.0) in at least one of the assays described above. The GH61A variants fall into the following Combinatorial Cohorts.

[1165] <u>Combinatorial Cohort 1</u>: Combinatorial GH61A variants having improved production (yield) as assessed by SEC and/or RPC as compared to wild-type: A165G-S185D; A168K-L290D-S297D-R304A; A168K-L290K; A168K-L290K-R304D; A168K-R304A; A168K-R304A-T313K; A168K-R304A-T313K; A168K-S187D-L290D-S297D-R304D; A168K-S187D-L290K-T313K; A168K-S187D-R304D; A168K-S297D-R304D-T313K; A16N-A48R; A16N-A48R-G99E; A16N-A48R-G99E-L290A; A16N-A48R-N59K; A16N-A48R-S164M-L290M; A16N-G99E-S164M; A16N-L290A; A16N-L290A; A16N-L290S; A16N-N59K; A16N-N59K-L290S; A16N-N59K-S164M; A48R-A168K-L290D-S297R; A48R-A168K-R304D-T313K; A48R-A168K-S187D-

L290D-S297D-R304D; A48R-A168K-S297R; A48R-A168K-S297R-R304A; A48R-K70E-A168K-L290D-S297D-R304D; A48R-K70E-A168K-L290D-T313K; A48R-K70E-A168K-L290K-R304A-T313K; A48R-K70E-A168K-L290K-S297R-R304A-T313K; A48R-K70E-A168K-L290K-S297R-R304D; A48R-K70E-A168K-S187D-L290D-S297D; A48R-K70E-A168K-S187D-L290D-S297R-

5   R304D; A48R-K70E-A168K-S187D-S297D; A48R-K70E-A168K-S187D-S297D-R304D; A48R-K70E-A168K-S187D-S297D-R304D-T313K; A48R-K70E-A168K-S187D-S297R-R304D-T313K; A48R-K70E-L290D-S297R-R304A; A48R-K70E-L290D-S297R-R304A; A48R-K70E-R304D; A48R-K70E-R304D-T313K; A48R-K70L-A168K-L290D-S297D-R304A; A48R-K70L-A168K-L290D-S297D-R304D; A48R-K70L-A168K-L290D-S297D-R304D; A48R-K70L-A168K-L290K-

10   S297R-R304D; A48R-K70L-A168K-L290K-T313K; A48R-K70L-A168K-S297D-R304D; A48R-K70L-A168K-S297R-R304A; A48R-K70L-L290K; A48R-K70L-L290K-R304A; A48R-K70L-S187D-L290D-R304A; A48R-K70L-S187D-L290K-R304A; A48R-K70L-S187D-L290K-R304D-T313K; A48R-K70L-S187D-R304D; A48R-K70L-S297D-R304D-T313K; A48R-L290D-S297R-R304A; A48R-L290D-S297R-R304D-T313K; A48R-L290K-S297R-R304D-T313K; A48R-N59K;

15   A48R-R304A-T313K; A48R-R304D; A48R-S187D-L290D-S297D-R304A; A48R-S187D-L290D-S297D-R304D-T313K; A48R-S187D-L290D-T313K; A48R-S187D-L290K-S297D-R304D; A48R-S187D-L290K-S297D-T313K; A48R-S187D-L290K-S297R-R304D; A48R-S187D-S297D-R304D-T313K; D100Q-D124N; D100Q-D124N-S185H; D100Q-S185H; D100Q-S185H; D100Q-S185H; D100Q-S185H; D124N-Q167K-S185H; D124N-S185H; D197E-S297C; D197L-

20   G235F; D197L-P316F; D197M-G235F-P316F; D197M-P316F; D197M-S297K; D73A-D100Q; D73A-D100Q-D124N; D73A-D124N; D73A-E110Q-D124N; D73A-E110Q-D124N-Q167K; D73A-E110Q-D124N-S185H; D73A-E110Q-Q167K-S185H; D73A-Q167K; D73A-S185H; D73A-S185H; D73E-G99E-T107F-Q167I; D73E-Q167A-S300K; D73E-Q167I; D73E-T107E-Q167A-A233C; D73E-T107E-Q167A-S300K; D73E-T107E-Q167I; D73E-T107E-Q167I; D73E-

25   T107F-S300K; E102S-V193T; E110Q-D124N; E110Q-D124N-S185H; E110Q-S185H; G192A-G235F-S297K; G192A-T212K-G235A-S297K; G235A-S297C; G235F-P316F; G235F-S297C; G235F-S297K; G235F-S297K; G235K-S297C; G235S-S297C; G295C-G298C; G295C-G298C; G295C-N315W; G295T-G298C; G2W-A165G; G2W-A165G; G2W-A165G; G301A-P302G-T303G; G301A-P302G-T303G-N315W; G301A-P302G-T303P; G301A-R304P; G301P-T303P-

30   R304P-Q320A; G301S-P302G-T303P-N315W-Q320P; G301S-T303G-Q320A; G301S-T303P-N315W-Y317W-Y318W; G99E; G99E-T107E-Q167A; I30E-E102S-V132N; I30E-E102S-V193T; I30E-E102S-V193T; I30E-N96P; I30E-N96P-E102S; I30E-N96P-E102S-V132N; I30E-N96P-V193T; I30E-P87H-N96P; I30E-P87H-N96P-E102S; I30E-P87H-N96P-E102S; I30E-P87H-N96P-E102S-V193T; I30E-P87H-N96P-V193T; I30E-V132N; I30E-V132N-V193T; I30E-

35   V193T; I30E-V193T; I30E-V80T; I30E-V80T-E102S; I30E-V80T-E102S-V193T; I30E-V80T-N96P; I30E-V80T-N96P; I30E-V80T-N96P-E102S; I30E-V80T-N96P-E102S-V193T; I30E-V80T-N96P-V132N; I30E-V80T-N96P-V132N-V193T; I30E-V80T-P87H; I30E-V80T-P87H-

E102S-V193T; I30E-V80T-P87H-N96P; I30E-V80T-P87H-N96P-V193T; I30E-V80T-P87H-
N96P-V193T; I30E-V80T-P87H-V132N; I30E-V80T-P87H-V193T; I30E-V80T-V132N; I30E-
V80T-V132N; I30E-V80T-V132N-V193T; I30E-V80T-V132N-V193T; I30E-V80T-V193T; K106F-
G192A-D197M-G235F-S297K; K106F-S297E; K106F-V117I-G235F-S297K; K106H-D197M;

5   K106H-G235F; K106H-G235F-P316F; K106H-P316F; K106H-P316F; K106R-D197M-G235A;
K106R-G235F; K106R-G235F; K106R-P316F; K106R-P316F; K58V-A168E; K58V-A168E;
K58V-K64L-K70N; K58V-K64L-K70N-A168E; K58V-K64L-K70N-A168E; K58V-K64L-K70N-
S135E-A168E; K58V-K64L-K70N-S135E-H163Y; K58V-K64L-S135E; K58V-K64L-S135E-
H163Y; K58V-K70N-H163Y-A168E; K58V-S135E; K64L-H163Y-A168E; K64L-K70N; K64L-

10   K70N; K64L-K70N; K64L-K70N; K64L-K70N-A168E; K64L-K70N-S135E; K64L-K70N-S135E;
K64L-K70N-S135E; K70A; K70A; K70A-H200A; K70A-H200A-P316A; K70A-N96P-H200A;
K70A-N96P-T108E-H200A-P316A; K70A-N96P-V104A-T108E-H200A; K70A-N96P-V104A-
T108E-P316A; K70A-N96P-V104A-T108E-P316A; K70A-N96P-V104I-T108E-P316A; K70A-
P316A; K70A-P316A; K70A-T108C-T287A; K70A-T108E; K70A-T108E-H200A; K70A-T108E-

15   H200A; K70A-T108E-H200A-P316A; K70A-T108E-H200A-P316A; K70A-T108E-T287A; K70A-
T108E-T287A; K70A-T108H-T287A; K70A-T108I-T196C-T287H; K70A-T108I-T287A; K70A-
T108I-T287A; K70A-T108K; K70A-T108L; K70A-T108L; K70A-T108L-T287A; K70A-T196C-
T287A; K70A-T287H; K70A-V104A-H200A; K70A-V104A-H200A; K70A-V104A-P316A; K70A-
V104A-T108E; K70A-V104A-T108E; K70A-V104A-T108E-H200A; K70A-V104A-T108E-H200A-

20   P316A; K70A-V104A-T108E-P316A; K70A-V104A-T108E-P316A; K70A-V104A-T108E-P316A;
K70A-V104I; K70A-V104I-T108E-H200A; K70A-V104I-T108E-H200A; K70A-V104I-T108E-
H200A-P316A; K70E-A168K-L290D-S297D; K70E-A168K-L290K-R304A-T313K; K70E-A168K-
L290K-S297D-R304A-T313K; K70E-A168K-R304D-T313K; K70E-A168K-S187D-L290D;
K70E-A168K-S187D-L290K-R304D-T313K; K70E-A168K-S297R-R304D; K70E-A168K-

25   S297R-R304D-T313K; K70E-L290D-R304A; K70E-L290D-R304D; K70E-L290D-R304D-
T313K; K70E-S187D-L290D-S297D-R304D; K70E-S187D-L290D-S297R; K70E-S187D-
S297D-T313K; K70E-S187D-S297R-R304A; K70E-S297D-R304A-T313K; K70L-A168K-
L290D-R304A-T313K; K70L-A168K-L290D-S297D-R304A-T313K; K70L-A168K-L290K-
S297D-R304D; K70L-A168K-L290K-S297D-R304D-T313K; K70L-A168K-R304A-T313K; K70L-

30   A168K-S187D-S297D-T313K; K70L-A168K-S187D-S297R; K70L-A168K-S297D-R304A-
T313K; K70L-A168K-S297D-R304A-T313K; K70L-A168K-S297D-R304A-T313K; K70L-A168K-
S297R-R304D; K70L-L290D-R304D; K70L-L290D-S297R-R304A-T313K; K70L-L290K-S297D-
R304A; K70L-R304A; K70L-R304D; K70L-S187D-R304A; K70L-S297D; K70L-S297D-R304A-
T313K; K70L-S297D-R304D; K70N-A168E; K70N-H163Y; K70N-S135E; K70N-S135E; K70N-

35   S135E-A168E; K70N-S135E-A168E; K70N-S135E-H163Y; K70N-S135E-H163Y-A168E;
K70R-H200A-P316A; K70R-N96P-T108E-H200A; K70R-N96P-T108E-H200A-P316A; K70R-
N96P-V104A-T108E-P316A; K70R-N96P-V104I-T108E; K70R-N96P-V104I-T108E; K70R-

N96P-V104I-T108E-H200A; K70R-T108E-P316A; K70R-V104A-T108E-P316A; K70R-V104A-
T108E-P316A; K70R-V104I; K70R-V104I-H200A; K70R-V104I-T108E-P316A; K70R-V104I-
T108E-P316A; K70S; K70S-H200A; K70S-H200A; K70S-N96P; K70S-N96P; K70S-N96P;
K70S-N96P-H200A; K70S-N96P-H200A; K70S-N96P-H200A-P316A; K70S-N96P-T108E;

5   K70S-N96P-T108E-P316A; K70S-N96P-V104I-T108E-H200A-P316A; K70S-N96P-V104I-
T108E-H200A-P316A; K70S-T108E; K70S-T108E; K70S-T108E; K70S-T108E-H200A; K70S-
T108E-P316A; K70S-V104A-H200A; K70S-V104A-H200A-P316A; K70S-V104A-T108E-
H200A; K70S-V104A-T108E-H200A-P316A; K70S-V104A-T108E-P316A; K70S-V104A-T108E-
P316A; K70S-V104I-H200A; K70S-V104I-P316A; L290A; L290K-R304A; L290K-S297R-

10   R304A; L290K-S297R-R304D; N10D-D197A; N10D-D197A-A201D; N10D-N51T-A201D;
N10D-N51T-D197A; N10D-N51T-D197A; N10D-N51T-D197A-A201D; N10D-N51T-T108Q;
N10D-N51T-T108Q-A201D; N10D-N51T-T108Q-A201D; N10D-N51T-T108Q-D197A; N10D-
N51T-T108Q-D197A-A201D; N10D-N51T-T108Q-D197A-A201D; N10D-N51T-T108Q-T127I-
D197A; N10D-N51T-T127I-D197A; N10D-N51T-T127I-D197A-A201D; N10D-N51T-V82W;

15   N10D-N51T-V82W; N10D-N51T-V82W-A201D; N10D-N51T-V82W-D197A; N10D-N51T-V82W-
D197A-A201D; N10D-N51T-V82W-T108Q; N10D-N51T-V82W-T108Q-D197A; N10D-N51T-
V82W-T108Q-D197A-A201D; N10D-N51T-V82W-T108Q-T127I; N10D-N51T-V82W-T108Q-
T127I; N10D-N51T-V82W-T108Q-T127I; N10D-N51T-V82W-T108Q-T127I-A201D; N10D-
N51T-V82W-T108Q-T127I-D197A; N10D-N51T-V82W-T108Q-T127I-D197A; N10D-N51T-

20   V82W-T108Q-T127I-D197A; N10D-N51T-V82W-T108Q-T127I-D197A; N10D-N51T-V82W-
T108Q-T127I-D197A-A201D; N10D-N51T-V82W-T108Q-T127I-D197A-A201D; N10D-N51T-
V82W-T127I-A201D; N10D-N51T-V82W-T127I-A201D; N10D-N51T-V82W-T127I-A201D;
N10D-N51T-V82W-T127I-D197A; N10D-N51T-V82W-T127I-D197A; N10D-N51T-V82W-T127I-
D197A; N10D-N51T-V82W-T127I-D197A; N10D-N51T-V82W-T127I-D197A; N10D-N51T-

25   V82W-T127I-D197A-A201D; N10D-N51T-V82W-T127I-D197A-A201D; N10D-T108Q-A201D;
N10D-T108Q-D197A; N10D-T108Q-D197A; N10D-T108Q-D197A-A201D; N10D-T108Q-T127I;
N10D-T108Q-T127I-A201D; N10D-T108Q-T127I-D197A; N10D-T108Q-T127I-D197A; N10D-
T108Q-T127I-D197A-A201D; N10D-T127I; N10D-T127I-D197A; N10D-T127I-D197A; N10D-
T127I-D197A; N10D-T127I-D197A; N10D-T127I-D197A-A201D; N10D-T127I-D197A-A201D;

30   N10D-T127I-D197A-A201D; N10D-T127I-D197A-A201D; N10D-V82W; N10D-V82W-D197A;
N10D-V82W-D197A-A201D; N10D-V82W-T108Q-A201D; N10D-V82W-T108Q-D197A; N10D-
V82W-T108Q-D197A; N10D-V82W-T108Q-T127I; N10D-V82W-T108Q-T127I; N10D-V82W-
T108Q-T127I-A201D; N10D-V82W-T108Q-T127I-D197A; N10D-V82W-T108Q-T127I-D197A;
N10D-V82W-T108Q-T127I-D197A-A201D; N10D-V82W-T108Q-T127I-D197A-A201D; N10D-

35   V82W-T127I; N10D-V82W-T127I-A201D; N10D-V82W-T127I-D197A-A201D; N10D-V82W-
T127I-D197A-A201D; N51E-A168E; N51E-H163Y; N51E-H163Y; N51E-H163Y-A168E; N51E-
K58V; N51E-K58V; N51E-K58V-A168E; N51E-K58V-K64L-K70N; N51E-K58V-K64L-K70N;

N51E-K58V-K64L-K70N; N51E-K58V-K64L-K70N; N51E-K58V-K64L-K70N; N51E-K58V-K64L-K70N-H163Y-A168E; N51E-K58V-K64L-K70N-S135E; N51E-K58V-K64L-S135E; N51E-K58V-K64L-S135E; N51E-K58V-K64L-S135E-A168E; N51E-K58V-K70N-A168E; N51E-K58V-K70N-S135E-A168E; N51E-K58V-K70N-S135E-H163Y; N51E-K58V-K70N-S135E-H163Y-A168E;

5 N51E-K58V-S135E; N51E-K58V-S135E; N51E-K58V-S135E; N51E-K64L-K70N-A168E; N51E-K64L-K70N-A168E; N51E-K64L-K70N-S135E; N51E-K64L-K70N-S135E; N51E-K64L-K70N-S135E-A168E; N51E-K64L-K70N-S135E-H163Y-A168E; N51E-K64L-K70N-S135E-H163Y-A168E; N51E-K70N; N51E-K70N; N51E-K70N-A168E; N51E-K70N-H163Y; N51E-K70N-H163Y-A168E; N51E-K70N-S135E; N51E-K70N-S135E-A168E; N51E-K70N-S135E-A168E;

10 N51E-K70N-S135E-H163Y-A168E; N51H-D100Q; N51H-D100Q-E110Q; N51H-D100Q-E110Q; N51H-D100Q-E110Q-D124N-Q167K; N51H-D100Q-E110Q-D124N-Q167K-S185H; N51H-D100Q-E110Q-D124N-S185H; N51H-D100Q-E110Q-D124N-S185H; N51H-D100Q-S185H; N51H-D100Q-S185H; N51H-D124N; N51H-D124N-S185H; N51H-D73A-D100Q; N51H-D73A-D100Q; N51H-D73A-D100Q; N51H-D73A-D100Q; N51H-D73A-D100Q; N51H-

15 D73A-D100Q-D124N; N51H-D73A-D100Q-D124N-Q167K; N51H-D73A-D100Q-D124N-Q167K; N51H-D73A-D100Q-S185H; N51H-D73A-D124N; N51H-D73A-D124N-Q167K; N51H-D73A-D124N-Q167K-S185H; N51H-D73A-D124N-S185H; N51H-D73A-D124N-S185H; N51H-D73A-E110Q-D124N-S185H; N51H-D73A-E110Q-D124N-S185H; N51H-D73A-E110Q-Q167K; N51H-D73A-E110Q-S185H; N51H-D73A-E110Q-S185H; N51H-D73A-Q167K; N51H-D73A-

20 S185H; N51H-D73A-S185H; N51H-E110Q; N51H-E110Q-D124N; N51H-E110Q-D124N; N51H-E110Q-D124N-Q167K-S185H; N51H-E110Q-D124N-S185H; N51H-E110Q-D124N-S185H; N51H-E110Q-Q167K; N51H-E110Q-S185H; N51H-S185H; N51H-S185H; N51T-A201D; N51T-A201D; N51T-D197A; N51T-D197A; N51T-D197A; N51T-D197A-A201D; N51T-T108Q; N51T-T108Q-A201D; N51T-T108Q-D197A-A201D; N51T-T108Q-T127I; N51T-T108Q-

25 T127I-A201D; N51T-T108Q-T127I-A201D; N51T-T108Q-T127I-A201D; N51T-T108Q-T127I-D197A; N51T-T108Q-T127I-D197A-A201D; N51T-T108Q-T127I-D197A-A201D; N51T-T127I; N51T-V82W; N51T-V82W; N51T-V82W-A201D; N51T-V82W-D197A; N51T-V82W-D197A-A201D; N51T-V82W-T108Q; N51T-V82W-T108Q; N51T-V82W-T108Q-A201D; N51T-V82W-T108Q-D197A-A201D; N51T-V82W-T108Q-D197A-A201D; N51T-V82W-T108Q-T127I; N51T-

30 V82W-T108Q-T127I; N51T-V82W-T108Q-T127I-A201D; N51T-V82W-T108Q-T127I-D197A-A201D; N51T-V82W-T108Q-T127I-D197A-A201D; N51T-V82W-T108Q-T127I-D197A-A201D; N51T-V82W-T127I; N51T-V82W-T127I; N51T-V82W-T127I; N51T-V82W-T127I; N51T-V82W-T127I-A201D; N51T-V82W-T127I-D197A-A201D; N59K-L290A; N62S-K70A; N62S-K70A-T108A; N62S-K70A-T108A; N62S-K70A-T108E-T287H; N62S-K70A-T108H-T287H; N62S-

35 K70A-T108I-T287H; N62S-K70A-T108K; N62S-K70A-T108L; N62S-K70A-T108L-T287A; N62S-K70A-T108L-T287H; N62S-K70A-T108L-T287H; N62S-K70A-T108L-T287H; N62S-K70A-T287A; N62S-K70A-T287H; N62S-K70A-T287H; N62S-T108A; N62S-T108C; N62S-

T108C-T287H; N62S-T108E-T287A; N62S-T108H; N62S-T108H-T287A; N62S-T108I-T287H;

N62S-T108K; N62S-T108K; N62S-T108K; N62S-T108K-T196C-T287H; N62S-T108K-T196C-

T287H; N62S-T108K-T287H; N62S-T108L-T287A; N62S-T287A; N96D-G235L; N96D-G235L;

N96D-T107A; N96D-T107A; N96D-T107A-D197E; N96D-T107A-D197E-G235F; N96D-T107A-

D197E-G235K; N96D-T107A-D197E-G235K; N96D-T107A-G235K; N96D-T107A-G235S;

N96D-T107D-G235K; N96D-T107E-D197E; N96D-T107E-D197E-G235A; N96D-T107E-

D197E-G235A; N96D-T107E-G235S; N96D-T107G-G235A; N96D-T107G-G235L; N96D-

T107G-G235S; N96D-T107H-D197E-G235L; N96D-T107H-G235S; N96D-T107K-D197E-

G235F; N96D-T107K-D197E-G235K; N96D-T107K-G235L; N96D-T107L-D197E-G235A;

N96D-T107L-D197E-G235K; N96D-T107L-D197E-G235K; N96D-T107M; N96D-T107M; N96D-

T107M-D197E; N96D-T107M-D197E-G235A; N96D-T107M-D197E-G235A; N96D-T107M-

D197E-G235L; N96D-T107M-G235L; N96D-T107N-D197E-G235A; N96D-T107N-G235K;

N96D-T107N-G235S; N96D-T107N-G235S; N96D-T107Q-G235F; N96D-T107R-D197E-

G235A; N96D-T107R-D197E-G235K; N96P-E102S-V193T; N96P-T108E; N96P-T108E; N96P-

T108E-H200A; N96P-T108E-H200A-P316A; N96P-V104A; N96P-V104I; N96P-V104I-T108E-

P316A; N96P-V132N-V193T; N96P-V193T; P28K-T108A; P28K-T108A; P28K-T108A-G295T-

G298C; P28K-T108A-N315W; P28K-T108D-G295C; P28K-T108D-G295C; P28K-T108D-

G295C-N315W; P28K-T108E; P28K-T108H-G298C; P28K-T108I-N315W; P28K-T108M; P28K-

T108M; P28K-V104A-G295C; P28K-V104A-G295T; P28K-V104A-G298C; P28K-V104A-

T108A; P28K-V104A-T108A; P28K-V104A-T108D; P28K-V104A-T108H-G295C; P28K-V104A-

T108H-G295T; P28K-V104A-T108I; P28K-V104I-N315W; P28K-V104I-T108A-G295T; P28K-

V104I-T108A-G295T-N315W; P28K-V104I-T108D-G295C; P28K-V104I-T108D-N315W; P28K-

V104I-T108D-N315W; P28K-V104I-T108E; P28K-V104I-T108E-G295T; P28K-V104I-T108H;

P28K-V104I-T108M; P28K-V104I-T108M-G295C; P302G-T303G-R304P-Y317W-Y318W;

P52F-D197M; P52F-G235A-P316F; P52F-G235F-P316F; P52F-K106R-G235A; P52F-P316F;

P52I-D197M; P52I-G235A; P52I-G235F; P52I-G235F-P316F; P52I-G235F-P316F; P52I-

K106H-G235A-P316F; P52I-K106H-G235F; P87H-E102S; P87H-E102S; P87H-E102S-V193T;

P87H-N96P; P87H-N96P-E102S-V193T; P87H-N96P-V132N; P87H-N96P-V132N-V193T;

P87H-N96P-V193T; P87H-V193T; P87H-V193T; Q167A-S300K; Q50G-D197M-G235F-S297K;

Q50G-K106F-G235A-S297K; Q50G-K106F-S297E; Q50G-T108D-D197M-G235F-S297K;

Q50G-T212K-G235F-S297K; Q50G-V117I-D197M-S297K; R304A-T313K; R304P; S135E-

A168E; S135E-A168E; S187D-S297R-R304D; S297D-R304D; S297R-R304D-T313K; S300T-

G301A-T303G; S300T-G301A-T303P-R304P-N315W-Y317W-Q320A; S300T-G301A-Y317W-

Y318W; S300T-G301P-T303P-Y317W-Q320P; S300T-G301S-T303G-N315W-Y317W-Q320S;

S300T-G301S-T303G-R304P-N315W-Y317W-Y318W; S300T-G301S-T303G-R304P-Y317W-

Q320S; S300T-G301S-T303P-R304P-Y317W-Q320A; S300T-N315W-Y317W-Q320A; S300T-

R304P-Y317W-Q320P; S300T-T303G-R304P-Y317W; T107A-A233C; T107A-D197E-G235A-

S297C; T107A-D197E-G235K; T107A-D197E-G235S-S297C; T107A-Q167I-A233C-S300K; T107C-S231A; T107C-S231C; T107D-D197E-G235K; T107D-G235F; T107D-G235L; T107D-S231A; T107E-D197E-G235A-S297C; T107E-D197E-G235S; T107E-G235A-S297C; T107E-G235A-S297C; T107E-G235F; T107E-G235S; T107E-Q167A; T107E-Q167A; T107E-Q167A-

5   A233C; T107E-Q167A-S300K; T107E-Q167I; T107E-Q167I; T107E-S297C; T107E-S297C; T107F-A233C; T107G-D197E-G235F; T107G-G235S; T107G-S297C; T107H-D197E-G235F; T107H-D197E-G235S; T107H-G235A; T107H-G235F; T107H-G235K; T107H-G235K; T107K-D197E-G235A; T107K-D197E-G235K; T107K-D197E-G235L; T107K-G235A-S297C; T107L-D197E-G235L; T107L-G235A; T107M-G235F-S297C; T107M-G235F-S297C; T107M-S231A;

10   T107N-D197E-G235A; T107N-D197E-G235K; T107N-D197E-G235S-S297C; T107N-G235K; T107N-G235L-S297C; T107N-G235S; T107N-L194D-S231C; T107Q-D197E-G235A-S297C; T107Q-D197E-G235F; T107Q-D197E-G235F; T107Q-D197E-G235K-S297C; T107Q-D197E-S297C; T107Q-G235K; T107R-D197E; T107R-D197E-G235A-S297C; T107R-D197E-G235F-S297C; T107R-D197E-G235K; T107R-D197E-G235K-S297C; T108A-G295C; T108A-G295C;

15   T108A-G295C-G298C; T108A-G295T-G298C; T108A-N315W; T108A-T287A; T108C-T287H; T108D-G295C; T108D-G295T; T108D-G295T-G298C-N315W; T108D-G298C; T108D-N315W; T108E-G295C-N315W; T108E-G295C-N315W; T108E-G295T; T108E-G295T-N315W; T108E-P316A; T108E-T196C-T287H; T108E-T287A; T108E-T287H; T108H-G295C; T108H-G295C; T108H-G295C-N315W; T108H-G295T-N315W; T108H-G295T-N315W; T108H-G298C; T108H-

20   T287H; T108I-G295C; T108I-G295C; T108I-G295T; T108I-T287A; T108I-T287A; T108I-T287H; T108I-T287H; T108K-T196C; T108K-T196C-T287A; T108K-T196C-T287A; T108K-T196C-T287H; T108K-T287A; T108L-T287A; T108M-G295C; T108M-G295C; T108M-G295C-N315W; T108M-G295T-G298C; T108Q-A201D; T108Q-A201D; T108Q-D197A; T108Q-D197A; T108Q-D197A; T108Q-D197A-A201D; T108Q-T127I; T108Q-T127I-A201D; T127I-A201D; T127I-

25   D197A; T127I-D197A; T127I-D197A-A201D; T212D-G235F-S297E; T212D-G235F-S297K; T303P-R304P-N315W-Y317W-Y318W-Q320S; T61D-D73E-G99E-T107F-Q167I-A233C; T61D-D73E-T107A-Q167A-A233C; T61D-D73E-T107A-Q167I; T61D-D73E-T107E-Q167I; T61D-D73E-T107F-Q167A; T61D-G99E-A233C-S300K; T61D-Q167A-S300K; T61D-Q167A-S300K; T61D-Q167I; T61D-Q167I-A233C-S300K; T61D-Q167I-A233C-S300K; T61D-T107A;

30   T61D-T107A; T61D-T107A-Q167I; T61D-T107A-S300K; T61D-T107E-Q167A-A233C-S300K; T61D-T107E-Q167A-S300K; T61D-T107E-Q167A-S300K; T61D-T107E-Q167I-A233C-S300K; T61D-T107E-Q167I-A233C-S300K; T61D-T107E-Q167I-S300K; T61K-D73E; T61K-D73E-G99E-T107A-Q167A-A233C-S300K; T61K-D73E-Q167A-S300K; T61K-D73E-Q167I-S300K; T61K-D73E-Q167I-S300K; T61K-D73E-T107A-Q167A; T61K-D73E-T107A-S300K; T61K-

