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(71) Applicants (for all designated States except US):
DANISCO US INC. [US/US]; 925 Page Mill Road, Palo Alto, California 94304 (US). **ESTELL, David A.** [US/US]; DANISCO US INC., 925 Page Mill Road, Palo Alto, California 94304 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **CONCAR, Edward M.** [US/US]; DANISCO US INC., 925 Page Mill Road, Palo Alto, California 94304 (US). **ADAMS, Christian D.** [US/US]; DANISCO US INC., 925 Page Mill Road, Palo Alto, California 94304 (US). **JONES, Brian E.** [GB/US]; DANISCO US INC., 925 Page Mill Road, Palo Alto, California 94304 (US). **KOLKMAN, Marc** [NL/US]; DANISCO US INC., 925 Page Mill Road, Palo Alto, California 94304 (US).

(74) Agent: **TODD, Stephen**; DANISCO US INC., 925 Page Mill Road, Palo Alto, California 94304 (US).

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(54) Title: CLEANING SYSTEM COMPRISING AN ALPHA-AMYLASE AND A PROTEASE

(57) Abstract: Described are compositions and methods involving a Bacillus alpha-amylase and a protease. The compositions and methods are especially effective for cold water cleaning applications.

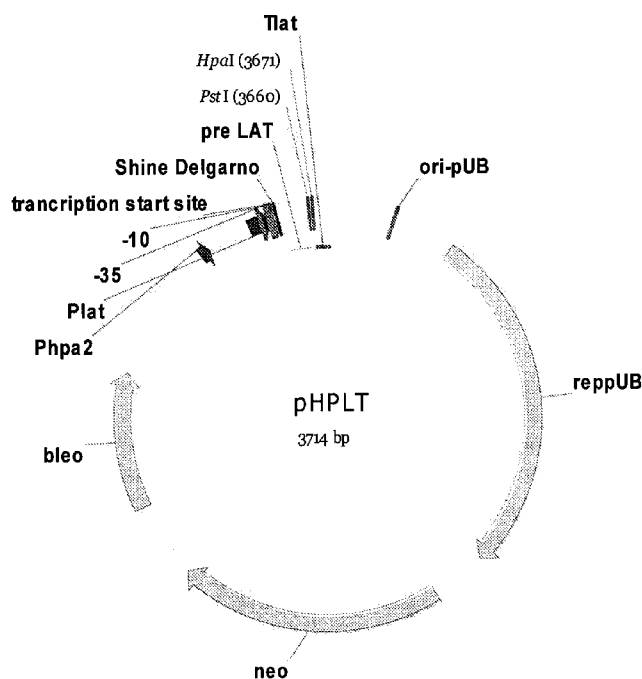


Figure 2



SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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CLEANING SYSTEM COMPRISING AN ALPHA-AMYLASE AND A PROTEASE

PRIORITY

5 [0001] The present application claims priority to U.S. Provisional Application Serial No. 61/165,813, filed on April 1, 2009, which is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

10 [0002] Described are compositions and methods involving a *Bacillus* alpha-amylase and a protease. The compositions and methods are especially effective for cold water cleaning applications.

BACKGROUND

15 [0003] Starch is of a mixture of amylose (15-30% w/w) and amylopectin (70-85% w/w). Amylose consists of linear chains of α -1,4-linked glucose units having a molecular weight (MW) from about 60,000 to about 800,000. Amylopectin is a branched polymer containing α -1,6 branch points every 24-30 glucose units. Its MW may be as high as 100 million.

[0004] Sugars from starch, in the form of concentrated dextrose syrups, are currently produced by an enzyme catalyzed process involving: (1) liquefaction (or thinning) of solid starch with an α -amylase into dextrins having an average degree of polymerization of about 7-10; and (2) saccharification of the resulting
20 liquefied starch (*i.e.*, starch hydrolysate) with amyloglucosidase (also called glucoamylase or GA). The resulting syrup has a high glucose content. Much of the glucose syrup, which is commercially produced, is subsequently enzymatically isomerized to a dextrose/fructose mixture known as isosyrup.