35   D73E-T107E-A233C; T61K-D73E-T107E-A233C; T61K-D73E-T107E-A233C; T61K-Q167A-A233C; T61K-Q167A-A233C; T61K-Q167A-A233C-S300K; T61K-Q167I-A233C; T61K-Q167I-A233C; T61K-Q167I-A233C-S300K; T61K-T107A-Q167A-A233C; T61K-T107A-Q167A-A233C;

T61K-T107E-A233C; T61K-T107E-Q167A; T61K-T107E-Q167A; T61K-T107E-Q167A-S300K; T61K-T107E-Q167I-S300K; T61R-D73E-Q167A; T61R-D73E-T107A-Q167A-A233C; T61R-D73E-T107A-Q167A-S300K; T61R-D73E-T107A-Q167A-S300K; T61R-D73E-T107E; T61R-D73E-T107E; T61R-D73E-T107E; T61R-D73E-T107E-Q167A-A233C-S300K; T61R-D73E-

5   T107E-Q167A-A233C-S300K; T61R-D73E-T107E-Q167A-S300K; T61R-D73E-T107E-Q167A-S300K; T61R-G99E-T107E-Q167A-A233C-S300K; T61R-G99E-T107E-Q167A-S300K; T61R-G99E-T107F-Q167I-S300K; T61R-Q167A; T61R-Q167I; T61R-T107A-Q167A-A233C-S300K; T61R-T107A-Q167I-A233C; T61R-T107E-A233C-S300K; T61R-T107E-Q167A-A233C; T61R-T107E-Q167A-A233C; T61R-T107E-Q167I; T61R-T107E-Q167I-A233C; T61R-T107E-Q167I-

10   S300K; unknown; V104A-G295C-G298C; V104A-G295T; V104A-G295T-N315W; V104A-G295T-N315W; V104A-H200A; V104A-H200A-P316A; V104A-H200A-P316A; V104A-S231C; V104A-S231C; V104A-T107A-S231A; V104A-T107D; V104A-T107D; V104A-T107D; V104A-T107G; V104A-T107G; V104A-T107H-S231A; V104A-T107H-S231A; V104A-T107I; V104A-T107I; V104A-T107I-S231C; V104A-T107M; V104A-T107M-S231C; V104A-T107N-S231A;

15   V104A-T107N-S231C; V104A-T107Q; V104A-T107Q; V104A-T107Q-S231A; V104A-T107Q-S231C; V104A-T108A; V104A-T108A-G295C; V104A-T108A-G295T; V104A-T108A-G295T; V104A-T108A-G295T; V104A-T108A-G295T-N315W; V104A-T108A-G298C; V104A-T108A-G298C-N315W; V104A-T108D; V104A-T108D; V104A-T108D; V104A-T108D; V104A-T108E; V104A-T108E-G295T; V104A-T108E-G295T; V104A-T108E-G295T; V104A-T108E-G295T;

20   V104A-T108E-G295T-N315W; V104A-T108E-G295T-N315W; V104A-T108E-H200A-P316A; V104A-T108E-H200A-P316A; V104A-T108E-P316A; V104A-T108H; V104A-T108H; V104A-T108H-G295T; V104A-T108H-N315W; V104A-T108I; V104A-T108I; V104A-T108I-G295T; V104A-T108I-N315W; V104A-T108M-N315W; V104I-G295T; V104I-H200A; V104I-N315W; V104I-P316A; V104I-S231A; V104I-S231C; V104I-T107A; V104I-T107C-S231A; V104I-T107D;

25   V104I-T107D; V104I-T107H; V104I-T107H; V104I-T107H-S231A; V104I-T107H-S231A; V104I-T107M; V104I-T107M-S231A; V104I-T107M-S231A; V104I-T107M-S231C; V104I-T107N; V104I-T107N-S231C; V104I-T107Q; V104I-T107Q; V104I-T107Q; V104I-T108A; V104I-T108A; V104I-T108A; V104I-T108A-G295C; V104I-T108A-G295C; V104I-T108A-G295C; V104I-T108A-G295C; V104I-T108A-G295C-N315W; V104I-T108A-G295C-

30   N315W; V104I-T108A-G295T-N315W; V104I-T108A-N315W; V104I-T108D; V104I-T108D-G295T; V104I-T108D-N315W; V104I-T108E-G295C; V104I-T108E-G295C-G298C; V104I-T108E-P316A; V104I-T108H; V104I-T108H; V104I-T108H-G295T-N315W; V104I-T108H-N315W; V104I-T108M; V104I-T108M; V104I-T108M; V104I-T108M-G295T; V104I-V117I-G235A-S297K; V117I-D197M; V117I-D197M-G235F-S297K; V117I-T212K-G235A-S297K;

35   V132N-V193T; V80T-E102S; V80T-E102S; V80T-E102S; V80T-E102S-V193T; V80T-E102S-V193T; V80T-N96P; V80T-N96P; V80T-N96P; V80T-N96P; V80T-N96P; V80T-N96P-E102S; V80T-N96P-E102S; V80T-N96P-E102S; V80T-N96P-E102S-V132N; V80T-N96P-E102S-

V193T; V80T-N96P-V132N; V80T-N96P-V132N-V193T; V80T-N96P-V132N-V193T; V80T-N96P-V132N-V193T; V80T-N96P-V193T; V80T-N96P-V193T; V80T-N96P-V193T; V80T-P87H; V80T-P87H; V80T-P87H-E102S; V80T-P87H-E102S-V193T; V80T-P87H-N96P; V80T-P87H-N96P; V80T-P87H-N96P-E102S; V80T-P87H-N96P-E102S-V193T; V80T-P87H-N96P-V132N; V80T-P87H-N96P-V193T; V80T-P87H-V132N; V80T-V132N; V80T-V132N; V80T-V132N-V193T; V80T-V132N-V193T; V80T-V193T; V82W; V82W-A201D; V82W-D197A; V82W-D197A; V82W-T108Q; V82W-T108Q-A201D; V82W-T108Q-A201D; V82W-T108Q-D197A; V82W-T108Q-D197A; V82W-T108Q-T127I; V82W-T108Q-T127I-D197A; V82W-T108Q-T127I-D197A; V82W-T108Q-T127I-D197A; V82W-T127I; V82W-T127I-A201D; V82W-T127I-A201D; Y291F-G301A-P302G-T303P-Q320A; Y291F-G301P-P302G-T303G-Y317W-Y318W; Y291F-G301S-P302G-R304P-N315W-Q320A; Y291F-G301S-T303G-R304P-N315W-Y317W-Q320A; Y291F-P302G-T303P-N315W-Y317W-Q320P; Y291F-S300T-G301A-P302G-T303P-R304P-N315W; Y291F-S300T-G301A-T303G-R304P-Y317W-Y318W-Q320P; Y291F-S300T-G301S-P302G-N315W-Y317W; Y291F-S300T-G301S-P302G-T303G-N315W-Y318W-Q320P; Y291F-S300T-G301S-R304P-Q320S; Y291F-S300T-G301S-R304P-Y317W-Y318W-Q320P; Y291F-S300T-G301S-T303P-R304P-N315W-Q320P; Y291F-S300T-N315W-Y317W-Q320A; Y291F-S300T-N315W-Y317W-Q320A; Y291F-S300T-N315W-Y318W-Q320A; Y291F-S300T-P302G-T303G-R304P; Y291F-S300T-P302G-T303G-Y317W; Y291F-S300T-P302G-T303P-R304P-N315W-Y317W-Q320A; Y291F-S300T-P302G-Y318W-Q320S; Y291F-S300T-Q320A; Y291F-S300T-R304P-N315W-Q320A; Y291F-S300T-T303G-N315W-Y317W; Y291F-S300T-T303G-R304P-N315W-Y318W-Q320P; Y291F-S300T-T303P-R304P-N315W-Q320A; Y291F-S300T-T303P-R304P-Y317W-Y318W-Q320S; Y291F-S300T-Y317W-Y318W-Q320S; Y291W-G301P-P302G-T303G-R304P-Y317W-Q320A; Y291W-G301S-P302G-T303G-Y317W-Q320A; Y291W-G301S-T303G-N315W-Y317W-Q320S; Y291W-N315W-Y317W-Q320S; Y291W-S300T-G301A-Q320A; and Y291W-T303P-Q320S.

[1166] <u>Combinatorial Cohort 2</u>: Combinatorial GH61A variants having improved Tm as compared to wild-type: A168K-L290K; A168K-L290K-R304D; A168K-R304A; A168K-R304A-T313K; A168K-S187D-L290D-S297D-R304D; A168K-S187D-L290K-T313K; A168K-S187D-R304D; A168K-S297D-R304D-T313K; A16N-A48R; A16N-A48R-G99E; A16N-A48R-G99E-L290A; A16N-A48R-N59K; A16N-A48R-S164M-L290M; A16N-G99E-S164M; A16N-G99E-S164M-L290A; A16N-L290A; A16N-L290A; A16N-L290S; A16N-N59K; A16N-N59K-G99E-L290A; A16N-N59K-L290S; A16N-N59K-S164M; A48R-A168K-L290K-R304A-T313K; A48R-A168K-R304D-T313K; A48R-A168K-S187D-L290D-S297D-R304D; A48R-A168K-S297R; A48R-K70E-A168K-L290D-T313K; A48R-K70E-A168K-L290K-R304A-T313K; A48R-K70E-A168K-L290K-S297R-R304D; A48R-K70E-A168K-S187D-L290D-S297D; A48R-K70E-A168K-S187D-L290D-S297R-R304D; A48R-K70E-A168K-S187D-S297D; A48R-K70E-A168K-S187D-S297D-R304D; A48R-K70E-A168K-S187D-S297D-R304D-T313K; A48R-K70E-L290D-S297R-

R304A; A48R-K70E-R304A; A48R-K70E-R304D-T313K; A48R-K70L-A168K-L290D-S297D-
R304A; A48R-K70L-A168K-L290D-S297D-R304D; A48R-K70L-A168K-L290K-S297R-R304D;
A48R-K70L-A168K-S297R-R304A; A48R-K70L-L290K; A48R-K70L-S187D-L290D-R304A;
A48R-K70L-S187D-L290K-R304D-T313K; A48R-K70L-S187D-R304D; A48R-K70L-S297D-
R304D-T313K; A48R-L290K-S297R-R304D-T313K; A48R-N59K-S164M-L290A; A48R-R304A-
T313K; A48R-R304D; A48R-S187D-L290K-S297D-R304D; A48R-S187D-L290K-S297R-
R304D; A48R-S187D-S297D-R304D-T313K; D100Q-D124N; D100Q-D124N-S185H; D100Q-
E110Q-D124N; D100Q-E110Q-D124N-S185H; D124N-Q167K-S185H; D124N-S185H; D197E-
S297C; D197L-P316F; D197L-P316F; D197L-P316F; D197M-P316F; D197M-S297K; D73A-
D100Q-D124N; D73A-D100Q-D124N-Q167K-S185H; D73A-D100Q-E110Q-D124N; D73A-
D100Q-Q167K; D73A-D124N; D73A-D124N-S185H; D73A-E110Q-D124N; D73A-E110Q-
D124N-Q167K; D73A-E110Q-D124N-S185H; D73A-E110Q-Q167K-S185H; D73A-Q167K;
D73A-S185H; D73E-G99E-T107F-Q167I; D73E-Q167I; D73E-T107F-S300K; E110Q-D124N;
E110Q-D124N-S185H; G235K-S297C; G235S-S297C; G295C-G298C; G295C-G298C;
G295C-N315W; G295T-G298C; G99E; G99E-L290A; G99E-L290A; G99E-L290A; K106F-
G192A-D197M-G235F-S297K; K58V-A168E; K64L-K70N; K64L-K70N; K64L-K70N; K64L-
K70N; K64L-K70N-A168E; K64L-K70N-S135E; K64L-K70N-S135E; K64L-K70N-S135E; K70A;
K70A; K70A-N96P-V104A-T108E-P316A; K70A-P316A; K70A-P316A; K70A-T108E; K70A-
T108E-T287A; K70A-T108E-T287A; K70A-T108E-T287H; K70A-T108I-T196C-T287H; K70A-
T108I-T287A; K70A-T108L; K70A-T108L; K70A-T108L-T287H; K70A-T287H; K70A-T287H;
K70A-V104A-P316A; K70A-V104A-T108E; K70A-V104A-T108E; K70A-V104A-T108E-P316A;
K70A-V104I; K70E-A168K-L290D-S297D; K70E-A168K-S187D-L290D; K70E-A168K-S187D-
L290K-R304D-T313K; K70E-L290D-R304A; K70E-L290D-R304D; K70E-S187D-L290D-
S297D-R304D; K70E-S187D-L290D-S297R; K70E-S187D-S297D-T313K; K70E-S187D-
S297R-R304A; K70E-S297D-R304A-T313K; K70L-A168K-L290D-R304A-T313K; K70L-A168K-
L290D-S297D-R304A-T313K; K70L-A168K-L290K-S297D-R304D; K70L-A168K-L290K-
S297D-R304D-T313K; K70L-A168K-R304A; K70L-A168K-R304A-T313K; K70L-A168K-S187D-
S297D-T313K; K70L-A168K-S187D-S297R; K70L-A168K-S297D-R304A-T313K; K70L-A168K-
S297D-R304A-T313K; K70L-A168K-S297R-R304D; K70L-L290D-R304D; K70L-L290D-S297D;
K70L-L290D-S297R-R304A-T313K; K70L-R304A; K70L-R304D; K70L-S187D-R304A; K70L-
S297D; K70L-S297D-R304A-T313K; K70L-S297D-R304D; K70N-A168E; K70N-S135E; K70N-
S135E; K70N-S135E-A168E; K70R-N96P-V104A; K70R-N96P-V104A-T108E-P316A; K70R-
N96P-V104A-T108E-P316A; K70R-N96P-V104I-T108E; K70R-N96P-V104I-T108E; K70R-
T108E-P316A; K70R-V104A-T108E-P316A; K70R-V104A-T108E-P316A; K70R-V104I; K70R-
V104I-T108E-P316A; K70S; K70S-N96P; K70S-N96P; K70S-N96P-T108E-P316A; K70S-
T108E; K70S-T108E; K70S-V104A-T108E-P316A; K70S-V104A-T108E-P316A; K70S-V104I-
P316A; L290A; L290K-R304A; L290K-S297R-R304A; L290K-S297R-R304D; N10D-N51T-

D197A; N10D-N51T-T108Q; N10D-N51T-T108Q-D197A; N10D-N51T-T108Q-T127I-D197A; N10D-N51T-T127I-D197A; N10D-N51T-V82W-T108Q-T127I; N10D-N51T-V82W-T127I-D197A; N10D-T108Q-T127I; N10D-T108Q-T127I-D197A; N10D-T108Q-T127I-D197A; N10D-T127I; N10D-T127I-D197A; N10D-T127I-D197A; N10D-T127I-D197A; N10D-T127I-D197A; N51E-K58V-K64L-K70N; N51E-K70N; N51H-D100Q; N51H-D100Q-E110Q; N51H-D100Q-E110Q-D124N; N51H-D100Q-E110Q-D124N-Q167K; N51H-D100Q-E110Q-D124N-Q167K-S185H; N51H-D100Q-E110Q-D124N-S185H; N51H-D100Q-E110Q-D124N-S185H; N51H-D100Q-E110Q-D124N-S185H; N51H-D100Q-E110Q-D124N-S185H; N51H-D100Q-E110Q-Q167K-S185H; N51H-D100Q-S185H; N51H-D100Q-S185H; N51H-D100Q-S185H; N51H-D124N; N51H-D124N-S185H; N51H-D73A-D100Q; N51H-D73A-D100Q; N51H-D73A-D100Q; N51H-D73A-D100Q; N51H-D73A-D100Q; N51H-D73A-D100Q-D124N; N51H-D73A-D100Q-D124N; N51H-D73A-D100Q-D124N-Q167K; N51H-D73A-D100Q-D124N-Q167K; N51H-D73A-D100Q-D124N-Q167K; N51H-D73A-D100Q-D124N-Q167K-S185H; N51H-D73A-D100Q-D124N-S185H; N51H-D73A-D100Q-E110Q; N51H-D73A-D100Q-E110Q-D124N-Q167K; N51H-D73A-D100Q-E110Q-D124N-S185H; N51H-D73A-D100Q-E110Q-S185H; N51H-D73A-D100Q-E110Q-S185H; N51H-D73A-D100Q-Q167K-S185H; N51H-D73A-D100Q-S185H; N51H-D73A-D100Q-S185H; N51H-D73A-D124N; N51H-D73A-D124N-Q167K; N51H-D73A-D124N-Q167K-S185H; N51H-D73A-D124N-S185H; N51H-D73A-D124N-S185H; N51H-D73A-E110Q; N51H-D73A-E110Q-D124N-S185H; N51H-D73A-E110Q-D124N-S185H; N51H-D73A-E110Q-D124N-S185H; N51H-D73A-E110Q-Q167K; N51H-D73A-E110Q-S185H; N51H-D73A-E110Q-S185H; N51H-D73A-Q167K; N51H-D73A-S185H; N51H-D73A-S185H; N51H-D73A-S185H; N51H-E110Q; N51H-E110Q-D124N; N51H-E110Q-D124N; N51H-E110Q-D124N-Q167K-S185H; N51H-E110Q-D124N-S185H; N51H-E110Q-D124N-S185H; N51H-E110Q-D124N-S185H; N51H-E110Q-Q167K; N51H-E110Q-S185H; N51H-S185H; N51H-S185H; N51T-D197A; N51T-D197A; N51T-D197A; N51T-T108Q; N51T-T108Q-T127I; N51T-T108Q-T127I-D197A; N51T-T127I; N51T-V82W-T108Q-T127I; N51T-V82W-T108Q-T127I; N51T-V82W-T127I; N51T-V82W-T127I; N51T-V82W-T127I; N51T-V82W-T127I; N96D-T107A; N96D-T107A-D197E-G235K; N96D-T107A-D197E-G235K; N96D-T107A-D197E-G235K-S297C; N96D-T107A-G235K; N96D-T107D-G235K; N96D-T107D-S297C; N96D-T107E-D197E; N96D-T107E-D197E-S297C; N96D-T107E-G235K-S297C; N96D-T107L-D197E-G235K; N96D-T107M; N96D-T107M; N96D-T107M-D197E; N96D-T107M-D197E-S297C; N96D-T107N-G235K; N96D-T107N-G235K-S297C; N96D-T107Q-D197E-S297C; N96D-T107Q-D197E-S297C; N96D-T107R-D197E-G235K; N96P-T108E; N96P-T108E; N96P-V104A; N96P-V104I; N96P-V104I-T108E-P316A; P28K-V104A-T108A; R304A-T313K; S135E-A168E; S297D-R304D; S297R-R304D-T313K; T107A-D197E-G235K; T107D-D197E-G235K; T107D-G235K-S297C; T107E-Q167I; T107E-Q167I; T107E-S297C; T107E-S297C; T107G-D197E-G235K-S297C; T107G-S297C; T107G-S297C; T107H-G235K; T107H-G235K; T107K-D197E-G235K; T107L-D197E-S297C; T107M-G235F-S297C;

T107M-G235K-S297C; T107N-D197E-G235K; T107N-D197E-G235S-S297C; T107N-D197E-G235S-S297C; T107N-G235K; T107N-G235K-S297C; T107N-S297C; T107Q-D197E-G235K-S297C; T107Q-D197E-S297C; T107Q-G235K; T107R-D197E; T107R-D197E-G235K; T107R-D197E-G235K-S297C; T107R-D197E-G235K-S297C; T108A-G295C; T108A-G295C; T108A-G295T-G298C; T108A-T287A; T108A-T287A; T108C-T287H; T108D-G295T-G298C; T108D-G295T-G298C-N315W; T108D-G295T-G298C-N315W; T108D-N315W; T108E-G295C-N315W; T108E-G295T; T108E-P316A; T108E-T287A; T108E-T287A; T108E-T287H; T108H-G295C; T108H-G295T-N315W; T108H-G298C; T108H-N315W; T108H-T287H; T108H-T287H; T108I-G295C; T108I-G295C; T108I-G295C-N315W; T108I-G295T; T108I-G298C; T108I-G298C; T108I-T287A; T108I-T287A; T108I-T287H; T108I-T287H; T108K-T287A; T108L-T287A; T108M-G295C; T108M-G295C-N315W; T108M-G295T-G298C; T108M-G295T-G298C-N315W; T108M-G298C; T108Q-D197A; T108Q-D197A; T108Q-T127I; T127I-D197A; T127I-D197A; T61D-D73E-T107A-Q167A-A233C; T61D-G99E-A233C-S300K; T61D-Q167I; T61K-D73E-G99E-T107F; T61R-G99E-T107E-Q167A-A233C-S300K; T61R-G99E-T107F-Q167I-S300K; V104A-G295C-G298C; V104A-G295T; V104A-G295T-N315W; V104A-T107C; V104A-T107D; V104A-T107G; V104A-T107I; V104A-T107M; V104A-T107Q; V104A-T107Q; V104A-T108A; V104A-T108A-G295C; V104A-T108A-G295T; V104A-T108A-G295T; V104A-T108A-G295T; V104A-T108A-G295T-N315W; V104A-T108A-G298C; V104A-T108A-G298C-N315W; V104A-T108D-G295T-N315W; V104A-T108D-G298C-N315W; V104A-T108E; V104A-T108E-G295T; V104A-T108E-G295T; V104A-T108E-G295T; V104A-T108E-G295T-G298C; V104A-T108E-G295T-N315W; V104A-T108E-G295T-N315W; V104A-T108E-G298C; V104A-T108E-G298C-N315W; V104A-T108E-P316A; V104A-T108H; V104A-T108H-G295T; V104A-T108H-G295T; V104A-T108H-G295T-G298C; V104A-T108H-G298C; V104A-T108H-N315W; V104A-T108I; V104A-T108I; V104A-T108I-G295T; V104A-T108I-G295T-G298C; V104A-T108I-G295T-G298C; V104A-T108M-G298C-N315W; V104A-T108M-N315W; V104I-P316A; V104I-T107D; V104I-T107D; V104I-T107M; V104I-T107N; V104I-T107Q; V104I-T107Q; V104I-T108A; V104I-T108A; V104I-T108A-G295C; V104I-T108A-G295C; V104I-T108A-G295T-N315W; V104I-T108D; V104I-T108D-N315W; V104I-T108E-G295C-G298C; V104I-T108E-G298C; V104I-T108E-P316A; V104I-T108H-G295T-G298C; V104I-T108H-G295T-N315W; V104I-T108H-G295T-N315W; V104I-T108H-G298C-N315W; V104I-T108I-G295C-G298C; V104I-T108I-G295C-N315W; V104I-T108I-G295T-N315W; V104I-T108I-G298C; V104I-T108M; V104I-T108M-G295T; V104I-T108M-G298C-N315W; V104K-T107M; V104K-T107N; V82W-T108Q-T127I; V82W-T127I; Y291F-G301S-P302G-R304P-N315W-Q320A; Y291F-S300T-Q320A; and Y291W-G301S-N315W-Y317W-Y318W-Q320P.

[1167] <u>Combinatorial Cohort 3</u>: Combinatorial GH61A variants having improved performance in a whPCS assay (using SEC and/or RPC purified enzyme) as compared to wild-type: A16N-S164M; A48R-A168K-L290K-R304A-T313K; A48R-K70E-R304D-T313K; A48R-L290K-S297R-

R304D-T313K; A48R-N59K-S164M-L290A; A48R-R304A-T313K; A48R-S187D-L290D-S297D-
R304D-T313K; D197L-P316F; D197M-G235A-P316C; D197M-T212D; D197M-T212K-G235A-
S297K; G235A-S297C; G235F-S297C; G235K-S297C; G235S-S297C; G295C-N315W;
G301S-P302G-T303P-N315W-Q320P; G99E; G99E-S164M; I30E-N96P; K106C-G235F;

5   K106F-D197M-S297E; K106H-D197M-G235F-P316C; K106H-P316F; K58V-A168E; K58V-
K64L-K70N-A168E; K58V-K64L-K70N-A168E; K64L-K70N-A168E; K70L-R304D; K70R-
T108E-P316A; K70S-N96P-H200A; K70S-N96P-V104A-H200A; K70S-N96P-V104A-T108E;
K70S-T108E-P316A; K70S-V104I-H200A; N10D-N51T-V82W-T108Q-D197A; N10D-N51T-
V82W-T108Q-T127I-D197A; N10D-N51T-V82W-T108Q-T127I-D197A-A201D; N10D-T108Q-

10   T127I-D197A; N10D-T127I-D197A; N10D-T127I-D197A-A201D; N10D-V82W-T127I-D197A-
A201D; N51E-K58V-K64L-K70N; N51E-K58V-K64L-K70N; N51E-K58V-K64L-K70N-S135E;
N51E-K64L-K70N-A168E; N51H-D73A-D100Q-E110Q-S185H; N51H-D73A-D100Q-S185H;
N51H-D73A-D124N-S185H; N51H-D73A-S185H; N51T-D197A; N51T-V82W-T127I-D197A-
A201D; N62S-K70A-T108K-T196C-T287H; N62S-K70A-T287H; N96D-G235F-S297C; N96D-

15   T107D-S297C; N96D-T107K-D197E-G235A-S297C; N96D-T107K-D197E-G235S-S297C;
N96D-T107M-G235F-S297C; N96D-T107Q-D197E-S297C; N96D-T107Q-D197E-S297C;
P28K-V104A-T108D; P52F-D197L; P52F-G235A-P316F; P52F-G235F-P316F; P52F-K106H-
G235A-P316F; P52F-K106R-G235F-P316F; P52F-P316F; P52I-D197L-G235F-P316F; P52I-
D197L-P316F; P52I-D197M-G235F-P316F; P52I-G235F-P316F; P52I-G235F-P316F; P52I-

20   K106C-G235F; P52I-K106C-P316F; Q50G-D197M-T212K; Q50G-D197M-T212K-G235A-
S297E; Q50G-K106F-D197M-S297K; Q50G-T108D-T212K-S297E; Q50G-V117I-D197M-
T212K-G235F-S297K; Q50G-V117I-T212D-S297K; S300T-G301A-Y317W-Y318W; T107A-
D197E-G235A-S297C; T107D-G235A-S297C; T107E-D197E-G235A-S297C; T107E-G235A-
S297C; T107E-S297C; T107E-S297C; T107H-D197E-G235L-S297C; T107K-G235A-S297C;

25   T107M-G235K-S297C; T107N-D197E-G235S-S297C; T107N-S297C; T107Q-G235A-S297C;
T107R-D197E-G235K-S297C; T108A-G295T-G298C; T108A-T287A; T108H-N315W; T108Q-
D197A; T61D-G99E-A233C-S300K; T61R-D73E-T107A-Q167A-A233C; T61R-D73E-T107E;
T61R-T107E-A233C-S300K; V104A-T107H-S231A; V104A-T107I; V104A-T108E-G298C-
N315W; V104I-V117I-T212D-S297K; V80T-V132N; V82W-D197A; V82W-T108Q-T127I; V82W-

30   T127I; V82W-T127I-A201D; Y291F-S300T-G301S-P302G-N315W-Y317W; Y291F-S300T-
Q320A; and Y291F-S300T-Y317W-Y318W-Q320S.

[1168] <u>Combinatorial Cohort 4</u>: Combinatorial GH61A variants having improved performance
in a daCS assay (using SEC and/or RPC purified enzyme) as compared to wild-type: A165G-
S185D; A168K-L290D-S297D-R304A; A16N-S164M; A48R-A168K-L290K-R304A-T313K;

35   A48R-K70L-S187D-L290K-R304D-T313K; A48R-L290K-S297R-R304D-T313K; A48R-N59K-
S164M-L290A; A48R-R304A-T313K; A48R-S187D-L290D-S297D-R304D-T313K; D197M-
G235A-P316C; D197M-P316F; D197M-T212D; D197M-T212K-G235A-S297K; D73A-D100Q;

G235A-S297C; G235K-S297C; G235S-S297C; G295C-G298C; G295C-N315W; G295T-
G298C; G301A-P302G-T303G-N315W-Y317W-Y318W-Q320P; G301A-P302G-T303G-R304P-
N315W; G301A-R304P; G301A-R304P-N315W-Y317W-Q320A; G301P-T303P-R304P-Q320A;
G301S-P302G-T303G-R304P-Y317W-Q320S; G301S-P302G-T303P-N315W-Q320P; G301S-

5   T303G-Q320A; G301S-T303G-Y317W-Y318W-Q320P; G301S-T303P-N315W-Y317W-
Y318W; G99E; G99E-L290A; G99E-S164M; I30E-N96P; K106C-G235A-P316C; K106C-
G235F; K106C-P316F; K106C-P316F; K106F-D197M-S297E; K106F-T108D-D197M-S297K;
K106H-D197M; K106H-P316F; K58V-A168E; K58V-K64L-K70N-A168E; K58V-K64L-K70N-
A168E; K58V-K64L-S135E; K64L-K70N-A168E; K70A-H200A; K70A-N96P-V104I-T108E-

10   P316A; K70A-T108E-T287A; K70A-T108E-T287H; K70A-V104A-T108E-H200A; K70A-V104I;
K70A-V104I-T108E; K70A-V104I-T108E-H200A-P316A; K70E-S187D-L290D-S297R; K70L-
A168K-L290D-S297D-R304A-T313K; K70L-R304D; K70N-S135E; K70R-H200A-P316A; K70R-
N96P-T108E-H200A-P316A; K70R-N96P-V104A-T108E-H200A; K70R-N96P-V104A-T108E-
H200A-P316A; K70R-N96P-V104A-T108E-P316A; K70R-T108E-P316A; K70S; K70S-N96P-

15   T108E-P316A; K70S-N96P-V104A-H200A; K70S-N96P-V104A-T108E; K70S-V104A-H200A-
P316A; K70S-V104A-T108E-H200A; K70S-V104A-T108E-P316A; K70S-V104I-H200A; N10D-
N51T-V82W-T108Q-D197A; N10D-N51T-V82W-T108Q-T127I-D197A; N10D-N51T-V82W-
T108Q-T127I-D197A-A201D; N10D-T108Q-T127I-D197A; N10D-T127I-D197A-A201D; N10D-
V82W-T108Q-T127I-A201D; N10D-V82W-T127I-D197A-A201D; N51E-K58V-K64L-A168E;

20   N51E-K58V-K64L-K70N; N51E-K58V-K64L-K70N-S135E; N51E-K58V-K70N-A168E; N51E-
K58V-S135E; N51E-K64L-K70N-A168E; N51H-D73A-D100Q-E110Q-S185H; N51H-D73A-
D100Q-S185H; N51H-D73A-S185H; N51T-D197A; N62C-K70A-P316A; N62C-K70R-H200A-
P316A; N62C-K70R-N96P-T108E-H200A; N62C-K70R-N96P-V104I-T108E-P316A; N62C-
K70S-N96P; N62C-N96P-H200A; N62C-N96P-T108E-P316A; N62S-K70A-T108I-T287H;

25   N62S-K70A-T287H; N96D-T107E-G235K-S297C; N96D-T107L-D197E-G235L-S297C; N96D-
T107N-D197E-G235F-S297C; N96D-T107Q; N96D-T107Q-D197E-G235A-S297C; N96D-
T107Q-D197E-S297C; N96D-T107Q-D197E-S297C; N96D-T107R-D197E-G235A; N96D-
T107R-D197E-G235S-S297C; N96D-T107R-D197E-G235S-S297C; P28K-T108A-G295T-
G298C; P28K-T108A-G298C-N315W; P28K-T108A-N315W; P28K-T108D-G295C-N315W;

30   P28K-T108E-G295T-G298C-N315W; P28K-T108H-G295C-G298C; P28K-T108H-G295C-
G298C; P28K-T108M-G295C-G298C-N315W; P28K-V104A-T108A-G295C-G298C-N315W;
P28K-V104A-T108D; P28K-V104A-T108D; P28K-V104A-T108D-G295T-G298C; P28K-V104A-
T108I-G295T-G298C-N315W; P28K-V104A-T108I-G295T-N315W; P28K-V104I-N315W;
P28K-V104I-T108A-G295C-N315W; P28K-V104I-T108A-G295T-G298C; P28K-V104I-T108A-

35   G295T-N315W; P28K-V104I-T108D-G295C-G298C; P28K-V104I-T108D-G295T-N315W;
P28K-V104I-T108D-N315W; P28K-V104I-T108E-G298C; P28K-V104I-T108H-G295C-N315W;
P28K-V104I-T108H-G295T-G298C-N315W; P28K-V104I-T108H-G295T-N315W; P28K-V104I-

T108H-N315W; P28K-V104I-T108I-G295T; P28K-V104I-T108M-G295T-G298C-N315W;
P302G-T303G-R304P-Y317W-Y318W; P302G-T303G-R304P-Y317W-Y318W; P302G-T303P-
Y317W-Q320S; P52C-G235F-P316F; P52F-G235A-P316F; P52F-G235F-P316F; P52F-
K106H-G235A-P316F; P52F-P316F; P52I-D197L-G235F-P316F; P52I-D197L-P316F; P52I-
5   D197M-G235F-P316F; P52I-G235F-P316F; P52I-G235F-P316F; P52I-K106C-G235F; P52I-
K106C-P316F; P52I-K106R-D197M-P316C; Q50G-D197M-T212K; Q50G-D197M-T212K-
G235A-S297E; Q50G-K106F-G235A-S297K; Q50G-K106F-S297E; Q50G-K106F-T212K-
S297K; Q50G-T108D-T212K-S297E; Q50G-V117I-T212D-S297K; R304P-Q320P; S300T-
G301A-P302G-R304P-Q320P; S300T-G301A-P302G-T303G-Q320P; S300T-G301A-P302G-
10   T303P-R304P-N315W-Y317W-Q320A; S300T-G301A-R304P-N315W-Y317W-Y318W; S300T-
G301A-Y317W-Y318W; S300T-G301P-P302G-T303G-R304P-N315W-Q320P; S300T-G301P-
T303G-N315W-Y318W-Q320A; S300T-G301P-T303P-Y317W-Q320P; S300T-G301S-P302G-
R304P-N315W-Y317W-Q320P; S300T-G301S-P302G-T303G-R304P-N315W-Y318W-Q320S;
S300T-G301S-P302G-T303P-N315W-Y317W-Y318W-Q320A; S300T-G301S-P302G-T303P-
15   Q320P; S300T-G301S-T303G-N315W-Y317W-Q320S; S300T-G301S-T303G-N315W-Y317W-
Y318W-Q320P; S300T-G301S-T303G-R304P-N315W-Y317W-Y318W; S300T-G301S-T303G-
R304P-Y317W-Q320S; S300T-G301S-T303G-Y317W-Y318W-Q320S; S300T-G301S-T303P-
N315W; S300T-G301S-T303P-R304P-Y317W-Q320A; S300T-P302G-T303P-N315W-Q320S;
S300T-P302G-T303P-R304P-N315W-Y317W-Y318W-Q320A; S300T-T303P-R304P-N315W-
20   Y317W-Y318W; T107A-D197E-G235A-S297C; T107D-G235K-S297C; T107E-G235A-S297C;
T107E-S297C; T107E-S297C; T107G-S297C; T107H-D197E-G235L-S297C; T107L-D197E-
S297C; T107M-G235K-S297C; T107N-D197E-G235S-S297C; T107N-S297C; T107Q-D197E-
G235F; T107Q-G235A-S297C; T108A-G295C-G298C; T108A-G295C-N315W; T108A-G295T-
G298C; T108A-N315W; T108A-T287A; T108D-G295T; T108D-G295T-G298C; T108D-G295T-
25   G298C-N315W; T108D-G295T-G298C-N315W; T108D-G298C; T108D-N315W; T108E-
G295C-N315W; T108E-G295C-N315W; T108E-G295T-N315W; T108H-G295C-N315W;
T108H-G295T-N315W; T108H-G295T-N315W; T108H-N315W; T108H-N315W; T108I-G295C;
T108I-G295C-N315W; T108I-G298C; T108M-G295C; T108M-G295C-N315W; T108M-G295T-
G298C-N315W; T108M-G298C; T108Q-D197A; T303P-R304P-N315W-Y317W-Y318W-
30   Q320S; T61K-G99E-Q167I-S300K; T61K-G99E-T107A-Q167I-A233C; T61K-T107F-Q167A-
S300K; T61R-D73E-Q167A; T61R-D73E-T107A-Q167A-S300K; T61R-D73E-T107F-A233C-
S300K; T61R-T107E-A233C-S300K; unknown; V104A-G295C-G298C; V104A-G295T; V104A-
G295T-N315W; V104A-H200A-P316A; V104A-N315W; V104A-T108A-G295T-N315W; V104A-
T108A-G298C; V104A-T108A-G298C-N315W; V104A-T108D; V104A-T108D-G295T-N315W;
35   V104A-T108D-G298C; V104A-T108E-G295T; V104A-T108E-G295T; V104A-T108E-G295T-
G298C; V104A-T108E-G295T-N315W; V104A-T108E-G298C; V104A-T108E-G298C-N315W;
V104A-T108H-G295C-N315W; V104A-T108H-G295T; V104A-T108H-G295T; V104A-T108H-

G295T-G298C; V104A-T108H-G295T-G298C; V104A-T108H-G298C; V104A-T108H-N315W; V104A-T108I; V104A-T108I-G295C-N315W; V104A-T108I-G295T-G298C; V104A-T108I-G295T-G298C; V104A-T108I-N315W; V104A-T108M-G298C; V104I-D197M-T212D-S297K; V104I-G295C-G298C; V104I-K106F-G235F-S297E; V104I-N315W; V104I-T108A-G295C;

V104I-T108A-G295C-G298C-N315W; V104I-T108A-G295C-N315W; V104I-T108A-G295C-N315W; V104I-T108A-G295T-G298C-N315W; V104I-T108A-G295T-N315W; V104I-T108A-G298C; V104I-T108A-N315W; V104I-T108D-G295C-G298C-N315W; V104I-T108D-G295C-N315W; V104I-T108D-G295C-N315W; V104I-T108D-G295C-N315W; V104I-T108D-G295T; V104I-T108D-G295T-G298C-N315W; V104I-T108D-G298C-N315W; V104I-T108D-N315W;

V104I-T108D-N315W; V104I-T108E-G295T-G298C-N315W; V104I-T108E-G295T-G298C-N315W; V104I-T108E-G298C; V104I-T108E-P316A; V104I-T108H-G295C-N315W; V104I-T108H-G295T-G298C; V104I-T108H-G295T-G298C-N315W; V104I-T108H-G295T-N315W; V104I-T108H-G295T-N315W; V104I-T108H-G298C-N315W; V104I-T108H-N315W; V104I-T108I-G295C-N315W; V104I-T108I-G295T-N315W; V104I-T108I-G295T-N315W; V104I-T108M; V104I-T108M; V104I-T108M-G298C-N315W; V104I-T108M-N315W; V104I-V117I-T212D-S297K; V82W-T108Q-T127I; Y291F-G301A-P302G-T303P-Q320A; Y291F-G301A-T303P-R304P-N315W-Y318W-Q320S; Y291F-G301P-P302G-T303G-R304P-N315W-Y317W-Q320S; Y291F-G301P-P302G-T303G-Y317W-Y318W; Y291F-G301S-P302G-T303G-R304P-Y317W-Y318W-Q320P; Y291F-G301S-P302G-T303P-R304P-Y317W-Y318W-Q320S; Y291F-G301S-T303G-R304P-N315W-Y317W-Q320A; Y291F-G301S-T303P-R304P-N315W-Y317W-Q320S; Y291F-P302G-R304P-Y317W-Y318W-Q320P; Y291F-P302G-T303P-N315W-Y317W-Q320P; Y291F-R304P-N315W-Y317W-Q320A; Y291F-R304P-N315W-Y317W-Y318W-Q320P; Y291F-S300T-G301A-P302G-T303P-R304P-N315W; Y291F-S300T-G301A-T303G-R304P-Y317W-Y318W-Q320P; Y291F-S300T-G301P-N315W-Q320A; Y291F-S300T-G301P-R304P-N315W-Y317W-Y318W; Y291F-S300T-G301S-P302G-N315W-Y317W; Y291F-S300T-G301S-P302G-T303G-N315W-Y318W-Q320P; Y291F-S300T-G301S-P302G-T303G-N315W-Y318W-Q320P; Y291F-S300T-G301S-P302G-T303P-Y318W-Q320S; Y291F-S300T-G301S-R304P-Q320S; Y291F-S300T-G301S-R304P-Y317W-Y318W-Q320P; Y291F-S300T-G301S-T303P-R304P-N315W-Q320P; Y291F-S300T-N315W-Y318W-Q320A; Y291F-S300T-P302G-T303G-R304P; Y291F-S300T-P302G-T303G-Y317W; Y291F-S300T-P302G-T303P-R304P-N315W-Y317W-Q320A; Y291F-S300T-P302G-Y318W-Q320S; Y291F-S300T-Q320A; Y291F-S300T-R304P-N315W-Q320A; Y291F-S300T-R304P-N315W-Y317W-Q320P; Y291F-S300T-T303G-R304P-N315W-Y318W-Q320P; Y291F-S300T-T303P-R304P-Y317W-Y318W-Q320S; Y291F-S300T-T303P-Y317W; Y291F-S300T-Y317W-Y318W-Q320S; Y291F-T303G-N315W-Y317W-Y318W; Y291F-Y317W-Q320S; Y291W-G301A-P302G-T303P-R304P-N315W-Y318W-Q320A; Y291W-G301P-P302G-T303G-R304P-Y317W-Q320A; Y291W-G301S-N315W-Y317W-Y318W-Q320P; Y291W-G301S-P302G-T303G-Q320A; Y291W-G301S-P302G-T303G-

Y317W-Q320A; Y291W-G301S-T303G-N315W-Y317W-Q320S; Y291W-N315W-Y317W-
Q320S; Y291W-P302G-R304P-N315W-Y317W-Y318W; Y291W-S300T-G301A-Q320A;
Y291W-S300T-G301S-T303G-Y317W-Y318W-Q320S; Y291W-S300T-G301S-T303P-N315W-
Y317W-Y318W-Q320P; Y291W-S300T-P302G-T303P-R304P-N315W-Y317W-Y318W;

5   Y291W-S300T-T303G-R304P-N315W-Y317W-Y318W-Q320S; and Y291W-T303P-Q320S.

[1169] <u>Combinatorial Cohort 5</u>: Combinatorial GH61A variants having improved performance
in an Avicel assay (using SEC and/or RPC purified enzyme) as compared to wild-type:

[1170] A165G-S185D; A168K-R304A; A168K-S187D-R304D; A16N-L290S; A16N-S164M;
A48R-A168K-S297R-R304A; A48R-K70E-A168K-L290K-S297R-R304A; A48R-K70E-R304A;

10   A48R-K70E-S187D-S297D-R304D; A48R-K70L-L290K; A48R-N59K-S164M-L290A; D124N-
Q167K-S185H; D197E-S297C; D197L-G235F; D197L-P316F; D197L-P316F; D197M-G235A-
P316C; D197M-G235F-P316F; D197M-G235F-P316F; D197M-P316F; D197M-S297K; D197M-
T212D; D197M-T212K-G235A; D197M-T212K-G235A-S297K; D73A-D100Q; D73A-D100Q;
D73A-D100Q-D124N-Q167K-S185H; D73A-D100Q-E110Q; D73A-D100Q-E110Q-D124N;

15   D73A-D100Q-E110Q-S185H; D73A-D100Q-E110Q-S185H; D73A-D100Q-S185H; D73A-
D124N; D73A-D124N-S185H; D73A-E110Q-D124N; D73A-Q167K; D73A-S185H; D73A-
S185H; D73E-G99E-T107A-Q167A; D73E-G99E-T107A-Q167A-A233C; D73E-G99E-T107A-
Q167I; D73E-G99E-T107A-Q167I-S300K; D73E-G99E-T107E-Q167I-A233C-S300K; D73E-
G99E-T107F-Q167I; D73E-T107E-Q167A-A233C; D73E-T107F-Q167I-S300K; E102S-V193T;

20   G192A-D197M-G235A-S297K; G192A-D197M-G235F-S297K; G235A-S297C; G235A-S297C;
G235A-S297K; G235F-S297C; G235K-S297C; G235S-S297C; G295C-N315W; G301S-
P302G-T303P-N315W-Q320P; G99E; G99E-Q167A-A233C-S300K; G99E-S164M; G99E-
T107E-Q167A-A233C-S300K; G99E-T107F-Q167A-A233C; I30E-N96P; I30E-N96P-E102S;
I30E-N96P-V193T; I30E-V193T; I30E-V80T-E102S; I30E-V80T-E102S-V193T; I30E-V80T-

25   N96P; I30E-V80T-N96P-E102S; I30E-V80T-P87H-E102S-V193T; I30E-V80T-P87H-N96P-
E102S-V193T; I30E-V80T-P87H-V132N; K106C-G235A-P316C; K106C-G235F; K106C-
G235F; K106C-P316F; K106E-D197M-G235F-S297K; K106E-T108D-G235A-S297K; K106E-
T108D-V117I-S297E; K106F-D197M-G235F-S297E; K106F-D197M-S297E; K106F-G235A-
S297K; K106F-S297E; K106F-T108D-D197M-S297K; K106F-T108D-V117I-G235F-S297K;

30   K106F-T212K-G235A; K106F-V117I-G235F-S297K; K106F-V117I-T212K-G235F-S297K;
K106H-D197L-G235F-P316C; K106H-D197M-G235A; K106H-D197M-G235A-P316C; K106H-
D197M-G235F; K106H-D197M-G235F; K106H-D197M-G235F-P316C; K106H-D197M-G235F-
P316C; K106H-D197M-P316C; K106H-G235A-P316F; K106H-G235F-P316F; K106H-P316C;
K106H-P316F; K106R-D197M-G235A; K106R-D197M-G235A-P316F; K106R-D197M-G235F-

35   P316C; K106R-D197M-P316F; K106R-G235A-P316C; K106R-G235A-P316C; K58V-A168E;
K58V-K64L-K70N-A168E; K58V-K64L-K70N-A168E; K64L-K70N-A168E; K70A-H200A-P316A;
K70A-N96P-T108E-H200A-P316A; K70A-T108E-H200A; K70A-T108E-H200A-P316A; K70A-

T108H-T196C-T287H; K70A-T108I-T196C; K70A-T108I-T196C; K70A-T108L-T196C; K70A-
T287H; K70A-V104A-H200A; K70A-V104A-H200A; K70A-V104A-H200A-P316A; K70A-V104A-
T108E-H200A; K70A-V104I-T108E; K70A-V104I-T108E-H200A-P316A; K70L-A168K-R304A;
K70L-A168K-S187D-L290K-S297D-R304A-T313K; K70L-A168K-S187D-S297R; K70L-S187D-
R304A; K70R-H200A-P316A; K70R-N96P-T108E-H200A-P316A; K70R-N96P-V104A-T108E-
H200A; K70R-N96P-V104A-T108E-H200A-P316A; K70R-N96P-V104I-T108E-H200A; K70R-
T108E-P316A; K70R-V104A-T108E-P316A; K70R-V104I-T108E-P316A; K70S; K70S-H200A;
K70S-H200A; K70S-N96P; K70S-N96P-H200A; K70S-N96P-H200A; K70S-N96P-H200A-
P316A; K70S-N96P-T108E-P316A; K70S-N96P-V104A-H200A; K70S-T108E; K70S-T108E-
P316A; K70S-V104A-H200A-P316A; K70S-V104A-T108E-H200A; K70S-V104A-T108E-
H200A-P316A; K70S-V104A-T108E-H200A-P316A; K70S-V104I-H200A; L194D-S231C;
N10D-N51T-T108Q-D197A; N10D-N51T-T108Q-D197A-A201D; N10D-N51T-T108Q-T127I-
D197A; N10D-N51T-T127I-D197A; N10D-N51T-V82W-T108Q-D197A; N10D-N51T-V82W-
T108Q-T127I-D197A; N10D-N51T-V82W-T108Q-T127I-D197A-A201D; N10D-N51T-V82W-
T127I-D197A; N10D-T108Q-T127I; N10D-T108Q-T127I-D197A; N10D-T108Q-T127I-D197A-
A201D; N10D-T127I-D197A-A201D; N10D-T127I-D197A-A201D; N10D-V82W-T108Q-T127I-
D197A-A201D; N10D-V82W-T127I-D197A-A201D; N51E-K58V-K64L-K70N; N51E-K58V-
K64L-K70N-H163Y; N51E-K58V-K64L-K70N-S135E; N51E-K58V-K64L-K70N-S135E-H163Y;
N51E-K58V-K64L-S135E-H163Y-A168E; N51E-K64L-K70N-A168E; N51E-K64L-K70N-H163Y;
N51E-K64L-K70N-H163Y-A168E; N51H-D73A-D100Q; N51H-D73A-D100Q; N51H-D73A-
D100Q; N51H-D73A-D100Q-D124N-Q167K-S185H; N51H-D73A-D100Q-E110Q-D124N-
S185H; N51H-D73A-D100Q-E110Q-S185H; N51H-D73A-D100Q-E110Q-S185H; N51H-D73A-
D100Q-E110Q-S185H; N51H-D73A-D100Q-S185H; N51H-D73A-D100Q-S185H; N51H-D73A-
D124N-Q167K-S185H; N51H-D73A-D124N-S185H; N51H-D73A-E110Q; N51H-D73A-E110Q-
D124N-S185H; N51H-D73A-E110Q-S185H; N51H-D73A-S185H; N51H-D73A-S185H; N51H-
D73A-S185H; N51H-E110Q-D124N-S185H; N51T-A201D; N51T-D197A; N51T-T108Q-A201D;
N51T-T108Q-T127I-A201D; N51T-T108Q-T127I-A201D; N51T-T108Q-T127I-D197A; N51T-
T108Q-T127I-D197A-A201D; N51T-T108Q-T127I-D197A-A201D; N51T-V82W-A201D; N51T-
V82W-T108Q-T127I-D197A-A201D; N51T-V82W-T108Q-T127I-D197A-A201D; N51T-V82W-
T127I; N51T-V82W-T127I-D197A-A201D; N62C-K70A-N96P-H200A-P316A; N62C-K70A-
N96P-T108E; N62C-K70A-N96P-T108E-P316A; N62C-K70A-T108E; N62C-K70R-H200A-
P316A; N62C-K70R-N96P-T108E-H200A; N62C-K70R-N96P-V104I-T108E-P316A; N62C-
K70R-V104A-P316A; N62C-K70R-V104A-T108E-P316A; N62C-K70S-H200A; N62C-K70S-
N96P; N62C-K70S-N96P-P316A; N62C-N96P-H200A; N62C-N96P-H200A; N62C-T108E-
H200A-P316A; N62C-T108E-P316A; N62C-V104I-T108E-P316A; N62S-K70A-T108A; N62S-
K70A-T108E-T196C; N62S-K70A-T108K; N62S-K70A-T287H; N62S-T108A; N62S-T108C-
T287H; N62S-T108C-T287H; N62S-T108E-T287A; N62S-T108H-T287A; N62S-T108I-T287H;

N62S-T108K; N62S-T108L-T287A; N96D-G235F-S297C; N96D-T107A-D197E-G235K-S297C;

N96D-T107D-G235A-S297C; N96D-T107D-S297C; N96D-T107E-D197E-G235A-S297C;

N96D-T107E-D197E-S297C; N96D-T107E-G235K-S297C; N96D-T107G-D197E-G235A-

S297C; N96D-T107G-D197E-G235S-S297C; N96D-T107G-G235S-S297C; N96D-T107G-

G235S-S297C; N96D-T107H-D197E-G235A-S297C; N96D-T107H-D197E-G235A-S297C;

N96D-T107H-D197E-G235S-S297C; N96D-T107H-G235S-S297C; N96D-T107K-D197E-

G235A-S297C; N96D-T107K-D197E-G235S-S297C; N96D-T107L-D197E-G235L-S297C;

N96D-T107L-G235F-S297C; N96D-T107M-D197E-G235A; N96D-T107M-D197E-S297C;

N96D-T107M-G235F-S297C; N96D-T107N-G235K-S297C; N96D-T107Q-D197E-G235A-

S297C; N96D-T107Q-D197E-G235F-S297C; N96D-T107Q-D197E-S297C; N96D-T107Q-

D197E-S297C; N96D-T107R-D197E-G235S-S297C; N96P-E102S-V193T; N96P-T108E;

N96P-T108E-H200A-P316A; N96P-V104A; N96P-V104I; P28K-T108A-G295T-G298C; P28K-

T108A-G298C-N315W; P28K-T108D-G295C-N315W; P28K-T108E-G295T-G298C-N315W;

P28K-T108H-G298C; P28K-V104A-G298C; P28K-V104A-T108D; P28K-V104A-T108D; P28K-

V104A-T108E-G298C; P28K-V104A-T108H; P28K-V104A-T108I-G295T-G298C-N315W;

P28K-V104A-T108I-G295T-N315W; P28K-V104A-T108I-G298C; P28K-V104I-T108A-G295C-

N315W; P28K-V104I-T108A-G295T-G298C; P28K-V104I-T108A-G295T-N315W; P28K-V104I-

T108D-G295T-N315W; P28K-V104I-T108D-G298C-N315W; P28K-V104I-T108E; P28K-V104I-

T108E-G298C; P28K-V104I-T108E-G298C; P28K-V104I-T108H-G295C-N315W; P28K-V104I-

T108H-G295T-G298C-N315W; P28K-V104I-T108H-G295T-N315W; P28K-V104I-T108M-

G298C; P52C-D197L-P316F; P52C-G235F-P316F; P52C-K106R-G235A-P316C; P52F-D197L;

P52F-D197L-G235A; P52F-D197M-G235A; P52F-D197M-P316C; P52F-G235A-P316F; P52F-

G235F-P316F; P52F-K106H-D197L; P52F-K106H-D197L; P52F-K106H-D197L-P316F; P52F-

K106H-D197L-P316F; P52F-K106H-D197M-G235F-P316C; P52F-K106H-D197M-G235F-

P316F; P52F-K106H-D197M-G235F-P316F; P52F-K106H-D197M-P316C; P52F-K106H-

G235A-P316F; P52F-K106H-G235F-P316C; P52F-K106H-P316C; P52F-K106R-D197L-

G235F-P316C; P52F-K106R-D197L-P316C; P52F-K106R-D197M-G235F-P316F; P52F-

K106R-D197M-P316C; P52F-K106R-D197M-P316C; P52F-K106R-G235A-P316F; P52F-

K106R-P316F; P52F-P316C; P52F-P316F; P52I-D197L-G235F-P316F; P52I-D197L-P316F;

P52I-D197M; P52I-D197M; P52I-D197M-G235F-P316C; P52I-D197M-G235F-P316F; P52I-

G235A; P52I-G235F-P316F; P52I-G235F-P316F; P52I-K106C-D197L; P52I-K106C-G235F;

P52I-K106C-G235F; P52I-K106C-G235F-P316F; P52I-K106C-P316F; P52I-K106H-D197L;

P52I-K106H-D197L-G235F; P52I-K106H-D197L-G235F-P316C; P52I-K106H-D197L-P316F;

P52I-K106H-D197L-P316F; P52I-K106H-D197M-G235A-P316C; P52I-K106H-D197M-P316C;

P52I-K106H-G235A-P316F; P52I-K106H-G235F-P316C; P52I-K106H-P316F; P52I-K106R-

D197L-G235A-P316F; P52I-K106R-D197L-G235F; P52I-K106R-D197L-G235F-P316C; P52I-

K106R-D197L-P316C; P52I-K106R-D197L-P316F; P52I-K106R-D197M-P316C; Q167A-

S300K; Q50G-D197M-G235F-S297K; Q50G-D197M-T212K; Q50G-D197M-T212K-G235A-
S297E; Q50G-D197M-T212K-G235F-S297K; Q50G-G192A-D197M-G235F-S297K; Q50G-
K106E-T212K-S297K; Q50G-K106E-V117I-G235F-S297K; Q50G-K106F-D197M-S297K;
Q50G-K106F-G235A-S297K; Q50G-K106F-S297E; Q50G-K106F-T108D-D197M-S297K;

5   Q50G-K106F-T212K-S297K; Q50G-T108D-D197M-G235F-S297K; Q50G-T108D-T212K-
G235F-S297K; Q50G-T108D-T212K-S297E; Q50G-V104I-D197M-T212K-G235F-S297K;
Q50G-V104I-G192A; Q50G-V104I-G192A-G235A-S297K; Q50G-V104I-G192A-G235A-S297K;
Q50G-V104I-G192A-S297K; Q50G-V104I-K106E; Q50G-V104I-K106F-T108D-G235A-S297K;
Q50G-V104I-K106F-T108D-G235F-S297K; Q50G-V104I-T108D-T212K-S297K; Q50G-V104I-

10 T108D-V117I-T212K-S297K; Q50G-V104I-T212D-G235F-S297K; Q50G-V117I-D197M-T212K-
G235F-S297K; Q50G-V117I-D197M-T212K-G235F-S297K; Q50G-V117I-G192A-D197M-
G235F-S297K; Q50G-V117I-T212D-S297K; R304A-T313K; S187D-S297R-R304D; S300T-
G301P-T303G-N315W-Y318W-Q320A; S300T-G301P-T303P-R304P-N315W-Y317W-Q320P;
S300T-P302G-T303P-N315W-Q320S; S300T-T303P-R304P-N315W-Y317W-Y318W; T107A-

15 A233C; T107A-D197E-G235A-S297C; T107A-D197E-G235S-S297C; T107D-G235A-S297C;
T107D-G235A-S297C; T107D-G235K-S297C; T107E-D197E-G235A-S297C; T107E-G235A-
S297C; T107E-G235A-S297C; T107E-Q167A-A233C; T107E-S297C; T107E-S297C; T107F-
Q167I-A233C; T107G-D197E-G235K-S297C; T107G-D197E-G235L-S297C; T107G-G235A-
S297C; T107G-G235S; T107G-S297C; T107G-S297C; T107H-D197E-G235L-S297C; T107H-