[0005] Alpha (α)-Amylases (α -1,4-glucan-4-glucanohydrolases, E.C. 3.2.1.1) are a group of enzymes that hydrolyze starch, glycogen, and related polysaccharides by cleaving internal α -1,4-glucosidic bonds at
25 random. This enzyme class has a number of important commercial applications in, for example, in the initial stages (liquefaction) of starch processing, in textile desizing, in deinking of recycled paper, in starch modification in the paper and pulp industry, in wet corn milling, in alcohol production, in sweetener (*e.g.*, sugar) manufacture, in the beverage industry, in brewing, in oilfields, in animal feed, and as cleaning agents in detergent matrices. For example, such enzymes can be used to remove starchy stains during dishwashing and
30 laundry washing.

[0006] α -amylases are isolated from a wide variety of bacterial, fungal, plant and animal sources. Industrially, many important α -amylases are those isolated from Bacilli. One characterized α -amylase is that of an alkaliphilic *Bacillus sp.* strain TS-23 which produces at least five kinds of enzymes exhibiting starch hydrolyzing activity. (*Lin et al., Biotechnol Appl Biochem*, 28:61-68, 1998). The α -amylase of *Bacillus sp.* no.
35 TS-23 has a pH optimum of 9 although it is stable over a broad pH range (*i.e.*, pH 4.7 to 10.8). Its temperature optimum is 45°C, although the enzyme has activity at lower temperatures, *e.g.*, 15-20°C.

[0007] There remains a need for variant amylases (*e.g.*, α -amylases) that possess altered biochemical characteristics and offer improved performance in industrial applications.

SUMMARY

5 [0008] Described are compositions, systems, and methods relating to cleaning compositions comprising, consisting of, or consisting essentially of a *Bacillus* alpha-amylases and a protease.

[0009] In one aspect, a cleaning composition is provided, comprising: (a) an alpha-amylase having an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 2; and (b) a protease; wherein the composition provides a level of cleaning greater than that observed for corresponding
10 compositions lacking the alpha amylase or lacking the protease. In some embodiments, the system is a cold-water cleaning system.

[0010] In some embodiments, the at least one additional enzyme is a subtilisin. In some embodiments, the at least one additional enzyme is subtilisin BPN' or a variant, thereof. In some embodiments, the at least one additional enzyme is the Y217L variant of subtilisin BPN'.

15 [0011] In some embodiments, the cleaning composition further comprising at least one surfactant. In some embodiments, the cleaning composition further comprising at least one additional enzyme selected from the group consisting of a lipase, a cutinase, a carbohydrase, a cellulase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, an oxidase, a perhydrolase, a pectate lyase, and a peroxidase.

[0012] In some embodiments, the alpha-amylase is derived from a parent alpha-amylase selected from the
20 group consisting of BASE, ACE, ACE-Q, and ACE-QK.

[0013] In some embodiments, the alpha-amylase is a mature form of a variant alpha-amylase having amylase activity, and comprising a substitution at one or more positions selected from the group consisting of 1, 2, 3, 4, 5, 7, 15, 16, 17, 18, 19, 22, 25, 26, 28, 29, 30, 32, 35, 36, 37, 50, 51, 52, 53, 54, 55, 56, 59, 60, 70, 71, 72, 73, 75, 78, 83, 87, 90, 91, 93, 94, 95, 104, 105, 107, 108, 110, 112, 113, 116, 118, 125, 126, 128, 129,
25 130, 131, 134, 136, 138, 142, 144, 147, 149, 150, 152, 154, 156, 158, 160, 161, 162, 165, 166, 168, 169, 170, 172, 174, 177, 178, 182, 183, 185, 189, 192, 195, 197, 201, 202, 203, 207, 210, 214, 217, 221, 228, 234, 236, 237, 246, 250, 254, 255, 257, 264, 267, 269, 270, 272, 275, 279, 283, 284, 298, 301, 303, 305, 306, 310, 311, 314, 318, 319, 320, 322, 323, 336, 337, 338, 339, 340, 344, 359, 374, 375, 376, 377, 379, 381, 382, 393, 394, 399, 401, 407, 408, 419, 433, 436, 438, 444, 447, 448, 451, 453, 459, 465, 470, 475, 476, 483, and 484;
30 wherein the positions correspond to amino acid residues in the amino acid sequence set forth in SEQ ID NO: 2; and wherein the substitution of the naturally-occurring amino acid residue at the one or more positions for a different amino acid residue produces an alpha-amylase variant having a performance index > 1.0 for a measure of stability, and a performance index >1.0 for a measure of activity.