20 G235S-S297C; T107K-G235A-S297C; T107L-D197E-S297C; T107M-D197E-G235L-S297C;
T107M-G235A-S297C; T107M-G235F-S297C; T107M-G235K-S297C; T107N-D197E-G235K;
T107N-D197E-G235S-S297C; T107N-D197E-G235S-S297C; T107N-G235K-S297C; T107N-
G235L-S297C; T107N-L194D; T107N-L194G-S231A; T107N-S297C; T107Q-D197E-G235A-
S297C; T107Q-D197E-G235K-S297C; T107Q-D197E-S297C; T107Q-G235A-S297C; T107Q-

25 G235S-S297C; T107Q-L194G-S231A; T107R-D197E-G235A-S297C; T107R-D197E-G235F-
S297C; T107R-D197E-G235K-S297C; T107R-D197E-G235K-S297C; T108A-G295C-N315W;
T108A-G295T-G298C; T108D-G298C; T108E-N315W; T108H-G295C-N315W; T108H-G298C;
T108H-N315W; T108I-G295C-N315W; T108I-G298C; T108I-G298C; T108I-T287A; T108M-
G298C; T108Q-D197A; T108Q-D197A; T108Q-T127I-A201D; T212D-G235F-S297K; T61D-

30 D73E-G99E-T107F-Q167I-A233C; T61D-D73E-T107A-Q167A-A233C; T61D-G99E-A233C-
S300K; T61D-G99E-Q167I; T61D-G99E-T107A-Q167I; T61D-G99E-T107E-Q167A-S300K;
T61D-G99E-T107E-Q167I; T61D-Q167I-A233C-S300K; T61D-T107A-A233C; T61D-T107E-
Q167A-A233C-S300K; T61D-T107E-Q167I-A233C-S300K; T61D-T107E-Q167I-A233C-S300K;
T61K-D73E-G99E-A233C-S300K; T61K-D73E-G99E-T107A-A233C; T61K-D73E-G99E-

35 T107E-Q167I-A233C-S300K; T61K-D73E-Q167A-A233C; T61K-D73E-T107A-A233C-S300K;
T61K-D73E-T107A-Q167I-S300K; T61K-D73E-T107E-A233C; T61K-D73E-T107E-A233C;
T61K-D73E-T107E-A233C; T61K-D73E-T107F; T61K-D73E-T107F-A233C; T61K-D73E-

T107F-Q167A-A233C-S300K; T61K-G99E-Q167A-A233C; T61K-G99E-Q167I-S300K; T61K-
G99E-T107A-A233C-S300K; T61K-Q167A-A233C; T61K-Q167A-A233C; T61K-Q167A-A233C-
S300K; T61K-Q167I-A233C; T61K-Q167I-A233C; T61K-Q167I-A233C-S300K; T61K-T107A-
A233C-S300K; T61K-T107A-Q167A-A233C; T61K-T107A-Q167I-A233C-S300K; T61K-T107E-

5   A233C; T61K-T107F-Q167I-A233C; T61R-D73E-Q167I-A233C; T61R-D73E-T107A-Q167A-
A233C; T61R-D73E-T107E-Q167A-A233C-S300K; T61R-D73E-T107E-Q167A-A233C-S300K;
T61R-D73E-T107F-Q167I; T61R-G99E-T107A-A233C-S300K; T61R-G99E-T107E-A233C;
T61R-G99E-T107E-Q167A; T61R-T107A-Q167A-A233C-S300K; T61R-T107A-Q167I-A233C;
T61R-T107E-A233C-S300K; T61R-T107E-A233C-S300K; T61R-T107E-Q167A-A233C; T61R-

10   T107E-Q167A-A233C; T61R-T107E-Q167I; T61R-T107E-Q167I-A233C; T61R-T107F-A233C;
T61R-T107F-Q167I-A233C; V104A-H200A; V104A-H200A-P316A; V104A-L194G-S231C;
V104A-T107C; V104A-T107D-S231C; V104A-T107G; V104A-T107H-S231A; V104A-T107I;
V104A-T107I; V104A-T107I-S231C; V104A-T107I-S231C; V104A-T107M; V104A-T107M-
S231C; V104A-T107N-L194G; V104A-T107Q; V104A-T107Q; V104A-T107Q-S231C; V104A-

15   T108A-G298C; V104A-T108A-G298C-N315W; V104A-T108D; V104A-T108D-G298C; V104A-
T108D-G298C-N315W; V104A-T108E-G298C-N315W; V104A-T108E-H200A-P316A; V104A-
T108E-H200A-P316A; V104A-T108H-G295C-N315W; V104A-T108H-G295T-G298C; V104A-
T108H-G295T-G298C; V104A-T108H-G298C; V104A-T108I; V104A-T108I-G295C-G298C-
N315W; V104A-T108I-G295C-N315W; V104A-T108I-G295T-G298C; V104A-T108I-G295T-

20   G298C; V104A-T108M-G298C; V104I-D197M-G235A-S297K; V104I-D197M-T212D-S297K;
V104I-G192A-T212K-G235F; V104I-H200A; V104I-K106F; V104I-K106F-G235F-S297E;
V104I-T107A; V104I-T107F; V104I-T107H; V104I-T107I-S231C; V104I-T107N; V104I-T107N-
L194G-S231A; V104I-T107N-S231C; V104I-T107Q; V104I-T108A; V104I-T108A-G295C-
N315W; V104I-T108A-G295C-N315W; V104I-T108A-G295T-N315W; V104I-T108A-G298C;

25   V104I-T108D-G295C-N315W; V104I-T108D-G295C-N315W; V104I-T108D-G298C-N315W;
V104I-T108E-G295T-G298C-N315W; V104I-T108E-G295T-G298C-N315W; V104I-T108E-
G298C; V104I-T108E-P316A; V104I-T108H-G295C-N315W; V104I-T108H-G295T-N315W;
V104I-T108H-G298C-N315W; V104I-T108I; V104I-T108I-G295C-N315W; V104I-T108I-G295T-
N315W; V104I-T108I-G298C; V104I-T108M; V104I-T108M; V104I-T108M-G298C-N315W;

30   V104I-V117I-T212D-S297K; V104K-T107D-S231A; V104K-T107G-S231A; V104K-T107I-
S231A; V104R-T107A-S231A; V104R-T107D; V104R-T107G-S231C; V104R-T107I; V104R-
T107I-S231A; V104R-T107M; V104R-T107N; V104R-T107Q-S231C; V117I-D197M; V117I-
D197M-G235F-S297K; V117I-G192A-T212K-G235A-S297K; V80T-E102S; V80T-E102S;
V80T-E102S; V80T-E102S-V193T; V80T-E102S-V193T; V80T-N96P; V80T-N96P; V80T-

35   N96P; V80T-N96P; V80T-N96P-E102S; V80T-N96P-E102S; V80T-N96P-E102S; V80T-N96P-
E102S; V80T-N96P-V193T; V80T-N96P-V193T; V80T-V132N; V80T-V132N-V193T; V80T-
V193T; V82W-D197A; V82W-T108Q-T127I; V82W-T127I; V82W-T127I-A201D; Y291F-R304P-

N315W-Y317W-Q320A; Y291W-N315W-Y317W-Q320S; Y291W-S300T-G301P-T303G-
Y318W-Q320A; and Y291W-S300T-P302G-T303P-R304P-N315W-Y317W-Y318W.

[1171] Combinatorial Cohort 6: Combinatorial GH61A variants having improved performance
in an Avicel After assay (using SEC and/or RPC purified enzyme) as compared to wild-type:

5   A168K-R304A-T313K; A16N-A48R-G99E-L290A; A16N-G99E-S164M; A16N-G99E-S164M-
       L290A; A16N-L290S; A16N-N59K; A16N-N59K-S164M; A16N-S164M; A48R-K70E-A168K-
       L290K-S297R-R304A; A48R-K70E-R304A; A48R-K70L-A168K-L290D-S297D-R304D; A48R-
       N59K-S164M-L290A; A48R-S187D-L290K-S297D-T313K; D100Q-E110Q-D124N-S185H;
       D124N-Q167K-S185H; D197L-P316F; D197M-P316F; D197M-S297K; D197M-T212K-G235A-
10   S297K; D73A-D100Q; D73A-D100Q-D124N-Q167K-S185H; D73A-D100Q-E110Q; D73A-
       D124N; D73A-D124N-S185H; D73A-E110Q-D124N; D73A-E110Q-D124N-Q167K; D73A-
       Q167K; D73A-S185H; D73E-G99E-T107A-S300K; D73E-G99E-T107F-Q167I; E110Q-D124N;
       E110Q-D124N-S185H; G192A-D197M-G235F-S297K; G235F-S297C; G235K-S297C; G2V-
       A165G; G301S-P302G-T303G-R304P-Y317W-Q320S; G301S-T303G-Y317W-Y318W-Q320P;
15   G99E; G99E-L290A; G99E-S164M; G99E-T107F-Q167A-A233C; I30E-E102S-V132N-V193T;
       I30E-N96P-E102S-V132N-V193T; I30E-V80T-P87H-E102S-V193T; I30E-V80T-P87H-N96P-
       E102S-V193T; I30E-V80T-P87H-N96P-V132N; I30E-V80T-P87H-V132N; I30E-V80T-P87H-
       V132N; I30E-V80T-P87H-V132N-V193T; K106C-G235F; K106E-T108D-G235A-S297K;
       K106E-T108D-V117I-S297E; K106F-G235A-S297K; K106F-T108D-D197M-S297K; K106F-
20   T108D-V117I-G235F-S297K; K106F-T212K-G235A; K58V-A168E; K58V-K64L-H163Y-A168E;
       K58V-K64L-K70N-A168E; K58V-K64L-K70N-A168E; K58V-K64L-K70N-S135E-H163Y; K58V-
       K64L-S135E-H163Y; K58V-S135E-H163Y-A168E; K64L-K70N; K64L-K70N-A168E; K70A-
       T108E-T287H; K70A-T108I-T196C; K70A-T108I-T196C; K70A-T108K-T287H; K70A-T108L;
       K70E-A168K-S297R-R304D-T313K; K70L-A168K-S187D-L290K-S297D-R304A-T313K; K70L-
25   A168K-S187D-L290K-S297R-R304D-T313K; K70L-L290D-S297D; K70R-N96P-V104A-T108E-
       P316A; K70R-T108E-P316A; K70R-V104A-T108E-P316A; K70S; L290K-S297R-R304D;
       N10D-N51T-T108Q-T127I-D197A; N10D-N51T-V82W-T108Q-D197A; N10D-N51T-V82W-
       T108Q-T127I-D197A; N10D-T108Q-T127I-D197A; N10D-V82W-T108Q-T127I; N51E-K58V-
       K64L-K70N; N51E-K58V-K64L-K70N-H163Y; N51E-K58V-K64L-K70N-H163Y; N51E-K58V-
30   K64L-K70N-S135E; N51E-K58V-K64L-K70N-S135E-H163Y; N51E-K58V-S135E-H163Y;
       N51E-K64L-K70N-A168E; N51E-K64L-K70N-H163Y-A168E; N51E-K70N-S135E-H163Y-
       A168E; N51H-D100Q-E110Q-D124N; N51H-D100Q-E110Q-D124N-Q167K; N51H-D100Q-
       E110Q-D124N-S185H; N51H-D124N; N51H-D124N-S185H; N51H-D73A-D100Q; N51H-D73A-
       D100Q-D124N; N51H-D73A-D100Q-D124N-Q167K; N51H-D73A-D100Q-D124N-Q167K;
35   N51H-D73A-D100Q-D124N-Q167K; N51H-D73A-D100Q-D124N-Q167K-S185H; N51H-D73A-
       D100Q-D124N-S185H; N51H-D73A-D100Q-D124N-S185H; N51H-D73A-D100Q-E110Q-
       D124N-S185H; N51H-D73A-D100Q-E110Q-S185H; N51H-D73A-D100Q-E110Q-S185H;

N51H-D73A-D100Q-E110Q-S185H; N51H-D73A-D100Q-E110Q-S185H; N51H-D73A-D100Q-
S185H; N51H-D73A-D100Q-S185H; N51H-D73A-D124N; N51H-D73A-D124N-Q167K; N51H-
D73A-D124N-Q167K-S185H; N51H-D73A-D124N-S185H; N51H-D73A-D124N-S185H; N51H-
D73A-E110Q; N51H-D73A-E110Q-D124N-S185H; N51H-D73A-E110Q-D124N-S185H; N51H-
D73A-E110Q-Q167K; N51H-D73A-S185H; N51H-D73A-S185H; N51H-E110Q; N51H-E110Q-
D124N; N51H-E110Q-D124N-Q167K-S185H; N51H-E110Q-D124N-S185H; N51H-E110Q-
Q167K; N51H-E110Q-S185H; N51T-D197A; N51T-T108Q-T127I-D197A; N51T-T108Q-T127I-
D197A-A201D; N51T-V82W-T108Q; N51T-V82W-T127I; N51T-V82W-T127I; N59K-G99E-
L290A; N62C-K70A-N96P-T108E-P316A; N62C-K70A-T108E; N62C-K70R-N96P-T108E-
H200A; N62C-K70R-N96P-V104I-T108E-P316A; N62C-K70R-V104A-P316A; N62C-K70R-
V104A-T108E-P316A; N62C-K70S-H200A; N62C-K70S-N96P; N62C-K70S-N96P-T108E;
N62C-V104I-T108E-P316A; N62S-K70A-T108E-T196C; N62S-K70A-T196C-T287A; N96D-
G235F-S297C; N96D-T107D-G235K; N96D-T107D-S297C; N96D-T107E-G235K-S297C;
N96D-T107G; N96D-T107G-G235S-S297C; N96D-T107K-D197E-G235A-S297C; N96D-
T107K-D197E-G235S-S297C; N96D-T107M-D197E-G235A; N96D-T107M-G235F-S297C;
N96D-T107Q-D197E-S297C; N96P-V104A; N96P-V104I; P28K-T108A-G298C-N315W; P28K-
T108E-G295T-G298C-N315W; P28K-V104A-T108I-G295T-N315W; P28K-V104I-T108A-
G295C-N315W; P28K-V104I-T108D-G295T-N315W; P28K-V104I-T108E-G298C; P28K-V104I-
T108H-G295C-N315W; P28K-V104I-T108H-G295T-G298C-N315W; P28K-V104I-T108H-
G295T-N315W; P28K-V104I-T108H-N315W; P28K-V104I-T108M-G295T-G298C-N315W;
P52C-D197L-P316F; P52F-K106R-D197L-P316C; Q50G-D197M-T212K; Q50G-K106E-T212K-
S297K; Q50G-K106F-T108D-D197M-S297K; Q50G-K106F-T212K-S297K; Q50G-V104I-
G192A; Q50G-V104I-G192A-G235A-S297K; Q50G-V104I-G192A-G235A-S297K; Q50G-
V104I-K106F-T108D-G235A-S297K; Q50G-V104I-T108D-V117I-T212K-S297K; Q50G-V104I-
T212D-G235F-S297K; Q50G-V117I-G192A-D197M-G235F-S297K; R304A-T313K; S187D-
S297R-R304D; S300T-G301P-P302G-N315W-Y317W; S300T-G301P-T303P-R304P-N315W-
Y317W-Q320P; S300T-G301S-P302G-T303G-R304P-N315W-Y318W-Q320S; T107A-D197E-
G235S-S297C; T107D-D197E-G235K; T107E-D197E-G235A-S297C; T107E-G235A-S297C;
T107E-S297C; T107H-D197E-G235L-S297C; T107K-G235A-S297C; T107L-D197E-S297C;
T107M-G235A-S297C; T107N-D197E-G235S-S297C; T107N-D197E-G235S-S297C; T107N-
G235K-S297C; T107N-L194D; T107N-L194D-S231C; T107N-S297C; T107Q-D197E-G235A-
S297C; T107R-D197E-G235A-S297C; T107R-D197E-G235K; T107R-D197E-G235K-S297C;
T107R-D197E-G235K-S297C; T108A-G295C-N315W; T108A-G295T-G298C; T108A-T287A;
T108D-G295T-G298C; T108D-G295T-G298C-N315W; T108E-N315W; T108E-T287A; T108H-
G295T-N315W; T108H-G298C; T108H-N315W; T108I-G295C-N315W; T108I-G298C; T108I-
G298C; T108I-T287H; T108M-G295C; T108M-G295C-N315W; T108M-G295T-G298C-N315W;
T108M-G298C; T108Q-D197A; T108Q-T127I; T127I-D197A; T127I-D197A; T61D-D73E-G99E-

T107F-Q167I-A233C; T61D-G99E-A233C-S300K; T61D-G99E-Q167I; T61D-G99E-T107A-Q167I; T61D-G99E-T107E-Q167A-S300K; T61D-G99E-T107E-Q167I; T61K-D73E-G99E-A233C-S300K; T61K-D73E-G99E-T107E-Q167I-A233C-S300K; T61K-D73E-T107F-Q167A-A233C-S300K; T61K-G99E-T107A-A233C-S300K; T61R-D73E-T107A-Q167A-A233C; T61R-G99E-Q167I-A233C; T61R-T107E-A233C-S300K; T61R-T107E-Q167I; V104A-G295T-N315W; V104A-T107D-L194D; V104A-T107D-S231C; V104A-T107F; V104A-T107G; V104A-T107I; V104A-T107N-L194G; V104A-T107Q; V104A-T108A-G295C; V104A-T108A-G295T-N315W; V104A-T108A-G298C-N315W; V104A-T108D-G298C; V104A-T108D-G298C-N315W; V104A-T108E-G295C; V104A-T108E-G295T-G298C; V104A-T108E-G295T-N315W; V104A-T108E-G298C-N315W; V104A-T108H; V104A-T108H-G295T-G298C; V104A-T108H-G295T-G298C; V104A-T108H-G298C; V104A-T108I; V104A-T108I; V104A-T108I-G295T-G298C; V104A-T108M-G298C; V104I-D197M-T212D-S297K; V104I-G192A-T212K-G235F; V104I-T107D; V104I-T107I-S231C; V104I-T107Q; V104I-T108A-G295T-N315W; V104I-T108A-G298C; V104I-T108D; V104I-T108D-G295T-G298C-N315W; V104I-T108D-N315W; V104I-T108E-G295T-G298C-N315W; V104I-T108E-G295T-G298C-N315W; V104I-T108H-G295C-N315W; V104I-T108H-G295T-G298C-N315W; V104I-T108H-G295T-N315W; V104I-T108H-G295T-N315W; V104I-T108H-G298C-N315W; V104I-T108I; V104I-T108I-G295C-N315W; V104I-T108I-G295T-N315W; V104I-T108M; V104I-T108M; V104I-T108M-G298C-N315W; V104I-V117I-T212D-S297K; V104K-T107M; V104K-T107Q; V104K-T107Q; V104R-T107Q-S231C; V80T-E102S; V80T-E102S-V132N-V193T; V80T-P87H-E102S-V193T; V82W-D197A; V82W-T127I; V82W-T127I-A201D; Y291F-G301S-T303P-R304P-N315W-Y317W-Q320S; Y291F-P302G-R304P-Y317W-Y318W-Q320P; Y291F-S300T-G301S-P302G-T303G-N315W-Y318W-Q320P; Y291F-S300T-G301S-R304P-N315W-Q320P; Y291F-Y317W-Y318W-Q320A; Y291W-G301S-P302G-T303G-Q320A; Y291W-P302G-R304P-N315W-Y317W-Y318W; and Y291W-S300T-G301A-Y317W-Y318W-Q320P.

[1172] **Combinatorial Cohort 7**: Combinatorial GH61A variants having improved stability (using SEC and/or RPC purified enzyme) as compared to wild-type: A165Y-S185D; A168K-L290D-S297D-R304A; A168K-L290K; A168K-L290K-R304D; A168K-R304A-T313K; A168K-R304A-T313K; A168K-S187D-L290D-S297D-R304D; A168K-S187D-L290K-T313K; A168K-S297D-R304D-T313K; A16N-A48R-G99E-L290A; A16N-A48R-N59K-G99E; A16N-A48R-S164M-L290M; A16N-G99E-S164M; A16N-G99E-S164M-L290A; A16N-L290A; A16N-L290A; A16N-N59K; A16N-N59K-G99E-L290A; A16N-N59K-G99E-S164M; A16N-N59K-L290S; A16N-N59K-S164M; A16N-S164M; A48R-A168K-L290D-S297R; A48R-A168K-L290K-R304A-T313K; A48R-A168K-R304D-T313K; A48R-A168K-S187D-L290D-S297D-R304D; A48R-A168K-S297R; A48R-G99E-L290A; A48R-K70E-A168K-L290D-S297D-R304D; A48R-K70E-A168K-L290D-T313K; A48R-K70E-A168K-L290K-R304A-T313K; A48R-K70E-A168K-L290K-S297R-R304A; A48R-K70E-A168K-L290K-S297R-R304A-T313K; A48R-K70E-A168K-S187D-L290D-

S297D; A48R-K70E-A168K-S187D-S297D; A48R-K70E-A168K-S187D-S297D-R304D; A48R-
K70E-A168K-S187D-S297D-R304D-T313K; A48R-K70E-L290D-S297R-R304A; A48R-K70E-
L290D-S297R-R304A; A48R-K70E-R304A; A48R-K70L-A168K-L290D-S297D-R304A; A48R-
K70L-A168K-L290D-S297D-R304D; A48R-K70L-A168K-L290D-S297D-R304D; A48R-K70L-

A168K-L290K-T313K; A48R-K70L-S187D-L290D-R304A; A48R-K70L-S187D-L290K-R304D-
T313K; A48R-K70L-S187D-R304D; A48R-K70L-S297D-R304D-T313K; A48R-L290D-S297R-
R304A; A48R-L290D-S297R-R304D-T313K; A48R-L290K-S297R-R304D-T313K; A48R-N59K-
S164M; A48R-N59K-S164M-L290A; A48R-R304A-T313K; A48R-S187D-L290D-S297D-R304A;
A48R-S187D-L290D-S297D-R304D-T313K; A48R-S187D-L290K-S297D-R304D; A48R-

S187D-L290K-S297D-T313K; A48R-S187D-L290K-S297R-R304D; A48R-S187D-S297D-
R304D-T313K; D100Q-E110Q-D124N-S185H; D73A-D100Q-D124N-Q167K-S185H; D73A-
D124N-S185H; D73A-E110Q-D124N; D73A-E110Q-D124N-Q167K; D73A-E110Q-Q167K-
S185H; D73E-G99E-T107A-S300K; D73E-T107F-S300K; E110Q-D124N; E110Q-D124N-
S185H; G295C-G298C; G295T-G298C; G2V-A165G; G2V-A165G-S185D; G2V-A165G-

V182R-S185D; G2W-A165G; G2W-A165G; G2W-A165G; G301A-P302G-T303G; G301A-
P302G-T303G-N315W; G301A-P302G-T303G-N315W-Y317W-Y318W-Q320P; G301A-
P302G-T303P; G301P-T303P-R304P-Q320A; G301S-P302G-T303G-R304P-Y317W-Q320S;
G301S-P302G-T303P-N315W-Q320P; G301S-T303G-Y317W-Y318W-Q320P; G301S-T303P-
N315W-Y317W-Y318W; G99E; G99E; G99E-L290A; G99E-L290A; G99E-L290A; G99E-

S164M; G99E-T107E-Q167A; I30E-E102S-V132N; I30E-E102S-V132N-V193T; I30E-N96P-
E102S-V132N-V193T; I30E-P87H-E102S; I30E-P87H-N96P; I30E-P87H-N96P-E102S; I30E-
P87H-N96P-E102S-V193T; I30E-P87H-N96P-E102S-V193T; I30E-P87H-N96P-E102S-V193T;
I30E-P87H-N96P-V193T; I30E-V132N; I30E-V132N-V193T; I30E-V80T-E102S-V132N; I30E-
V80T-N96P-V132N-V193T; I30E-V80T-P87H; I30E-V80T-P87H-E102S-V193T; I30E-V80T-

P87H-N96P; I30E-V80T-P87H-N96P-V132N; I30E-V80T-P87H-N96P-V193T; I30E-V80T-
P87H-N96P-V193T; I30E-V80T-P87H-V132N; I30E-V80T-P87H-V132N; I30E-V80T-P87H-
V132N-V193T; I30E-V80T-P87H-V193T; I30E-V80T-V132N; I30E-V80T-V132N-V193T; I30E-
V80T-V132N-V193T; K106E-T108D-V117I-S297E; K106R-D197M-P316C; K58V-A168E;
K58V-H163Y-A168E; K58V-K64L-H163Y-A168E; K58V-K64L-K70N; K58V-K64L-K70N-H163Y-

A168E; K58V-K64L-K70N-S135E-H163Y; K58V-K64L-K70N-S135E-H163Y; K58V-K64L-
S135E-H163Y; K58V-K64L-S135E-H163Y; K58V-K64L-S135E-H163Y-A168E; K58V-K64L-
S135E-H163Y-A168E; K58V-K70N-H163Y; K58V-K70N-H163Y-A168E; K58V-K70N-S135E;
K58V-S135E-H163Y-A168E; K64L-H163Y-A168E; K64L-K70N; K64L-K70N; K64L-K70N-
A168E; K64L-K70N-H163Y; K64L-K70N-H163Y; K64L-K70N-H163Y; K64L-K70N-H163Y-

A168E; K64L-K70N-H163Y-A168E; K64L-K70N-S135E; K64L-K70N-S135E-H163Y; K64L-
S135E-H163Y; K70A-N96P-V104A-T108E-P316A; K70A-N96P-V104I-H200A-P316A; K70A-
P316A; K70A-T108E-T196C-T287H; K70A-T108E-T287A; K70A-T108E-T287H; K70A-T108I-

T196C; K70A-T108K-T287H; K70A-T108L; K70A-V104A-P316A; K70A-V104A-T108E; K70A-
V104A-T108E-H200A; K70A-V104A-T108E-P316A; K70A-V104I-T108E-H200A; K70A-V104I-
T108E-P316A; K70E-A168K-L290D-S297D; K70E-A168K-L290K-S297D-R304A-T313K; K70E-
A168K-S187D-L290D; K70E-A168K-S187D-L290K-R304D-T313K; K70E-A168K-S297R-

5   R304D-T313K; K70E-L290D-R304A; K70E-L290D-R304D; K70E-S187D-L290D-S297D-
R304D; K70E-S187D-S297D-T313K; K70E-S297D-R304A-T313K; K70L-A168K-L290D-
R304A-T313K; K70L-A168K-L290D-S297D-R304A-T313K; K70L-A168K-L290K-S297D-
R304D; K70L-A168K-L290K-S297D-R304D-T313K; K70L-A168K-R304A-T313K; K70L-A168K-
S187D-L290K-S297D-R304A-T313K; K70L-A168K-S187D-L290K-S297R-R304D-T313K;

10   K70L-A168K-S187D-S297D-T313K; K70L-A168K-S297D-R304A-T313K; K70L-A168K-S297D-
R304A-T313K; K70L-A168K-S297D-R304A-T313K; K70L-L290D-R304D; K70L-L290D-S297D;
K70L-L290D-S297R-R304A-T313K; K70L-L290K-S297D-R304A; K70L-R304D; K70L-S297D-
R304D; K70N-A168E; K70N-H163Y; K70N-S135E-A168E; K70N-S135E-H163Y; K70N-S135E-
H163Y-A168E; K70R-N96P-V104A; K70R-N96P-V104A-T108E-P316A; K70R-N96P-V104A-

15   T108E-P316A; K70R-N96P-V104I-T108E; K70R-V104A-T108E-P316A; K70S-N96P; K70S-
N96P-V104A-T108E; K70S-N96P-V104I-H200A; K70S-V104A-T108E-P316A; K70S-V104I-
P316A; L290A; L290K-S297R-R304A; L290K-S297R-R304D; N10D-N51T-V82W-T108Q-
T127I-D197A; N10D-N51T-V82W-T108Q-T127I-D197A; N10D-N51T-V82W-T127I-D197A;
N10D-N51T-V82W-T127I-D197A; N10D-T108Q-T127I-D197A; N10D-T108Q-T127I-D197A;

20   N10D-V82W-T108Q-T127I-D197A; N51E-H163Y; N51E-H163Y; N51E-H163Y-A168E; N51E-
K58V; N51E-K58V-A168E; N51E-K58V-H163Y; N51E-K58V-H163Y; N51E-K58V-K64L-K70N-
H163Y; N51E-K58V-K64L-K70N-H163Y; N51E-K58V-K64L-K70N-H163Y-A168E; N51E-K58V-
K64L-K70N-H163Y-A168E; N51E-K58V-K64L-K70N-S135E-H163Y; N51E-K58V-K64L-K70N-
S135E-H163Y; N51E-K58V-K64L-S135E-H163Y; N51E-K58V-K64L-S135E-H163Y-A168E;

25   N51E-K58V-K70N-H163Y; N51E-K58V-K70N-S135E-H163Y; N51E-K58V-K70N-S135E-
H163Y-A168E; N51E-K58V-K70N-S135E-H163Y-A168E; N51E-K58V-S135E-H163Y; N51E-
K58V-S135E-H163Y-A168E; N51E-K64L; N51E-K64L-H163Y-A168E; N51E-K64L-K70N-
A168E; N51E-K64L-K70N-H163Y; N51E-K64L-K70N-H163Y-A168E; N51E-K64L-K70N-
S135E; N51E-K64L-K70N-S135E-H163Y-A168E; N51E-K64L-K70N-S135E-H163Y-A168E;

30   N51E-K70N-H163Y; N51E-K70N-H163Y; N51E-K70N-H163Y; N51E-K70N-H163Y; N51E-
K70N-H163Y-A168E; N51E-K70N-H163Y-A168E; N51E-K70N-S135E-A168E; N51E-K70N-
S135E-H163Y-A168E; N51E-K70N-S135E-H163Y-A168E; N51H-D100Q-E110Q-D124N;
N51H-D100Q-E110Q-D124N-Q167K; N51H-D100Q-E110Q-D124N-Q167K-S185H; N51H-
D100Q-E110Q-Q167K-S185H; N51H-D124N; N51H-D124N-S185H; N51H-D73A-D100Q-

35   D124N; N51H-D73A-D100Q-D124N-Q167K; N51H-D73A-D100Q-D124N-Q167K; N51H-D73A-
D100Q-E110Q-S185H; N51H-D73A-D124N; N51H-D73A-D124N-Q167K; N51H-D73A-D124N-
Q167K-S185H; N51H-D73A-D124N-S185H; N51H-D73A-E110Q-D124N-S185H; N51H-D73A-

E110Q-D124N-S185H; N51H-E110Q-D124N; N51H-E110Q-D124N; N51H-E110Q-D124N-
S185H; N51H-E110Q-Q167K; N51T-T108Q-T127I-D197A; N51T-V82W-T108Q; N51T-V82W-
T127I; N59K-G99E-L290A; N59K-L290A; N62C-K70A-N96P-T108E-P316A; N62C-K70A-
P316A; N62C-K70S-N96P; N62C-K70S-N96P-T108E; N62C-N96P-V104I-T108E-P316A;

5   N62C-V104I; N62C-V104I-T108E-P316A; N62S-K70A-T108A-T196C; N62S-K70A-T108A-
T287A; N62S-K70A-T108C-T287A; N62S-K70A-T108E-T196C; N62S-K70A-T108I-T287H;
N62S-K70A-T196C-T287A; N62S-T196C-T287H; N96D-T107D-G235K; N96D-T107D-G235S-
S297C; N96D-T107G; N96D-T107H-D197E-G235K-S297C; N96D-T107M; N96D-T107M-
D197E; P28K-T108A-G295T-G298C; P28K-T108A-G298C-N315W; P28K-T108D-G295C;

10   P28K-T108M-G295C-G298C-N315W; P28K-V104A-T108A; P28K-V104A-T108D-G295C-
G298C-N315W; P28K-V104A-T108H-G295C; P28K-V104A-T108I-G295T-N315W; P28K-
V104I-N315W; P28K-V104I-T108A-G295C-N315W; P28K-V104I-T108A-G295T; P28K-V104I-
T108A-G295T-N315W; P28K-V104I-T108D-G295T-N315W; P28K-V104I-T108D-N315W;
P28K-V104I-T108E-G298C; P28K-V104I-T108H-G295C-N315W; P28K-V104I-T108H-G295T-

15   G298C-N315W; P28K-V104I-T108H-G295T-N315W; P28K-V104I-T108H-N315W; P28K-
V104I-T108M-G295C-G298C; P28K-V104I-T108M-G295T-G298C-N315W; P302G-T303G-
R304P-N315W-Y317W-Q320A; P302G-T303G-R304P-Y317W-Y318W; P302G-T303G-R304P-
Y317W-Y318W; P52C-G235F-P316F; P52F-K106H-G235A-P316C; P87H-E102S-V193T;
P87H-N96P-E102S-V193T; P87H-N96P-V132N-V193T; P87H-N96P-V193T; R304A-T313K;

20   R304P-Q320P; S130L-A165Y-V182N-S185D; S135E-A168E; S187D-S297R-R304D; S297D-
R304D; S297R-R304D-T313K; S300T-G301A-P302G-T303G-Q320P; S300T-G301A-P302G-
T303P-R304P-N315W-Y317W-Q320A; S300T-G301A-T303P-R304P-N315W-Y317W-Q320A;
S300T-G301A-Y317W-Y318W; S300T-G301P-P302G-N315W-Y317W; S300T-G301P-P302G-
T303G-R304P-N315W-Q320P; S300T-G301P-T303P-R304P-N315W-Y317W-Q320P; S300T-

25   G301P-T303P-Y317W-Q320P; S300T-G301S-P302G-T303G-R304P-N315W-Y318W-Q320S;
S300T-G301S-P302G-T303P-Q320P; S300T-G301S-T303G-N315W-Y317W-Q320S; S300T-
G301S-T303G-R304P-N315W-Y317W-Y318W; S300T-G301S-T303G-R304P-Y317W-Q320S;
S300T-G301S-T303P-R304P-Y317W-Q320A; S300T-N315W-Y317W-Q320A; S300T-R304P-
N315W-Y317W-Y318W; S300T-R304P-Y317W-Q320P; S300T-T303P-R304P-N315W-Y317W-

30   Y318W; T107A-D197E-G235K; T107D-D197E-G235K; T107E-D197E-G235A-S297C; T107E-
Q167I; T107E-S297C; T107F-Q167I-S300K; T107N-D197E-G235S-S297C; T107N-L194D-
S231C; T107Q-D197E-G235F; T107R-D197E-G235K-S297C; T108A-G295C; T108A-G295C;
T108A-G295C-G298C; T108A-G295C-N315W; T108A-G295T-G298C; T108A-T287A; T108C-
T287H; T108D-G295T-G298C; T108D-G295T-G298C-N315W; T108D-N315W; T108E-G295C-

35   N315W; T108E-G295T; T108E-N315W; T108E-P316A; T108E-T287A; T108H-G295C; T108H-
G295T-N315W; T108H-G298C; T108H-N315W; T108H-N315W; T108I-G295C-N315W; T108I-
G298C; T108I-G298C; T108I-T287H; T108M-G295C; T108M-G295C; T108M-G295C-N315W;

T108M-G295T-G298C-N315W; T108M-G298C; T108Q-T127I; T127I-D197A; T127I-D197A; T303P-R304P-N315W-Y317W-Y318W-Q320S; T61D-T107E-Q167I-A233C-S300K; T61K-G99E-T107A-A233C-S300K; T61R-G99E-Q167I-A233C; T61R-T107F-Q167A; unknown; V104A-G295C-G298C; V104A-G295T; V104A-G295T-N315W; V104A-T107D; V104A-T107D;

5   V104A-T107D-L194D; V104A-T107F; V104A-T107F; V104A-T107G; V104A-T107I; V104A-T107I-S231C; V104A-T107I-S231C; V104A-T107I-S231C; V104A-T107N-S231C; V104A-T108A-G295C; V104A-T108A-G295T; V104A-T108A-G295T; V104A-T108A-G295T; V104A-T108A-G295T-N315W; V104A-T108A-G298C-N315W; V104A-T108D-G295T; V104A-T108D-G298C-N315W; V104A-T108E; V104A-T108E-G295C; V104A-T108E-G295T; V104A-T108E-

10   G295T-G298C; V104A-T108E-G295T-N315W; V104A-T108E-G295T-N315W; V104A-T108E-G298C; V104A-T108E-P316A; V104A-T108H-G295C-N315W; V104A-T108H-G295T; V104A-T108H-G295T; V104A-T108H-G295T-G298C; V104A-T108H-G295T-G298C; V104A-T108H-G298C; V104A-T108H-N315W; V104A-T108I; V104A-T108I; V104A-T108I-G295T; V104A-T108I-G295T-G298C; V104A-T108I-N315W; V104A-T108M-G298C; V104A-T108M-G298C-

15   N315W; V104A-T108M-N315W; V104I-G295C-G298C; V104I-G295T; V104I-P316A; V104I-T107A; V104I-T107D; V104I-T107H; V104I-T107I-S231C; V104I-T107Q; V104I-T107Q-L194D; V104I-T108A-G295C; V104I-T108A-G295C; V104I-T108A-G295C; V104I-T108A-G295C-G298C-N315W; V104I-T108A-G295C-N315W; V104I-T108A-G295C-N315W; V104I-T108A-G295T-N315W; V104I-T108A-G298C; V104I-T108A-N315W; V104I-T108D; V104I-T108D-

20   G295C-N315W; V104I-T108D-G295T; V104I-T108D-G295T; V104I-T108D-G295T-G298C-N315W; V104I-T108D-N315W; V104I-T108D-N315W; V104I-T108E-G295C-G298C; V104I-T108E-G295T-G298C-N315W; V104I-T108H-G295T-G298C; V104I-T108H-G295T-G298C-N315W; V104I-T108H-G295T-N315W; V104I-T108H-G295T-N315W; V104I-T108H-G298C-N315W; V104I-T108H-N315W; V104I-T108I; V104I-T108I-G295C-G298C; V104I-T108I-

25   G295C-N315W; V104I-T108I-G295T-N315W; V104I-T108I-G295T-N315W; V104I-T108M; V104I-T108M; V104I-T108M-G298C-N315W; V104K-T107H-S231C; V104K-T107M; V104K-T107Q; V104K-T107Q; V104K-T107Q; V104R-T107G-S231C; V104R-T107H; V80T-E102S-V132N-V193T; V80T-N96P-E102S-V132N; V80T-P87H; V80T-P87H; V80T-P87H-E102S; V80T-P87H-E102S; V80T-P87H-E102S-V193T; V80T-P87H-N96P; V80T-P87H-N96P-E102S;

30   V80T-P87H-N96P-E102S-V193T; V80T-P87H-N96P-V132N-V193T; V80T-P87H-N96P-V193T; V80T-P87H-V132N; V82W-T127I; Y291F-G301A-P302G-T303P-Q320A; Y291F-G301S-T303G-R304P-N315W-Y317W-Q320A; Y291F-G301S-T303P-R304P-N315W-Y317W-Q320S; Y291F-P302G-R304P-Y317W-Y318W-Q320P; Y291F-P302G-T303P-N315W-Y317W-Q320P; Y291F-R304P; Y291F-S300T-G301A-T303G-R304P-Y317W-Y318W-Q320P; Y291F-S300T-

35   G301P-N315W-Q320A; Y291F-S300T-G301S-P302G-N315W-Y317W; Y291F-S300T-G301S-P302G-T303G-N315W-Y318W-Q320P; Y291F-S300T-G301S-P302G-T303G-N315W-Y318W-Q320P; Y291F-S300T-G301S-R304P-N315W-Q320P; Y291F-S300T-G301S-R304P-Q320S;

Y291F-S300T-G301S-R304P-Y317W-Y318W-Q320P; Y291F-S300T-N315W-Y317W-Q320A; Y291F-S300T-P302G-T303G-R304P; Y291F-S300T-P302G-T303G-Y317W; Y291F-S300T-P302G-T303P-R304P-N315W-Y317W-Q320A; Y291F-S300T-P302G-Y318W-Q320S; Y291F-S300T-Q320A; Y291F-S300T-R304P-N315W-Q320A; Y291F-S300T-R304P-N315W-Y317W-Q320P; Y291F-S300T-T303G-N315W-Y317W; Y291F-S300T-T303G-R304P-N315W-Y318W-Q320P; Y291F-S300T-T303G-Y317W-Y318W; Y291F-S300T-T303P-R304P-N315W-Q320A; Y291F-S300T-Y317W-Y318W-Q320S; Y291F-T303G-N315W-Y317W-Y318W; Y291F-Y317W-Y318W-Q320A; Y291W-G301A-P302G-T303P-R304P-N315W-Y318W-Q320A; Y291W-G301P-P302G-T303G-R304P-Y317W-Q320A; Y291W-G301S-P302G-T303G-Q320A; Y291W-G301S-P302G-T303G-Y317W-Q320A; Y291W-G301S-T303G-N315W-Y317W-Q320S; Y291W-N315W-Y317W-Q320S; Y291W-N315W-Y317W-Y318W; Y291W-P302G-R304P-N315W-Y317W-Y318W; Y291W-S300T-G301A-P302G-T303P-N315W; Y291W-S300T-G301A-Q320A; Y291W-S300T-G301A-Y317W-Y318W-Q320P; Y291W-S300T-G301S-T303G-Y317W-Y318W-Q320S; Y291W-S300T-P302G-T303P-R304P-N315W-Y317W-Y318W; and Y291W-T303P-Q320S.

[1173] Any one of the combinatorial GH61A variants in Combinatorial Cohorts 1 to 7 finds use in aspects of the subject invention. As with the single SEL variants described in the previous section, a number of combinatorial GH61A variants fall into more than one Combinatorial Cohort above. Thus, combinatorial GH61A variants that fall into 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, or all 7 of the Combinatorial Cohorts above find use in aspects of the subject invention (e.g., combinatorial GH61A variant K64L-K70N-A168E is in all 7 of the Combinatorial Cohorts above). As such, GH61A combinatorial variants having a similar combination of improved properties/PIs (i.e., that fall into the same Combinatorial Cohorts) form unique functional groups.

[1174] The data obtained for each of the combinatorial GH61A variants generated above were analyzed using a linear model deconvolution method, wherein the individual impact of substitutions on each property was deconvoluted using simple linear model regression assuming additivity of all substitutions (using no interaction terms). Ihaka et al., "A Language for Data Analysis and Graphics" J. Comput. Graphical Statistics 1996, 5(3): 299-314. Specifically, linear modeling was conducted using the *lm* function in the base package of R (reference below) after converting all sites of substitutions into factor variables with the wild-type amino acid as the first factor. The effect of each specific substitution in GH61A on Tm, protein production (yield), performance in a daCS assay, and performance in a whPCS assay were thus deconvoluted. GH61A substitutions that correlate with an improvement in one or more of Tm, protein production (yield), performance in a daCS assay, and performance in a whPCS assay are listed in the following Deconvoluted Cohorts.

[1175] **Deconvoluted Cohort 1:** Substitutions in GH61A that correlate with improved activity in a daCS assay: N10D, N51E, K58V, K64L, K70S, K70E, V104A, K106C, T108K, T108A, T127I, A129N, Q167K, D197A, D197M, A201D, T287A, Y291F, Y291W, G295T, G295C, S297E, S300T, T303G, T303P, R304D, R304P, T313K, N315W, P316F, Y317W, Q320A, Q320P, and Q320S.

[1176] **Deconvoluted Cohort 2:** Substitutions in GH61A that correlate with improved activity in a whPCS assay: N10D, A16N, N51E, P52F, K58V, K64L, K70N, T107G, T107Q, T107E, T107N, T108K, T127I, S185D, D197L, H200A, T287A, Y291F, S297E, T313K, P316A, and P316F.

[1177] **Deconvoluted Cohort 3:** Substitutions in GH61A that correlate with improved Tm: A16N, N51H, N51T, K70N, K70L, T107N, T107M, D124N, T127I, S164M, Q167K, A168K, S187D, D197E, G235K, T287A, L290A, S297C, G298C, G301S, G301P, T313K, P316A, Y318W, and Q320A.

[1178] **Deconvoluted Cohort 4:** Substitutions in GH61A that correlate with improved production (yield): A16N, N51H, N51T, K70N, V80T, T107E, T108K, S135E, S187D, D197E, G235K, G235L, G235S, T287A, L290M, and P316A.

[1179] Any one or any combination of GH61A substitutions in Deconvoluted Cohorts 1, 2, 3 and/or 4 find use in aspects of the subject invention. As with the single SEL variants described in the previous section, a number of GH61A substitutions fall into more than one Deconvoluted Cohort above. Thus, GH61A substitutions that fall into 2 or more, 3 or more, or all 4 of the Deconvoluted Cohorts above find use in aspects of the subject invention (e.g., GH61A substitution T287A is in all 4 of the Deconvoluted Cohorts above). As such, GH61A combinatorial variants having a similar combination of improved properties (i.e., that fall into the same Deconvoluted Cohorts) form unique functional groups. As but one example, the following GH61A substitutions fall into both Deconvoluted Cohorts 1 and 2 above (but not necessarily exclusively; e.g., T287A also falls into Deconvoluted Cohorts 3 and 4): N10D, N51E, K58V, K64L, T108K, T127I, T287A, Y291F, S297E, T313K, and P316F.

[1180] An addition combinatorial GH61A variant library was constructed and tested in: (i) Thermostability Assay as described section V.B in Example 1 (Tm) (ii) whPCS Hydrolysis Assay as described in section VI.A in Example 1 (whPCS-A); (iii) whPCS Hydrolysis Assay as described in section VI.B in Example 1 (whPCS-B) (iv) daCS Hydrolysis Assay as described in section VII in Example 1 (daCS).

[1181] Below is a listing of combinatorial GH61A variants from this additional combinatorial GH61A variant library having an improved property (e.g., improved Tm) / improved PI over wild type GH61A (i.e., a PI>1.0) in at least one of the assays described above. The GH61A variants fall into the following Combinatorial Cohorts. Some Combinatorial Cohorts below are the same

as the Combinatorial Cohorts described for the previous combinatorial GH61A library above and thus are noted as being a "Supplement" to the previous Combinatorial Cohort.

[1182] <u>Combinatorial Cohort 8 (Supplement to Combinatorial Cohort 2, above)</u>:

Combinatorial GH61A variants having improved Tm as compared to wild-type: C294S-C311S; D038N-S135H-Q167R-S213R; D038N-S164L-G166F-Q167R-A168L-V206I; D100L-S185K; D124S-S164R; G166F-A168Y; I004V-D038N-G166W-N216I; I004V-D038N-G166W-Q167R-A168L-V206I-N216I; I004V-D038N-H163Q-S164R-G166W-Q167R-A168L-V206I-N216I; I004V-D038N-S164K-G166W-Q167R-A168L-V206I-N216I; I004V-G166W; I004V-S164K-G166W; K070N-T107E-N147E-S187E; N005E-K064Q-S135E-S187E; N005R-P028A-T061D-V104A-H200A-G235A-S297E; N147E-S187E; P052H-V141R; P083K-Q167R; S164K-G166W; S164K-G166W-Q167R-A168L-V206I; S297E-R304D; T287D-S297E; and Y024S-V132L-T202A-A233C-L290M-P316C.

[1183] <u>Combinatorial Cohort 9 (Supplement to Combinatorial Cohort 3, above)</u>:

Combinatorial GH61A variants having improved performance in a whPCS-A assay as compared to wild-type: D038N-T212R; D100L-S185K; E025M-Q050G-G192A-G235A; E025M-Q050G-V104A-T108A; E025M-T107C-G192A-G235F; E025M-T107F-G235F; E110M-S187H; F022D-Q050G-T107C; F112W-K113P-I114F; I004V-K106E-V117I-Y153A; I004V-V104A-T107A-A171E; I004V-V104A-T108E-N173S-T287A; I114F-G151D-E158G; I114F-H163Y; I114F-V154K; K058I-K070N-T107E-S164E-Q189D-S213E; K064Q-L076E; K070N-T107E-N147E-S187E; K106C-T107A-T108E; K113P-E158G; N005A-T061A-V104K-T107F-T108C; N005E-K058I-S135E-Q167E; N005E-N027E-K058I-L076E-T103E-Q167E-S185D-Q189D; N005R-P028A-T061D-N062C-V104A-T107D-T108E-H200A-G235A-G295W-S297E-G298A; N027H-D197T; N027H-P052H-V141R-I218R; N096D-T107A-G235A-T287A; P052H-V141R; P083K-Q167R; Q050G-V104A-C167R; Q050G-V104A-K106C; S135E-S213E; S135H-S213R; S183R-S191R; T061R-I218R; T103E-Q189D; T103E-S185D-Q189D-T223E; T107A-T108E-G166F-A171E; T107A-V117I-S187K-T212D; T107E-T223E; T108D-H163I-G166W-T212D; T287D-S297E; T287H-S297R; V104A-K106C-T107A; V104A-K106C-T108E; V104A-T107A-G235F; V104A-T107A-T108E; V104A-T107A-T108E-A171E-T287A; and V104A-T108E-A171E-N173S.

[1184] <u>Combinatorial Cohort 10</u>: Combinatorial GH61A variants having improved performance in a whPCS-B assay as compared to wild-type: D038N-T212R; D100L-S185K; E025L-V104A-T108L; E025M-Q050G-G192A-G235A; E025M-Q050G-V104A-T108A; E025M-T107F-G235F; F022A-K106F-V117I-T212K-S297C; F022D-Q050G-T107C; I004V-V104A-T108E-N173S-T287A; K058I-S164E; K064Q-L076E; K113P-E158G; N005A-T061A-V104K-T107F-T108C; N005E-L039D-T103E-S135E-S185D-T223E; N010D-N027E; N027H-D197T; N096D-T107A-G235A-T287A; Q050G-T107A-T108E; Q050G-V104A-C167R; Q050G-V104A-

G235F; S135E-S213E; S135H-S213R; S183R-S191R; S297E-R304D; T061R-I218R; T103E-Q189D; T107E-T223E; T287D-S297E; T287H-S297R; and V104A-T107A-G235F.

[1185] Combinatorial Cohort 11 (Supplement to Combinatorial Cohort 4, above):

Combinatorial GH61A variants having improved performance in a daCS assay as compared to wild-type: C294S-C311S; C305E-C321E; C305G-C321G; C305K-C321R; D006S-E025L-D105S-T108K-H200D-G298H; D038N-S135H-Q167R-S213R; D038N-S164L-G166F-Q167R-A168L-V206I; D038N-T212R; D038N-V142S-V154D-S185H; D100L-S183R-S187H-S191R; D100L-S185K; E025L-V104A-T108L; E025M-Q050G-G192A-G235A; E025M-Q050G-V104A-T108A; E025M-T107C-G192A-G235F; E025M-T107F-G235F; E110M-S187H; F022A-K106F-V117I-T212K-S297C; F022D-Q050G-T107C; F022D-V104A-T107A-T287H; F022G-A060G-N096A-V142S-N173S-A201F; F112W-H163Y; F112W-I144Y-V154K; F112W-K113P-I114F; I004V-D038N-G166W-N216I; I004V-D038N-G166W-Q167R-A168L-V206I-N216I; I004V-G166W; I004V-K106E-V117I-Y153A; I004V-V104A-T107A-A171E; I004V-V104A-T108E-N173S-T287A; I004V-V154K-S187P-T212D; I004V-Y024A-G151P-N315W; I114F-G151D-E158G; I114F-H163Y; I114F-V154K; I144Y-P145D-G151D-V154K-E158G-H163Y; K058I-K070N-T107E-S164E-Q189D-S213E; K064Q-L076E; K070A-N096D-K106L-E110L-A201E-G235D-G295D; K070N-T107E-N147E-S187E; K106C-T107A-T108E; K113P-E158G; K113P-P145D-H163Y; L039D-Q167E; L039D-T103E-Q167E-T223E; N005A-T061A-V104K-T107F-T108C; N005E-K058I-S135E-Q167E; N005E-K064Q-S135E-S187E; N005E-L039D-T103E-S135E-S185D-T223E; N005E-N027E-K058I-L076E-T103E-Q167E-S185D-Q189D; N005R-P028A-T061D-N062C-V104A-T107D-T108E-H200A-G235A-G295W-S297E-G298A; N005R-P028A-T061D-V104A-H200A-G235A-S297E; N010D-L076D-N147E-H163Y-Q167E-S187E; N010D-N027E; N010R-E110M-S185K-Q189R; N010R-Q189R; N027E-L076E-T107E-S213E; N027H-D197T; N027H-P052H-V141R-I218R; N062H-K070A-N096H-E110L-A201E-S297K; N096D-T107A-G235A-T287A; P028K-N062C-T107D-T108E-G295W-G298A; P052H-V141R; Q050G-K106C-A171E-T287A; Q050G-T107A-T108E; Q050G-V104A-C167R; Q050G-V104A-G235F; Q050G-V104A-K106C; S135E-S213E; S135H-S213R; S183R-S191R; T061R-I218R; T103E-Q189D; T103E-S185D-Q189D-T223E; T107A-T108E-G166F-A171E; T107A-V117I-S187K-T212D; T107E-T223E; T108D-H163I-G166W-T212D; T287D-S297E; T287H-S297R; V008I-D038N-P145F-S187P; V008I-K106E-V142S-G235F; V104A-K106C-T107A; V104A-K106C-T108E; V104A-K106R-T107A-T108A; V104A-T107A-G166F-N173S; V104A-T107A-G235F; V104A-T107A-T108E; V104A-T107A-T108E-A171E-T287A; V104A-T108E-A171E-N173S; V104A-T108E-G166F-T287A; Y024A-D038N-V104A-E158W; Y024S-T108D-V142S-G235A; and Y024S-V132L-T202A-A233C-L290M-P316C.

[1186] Any one of the combinatorial GH61A variants in Combinatorial Cohorts 8 to 11 finds use in aspects of the subject invention. As with the single SEL variants described in the previous section, a number of combinatorial GH61A variants fall into more than one Combinatorial

Cohort 8 to 11. Thus, combinatorial GH61A variants that fall into 2 or more, 3 or more, or all 4 of Combinatorial Cohorts 8 to 11 above find use in aspects of the subject invention (e.g., combinatorial GH61A variant T287D-S297E is in all 4 of the Combinatorial Cohorts 8 to 11 above). As such, GH61A combinatorial variants having a similar combination of improved

5   properties/PIs (i.e., that fall into the same Combinatorial Cohorts) form unique functional groups.