[0014] In some embodiments, the variant alpha-amylase comprises a substitution at one or more positions
35 selected from the group consisting of 7, 29, 35, 53, 60, 72, 87, 108, 116, 126, 128, 129, 130, 131, 134, 136, 138, 142, 156, 161, 165, 178, 182, 185, 189, 192, 195, 197, 202, 210, 214, 217, 221, 234, 246, 269, 303, 310, 337, 340, 374, 401, and 438, and wherein the substitution of the naturally-occurring amino acid residue for a

different amino acid residue, produces an alpha-amylase variant having a performance index > 1.5 for a measure of activity and a performance index > 1.0 for a measure of stability.

[0015] In some embodiments, the variant alpha-amylase comprises a substitution at one or more positions selected from the group consisting of 2, 7, 22, 25, 28, 30, 37, 70, 75, 83, 87, 91, 93, 108, 128, 160, 165, 178, 182, 183, 217, 269, 270, 279, 283, 298, 305, 306, 310, 320, 374, 375, 376, 407, 419, 475, and 476, wherein the substitution of the naturally-occurring amino acid residue for a different amino acid residue, produces an alpha-amylase variant having a performance index > 1.5 for a measure of stability and a performance index > 1.0 for a measure of activity.

[0016] In some embodiments, the variant alpha-amylase comprises a substitution at one or more positions selected from the group consisting of 83, 125, 128, 131, 160, 178, 182, 183, 185, 189, 279, 305, 319, 320, 379, 407, 433, 453, 475, 476, and 483.

[0017] In some embodiments, the alpha-amylase is a mature form of a variant alpha-amylase comprising a substitution at one or more positions selected from the group consisting of 83, 125, 128, 131, 160, 178, 182, 183, 185, 189, 279, 305, 319, 320, 379, 407, 433, 453, 475, 476, and 483, wherein the positions correspond to amino acid residues in the amino acid sequence set forth in SEQ ID NO: 2, and wherein the substitution provides at least one beneficial effect selected from the group consisting of improved cleaning performance, improved detergent stability, improved thermostability, and improved protein expression.

[0018] In some embodiments, the alpha-amylase is a mature form of a variant alpha-amylase comprising a substitution at one or more positions selected from the group consisting of 5, 32, 83, 95, 154, 214, 221, 228, 322, 401, 407, 419, 444, 447, 459, 470, 483, and 484; wherein the positions correspond to amino acid residues in the amino acid sequence set forth in SEQ ID NO: 2; and wherein the substitution of the naturally-occurring amino acid residue for a different amino acid residue, produces an alpha-amylase variant having a performance index value of 0.5 or better for activity at pH 8, activity at pH 10, activity at 16°C, and activity at 32°C, and a performance index value of 0.5 or better for stability in detergent and for thermostability.

[0019] In some embodiments, the alpha-amylase variant further comprises a substitution at position 243 corresponding to the amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the alpha-amylase variant further comprises a deletion at position 180 and/or position 181, corresponding to the amino acid sequence set forth in SEQ ID NO: 2.

[0020] In some embodiments, the alpha-amylase variant is derived from a parent alpha-amylase having an amino acid sequence that is at least 75% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14. In some embodiments, the alpha-amylase variant has at least 75% sequence identity with the amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the alpha-amylase variant has at least 80% sequence identity with the amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the alpha-amylase variant has at least 90% sequence identity with the amino acid sequence set forth in SEQ ID NO: 2.