[1187] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application

10   and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

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Sequences

SEQ ID NO	Description	Sequence
1	*H. jecorina GH61A* coding DNA sequence	ATGATCCAGAAGCTTTCCAACCTCCTTGTCACCGCACTGG CGGTGGCTACTGGCGTTGTCGGACATGGACATATTAATG ACATTGTCATCAACGGGGTGTGGTATCAGGCCTATGATC CTACAACGTTTCCATACGAGTCAAACCCCCCCATAGTAGT GGGCTGGACGGCTGCCGACCTTGACAACGGCTTCGTTTC ACCCGACGCATACCAAAACCCTGACATCATCTGCCACAA GAATGCTACGAATGCCAAGGGGCACGCGTCTGTCAAGGC CGGAGACACTATTCTCTTCCAGTGGGTGCCAGTTCCATG GCCGCACCCTGGTCCCATTGTCGACTACCTGGCCAACTG CAATGGTGACTGCGAGACCGTTGACAAGACGACGCTTGA GTTCTTCAAGATCGATGGCGTTGGTCTCCTCAGCGGCGG GGATCCGGGCACCTGGGCCTCAGACGTGCTGATCTCCAA CAACACCTGGGTCGTCAAGATCCCCGACAATCTTGC GCCAGGCAATTACGTGCTCCGCCACGAGATCATCGCGTT ACACAGCGCCGGGCAGGCAAACGGCGCTCAGAACTACC CCCAGTGCTTCAACATTGCCGTCTCAGGCTCGGGTTCTC TGCAGCCCAGCGGCGTTCTAGGGACCGACCTCTATCACG CGACGGACCCTGGTGTTCTCATCAACATCTACACCAGCC CGCTCAACTACATCATCCCTGGACCTACCGTGGTATCAG GCCTGCCAACGAGTGTTGCCCAGGGGAGCTCCGCCGCG ACGGCCACCGCCAGCGCCACTGTTCCTGGAGGCGGTAG CGGCCCGACCAGCAGAACCACGACAACGGCGAGGACGA CGCAGGCCTCAAGCAGGCCCAGCTCTACGCCTCCCGCA ACCACGTCGGCACCTGCTGGCGGCCCAACCCAGACTCT GTACGGCCAGTGTGGTGGCAGCGGTTACAGCGGGCCTA CTCGATGCGCGCCGCCAGCCACTTGCTCTACCTTGAACC CCTACTACGCCCAGTGCCTTAAC
2	*H. jecorina GH61A* full length protein	MIQKLSNLLVTALAVATGVVGHGHINDIVINGVWYQAYDPTT FPYESNPPIVVGWTAADLDNGFVSPDAYQNPDIICHKNATNA KGHASVKAGDTILFQWVPVPWPHPGPIVDYLANCNGDCET VDKTTLEFFKIDGVGLLSGGDPGTWASDVLISNNNTWVVKIP DNLAPGNYVLRHEIIALHSAGQANGAQNYPQCFNIAVSGSG SLQPSGVLGTDLYHATDPGVLINIYTSPLNYIIPGPTVVSGLP TSVAQGSSAATATASATVPGGGSGPTSRTTTTARTTQASSR PSSTPPATTSAPAGGPTQTLYGQCGGSGYSGPTRCAPPAT CSTLNPYYAQCLN
3	*H. jecorina GH61A* mature protein	HGHINDIVINGVWYQAYDPTTFPYESNPPIVVGWTAADLDN GFVSPDAYQNPDIICHKNATNAKGHASVKAGDTILFQWVPV PWPHPGPIVDYLANCNGDCETVDKTTLEFFKIDGVGLLSGG DPGTWASDVLISNNNTWVVKIPDNLAPGNYVLRHEIIALHSA GQANGAQNYPQCFNIAVSGSGSLQPSGVLGTDLYHATDPG VLINIYTSPLNYIIPGPTVVSGLPTSVAQGSSAATATASATVP GGGSGPTSRTTTTARTTQASSRPSSTPPATTSAPAGGPTQT LYGQCGGSGYSGPTRCAPPATCSTLNPYYAQCLN
4	Endoglucanase IV *Hypocrea rufa* full length protein	MIQKLSNLLVTALAVATGVVGHGHINDIVINGVWYQAYDPTT FPYESNPPIVVGWTAADLDNGFVSPDAYQNPDIICHKNATNA KGHASVKARDTILFQWVPVPWPHPGPIVDYLANCNGDCETV DKTTLEFFKIDGVGLLSGGDPGTWASDVLISNNNTWVVKIPD NLAPGNYVLRHEIIALHSAGQANGAQNYPQCFNIAVSGSGSL QPSGVLGTDLYHATDPGVPINIYTSPLNYIIPGPTVVSGLPTS VAQGSSAATATASATAPGGGSGPTSRTTTTARTTQASSRPS STPPATTSAPAGGPTQTLYGQCGGSGYSGPTRCAPPATCS

		TLNPYYAQCLN
5	Type IV endoglucanase Trichoderma saturnisporum full length protein	MIQKLSNLLVAALTVATGVVGHGHINNIVINGVYYQAYDPTSF PYESNPPIVVGWTAADLDNGFVSPDAYGSPDIICHKNATNAK GHASVRAGDTVLFQWVPLPWPHPGPIVDYLANCNGDCETV DKTSLEFFKIDGVGLISGGDPGNWASDVLIANNNTWVVKIPD DLAPGNYVLRHEIIALHSAGQANGAQNYPQCFNLAVSGSGS LKPSGVKGTALYHATDPGVLINIYTSPLNYIIPGPTVVSGLPTS VAQRSSAATATASATLPGGGGSPPGGPTSRPTTTARSTSQ ASSRPSPPATTSAPAGGPTQTLYGQCGGSGYSGPTRCAPP ATVSTLNPYYARLN
6	Endoglucanase IV Hypocrea orientalis full length protein	MIQKLSNLLLTALAVATGVVGHGHINNIVVNGVYYQGYDPTS FPYESDPPIVVGWTAADLDNGFVSPDAYQSPDIICHKNATNA KGHASVKAGDTILFQWVPVPWPHPGPIVDYLANCNGDCET VDKTSLEFFKIDGVGLISGGDPGNWASDVLIANNNTWVVKIP EDLAPGNYVLRHEIIALHSAGQADGAQNYPQCFNLAVSGSG SLQPSGVKGTALYHSDDPGVLINIYTSPLAYTIPGPSVVSGLP TSVAQGSSAATATASATVPGGSGPGNPTSKTTTTARTTQAS SSRASSTPPATTSAPGGGPTQTLYGQCGGSGYSGPTRCAP PATCSTLNPYYAQCLN
7	Endoglucanase IV Trichoderma sp. full length protein	MIQKLSNLLLTALAVATGVVGHGHINNIVVNGVYYQGYDPTS FPYESDPPIVVGWTAADLDNGFVSPDAYQSPDIICHKNATNA KGHASVKAGDTIPLQWVPVPWPHPGPIVDYLANCNGDCET VDKTSLEFFKIDGVGLISGGDPGNWASDVLIANNNTWVVKIP EDLAPGNYVLRHEIIALHSAGQADGAQNYPQCFNLAVPGSG SLQPSGVKGTALYHSDDPGVLINIYTSPLAYTIPGPSVVSGLP TSVAQGSSAATATASATVPGGSGPGNPTSKTTTTARTTQAS SSRASSTPPATTSAPGGGPTQTLYGQCGGSGYSGPTRCAP PATCSTLNPYYAQCLN
8	Glycoside hydrolase family 61 protein Hypocrea atroviridis full length protein	MAQKLSNLFAIALTVATGVVGHGHVNNIVVNGVYYQGYDPT SFPYMPDPPIVVGWTAADTDNGFVSPDAYQTPDIVCHKNGT NAKGHASVKAGDSVLFQWVPVPWPHKSTVVDYLANCNGP CETVDKTTLEFFKIDGIGLLSGGNPGTWGSDVLIGNNNTWVI QIPEDLQTGNYVLRHELIALHSAEQADGAQNYPQCFNLAVT GTGSLQPSGVLATDLYHETDPGILFNIYTSPLTYIIPGPTVVS GLPSSVAQASSAATATSSATVSGGGGGSSTGGSTSKTTTV VRSTTSVTSKASSSTAVTTPPPAGGTQTLYGQCGGSGYSG PTKCASPAVCTTLNPYYAQCLN
9	Glycoside hydrolase family 61 protein Hypocrea virens full length protein	MTQKLTSLLVTALTVATGVIGHGHVNNIVINGAYYQGYDPTL FPYEPNPPIVVGWTASDTDNGFVAPDAYQSPDIICHRNATN ARGHASVMAGSSVLIQWVPIPWPHPGPVLDYLANCNGDCE TVDKTTLEFFKIDGIGLISGGNPGRWASDVLIGNNGTWVVQI PADLETGNYVLRHELIALHSAGSVDGAQNYPQCFNLAVTGT GSLQPTGVLGTKLYQESDPGILFNIYTSPLTYTIPGPTVVSGL PSSVTQRSSTATATSIATVPGSVSTGGTSSKTTTVPRSTSSA TTRRSSSSAITTSAPAGPSQTLYGQCGGSGYSGPTICASPA VCSTLNPYYAQCLTR
10	Glycoside hydrolase family 61 protein Thielavia terrestris full length protein	MPSFASKTLLSTLAGAASVAAHGHVSNIVINGVSYQGYDPTS FPYMQNPPIVVGWTAADTDNGFVAPDAFASGDIICHKNATN AKGHAVVAAGDKIFIQWNTWPESHHGPVIDYLASCGSASCE TVDKTKLEFFKIDEVGLVDGSSAPGVWGSDQLIANNNSWLV EIPPTIAPGNYVLRHEIIALHSAENADGAQNYPQCFNLQITGT GTATPSGVPGTSLYPTDPGILVNIYSAPITYTVPGPALISGA VSIAQSSSAITASGTALTGSATAPAAAATTTSTTNAAAAATS AAAAGTSTTTSAAAVVQTSSSSSSAPSSAAAAATTTAAAS ARPTGCSSGRSRKQPRRHARDMVVARGAEEAN

11	Endoglucanase IV Neurospora tetrasperma full length protein	MARKSILTALAGASLVAAHGHVSKVIVNGVEYQNYDPTSFPY NSNPPTVIGWTIDQKDNGFVSPDAFDSGDIICHKSATPAGGH ATVKAGDKISLQWDQWPESHKGPVIDYLAACDGDCESVDK TALKFFKIDGAGYDATNGWASDVLIKDGNSWVVEIPENIKPG NYVLRHEIIALHSAGQANGAQNYPQCFNLKVEGSGSTVPAG VAGTELYKATDAGILFDIYKNDISYPVPGPSLIAGASSSIAQSK MAATATASATLPGATGGSNSPATSAAAAAPAPSTTLVTSTK AAAPATSAAPAAPATSAAAGSGQVQAKQTKWGQCGGNGY TGATECESGSTCTKYNDWYSQCV
12	uncharacterized protein Neurospora tetrasperma full length protein	MARKSILTALAGASLVAAHGHVSKVIVNGVEYQNYDPTSFPY NSNPPTVIGWTIDQKDNGFVSPDAFDSGDIICHKSATPAGGH ATVKAGDKISLQWDQWPESHKGPVIDYLAACDGDCESVDK TALKFFKIDGAGYDATNGWASDVLIKDGNSWVVEIPENIKPG NYVLRHEIIALHSAGQANGAQNYPQCFNLKVEGSGSTVPAG VAGTELYKATDAGILFDIYKNDISYPVPGPSLIAGASSSIAQSK MAATATASATLPGATGGSNSPATSAAAAAPAPSTTLVTSTK AAAPATSAAPAAPATSAAAGSGQVQAKQTKWGQCGGNGY TGATECESGSTCTKYNDWYSQCV
13	Glycoside hydrolase family 61 protein Thielavia heterothallica full length protein	MSSFTSKGLLSALMGAATVAAHGHVTNIVINGVSYQNFDPFT HPYMQNPPTVVGWTASNTDNGFVGPESFSSPDIICHKSATN AGGHAVVAAGDKVFIQWDTWPESHHGPVIDYLADCGDAGC EKVDKTTLKFFKISESGLLDGTNAPGKWASDTLIANNNSWLV QIPPNIAPGNYVLRHEIIALHSAGQQNGAQNYPQCFNLQVTG SGTQKPSGVLGTELYKATDAGILANIYTSPVTYQIPGPAIISG ASAVQQTTSAITASASAITGSATAAPTAATTTAAAAATTTTTA GSRCYRHALDRRLSFFRPACSYHRCRYLQPCSPDPLRWSE EAPSPRP
14	Endoglucanase IV Neurospora crassa full length protein	MARMSILTALAGASLVAAHGHVSKVIVNGVEYQNYDPTSFP YNSNPPTVIGWTIDQKDNGFVSPDAFDSGDIICHKSAKPAGG HATVKAGDKISLQWDQWPESHKGPVIDYLAACDGDCESVD KTALKFFKIDGAGYDATNGWASDTLIKDGNSWVVEIPESIKP GNYVLRHEIIALHSAGQANGAQNYPQCFNLKVEGSGSTVPA GVAGTELYKATDAGILFDIYKNDISYPVPGPSLIAGASSSIAQ SKMAATATASATLPGATGGSNSPATSAAAAAPATSAAAATS QVQAAPATTLVTSTKAAAPATSAAAPAAPATSAAAGGAGQV QAKQTKWGQCGGNGFTGPTECESGSTCTKYNDWYSQCV
15	Uncharacterized protein Sordaria macrospora full length protein	MARKSIITALAGASLVAAHGHVSKVIVNGVEYQNYDPAVFPY LSNPPTVIGWTADQKDNGFVSPDAFGTPDIICHRSATPAGG HATVKAGDKISLKWDPVWPDSHKGPVIDYLAACNGDCETVD KTSLRFFKIDGAGYNNGVWAADALVNNGNSWLVQIPADLKP GNYVLRHEIIALHGAGSANGAQAYPQCFNLKVEGSGNNLPS GVPLYKATDAGILFNMYQNDFTYPVPGPALIAGAVSSIPQSS SAATATASATVPGGGGSGGSPVTTTAAGATTTKATTTLVTS TKATTSDAQVTTTAPPATGGGGGAAQKYGQCGGNGWTGP TTCVSGSVCTKVNDWYSQCL

| 16 | Endoglucanase IV Gaeumannomyces graminis var. tritici full length protein | MGFKSRALVSALGSAATVLAHGHVSNIVVNGVFYPGYDVTK YPWQPNAPTVVGWSATNTDNGFVEPNNFGHPDIICHRGAQ PAKGHARVRAGDKILLQWDTWPESHKGPVLDYLARCPGDC ETVDKTALRFFKIGEGSYISGAAPGHWAADVLLGNGFSWVV QIPEDVAPGNYVLRHEIIALHGSPNPNGAQAYPQCFNLEISG SGSRQPAGVAGTSLYRAGDPGIHFPLYNSPIVYPVPGPALIP GVPSTVAQVSTRATATSSPFLPGGGGGGGGGGGGGNPGP TSAPGGGNGGGGGGGQQPPQTTTAPGNGGGGGGGGGGG GGGQTRWGQCGGSGWNGPTACAQGACSTLNPYYAQCV |
| 17 | uncharacterized protein Nectria haematococca

full length protein | MTFFTAMSTLCASAWLYLLFSAVSVSAHGHVTQVIINGVAYG GYLSTSFPLQRKPPVVLGWTIEQRDNGFVSPDKYDHPDIICH RDATPAQGHVQVAAGDTITIKWSSWPENHRGPVMDYLANC NGPCETVDKTKLEFFKIDGMGLISQDRPGKYADGALRENGY TWSVRIPSNIAPGNYVLRHEIIALHSGLERNGAQNYPQCFNL KITGSGSDNPPGYLGTELYDANDPGILVNIYGNLPNYQVPGP TIVSGGVSSVRQSPSRATTTAKCTTRS |
| 18 | Uncharacterized protein Fusarium pseudograminearum

full length protein | MTFQSVHSSKASFWLTLFLPALGISAHGHVDEIIVNGVSYQG YGSTDFPYMQDPPVVAGWTIEQADNGFVSPDKYDDPDIICH RDATPAKGHIELAAGDTLTLRWSGWPENHSGPILNYLANCN GPCERVDKTKLEFFKIDGLGLLEQGTPGRYADKVLQDNGDR WNVRIPKNIAPGNYVLRHEIIALHNALDKGGAQNYPQCFNLKI TGDGSDSPSGYLGTELYDAADPGILVNVYSSSVDYEVPGPTI CEGGVSSVEQKPSEATTTAKCTTRY |
| 19 | Uncharacterized protein Gibberella zeae

full length protein | MAFQSINSSKASFWLTLLLPALGISAHGHVDEIIVNGVSYQG YGSTDFPYMQDPPVVAGWTIEQADNGFVSPDKYDDPDIICH RDATPAKGHIELAAGDTLTLRWSGWPENHSGPILNYLANCN GPCERVDKTKLEFFKIDGLGLLEQGTPGRYADKVLQDNGDR WNVRIPKNIAPGNYVLRHEIIALHNALDKGGAQNYPQCFNLKI TGDGSDSPSGYLGTELYDAADPGILVNVYSSSVDYEVPGPTI CEGGVSSVEQKPSEATTTAKCTTRY |
| 20 | Glycoside hydrolase family 28 protein Thielavia terrestris

full length protein | MKYRPSLSLAAAALFLLEPWVQAQLSGSVGPTTSRAAKAAK KVCNIMNYGGVASATTDNSAAITAAWNACKGGGEVYIPSGS YGLSSWVTLSGGSGVSINLEGVIYRITSATAGGTMISVSSTT DFEFYSGNSKGAIQGYGYLLNASDPRLVRLTQVTNFSFHDIA LVDAPEFSLVMDTCSNGEVYNSIVRAGSEGGLDGVDVWGQ NIWIHDIEVTNKDECVTVKSPASNILVESIFCNWSGGSAMGS LGANTDISNIYYRNVYSQNCNQMYMIKSWGGSGTVKNVKLE NFWGHSNAYTLDLNAYWTSMTQAPGDGVSYQNITFTGWK GTNSNGAQRGSIQVLCPSAVPCTGITISDVNIWTESGSTEKE ICENAYGTGGCLRAGSGGTYTTTVTRTTASNYAIQTMPNEIK AWGLGTEIPIPAIPTSFFPGLRPISALMAASSNGGGATPTTAG PTPTTTSAGTGGGVQSEYGQCGGSGYSGPTACAAPYACST LNPYYAQCL |

| 21 | Glycoside hydrolase family 45 protein Hypocrea atroviridis

full length protein | MKASLFVGSLIASSAAAYKATTTRYYDGQEGACGCGGANG GAAFSWQLGISSGVYTAAGSQALYDTAGASWCGAGCGKC YNLTSTGEPPCTSCGTGGVAGQSIIVMVTNLCPNNGNAQW CPTVGGTNQYGYSYHFDIMAQNEIFGDNVVVDFEPVACPG QATSDWQQCLCVGMQETDTTPVLGGGSSPPPGSSSSRPP ASATSSAPTGSGTQSLYGQCGGTGWAGPTACPPATCKVL NQYYSQCLD |
| 22 | putative Endoglucanase Neosartorya fumigata

full length protein | MLYFTLLHSMTDQRGSDTMTDRKELVAVEHRLLGISNGVYT AAGSQALFDTAGASWCGAGCGKCYNLTSTGSAPCTGCGT GGAAGESIIVMVTNLCPYNGNQQWCPQVGATNNYGYSYHF DIMAQSEVFGDNVVVNFEPVACPGQATSDWETCVCYGQTE TDETPVGMTPGGSNPSPLTSTTTTKTTTTETTITTTTGGATQ TLYGQCGGSGWTGPTACASGATCKVLNPYYSQCLS |
| 23 | Putative uncharacterized protein Aspergillus terreus

full length protein | MHTLQSAILLGGLLATQVAAHGHVTNIVINGVYYRGWNIDSD PYNSNPPLVAAWRTPNTANGFIAPDAFGTSDIICHLNALNGQ GHIQVAAGDRISLQWNTWPESHHGPVLDYLADCGGSCETV DKTTLKFFKIDGVGLVDDTTPPGIWGDDQLIANNNTWLVEIP SSIAPGNYVLRHELIALHGAGSANGAQNYPQCFNLQITGSGT VKPSGVLGTALYSPTDPGILVNIYNSLNYIVPGPTPIPQAVSV VQSSSAIRATGTATAPGATGGTTATTTSKATTTSSTTLVTTT SASTTSRTTTTTTAGAGGSQTVYGQCGGTGWTGPTACVAS ATCTTLNPYYAQCLPTST |
| 24 | Cip1 Hypocrea jecorina

full length protein | MVRRTALLALGALSTLSMAQISDDFESGWDQTKWPISAPDC NQGGTVSLDTTVAHSGSNSMKVVGGPNGYCGHIFFGTTQV PTGDVYVRAWIRLQTALGSNHVTFIIMPDTAQGGKHLRIGGQ SQVLDYNRESDDATLPDLSPNGIASTVTLPTGAFQCFEYHL GTDGTIETWLNGSLIPGMTVGPVDNPNDAGWTRASYIPEI TGVNFGWEAYSGDVNTVWFDDISIASTRVGCGPGSPGGPG SSTTGRSSTSGPTSTSRPSTTIPPPTSRTTTATGPTQTHYGQ CGGIGYSGPTVCASGTTCQVLNPYYSQCL |
| 25 | Exoglucanase 1 Hypocrea rufa

full length protein | MYQKLALISAFLATARAQSACTLQAETHPPLTWQKCSSGGT CTQQTGSVVIDANWRWTHATNSSTNCYDGNTWSSTLCPD NETCAKNCCLDGAAYASTYGVTTSADSLSIGFVTQSAQKNV GARLYLMASDTTYQEFTLLGNEFSFDVDVSQLPCGLNGALY FVSMDADGGVSKYPTNTAGAKYGTGYCDSQCPRDLKFING QANVEGWEPSSNNANTGIGGHGSCCSEMDIWEANSISEAL TPHPCTTVGQEICDGDSCGGTYSGDRYGGTCDPDGCDWN PYRLGNTSFYGPGSSFTLDTTKKLTVVTQFETSGAINRYYVQ NGVTFQQPNAELGDYSGNSLDDDYCAAEEAEFGGSSFSDK GGLTQFKKATSGGMVLVMSLWDDYYANMLWLDSTYPTNET SSTPGAVRGSCSTSSGVPAQLESNSPNAKVVYSNIKFGPIG STGNSSGGNPPGGNPPGTTTTRRPATSTGSSPGPTQTHYG QCGGIGYSGPTVCASGSTCQVLNPYYSQCL |
| 26 | Glycoside hydrolase family 7 protein Hypocrea virens

full length protein | MYQKLAAISAFLAAARAQQVCTQQAETHPPLTWQKCSSSG CTAQSGSVVLDANWRWTHDVKSTTNCYDGNTWSKTLCPD DATCAKNCCLDGAAYSSTYGITTSSDSLTINFVTQSNVGARL YLMATDTSYQEFTLSGNEFSFDVDVSQLPCGLNGALYFVSM DADGGQSKYPTNAAGAKYGTGYCDSQCPRDLKFINGQANV DGWQPSSNNANTGIGGHGSCCSEMDIWEANSISQAVTPHP CETVGQTMCSGDGCGGTYSSDRYGGTCDPDGCDWNPYR LGNTTFYGPGSGFTLDTTKKMTVVTQFATSGAISRYYVQNG VKFQQPNAQLSGYSGNTLNSDYCAAEQAAFGGTSFTDKGG LAQFNKALSGGMVLVMSLWDDYYANMLWLDSTYPTNATAS TPGAKRGSCSTSSGVPSQIESQSPNAKVVFSNIRFGPIGSTG GSTGNPPPGTSTTRLPPSSTGSSPGPTQTHYGQCGGIGYS GPTQCVSGTTCQVLNPYYSQCL |

27	Glycoside hydrolase family 5 protein Hypocrea atroviridis full length protein	MNKPMGPLLLAATLMASGAIAQTQTVWGQCGGQGYSGPT NCASGSACSTLNPYYAQCIPGATSFTTSTTSTKSPGSGSST TSSASQPTGSGQTRFAGINIAGFDFGCTTDGTCVTSQIYPPL KNFGGTNNHPDGVGQMQHFVNDDKLNIFRLPVGWQYLVN NNLGGTLDSTAISNYDQLVQGCLATGAYCIVDIHNYARWNG AIIGQGGPTNAQFVSLWTQLATKYASQSKIWFGIMNEPHDV DINTWGTTVQAVVTAIRNAGATTQFISLPGTDYQSAGNFLTD GSSTALSQVKNPDGSTTNLIFDLHKYLDSDNSGTHTECVTN NIATAFQPVATWLRQNKRQGILTETGGGNTQSCIQDVCQQN QFLNQNSDVFLGYVGWGAGSFDSTYQLTLTPTQNGNTWTD TALAAACFSRA
28	Glycosyl hydrolase family 45 protein Neosartorya fischeri full length protein	MTDRKELVAVEHHLVPTLGSNGVYTAAGSQALFDTAGASW CGAGCGKCYNLTSTGNPPCTGCGTGGAAGESIIVMVTNLCP YNGNQQWCPQVGATNNYGYSYHFDIMAQSEVFGDNVVVN FEPIACPGQATSDWETCVCYGKTATDETPVGMTPGGSNPS PPTSTTTTETTTTITTSGATQTLYGQCGGSGWTGPTACASG ATCKVLNSYYSQCLS
29	Exoglucanase 1 Trichoderma koningii full length protein	MYRKLAVISAFLATARAQSACTLQSETHPPLTWQKCSSGGT CTQQTGSVVIDANWRWTHATNSSTNCYDGNTWSSTLCPD NETCAKNCCLDGAAYASTYGVTTSGNSLSIGFVTQSAQKNV GARLYLMASDTTYQEFTLLGNEFSFDVDVSQLPCGLNGALY FVSMDADGGVSKYPTNTAGAKYGTGYCDSQCPRDLKFING QANVEGWEPSSNNANTGIGGHGSCCSEMDIWEANSISEAL TPHPCTTVGQEICEGDGCGGTYSDNRYGGTCDPDGCDWN PYRLGNTSFYGPGSSFTLDTTKKLTVVTQFETSGAINRYYVQ NGVTFQQPNAELGSYSGNELNDDYCTAEEAEFGGSSFSDK GGLTQFKKATSGGMVLVMSLWDDYYANMLWLDSTYPTNET SSTPGAVRGSCSTSSGVPAQVESQSPNAKVTFSNIKFGPIG STGNPSGGNPPGGNRGTTTTRRPATTTGSSPGPTQSHYG QCGGIGYSGPTVCASGTTCQVLNPYYSQCL
30	Glycosyl hydrolase family 61 Colletotrichum graminicola full length protein	MSYRSKTASFVAILASAATVAAHGHVTNIVINGVSYRNYIPVQ DPYTNNPPLVAGWTTDQRDNGFVAPDAYNAPDIICHRQAVA GKGRITVAAGDTVQLQWTEWPDSHKGPVIDWLANCNGPCN LVDKTDLRFFKIDGAGLIDPPQRTNRWAATALIENGNAWLVR IPANVAPGHYVLRHDIIALHSAGQQNGAQSYPQCINLEITGE GTDNPPGVLGTALYRANDAGILYNIYRDNLNDYVVPGDAIIP GGVSMLPQSRIQITASGSATPYGTTSVGSSSSTRIAPSSVTS AATSSSSRESASSVEAEASTISTTIRLTRTITATHTNSTSNNIP PSSTAAPTRTLAPTTLQTQTTTAPPSGEPTQKMYGQCGGVA YMGPTQCPAYATCSTVNPYYAQCTPLPVPPGVQPLYGQCG GLNWPPESPTECVPGARCSTINPYYAQCTPA
31	Putative uncharacterized protein Arthrobotrys oligospora full length protein	MLSSTLLLTALAVPAAFAQSNLDWDAAYTKATTMLGKLTLQ QKINMVTGVGWQKGPCVGNIAAISSAGFPGLCLQDGPVGV RYASGVTAFPAAIHLGATWDKDLMRAQGVAMGEEFRGKGV NIALAPVSGALGKIPQAGRNWEGYSNDPYHAGVGMTEVITG VQSVGVQACAKHYIGNEQERNRETMSSNIDDRTMHELYLW PFADAAKANVATFMCSYNKLNSSWACDNDYALNKLLKGEL GFRGQVLSDWNAKTTTGGATRGLDMTPGDNFGDNNFVW GQNLLNAVNQGSVSTSRLDDMVKRIFASWYLVGQDQNYPS VSFNSWNNNGGGDVSGNHKELARTVAGDGIILLKNVNNALP LKKPASLAIIGRDAINNPAGINSCTDRACNDGTLAMGWGSGT TNFPYLIDPLTAIRAQAQVDGTTVVTSTTDNASQGASAAQSA STAIVFINANSGEGYLTVQGNSGDRNNLDPWNNGNDLVKAV AAVNSKTIVVIHSVGPIILEQFVDLPNVIAVVWAGLPGQESGN GLVDVLYGSKAPGGKLPFTIAKSPSDYGTSIIINGDDNFSEGL FIDYRRFDAQGITPRYEFGFGLSYTTFSFSNLVISYTSTTTGPI

		SSTQNAPGGYPALYEPVATITARVTNTGGVAGSEVAQLYIGL PAGSPSTPPKQLRGFQKLKLASGASGTATFVLKRKDLAYWN TASQRWVVPTGNFNIFIGASSRDIRLQGTMGPSGSTTTTIGG STSSTTTAQTTTRVTTTPSTTVTTTRTTTAPTTTRTTTVATTT RATTTAVITTTAAPTGGPLQSKWGQCGGVGYTGASVCSPT ATCSTLNPYYAQCL
32	Cellobiohydrolase Trichoderma harzianum full length protein	MYRKLAAISAFLAAARAQQVCTQQAETHPPLTWQKCTASG CTAQSGSVVLDANWRWTHDTKSTTNCYDGNTWSSTLCPD DATCAKNCCLDGANYSGTYGVTTSGDALTLQFVTASNVGS RLYLMANDSTYQEFTLSGNEFSFDVDVSQLPCGLNGALYFV SMDADGGQSKYPGNAAGAKYGTGYCDSQCPRDLKFINGQ ANVEGWEPSSNNANTGVGGHGSCCSEMDIWEANSISEALT PHPCETVGQTMCSGDACGGTYSNDRYGGTCDPDGCDWN PYRLGNTSFYGPGSSFALDTTKKLTVVTQFATDGSISRYYVQ NGVKFQQPSASVGSYTGNTINTAYCAAEQTAFGGTSFTDKG GLAQINKAFQGGMVLVMSLWDDYAVNMLWLDSTYPTNATA STPGAKRGSCSTSSGVPAQVEAQSPNSKVIYSNIRFGPIGST GGNTGSNPPGTSTTRAPPSSTGSSPTATQTHYGQCGGTG WTGPTRCASGFTCQVLNPFYSQCL
33	Endoglucanase Penicillium sp. full length protein	MATRPLAFAAIAALIHQAASQQAPTPDNLASLPTWKCTTSGG CVQQSTSIVVDWVYHWIHTVNGSTSCTTSSGLDSTLCGTEE ECYTNCEISPATYDGLGIKTSGNALTLNQYVTSNGTTSNASP RVYLLDPAGKNYEMLQLLGQEISFDVDASNLPCGENGALYL SEMDATGGRSQYNPAGASYGSGYCDAQCGSSSWFNGSIN SAGLGSCCNEMDLWEANGEATALTPHPCSVDGPYGCSGS ACGSTGVCDKNGCGFNPYALGDQSYYGPGLTVDTSKPFTV TTQFVTNDGTKTGTLTEIRRSYTQNGKVIANAVASASSGFSG QSSITESFCTAMDSEAGTLGGLTTMGEALGRGMVLIFSIWN DAGGYMNWLDSGSSGPCSSTAGIPSTIQANDPGTSVTFSNI KWGDIGSTGSGTGGSSSSSSSTSTSPKTTSTTTTSATTKTS ATTTTTSTGATQTHYGQCGGMSYTGPTVCASPYTCQVQNP YYSQCL
34	*H. jecorina* GH61A Cat Domain	HGHINDIVINGVWYQAYDPTTFPYESNPPIVVGWTAADLDN GFVSPDAYQNPDIICHKNATNAKGHASVKAGDTILFQWVPV PWPHPGPIVDYLANCNGDCETVDKTTLEFFKIDGVGLLSGG DPGTWASDVLISNNNTWVVKIPDNLAPGNYVLRHEIIALHSA GQANGAQNYPQCFNIAVSGSGSLQPSGVLGTDLYHATDPG VLINIYTSPLNYIIPGPTVVSGLPTSVAQGSSAATATASATVP G
35	Endoglucanase IV Hypocrea rufa Cat Domain	HGHINDIVINGVWYQAYDPTTFPYESNPPIVVGWTAADLDN GFVSPDAYQNPDIICHKNATNAKGHASVKARDTILFQWVPV PWPHPGPIVDYLANCNGDCETVDKTTLEFFKIDGVGLLSGG DPGTWASDVLISNNNTWVVKIPDNLAPGNYVLRHEIIALHSA GQANGAQNYPQCFNIAVSGSGSLQPSGVLGTDLYHATDPG VPINIYTSPLNYIIPGPTVVSGLPTSVAQGSSAATATASATAP G
36	Type IV endoglucanase Trichoderma saturnisporum Cat Domain	HGHINNIVINGVYYQAYDPTSFPYESNPPIVVGWTAADLDNG FVSPDAYGSPDIICHKNATNAKGHASVRAGDTVLFQWVPLP WPHPGPIVDYLANCNGDCETVDKTSLEFFKIDGVGLISGGD PGNWASDVLIANNNTWVVKIPDDLAPGNYVLRHEIIALHSAG QANGAQNYPQCFNLAVSGSGSLKPSGVKGTALYHATDPGV LINIYTSPLNYIIPGPTVVSGLPTSVAQRSSAATATASATLPG
37	Endoglucanase IV Hypocrea orientalis	HGHINNIVVNGVYYQGYDPTSFPYESDPPIVVGWTAADLDN GFVSPDAYQSPDIICHKNATNAKGHASVKAGDTILFQWVPV PWPHPGPIVDYLANCNGDCETVDKTSLEFFKIDGVGLISGG

	Cat Domain	DPGNWASDVLIANNNTWVVKIPEDLAPGNYVLRHEIIALHSA GQADGAQNYPQCFNLAVSGSGSLQPSGVKGTALYHSDDP GVLINIYTSPLAYTIPGPSVVSGLPTSVAQGSSAATATASATV PG
38	Endoglucanase IV Trichoderma sp. Cat Domain	HGHINNIVVNGVYYQGYDPTSFPYESDPPIVVGWTAADLDN GFVSPDAYQSPDIICHKNATNAKGHASVKAGDTIPLQWVPV PWPHPGPIVDYLANCNGDCETVDKTSLEFFKIDGVGLISGG DPGNWASDVLIANNNTWVVKIPEDLAPGNYVLRHEIIALHSA GQADGAQNYPQCFNLAVPGSGSLQPSGVKGTALYHSDDP GVLINIYTSPLAYTIPGPSVVSGLPTSVAQGSSAATATASATV PG
39	Glycoside hydrolase family 61 protein Hypocrea atroviridis Cat Domain	HGHVNNIVVNGVYYQGYDPTSFPYMPDPPIVVGWTAADTD NGFVSPDAYQTPDIVCHKNGTNAKGHASVKAGDSVLFQWV PVPWPHKSTVVDYLANCNGPCETVDKTTLEFFKIDGIGLLSG GNPGTWGSDVLIGNNNTWVIQIPEDLQTGNYVLRHELIALHS AEQADGAQNYPQCFNLAVTGTGSLQPSGVLATDLYHETDP GILFNIYTSPLTYIIPGPTVVSGLPSSVAQASSAATATSSATVS G
40	Glycoside hydrolase family 61 protein Hypocrea virens Cat Domain	HGHVNNIVINGAYYQGYDPTLFPYEPNPPIVVGWTASDTDN GFVAPDAYQSPDIICHRNATNARGHASVMAGSSVLIQWVPIP WPHPGPVLDYLANCNGDCETVDKTTLEFFKIDGIGLISGGNP GRWASDVLIGNNGTWVVQIPADLETGNYVLRHELIALHSAG SVDGAQNYPQCFNLAVTGTGSLQPTGVLGTKLYQESDPGIL FNIYTSPLTYTIPGPTVVSGLPSSVTQRSSTATATSIATVPG
41	Glycoside hydrolase family 61 protein Thielavia terrestris Cat Domain	HGHVSNIVINGVSYQGYDPTSFPYMQNPPIVVGWTAADTDN GFVAPDAFASGDIICHKNATNAKGHAVVAAGDKIFIQWNTW PESHHGPVIDYLASCGSASCETVDKTKLEFFKIDEVGLVDGS SAPGVWGSDQLIANNNSWLVEIPPTIAPGNYVLRHEIIALHSA ENADGAQNYPQCFNLQITGTGTATPSGVPGTSLYTPTDPGI LVNIYSAPITYTVPGPALISGAVSIAQSSSAITASGTALTGS
42	Endoglucanase IV Neurospora tetrasperma Cat Domain	HGHVSKVIVNGVEYQNYDPTSFPYNSNPPTVIGWTIDQKDN GFVSPDAFDSGDIICHKSATPAGGHATVKAGDKISLQWDQW PESHKGPVIDYLAACDGDCESVDKTALKFFKIDGAGYDATN GWASDVLIKDGNSWVVEIPENIKPGNYVLRHEIIALHSAGQA NGAQNYPQCFNLKVEGSGSTVPAGVAGTELYKATDAGILFD IYKNDISYPVPGPSLIAGASSSIAQSKMAATATASATLPG
43	Putative uncharacterized protein Neurospora tetrasperma Cat Domain	HGHVSKVIVNGVEYQNYDPTSFPYNSNPPTVIGWTIDQKDN GFVSPDAFDSGDIICHKSATPAGGHATVKAGDKISLQWDQW PESHKGPVIDYLAACDGDCESVDKTALKFFKIDGAGYDATN GWASDVLIKDGNSWVVEIPENIKPGNYVLRHEIIALHSAGQA NGAQNYPQCFNLKVEGSGSTVPAGVAGTELYKATDAGILFD IYKNDISYPVPGPSLIAGASSSIAQSKMAATATASATLPG
44	Glycoside hydrolase family 61 protein Thielavia heterothallica Cat Domain	HGHVTNIVINGVSYQNFDPFTHPYMQNPPTVVGWTASNTD NGFVGPESFSSPDIICHKSATNAGGHAVVAAGDKVFIQWDT WPESHHGPVIDYLADCGDAGCEKVDKTTLKFFKISESGLLD GTNAPGKWASDTLIANNNSWLVQIPPNIAPGNYVLRHEIIALH SAGQQNGAQNYPQCFNLQVTGSGTQKPSGVLGTELYKATD AGILANIYTSPVTYQIPGPAIISGASAVQQTTSAITASASAITGS
45	Endoglucanase IV Neurospora crassa Cat Domain	HGHVSKVIVNGVEYQNYDPTSFPYNSNPPTVIGWTIDQKDN GFVSPDAFDSGDIICHKSAKPAGGHATVKAGDKISLQWDQW PESHKGPVIDYLAACDGDCESVDKTALKFFKIDGAGYDATN GWASDTLIKDGNSWVVEIPESIKPGNYVLRHEIIALHSAGQA NGAQNYPQCFNLKVEGSGSTVPAGVAGTELYKATDAGILFD IYKNDISYPVPGPSLIAGASSSIAQSKMAATATASATLPG

46	Uncharacterized protein Sordaria macrospora Cat Domain	HGHVSKVIVNGVEYQNYDPAVFPYLSNPPTVIGWTADQKDN GFVSPDAFGTPDIICHRSATPAGGHATVKAGDKISLKWDPV WPDSHKGPVIDYLAACNGDCETVDKTSLRFFKIDGAGYNNG VWAADALVNNGNSWLVQIPADLKPGNYVLRHEIIALHGAGS ANGAQAYPQCFNLKVEGSGNNLPSGVPLYKATDAGILFNMY QNDFTYPVPGPALIAGAVSSIPQSSSAATATASATVPG
47	Endoglucanase IV Gaeumannomyces graminis var. tritici Cat Domain	HGHVSNIVVNGVFYPGYDVTKYPWQPNAPTVVGWSATNTD NGFVEPNNFGHPDIICHRGAQPAKGHARVRAGDKILLQWDT WPESHKGPVLDYLARCPGDCETVDKTALRFFKIGEGSYISG AAPGHWAADVLLGNGFSWVVQIPEDVAPGNYVLRHEIIALH GSPNPNGAQAYPQCFNLEISGSGSRQPAGVAGTSLYRAGD PGIHFPLYNSPIVYPVPGPALIPGVPSTVAQVSTRATATSSPF LPG
48	Putative uncharacterized protein Nectria haematococca Cat Domain	HGHVTQVIINGVAYGGYLSTSFPLQRKPPVVLGWTIEQRDN GFVSPDKYDHPDIICHRDATPAQGHVQVAAGDTITIKWSSW PENHRGPVMDYLANCNGPCETVDKTKLEFFKIDGMGLISQD RPGKYADGALRENGYTWSVRIPSNIAPGNYVLRHEIIALHSG LERNGAQNYPQCFNLKITGSGSDNPPGYLGTELYDANDPGI LVNIYGNLPNYQVPGPTIVSGGVSSVRQSPSRATTTAKCTTR S
49	Uncharacterized protein Fusarium pseudograminearum Cat Domain	HGHVDEIIVNGVSYQGYGSTDFPYMQDPPVVAGWTIEQAD NGFVSPDKYDDPDIICHRDATPAKGHIELAAGDTLTLRWSG WPENHSGPILNYLANCNGPCERVDKTKLEFFKIDGLGLLEQ GTPGRYADKVLQDNGDRWNVRIPKNIAPGNYVLRHEIIALHN ALDKGGAQNYPQCFNLKITGDGSDSPSGYLGTELYDAADP GILVNVYSSSVDYEVPGPTICEGGVSSVEQKPSEATTTAKCT TRY
50	Uncharacterized protein Gibberella zeae Cat Domain	HGHVDEIIVNGVSYQGYGSTDFPYMQDPPVVAGWTIEQAD NGFVSPDKYDDPDIICHRDATPAKGHIELAAGDTLTLRWSG WPENHSGPILNYLANCNGPCERVDKTKLEFFKIDGLGLLEQ GTPGRYADKVLQDNGDRWNVRIPKNIAPGNYVLRHEIIALHN ALDKGGAQNYPQCFNLKITGDGSDSPSGYLGTELYDAADP GILVNVYSSSVDYEVPGPTICEGGVSSVEQKPSEATTTAKCT TRY
51	*H. jecorina* GH61A Carb bind domain	PTQTLYGQCGGSGYSGPTRCAPPATCSTLNPYYAQCL
52	Glycoside hydrolase family 61 protein Hypocrea virens Carb bind domain	PSQTLYGQCGGSGYSGPTICASPAVCSTLNPYYAQCL
53	Glycoside hydrolase family 28 protein Thielavia terrestris Carb bind domain	GVQSEYGQCGGSGYSGPTACAAPYACSTLNPYYAQCL

54	Glycoside hydrolase family 45 protein Hypocrea atroviridis Carb bind domain	GTQSLYGQCGGTGWAGPTACAPPATCKVLNQYYSQCL
55	Endoglucanase, putative Neosartorya fumigata Carb bind domain	ATQTLYGQCGGSGWTGPTACASGATCKVLNPYYSQCL
56	Putative uncharacterized protein Aspergillus terreus Carb bind domain	GSQTVYGQCGGTGWTGPTACVASATCTTLNPYYAQCL
57	Cip1 Hypocrea jecorina Carb bind domain	PTQTHYGQCGGIGYSGPTVCASGTTCQVLNPYYSQCL
58	Exoglucanase 1 Hypocrea rufa Carb bind domain	PTQTHYGQCGGIGYSGPTVCASGSTCQVLNPYYSQCL
59	Glycoside hydrolase family 7 protein Hypocrea virens Carb bind domain	PTQTHYGQCGGIGYSGPTQCVSGTTCQVLNPYYSQCL
60	Glycoside hydrolase family 5 protein Hypocrea atroviridis Carb bind domain	QTQTVWGQCGGQGYSGPTNCASGSACSTLNPYYAQCI
61	Glycosyl hydrolase family 45 protein Neosartorya fischeri Carb bind domain	ATQTLYGQCGGSGWTGPTACASGATCKVLNSYYSQCL
62	Exoglucanase 1 Trichoderma koningii Carb bind domain	PTQSHYGQCGGIGYSGPTVCASGTTCQVLNPYYSQCL

63	Glycosyl hydrolase family 61 Colletotrichum graminicola Carb bind domain	PTQKMYGQCGGVAYMGPTQCPAYATCSTVNPYYAQC
64	Glycosyl hydrolase family 61 Colletotrichum graminicola Carb bind domain	QPLYGQCGGLNWPPESPTECVPGARCSTINPYYAQC
65	Putative uncharacterized protein Arthrobotrys oligospora Carb bind domain	PLQSKWGQCGGVGYTGASVCSPTATCSTLNPYYAQCL
66	Cellobiohydrolase Trichoderma harzianum Carb bind domain	ATQTHYGQCGGTGWTGPTRCASGFTCQVLNPFYSQCL
67	Endoglucanase Penicillium sp. Carb bind domain	ATQTHYGQCGGMSYTGPTVCASPYTCQVQNPYYSQCL
68	Endoglucanase IV Hypocrea rufa MATURE	HGHINDIVINGVWYQAYDPTTFPYESNPPIVVGWTAADLDN GFVSPDAYQNPDIICHKNATNAKGHASVKARDTILFQWVPV PWPHPGPIVDYLANCNGDCETVDKTTLEFFKIDGVGLLSGG DPGTWASDVLISNNNTWVVKIPDNLAPGNYVLRHEIIALHSA GQANGAQNYPQCFNIAVSGSGSLQPSGVLGTDLYHATDPG VPINIYTSPLNYIIPGPTVVSGLPTSVAQGSSAATATASATAP GGGSGPTSRTTTTARTTQASSRPSSTPPATTSAPAGGPTQT LYGQCGGSGYSGPTRCAPPATCSTLNPYYAQCLN
69	Type IV endoglucanase Trichoderma saturnisporum MATURE	HGHINNIVINGVYYQAYDPTSFPYESNPPIVVGWTAADLDNG FVSPDAYGSPDIICHKNATNAKGHASVRAGDTVLFQWVPLP WPHPGPIVDYLANCNGDCETVDKTSLEFFKIDGVGLISGGD PGNWASDVLIANNNTWVVKIPDDLAPGNYVLRHEIIALHSAG QANGAQNYPQCFNLAVSGSGSLKPSGVKGTALYHATDPGV LINIYTSPLNYIIPGPTVVSGLPTSVAQRSSAATATASATLPGG GGSPPGGPTSRPTTTARSTSQASSRPSPPATTSAPAGGPT QTLYGQCGGSGYSGPTRCAPPATVSTLNPYYARLN

70	Endoglucanase IV Hypocrea orientalis MATURE	HGHINNIVVNGVYYQGYDPTSFPYESDPPIVVGWTAADLDN GFVSPDAYQSPDIICHKNATNAKGHASVKAGDTILFQWVPV PWPHPGPIVDYLANCNGDCETVDKTSLEFFKIDGVGLISGG DPGNWASDVLIANNNTWVVKIPEDLAPGNYVLRHEIIALHSA GQADGAQNYPQCFNLAVSGSGSLQPSGVKGTALYHSDDP GVLINIYTSPLAYTIPGPSVVSGLPTSVAQGSSAATATASATV PGGSGPGNPTSKTTTTARTTQASSSRASSTPPATTSAPGG GPTQTLYGQCGGSGYSGPTRCAPPATCSTLNPYYAQCLN
71	Endoglucanase IV Trichoderma sp. MATURE	HGHINNIVVNGVYYQGYDPTSFPYESDPPIVVGWTAADLDN GFVSPDAYQSPDIICHKNATNAKGHASVKAGDTIPLQWVPV PWPHPGPIVDYLANCNGDCETVDKTSLEFFKIDGVGLISGG DPGNWASDVLIANNNTWVVKIPEDLAPGNYVLRHEIIALHSA GQADGAQNYPQCFNLAVPGSGSLQPSGVKGTALYHSDDP GVLINIYTSPLAYTIPGPSVVSGLPTSVAQGSSAATATASATV PGGSGPGNPTSKTTTTARTTQASSSRASSTPPATTSAPGG GPTQTLYGQCGGSGYSGPTRCAPPATCSTLNPYYAQCLN
72	Glycoside hydrolase family 61 protein Hypocrea atroviridis MATURE	HGHVNNIVVNGVYYQGYDPTSFPYMPDPPIVVGWTAADTD NGFVSPDAYQTPDIVCHKNGTNAKGHASVKAGDSVLFQWV PVPWPHKSTVVDYLANCNGPCETVDKTTLEFFKIDGIGLLSG GNPGTWGSDVLIGNNNTWVIQIPEDLQTGNYVLRHELIALHS AEQADGAQNYPQCFNLAVTGTGSLQPSGVLATDLYHETDP GILFNIYTSPLTYIIPGPTVVSGLPSSVAQASSAATATSSATVS GGGGGSSTGGSTSKTTTVVRSTTSVTSKASSSTAVTTPPPA GGTQTLYGQCGGSGYSGPTKCASPAVCTTLNPYYAQCLN
73	Glycoside hydrolase family 61 protein Hypocrea virens MATURE	HGHVNNIVINGAYYQGYDPTLFPYEPNPPIVVGWTASDTDN GFVAPDAYQSPDIICHRNATNARGHASVMAGSSVLIQWVPIP WPHPGPVLDYLANCNGDCETVDKTTLEFFKIDGIGLISGGNP GRWASDVLIGNNGTWVVQIPADLETGNYVLRHELIALHSAG SVDGAQNYPQCFNLAVTGTGSLQPTGVLGTKLYQESDPGIL FNIYTSPLTYIPGPTVVSGLPSSVTQRSSTATATSIATVPGS VSTGGTSSKTTTVPRSTSSATTRRSSSSAITTSAPAGPSQTL YGQCGGSGYSGPTICASPAVCSTLNPYYAQCLTR
74	Glycoside hydrolase family 61 protein Thielavia terrestris MATURE	HGHVSNIVINGVSYQGYDPTSFPYMQNPPIVVGWTAADTDN GFVAPDAFASGDIICHKNATNAKGHAVVAAGDKIFIQWNTW PESHHGPVIDYLASCGSASCETVDKTKLEFFKIDEVGLVDGS SAPGVWGSDQLIANNNSWLVEIPPTIAPGNYVLRHEIIALHSA ENADGAQNYPQCFNLQITGTGTATPSGVPGTSLYTPTDPGI LVNIYSAPITYTVPGPALISGAVSIAQSSSAITASGTALTGSAT APAAAAATTTSTTNAAAAATSAAAAAGTSTTTTSAAAVVQTS SSSSSAPSSAAAAATTTAAASARPTGCSSGRSRKQPRRHA RDMVVARGAEEAN
75	Endoglucanase IV Neurospora tetrasperma MATURE	HGHVSKVIVNGVEYQNYDPTSFPYNSNPPTVIGWTIDQKDN GFVSPDAFDSGDIICHKSATPAGGHATVKAGDKISLQWDQW PESHKGPVIDYLAACDGDCESVDKTALKFFKIDGAGYDATN GWASDVLIKDGNSWVVEIPENIKPGNYVLRHEIIALHSAGQA NGAQNYPQCFNLKVEGSGSTVPAGVAGTELYKATDAGILFD IYKNDISYPVPGPSLIAGASSSIAQSKMAATATASATLPGATG GSNSPATSAAAAAPAPSTTLVTSTKAAAPATSAAPAAPATSA AAGSGQVQAKQTKWGQCGGNGYTGATECESGSTCTKYND WYSQCV
76	Putative uncharacterized protein Neurospora tetrasperma	HGHVSKVIVNGVEYQNYDPTSFPYNSNPPTVIGWTIDQKDN GFVSPDAFDSGDIICHKSATPAGGHATVKAGDKISLQWDQW PESHKGPVIDYLAACDGDCESVDKTALKFFKIDGAGYDATN GWASDVLIKDGNSWVVEIPENIKPGNYVLRHEIIALHSAGQA NGAQNYPQCFNLKVEGSGSTVPAGVAGTELYKATDAGILFD

	MATURE	IYKNDISYPVPGPSLIAGASSSIAQSKMAATATASATLPGATG GSNSPATSAAAAAPAPSTTLVTSTKAAAPATSAAPAAPATSA AAGSGQVQAKQTKWGQCGGNGYTGATECESGSTCTKYND WYSQCV
77	Glycoside hydrolase family 61 protein Thielavia heterothallica MATURE	HGHVTNIVINGVSYQNFDPFTHPYMQNPPTVVGWTASNTD NGFVGPESFSSPDIICHKSATNAGGHAVVAAGDKVFIQWDT WPESHHGPVIDYLADCGDAGCEKVDKTTLKFFKISESGLLD GTNAPGKWASDTLIANNNSWLVQIPPNIAPGNYVLRHEIIALH SAGQQNGAQNYPQCFNLQVTGSGTQKPSGVLGTELYKATD AGILANIYTSPVTYQIPGPAIISGASAVQQTTSAITASASAITGS ATAAPTAATTTAAAAATTTTTAGSRCYRHALDRRLSFFRPAC SYHRCRYLQPCSPDPLRWSEEAPSPRP
78	Endoglucanase IV Neurospora crassa MATURE	HGHVSKVIVNGVEYQNYDPTSFPYNSNPPTVIGWTIDQKDN GFVSPDAFDSGDIICHKSAKPAGGHATVKAGDKISLQWDQW PESHKGPVIDYLAACDGDCESVDKTALKFFKIDGAGYDATN GWASDTLIKDGNSWVVEIPESIKPGNYVLRHEIIALHSAGQA NGAQNYPQCFNLKVEGSGSTVPAGVAGTELYKATDAGILFD IYKNDISYPVPGPSLIAGASSSIAQSKMAATATASATLPGATG GSNSPATSAAAAAPATSAAAATSQVQAAPATTLVTSTKAAA PATSAAAPAAPATSAAAGGAGQVQAKQTKWGQCGGNGFT GPTECESGSTCTKYNDWYSQCV
79	Endoglucanase IV Gaeumannomyces graminis var. tritici MATURE	HGHVSNIVVNGVFYPGYDVTKYPWQPNAPTVVGWSATNTD NGFVEPNNFGHPDIICHRGAQPAKGHARVRAGDKILLQWDT WPESHKGPVLDYLARCPGDCETVDKTALRFFKIGEGSYISG AAPGHWAADVLLGNGFSWVVQIPEDVAPGNYVLRHEIIALH GSPNPNGAQAYPQCFNLEISGSGSRQPAGVAGTSLYRAGD PGIHFPLYNSPIVYPVPGPALIPGVPSTVAQVSTRATATSSPF LPGGGGGGGGGGGGGNPGPTSAPGGGNGGGGGGQQPP QTTTAPGNGGGGGGGGGGGGGGQTRWGQCGGSGWNG PTACAQGACSTLNPYYAQCV

CLAIMS

What is claimed is:

1. A variant of a parent glycosyl hydrolase 61 (GH61) enzyme, wherein said variant has cellulase augmenting activity, has at least 80% sequence identity to SEQ ID NO:3, and has at least one improved property over said parent GH61 enzyme selected from: (a) expression, (b) thermostability and/or Tm, (c) performance in a Whole Hydrolysate Dilute Acid Pretreated Corn Stover (whPCS) hydrolysis assay, (f) performance in a Dilute Ammonia Pretreated Corn Stover (daCS) hydrolysis assay.

2. The variant of claim 1, wherein said variant comprises at least one amino acid substitution at a position selected from the group consisting of: T287, W13, L288, P19, T20, P29, A37, L39, D47, A48, S68, T107, S121, G122, T127, S164, G166, Q167, A168, N216, I218, I219, V224, T289, Y291, S297, T303, R304, A306, P308, S312, T313, N315, Y317, Y318, N5, I7, V12, Q15, T21, F22, S26, P28, V32, G33, T35, A36, N41, Q50, N51, K58, N59, V80, P89, T108, G116, L120, G123, V132, S135, N147, A161, A181, S183, S185, Q189, G195, T196, H200, A201, T202, I208, P222, T223, S226, S231, G235, L290, G295, G296, G298, Y299, S300, G301, P302, P307, A309, T310, P316, A319, L322, I4, I9, N10, P23, Y24, E25, N27, D38, F43, V44, Y49, P52, I54, T61, N62, K64, V69, K70, A71, I90, V91, N96, N98, D100, T103, E110, V117, L119, D124, P125, G126, V141, D146, A149, I160, H163, A171, N173, P175, I180, S187, L188, S191, L194, D197, P204, V206, L207, N209, Y211, S213, P214, Y217, T230, V232, A233, G292, Q293, Q320, and N323, wherein the position of each amino acid substitution corresponds to SEQ ID NO:3.

3. The variant of any preceding claim, wherein said variant comprises one or more of:
an amino acid substitution at position T287 selected from the group consisting of: H, A, F, G, S, and I; an amino acid substitution at position W13 selected from the group consisting of: G, L, N, R, T, V, F, Q, Y, A, E, I, K, and M; an amino acid substitution at position L288 selected from the group consisting of: E, F, G, I, K, M, N, R, S, T, A, C, D, V, and W; an amino acid substitution at position P19 selected from the group consisting of: F, M, E, H, Q, T, K, and W; an amino acid substitution at position T20 selected from the group consisting of: K, P, G, M, R, Y, A, and N; an amino acid substitution at position P29 selected from the group consisting of: C, E, F, K, T, Y D, G, M, R, and S; an amino acid substitution at position A37 selected from the group consisting of: D, S, E, L, W, F, K, and Q; an amino acid substitution at position L39 selected from the group consisting of: A, D, E, M, T, Y, F, I, N, P, Q, S, and V; an amino acid substitution at position D47 selected from the group consisting of: A, K, T, H, R, P, and W; an amino acid

substitution at position A48 selected from the group consisting of: C, E, G, K, L, N, T, Y, Q, W, H, I, and S; an amino acid substitution at position S68 selected from the group consisting of: E, I, L, M, N, T, V, and Y; an amino acid substitution at position T107 selected from the group consisting of: A, D, E, K, M, R, S, and G; an amino acid substitution at position S121 selected from the group consisting of: E, F, N, T, C, L, V, D, G, K, M, and Y; an amino acid substitution at position G122 selected from the group consisting of: E, N, S, V, A, M, T, F, and L; an amino acid substitution at position T127 selected from the group consisting of: A, E, F, I, L, M, V, W, and Y; an amino acid substitution at position S164 selected from the group consisting of: A, D, E,G, K, L, M, Q, R, V, and Y; an amino acid substitution at position G166 selected from the group consisting of: A, D, E, F, H, K, M, N, Q, and S; an amino acid substitution at position Q167 selected from the group consisting of: E, A, D, F, G, K, L, N, R, V, and Y; an amino acid substitution at position A168 selected from the group consisting of: E, M, D, F, I, L, N, P, Q, R, T, and Y; an amino acid substitution at position N216 selected from the group consisting of: D, Q, T, K, and P; an amino acid substitution at position I218 selected from the group consisting of: T, A, D, E, K, M, N, P, S, and V; an amino acid substitution at position I219 selected from the group consisting of: M, A, D, L, N, P, and Q; an amino acid substitution at position V224 selected from the group consisting of: Q, I, A, E, N, P, S, and T; an amino acid substitution at position T289 selected from the group consisting of: A, D, K, L, M, R, and S; an amino acid substitution at position Y291 selected from the group consisting of: A, F, G, I, K, L, M, N, R, S, V, W, D, and E; an amino acid substitution at position S297 selected from the group consisting of: D, E, K, N, Q, R, and T; an amino acid substitution at position T303 selected from the group consisting of: K, M, P, R, S, V, and Y; an amino acid substitution at position R304 selected from the group consisting of: A, C, D, E, N, Q, T, and V; an amino acid substitution at position A306 selected from the group consisting of: E, F, G, I, K, L, M, Q, T, and Y; an amino acid substitution at position P308 selected from the group consisting of: A, G, H, I, K, L, Q, R, S, T, V, W, and Y; an amino acid substitution at position S312 selected from the group consisting of: Q, F, G, I, K, L, M, N, T, V, D, R, and Y; an amino acid substitution at position T313 selected from the group consisting of: S, D, G, L, P, V, E, F, I, K, M, and Y; an amino acid substitution at position N315 selected from the group consisting of: S, H, L, Q, R, V, Y, E, K, and M; an amino acid substitution at position Y317 selected from the group consisting of: Q, S, V, R, E, G, L, M, P, and T; a Y318T amino acid substitution; an amino acid substitution at position N5 selected from the group consisting of: A, D, G, and S; an amino acid substitution at position I7 selected from the group consisting of: L, M, and V; an amino acid substitution at position V12 selected from the group consisting of: M and T; an amino acid substitution at position Q15 selected from the group consisting of: H, K, and D; an amino acid substitution at position T21 selected from the group consisting of: I, K, M, and S; an amino acid substitution at position F22 selected from the group consisting of: K, I, L, and R; an amino acid substitution at position S26 selected from

the group consisting of: M, P, and T; an amino acid substitution at position P28 selected from the group consisting of: M, S, and V; an amino acid substitution at position V32 selected from the group consisting of: A and P; an amino acid substitution at position G33 selected from the group consisting of: Q, A, and S; an amino acid substitution at position T35 selected from the group consisting of: A, D, E, K, N, and S; an amino acid substitution at position A36 selected from the group consisting of: T, I, N, S, and Y; an amino acid substitution at position N41 selected from the group consisting of: D, E, L, and M; an amino acid substitution at position Q50 selected from the group consisting of: A, T, Y, D, E, and K; an amino acid substitution at position N51 selected from the group consisting of: S, E, H, K, L, and T; an amino acid substitution at position K58 selected from the group consisting of: I, R, and V; an amino acid substitution at position N59 selected from the group consisting of: G, E, Q, and S; an amino acid substitution at position V80 selected from the group consisting of: A, E, L, and M; an amino acid substitution at position P89 selected from the group consisting of: T, S, V, and L; an amino acid substitution at position T108 selected from the group consisting of: A, Q, R, and S; an amino acid substitution at position G116 selected from the group consisting of: H, Q, and S; an amino acid substitution at position L120 selected from the group consisting of: V, T, I, and S; an amino acid substitution at position G123 selected from the group consisting of: E, Q, and R; an amino acid substitution at position V132 selected from the group consisting of: D, E, M, and R; an amino acid substitution at position S135 selected from the group consisting of: A, E, G, H, M, and N; an amino acid substitution at position N147 selected from the group consisting of: E, M, S, and T; an amino acid substitution at position A161 selected from the group consisting of: E, G, L, and S; an amino acid substitution at position A181 selected from the group consisting of: K, N, and R; an amino acid substitution at position S183 selected from the group consisting of: E, K, N, and T; an amino acid substitution at position S185 selected from the group consisting of: D, G, and T; an amino acid substitution at position Q189 selected from the group consisting of: E, L, M, R, G, and T; an amino acid substitution at position G195 selected from the group consisting of: A, E, and S; an amino acid substitution at position T196 selected from the group consisting of: H, Y, and R; an amino acid substitution at position H200 selected from the group consisting of: R, A, Q, and S; an amino acid substitution at position A201 selected from the group consisting of: P, K, and Q; an amino acid substitution at position T202 selected from the group consisting of: S, E, and Q; an amino acid substitution at position I208 selected from the group consisting of: F, L, and V; an amino acid substitution at position P222 selected from the group consisting of: D and G; an amino acid substitution at position T223 selected from the group consisting of: S, E, and Q; an amino acid substitution at position S226 selected from the group consisting of: D, G, K, M, P, and Q; an amino acid substitution at position S231 selected from the group consisting of: C and I; an amino acid substitution at position G235 selected from the group consisting of: A, F, I, M, N, Q, V; an amino acid substitution at position

L290 selected from the group consisting of: K, M, and T; an amino acid substitution at position G295 selected from the group consisting of: A, F, H, Q, and R; an amino acid substitution at position G296 selected from the group consisting of: A and M; an amino acid substitution at position G298 selected from the group consisting of: K, A, I, N, and Q; an amino acid substitution at position Y299 selected from the group consisting of: N, S, F, and P; an amino acid substitution at position S300 selected from the group consisting of: F, L, N, T, and Q; an amino acid substitution at position G301 selected from the group consisting of: D, S, and Y; an amino acid substitution at position P302 selected from the group consisting of: G, L, T, V, Y, and F; an amino acid substitution at position P307 selected from the group consisting of: A, F, H, L, and V; an amino acid substitution at position A309 selected from the group consisting of: I, K, R, T, V, and Y; an amino acid substitution at position T310 selected from the group consisting of: L, M, Q, S, and W; an amino acid substitution at position P316 selected from the group consisting of: D, N, R, and T; an amino acid substitution at position A319 selected from the group consisting of: Q, W, D, F, and S; an amino acid substitution at position L322 selected from the group consisting of: A, E, S, T, and V; an amino acid substitution at position I4 selected from the group consisting of: V and T; an amino acid substitution at position I9 selected from the group consisting of: V and A; an N10D amino acid substitution; a P23G amino acid substitution; a Y24T amino acid substitution; an amino acid substitution at position E25 selected from the group consisting of: N and Q; an N27E amino acid substitution; an amino acid substitution at position D38 selected from the group consisting of: E and N; an F43Y amino acid substitution; a V44I amino acid substitution; a Y49W amino acid substitution; an amino acid substitution at position P52 selected from the group consisting of: A and N; an amino acid substitution at position I54 selected from the group consisting of: Q and E; an amino acid substitution at position T61 selected from the group consisting of: Q and S; an N62P amino acid substitution; an amino acid substitution at position K64 selected from the group consisting of: A and Q; a V69Y amino acid substitution; a K70R amino acid substitution; an amino acid substitution at position A71 selected from the group consisting of: Y and P; an I90V amino acid substitution; an amino acid substitution at position V91 selected from the group consisting of: L and I; an amino acid substitution at position N96 selected from the group consisting of: A and S; an N98H amino acid substitution; an amino acid substitution at position D100 selected from the group consisting of: P and S; a T103A amino acid substitution; an E110K amino acid substitution; a V117A amino acid substitution; an amino acid substitution at position L119 selected from the group consisting of: N and T; a D124N amino acid substitution; a P125D amino acid substitution; an amino acid substitution at position G126 selected from the group consisting of: M and Q; a V141T amino acid substitution; a D146E amino acid substitution; an A149E amino acid substitution; an amino acid substitution at position I160 selected from the group consisting of: F and M; an H163A amino acid substitution; an A171T amino acid

substitution; an N173H amino acid substitution; a P175F amino acid substitution; an I180L amino acid substitution; an amino acid substitution at position S187 selected from the group consisting of: A and D; an amino acid substitution at position L188 selected from the group consisting of: A and D; an S191D amino acid substitution; an amino acid substitution at position L194 selected from the group consisting of: D and M; an amino acid substitution at position D197 selected from the group consisting of: A and V; a P204A amino acid substitution; a V206I amino acid substitution; an L207F amino acid substitution; an N209D amino acid substitution; a Y211A amino acid substitution; an S213T amino acid substitution; a P214S amino acid substitution; a Y217H amino acid substitution; a T230S amino acid substitution; an amino acid substitution at position V232 selected from the group consisting of: F and Y; comprises an amino acid substitution at position A233 selected from the group consisting of: F and S; an amino acid substitution at position G292 selected from the group consisting of: D and K; an amino acid substitution at position Q293 selected from the group consisting of: K and P; a Q320R amino acid substitution; and an N323V amino acid substitution.

4. The variant of any preceding claim, wherein said variant comprises from 1 to 10 amino acid substitutions.

5. The variant of claim 4, wherein said variant is a combinatorial variant.

6. The variant of any preceding claim, wherein said parent GH61 polypeptide is a fungal glycosyl hydrolase 61a (GH61A).

7. The variant of claim 6, wherein said fungal GH61A is from *Hypocrea jecorina, Hypocrea rufa, Hypocrea orientalis, Hypocrea atroviridis, Hypocrea virens, Emericella nidulans, Aspergillus terreus, Aspergillus oryzae, Aspergillus niger, Aspergillus kawachii, Aspergillus flavus, Aspergillus clavatus, Gaeumannomyces graminis, Trichoderma saturnisporum, Neurospora tetrasperma, Neurospora crassa, Neosartorya fumigate, Neosartorya fumigate, Neosartorya fischeri, Thielavia terrestris, Talaromyces sp., Sporotricum sp,* and *Thielavia heterothallica.*

8. The variant of any preceding claim, wherein said variant has at least 90% sequence identity to SEQ ID NO:3.

9. The variant of any preceding claim, wherein said variant has at least 95% sequence identity to SEQ ID NO:3.

10. A polynucleotide comprising a polynucleotide sequence encoding a variant of a parent GH61 polypeptide according to any preceding claim.

11. A vector comprising the polynucleotide of claim 10.

12. The vector of claim 11, wherein said vector is an expression vector.

13. A host cell comprising the polynucleotide of claim 10, the vector of claim 11, or the expression vector of claim 12.

14. The host cell of claim 13, wherein said host cell is a fungal cell or a bacterial cell.

15. The host cell of claim 14, wherein said fungal cell is a filamentous fungal cell selected from the group consisting of: *Trichoderma reesei, Trichoderma longibrachiatum, Trichoderma viride, Trichoderma koningii, Trichoderma harzianum, Penicillium, Humicola, Humicola insolens, Humicola grisea, Chrysosporium, Chrysosporium lucknowense, Myceliophthora thermophilia, Gliocladium, Aspergillus, Fusarium, Neurospora, Hypocrea, Emericella, Aspergillus niger, Aspergillus awamori, Aspergillus aculeatus*, and *Aspergillus nidulans*.

16. The host cell of claim 13, 14 or 15, wherein said host cell expresses the variant of a parent GH61 polypeptide encoded by said polynucleotide, vector, or expression vector.

17. A method of producing a variant GH61 polypeptide comprising culturing a host cell according to claim 16 in a suitable culture medium under suitable conditions to produce said variant.

18. The method of claim 17, further comprising isolating said produced variant.

19. A composition comprising a GH61 variant according to any one of claims 1 to 9.

20. The composition of claim 19, wherein said composition is selected from the group consisting of: a detergent, an animal feed, a feed additive, and a cell culture supernatant.

21. The composition of claims 19 or 20, wherein said composition is enriched for said GH61 variant.

22. A method for hydrolyzing a cellulosic substrate, comprising contacting said substrate with a variant GH61 polypeptide according to any one of claims 1 to 9.

SEQ ID NO:1 (Top); SEQ ID NO:2 (Bottom: signal sequence underlined)

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1       atgatccagaagctttccaacctccttgtcaccgcactggcggtggctactggcgttgtc
1        M   I   Q   K   L   S   N   L   L   V   T   A   L   A   V   A   T   G   V   V

61      ggacatggacatattaatgacattgtcatcaacggggtgtggtatcaggcctatgatcct
21       G   H   G   H   I   N   D   I   V   I   N   G   V   W   Y   Q   A   Y   D   P

121     acaacgtttccatacgagtcaaacccccccatagtagtgggctggacggctgccgacctt
41       T   T   F   P   Y   E   S   N   P   P   I   V   V   G   W   T   A   A   D   L

181     gacaacggcttcgtttcacccgacgcataccaaaaccctgacatcatctgccacaagaat
61       D   N   G   F   V   S   P   D   A   Y   Q   N   P   D   I   I   C   H   K   N

241     gctacgaatgccaagggggcacgcgtctgtcaaggccggagacactattctcttccagtgg
81       A   T   N   A   K   G   H   A   S   V   K   A   G   D   T   I   L   F   Q   W

301     gtgccagttccatggccgcaccctggtcccattgtcgactacctggccaactgcaatggt
101      V   P   V   P   W   H   P   G   P   I   V   D   Y   L   A   N   C   N   G

361     gactgcgagaccgttgacaagacgacgcttgagttcttcaagatcgatggcgttggtctc
121      D   C   E   T   V   D   K   T   T   L   E   F   F   K   I   D   G   V   G   L

421     ctcagcggcgggggatccgggcacctgggcctcagacgtgctgatctccaacaacaacacc
141      L   S   G   G   D   P   G   T   W   A   S   D   V   L   I   S   N   N   N   T

481     tgggtcgtcaagatccccgacaatcttgcgccaggcaattacgtgctccgccacgagatc
161      W   V   V   K   I   P   D   N   L   A   P   G   N   Y   V   L   R   H   E   I

541     atcgcgttacacagcgccgggcaggcaaacggcgctcagaactaccccccagtgcttcaac
181      I   A   L   H   S   A   G   Q   A   N   G   A   Q   N   Y   P   Q   C   F   N

601     attgccgtctcaggctcgggttctctgcagcccagcggcgttctagggaccgacctctat
201      I   A   V   S   G   S   G   S   L   Q   P   S   G   V   L   G   T   D   L   Y

661     cacgcgacggaccctggtgttctcatcaacatctacaccagcccgctcaactacatcatc
221      H   A   T   D   P   G   V   L   I   N   I   Y   T   S   P   L   N   Y   I   I

721     cctggacctaccgtggtatcaggcctgccaacgagtgttgcccaggggagctccgccgcg
241      P   G   P   T   V   V   S   G   L   P   T   S   V   A   Q   G   S   S   A   A

781     acggccaccgccagcgccactgttcctggaggcggtagcggcccgaccagcagaaccacg
261      T   A   T   A   S   A   T   V   P   G   G   G   S   G   P   T   S   R   T   T

841     acaacggcgaggacgacgcaggcctcaagcaggcccagctctacgcctcccgcaaccacg
281      T   T   A   R   T   T   Q   A   S   S   R   P   S   S   T   P   P   A   T   T

901     tcggcacctgctggcggcccaacccagactctgtacggccagtgtggtggcagcggttac
301      S   A   P   A   G   G   P   T   Q   T   L   Y   G   Q   C   G   G   S   G   Y

961     agcgggcctactcgatgcgcgccgccagccacttgctctaccttgaaccccctactacgcc
321      S   G   P   T   R   C   A   P   P   A   T   C   S   T   L   N   P   Y   Y   A

1021    cagtgccttaac
341      Q   C   L   N
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FIG. 1

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SEQ ID NO:48 : HGHVTQVIINGVAYGGYLSTSFPLQRKPPVVLGWTIEQRDNGFVSPDKYD : 50
SEQ ID NO:49 : HGHVDEIIVNGVSYQGYGSTDFPYMQDPPVVAGWTIEQADNGFVSPDKYD : 50
SEQ ID NO:50 : HGHVDEIIVNGVSYQGYGSTDFPYMQDPPVVAGWTIEQADNGFVSPDKYD : 50
SEQ ID NO:47 : HGHVSNIVVNGVFYPGYDVTKYPWQPNAPTVVGWSATNTDNGFVEPNNFG : 50
SEQ ID NO:46 : HGHVSKVIVNGVEYQNYDPAVFPYLSNPPTVIGWTADQKDNGFVSPDAFG : 50
SEQ ID NO:42 : HGHVSKVIVNGVEYQNYDPTSFPYNSNPPTVIGWTIDQKDNGFVSPDAFD : 50
SEQ ID NO:43 : HGHVSKVIVNGVEYQNYDPTSFPYNSNPPTVIGWTIDQKDNGFVSPDAFD : 50
SEQ ID NO:45 : HGHVSKVIVNGVEYQNYDPTSFPYNSNPPTVIGWTIDQKDNGFVSPDAFD : 50
SEQ ID NO:34 : HGHINDIVINGVWYQAYDPTTFPYESNPPIVVGWTAADLDNGFVSPDAYQ : 50
SEQ ID NO:35 : HGHINDIVINGVWYQAYDPTTFPYESNPPIVVGWTAADLDNGFVSPDAYQ : 50
SEQ ID NO:36 : HGHINNIVINGVYYQAYDPTSFPYESNPPIVVGWTAADLDNGFVSPDAYG : 50
SEQ ID NO:37 : HGHINNIVVNGVYYQGYDPTSFPYESDPPIVVGWTAADLDNGFVSPDAYQ : 50
SEQ ID NO:38 : HGHINNIVVNGVYYQGYDPTSFPYESDPPIVVGWTAADLDNGFVSPDAYQ : 50
SEQ ID NO:40 : HGHVNNIVINGAYYQGYDPTLFPYEPNPPIVVGWTASDTDNGFVAPDAYQ : 50
SEQ ID NO:39 : HGHVNNIVVNGVYYQGYDPTSFPYMPDPPIVVGWTAADTDNGFVSPDAYQ : 50
SEQ ID NO:41 : HGHVSNIVINGVSYQGYDPTSFPYMQNPPIVVGWTAADTDNGFVAPDAFA : 50
SEQ ID NO:44 : HGHVTNIVINGVSYQNFDPFTHPYMQNPPTVVGWTASNTDNGFVGPESFS : 50



SEQ ID NO:48 : HPDIICHRDATPAQGHVQVAAGDTITIKWSS-WPENHRGPVMDYLANC-N : 98
SEQ ID NO:49 : DPDIICHRDATPAKGHIELAAGDTLTLRWSG-WPENHSGPILNYLANC-N : 98
SEQ ID NO:50 : DPDIICHRDATPAKGHIELAAGDTLTLRWSG-WPENHSGPILNYLANC-N : 98
SEQ ID NO:47 : HPDIICHRGAQPAKGHARVRAGDKILLQWDT-WPESHKGPVLDYLARC-P : 98
SEQ ID NO:46 : TPDIICHRSATPAGGHATVKAGDKISLKWDPVWPDSHKGPVIDYLAAC-N : 99
SEQ ID NO:42 : SGDIICHKSATPAGGHATVKAGDKISLQWDQ-WPESHKGPVIDYLAAC-D : 98
SEQ ID NO:43 : SGDIICHKSATPAGGHATVKAGDKISLQWDQ-WPESHKGPVIDYLAAC-D : 98
SEQ ID NO:45 : SGDIICHKSAKPAGGHATVKAGDKISLQWDQ-WPESHKGPVIDYLAAC-D : 98
SEQ ID NO:34 : NPDIICHKNATNAKGHASVKAGDTILFQWVP-VPWPHPGPIVDYLANC-N : 98
SEQ ID NO:35 : NPDIICHKNATNAKGHASVKARDTILFQWVP-VPWPHPGPIVDYLANC-N : 98
SEQ ID NO:36 : SPDIICHKNATNAKGHASVRAGDTVLFQWVP-LPWPHPGPIVDYLANC-N : 98
SEQ ID NO:37 : SPDIICHKNATNAKGHASVKAGDTILFQWVP-VPWPHPGPIVDYLANC-N : 98
SEQ ID NO:38 : SPDIICHKNATNAKGHASVKAGDTIPLQWVP-VPWPHPGPIVDYLANC-N : 98
SEQ ID NO:40 : SPDIICHRNATNARGHASVMAGSSVLIQWVP-IPWPHPGPVLDYLANC-N : 98
SEQ ID NO:39 : TPDIVCHKNGTNAKGHASVKAGDSVLFQWVP-VPWPHKSTVVDYLANC-N : 98
SEQ ID NO:41 : SGDIICHKNATNAKGHAVVAAGDKIFIQWNT-WPESHHGPVIDYLASCGS : 99
SEQ ID NO:44 : SPDIICHKSATNAGGHAVVAAGDKVFIQWDT-WPESHHGPVIDYLADCGD : 99
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FIG. 2A

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SEQ ID NO:48 : GPCETVDKTKLEFFKIDGMGLISQDR-PGKYADGALRENGYTWSVRIPSN : 147
SEQ ID NO:49 : GPCERVDKTKLEFFKIDGLGLLEQGT-PGRYADKVLQDNGDRWNVRIPKN : 147
SEQ ID NO:50 : GPCERVDKTKLEFFKIDGLGLLEQGT-PGRYADKVLQDNGDRWNVRIPKN : 147
SEQ ID NO:47 : GDCETVDKTALRFFKIGEGSYISGAA-PGHWAADVLLGNGFSWVVQIPED : 147
SEQ ID NO:46 : GDCETVDKTSLRFFKIDGAGYN-----NGVWAADALVNNGNSWLVQIPAD : 144
SEQ ID NO:42 : GDCESVDKTALKFFKIDGAGYDATN---G-WASDVLIKDGNSWVVEIPEN : 144
SEQ ID NO:43 : GDCESVDKTALKFFKIDGAGYDATN---G-WASDVLIKDGNSWVVEIPEN : 144
SEQ ID NO:45 : GDCESVDKTALKFFKIDGAGYDATN---G-WASDTLIKDGNSWVVEIPES : 144
SEQ ID NO:34 : GDCETVDKTTLEFFKIDGVGLLSGGD-PGTWASDVLISNNNTWVVKIPDN : 147
SEQ ID NO:35 : GDCETVDKTTLEFFKIDGVGLLSGGD-PGTWASDVLISNNNTWVVKIPDN : 147
SEQ ID NO:36 : GDCETVDKTSLEFFKIDGVGLISGGD-PGNWASDVLIANNNTWVVKIPDD : 147
SEQ ID NO:37 : GDCETVDKTSLEFFKIDGVGLISGGD-PGNWASDVLIANNNTWVVKIPED : 147
SEQ ID NO:38 : GDCETVDKTSLEFFKIDGVGLISGGD-PGNWASDVLIANNNTWVVKIPED : 147
SEQ ID NO:40 : GDCETVDKTTLEFFKIDGIGLISGGN-PGRWASDVLIGNNGTWVVQIPAD : 147
SEQ ID NO:39 : GPCETVDKTTLEFFKIDGIGLLSGGN-PGTWGSDVLIGNNNTWVIQIPED : 147
SEQ ID NO:41 : ASCETVDKTKLEFFKIDEVGLVDGSSAPGVWGSDQLIANNNSWLVEIPPT : 149
SEQ ID NO:44 : AGCEKVDKTTLKFFKISESGLLDGTNAPGKWASDTLIANNNSWLVQIPPN : 149


SEQ ID NO:48 : IAPGNYVLRHEIIALHSGLERNGAQNYPQCFNLKITGSGSDNPPGYLGTE : 197
SEQ ID NO:49 : IAPGNYVLRHEIIALHNALDKGGAQNYPQCFNLKITGDGSDSPSGYLGTE : 197
SEQ ID NO:50 : IAPGNYVLRHEIIALHNALDKGGAQNYPQCFNLKITGDGSDSPSGYLGTE : 197
SEQ ID NO:47 : VAPGNYVLRHEIIALHGSPNPNGAQAYPQCFNLEISGSGSRQPAGVAGTS : 197
SEQ ID NO:46 : LKPGNYVLRHEIIALHGAGSANGAQAYPQCFNLKVEGSGNNLPSGVP--- : 191
SEQ ID NO:42 : IKPGNYVLRHEIIALHSAGQANGAQNYPQCFNLKVEGSGSTVPAGVAGTE : 194
SEQ ID NO:43 : IKPGNYVLRHEIIALHSAGQANGAQNYPQCFNLKVEGSGSTVPAGVAGTE : 194
SEQ ID NO:45 : IKPGNYVLRHEIIALHSAGQANGAQNYPQCFNLKVEGSGSTVPAGVAGTE : 194
SEQ ID NO:34 : LAPGNYVLRHEIIALHSAGQANGAQNYPQCFNIAVSGSGSLQPSGVLGTD : 197
SEQ ID NO:35 : LAPGNYVLRHEIIALHSAGQANGAQNYPQCFNIAVSGSGSLQPSGVLGTD : 197
SEQ ID NO:36 : LAPGNYVLRHEIIALHSAGQANGAQNYPQCFNLAVSGSGSLKPSGVKGTA : 197
SEQ ID NO:37 : LAPGNYVLRHEIIALHSAGQADGAQNYPQCFNLAVSGSGSLQPSGVKGTA : 197
SEQ ID NO:38 : LAPGNYVLRHEIIALHSAGQADGAQNYPQCFNLAVPGSGSLQPSGVKGTA : 197
SEQ ID NO:40 : LETGNYVLRHELIALHSAGSVDGAQNYPQCFNLAVTGTGSLQPTGVLGTK : 197
SEQ ID NO:39 : LQTGNYVLRHELIALHSAEQADGAQNYPQCFNLAVTGTGSLQPSGVLATD : 197
SEQ ID NO:41 : IAPGNYVLRHEIIALHSAENADGAQNYPQCFNLQITGTGTATPSGVPGTS : 199
SEQ ID NO:44 : IAPGNYVLRHEIIALHSAGQQNGAQNYPQCFNLQVTGSGTQKPSGVLGTE : 199
```

FIG. 2B

```
SEQ ID NO:48 : LYDANDPGILVNIYGNLPNYQVPGPTIVSGGVSSVRQSPSRATTTAKCTT : 247
SEQ ID NO:49 : LYDAADPGILVNVYSSSVDYEVPGPTICEGGVSSVEQKPSEATTTAKCTT : 247
SEQ ID NO:50 : LYDAADPGILVNVYSSSVDYEVPGPTICEGGVSSVEQKPSEATTTAKCTT : 247
SEQ ID NO:47 : LYRAGDPGIHFPLYNSPIVYPVPGPALIPGVPSTVAQVSTRATATSSPFL : 247
SEQ ID NO:46 : LYKATDAGILFNMYQNDFTYPVPGPALIAGAVSSIPQSSSAATATASATV : 241
SEQ ID NO:42 : LYKATDAGILFDIYKNDISYPVPGPSLIAGASSSIAQSKMAATATASATL : 244
SEQ ID NO:43 : LYKATDAGILFDIYKNDISYPVPGPSLIAGASSSIAQSKMAATATASATL : 244
SEQ ID NO:45 : LYKATDAGILFDIYKNDISYPVPGPSLIAGASSSIAQSKMAATATASATL : 244
SEQ ID NO:34 : LYHATDPGVLINIYTSPLNYIIPGPTVVSGLPTSVAQGSSAATATASATV : 247
SEQ ID NO:35 : LYHATDPGVPINIYTSPLNYIIPGPTVVSGLPTSVAQGSSAATATASATA : 247
SEQ ID NO:36 : LYHATDPGVLINIYTSPLNYIIPGPTVVSGLPTSVAQRSSAATATASATL : 247
SEQ ID NO:37 : LYHSDDPGVLINIYTSPLAYTIPGPSVVSGLPTSVAQGSSAATATASATV : 247
SEQ ID NO:38 : LYHSDDPGVLINIYTSPLAYTIPGPSVVSGLPTSVAQGSSAATATASATV : 247
SEQ ID NO:40 : LYQESDPGILFNIYTSPLTYTIPGPTVVSGLPSSVTQRSSTATATSIATV : 247
SEQ ID NO:39 : LYHETDPGILFNIYTSPLTYIIPGPTVVSGLPSSVAQASSAATATSSATV : 247
SEQ ID NO:41 : LYTPTDPGILVNIYSAPITYTVPGPALISGAV-SIAQSSSAITASGTALT : 248
SEQ ID NO:44 : LYKATDAGILANIYTSPVTYQIPGPAIISGA-SAVQQTTSAITASASAIT : 248
```

```
SEQ ID NO:48 : RS : 249
SEQ ID NO:49 : RY : 249
SEQ ID NO:50 : RY : 249
SEQ ID NO:47 : PG : 249
SEQ ID NO:46 : PG : 243
SEQ ID NO:42 : PG : 246
SEQ ID NO:43 : PG : 246
SEQ ID NO:45 : PG : 246
SEQ ID NO:34 : PG : 249
SEQ ID NO:35 : PG : 249
SEQ ID NO:36 : PG : 249
SEQ ID NO:37 : PG : 249
SEQ ID NO:38 : PG : 249
SEQ ID NO:40 : PG : 249
SEQ ID NO:39 : SG : 249
SEQ ID NO:41 : GS : 250
SEQ ID NO:44 : GS : 250
```

FIG. 2C

```
SEQ ID NO:51 : PTQTLYGQCGGSGYS--GPTRCAPPATCSTLNPYYAQCL :
SEQ ID NO:64 : --QPLYGQCGGLNWPPESPTECVPGARCSTINPYYAQC- :
SEQ ID NO:65 : PLQSKWGQCGGVGYT--GASVCSPTATCSTLNPYYAQCL :
SEQ ID NO:63 : PTQKMYGQCGGVAYM--GPTQCPAYATCSTVNPYYAQC- :
SEQ ID NO:56 : GSQTVYGQCGGTGWT--GPTACVASATCTTLNPYYAQCL :
SEQ ID NO:60 : QTQTVWGQCGGQGYS--GPTNCASGSACSTLNPYYAQCI :
SEQ ID NO:67 : ATQTHYGQCGGMSYT--GPTVCASPYTCQVQNPYYSQCL :
SEQ ID NO:59 : PTQTHYGQCGGIGYS--GPTQCVSGTTCQVLNPYYSQCL :
SEQ ID NO:62 : PTQSHYGQCGGIGYS--GPTVCASGTTCQVLNPYYSQCL :
SEQ ID NO:57 : PTQTHYGQCGGIGYS--GPTVCASGTTCQVLNPYYSQCL :
SEQ ID NO:58 : PTQTHYGQCGGIGYS--GPTVCASGSTCQVLNPYYSQCL :
SEQ ID NO:54 : GTQSLYGQCGGTGWA--GPTACAPPATCKVLNQYYSQCL :
SEQ ID NO:66 : ATQTHYGQCGGTGWT--GPTRCASGFTCQVLNPFYSQCL :
SEQ ID NO:55 : ATQTLYGQCGGSGWT--GPTACASGATCKVLNPYYSQCL :
SEQ ID NO:61 : ATQTLYGQCGGSGWT--GPTACASGATCKVLNSYYSQCL :
SEQ ID NO:53 : GVQSEYGQCGGSGYS--GPTACAAPYACSTLNPYYAQCL :
SEQ ID NO:52 : PSQTLYGQCGGSGYS--GPTICASPAVCSTLNPYYAQCL :
```

FIG. 3