[0021] In some embodiments, the alpha-amylase variant comprises a substitution at one or more positions selected from the group consisting of 128, 178, 182, 185, and 189 corresponding to the amino acid sequence set forth in SEQ ID: 2, wherein the substitution provides improved cleaning performance or improved detergent stability.

5 [0022] In some embodiments, the alpha-amylase variant comprises:

- (a) an alanine at position 125,
a cysteine at position 128,
an isoleucine at position 131,
an isoleucine at position 165,
10 a leucine at position 178,
a glycine at position 182,
a tyrosine at position 202,
an arginine at position 305,
a threonine at position 319, or
15 an arginine at position 475;
- (b) the substitutions N128C+K178L+T182G+Y305R+G475K, and
at least one additional substitution selected from the group consisting of S125A, T131I, T165I,
F202Y, and D319T; or

(c) the substitutions

20 N128C+K178L+T182G+F202Y+Y305R+D319T+G475K,
S125A+N128C+K178L+T182G+Y305R+G475K, or
S125A+N128C+T131I+T165I+K178L+T182G+Y305R+G475K;

wherein the variant optionally further comprises a substitution at position 243 and/or a deletion at position 180 and/or position 181; and

25 wherein the positions correspond to the amino acid sequence set forth in SEQ ID NO: 2.

[0023] In some embodiments, the alpha-amylase variant comprises a substitution at position 475. In some embodiments, the alpha-amylase variant comprises a substitution at position 243 and/or a deletion at position 180 and/or position 181. In some embodiments, the alpha-amylase variant comprises a substitution at position 243 and/or a deletion at position 180 and/or position 181.

30 [0024] In some embodiments, the cleaning composition provides a level of cleaning greater than that observed for corresponding compositions lacking the alpha amylase or lacking the protease, as measured at a temperature below about 25°C. In some embodiments, the cleaning composition provides a level of cleaning greater than that observed for corresponding compositions lacking the alpha amylase or lacking the protease, as measured at a temperature below about 20°C.

35 [0025] In some embodiments, the alpha-amylase and protease are co-formulated.

[0026] In another aspect, a method of cleaning a fabric or hard surface is provided, comprising contacting the fabric or hard surface with any of the described cleaning compositions. In some embodiments, the method

is performed at a temperature below about 25°C. In some embodiments, the method is performed at a temperature below about 20°C.

[0027] In related embodiments, the alpha-amylase variant is a mature form having amylase activity and comprising a substitution at one or more (preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) positions selected from the group consisting of: 1, 2, 3, 4, 5, 7, 15, 16, 17, 18, 19, 22, 25, 26, 28, 29, 30, 32, 35, 36, 37, 50, 51, 52, 53, 54, 55, 56, 57, 59, 60, 70, 71, 72, 73, 75, 78, 82, 83, 87, 90, 91, 93, 94, 95, 103, 104, 105, 107, 108, 110, 112, 113, 114, 115, 116, 118, 121, 123, 125, 126, 127, 128, 129, 130, 131, 132, 134, 135, 136, 138, 140, 142, 144, 147, 149, 150, 152, 154, 156, 158, 159, 160, 161, 162, 164, 165, 166, 167, 168, 169, 170, 171, 172, 174, 175, 176, 177, 178, 179, 182, 183, 185, 186, 188, 189, 190, 191, 192, 193, 195, 197, 199, 200, 201, 202, 203, 207, 210, 214, 217, 221, 228, 234, 237, 238, 239, 240, 243, 246, 250, 254, 255, 257, 264, 266, 267, 268, 269, 270, 272, 273, 275, 279, 283, 284, 298, 301, 303, 305, 306, 310, 311, 314, 318, 319, 320, 322, 323, 336, 337, 338, 339, 340, 344, 359, 374, 375, 376, 377, 379, 381, 382, 393, 394, 399, 401, 407, 408, 419, 433, 436, 438, 444, 447, 448, 451, 453, 459, 465, 479, 475, 483, and 484, and wherein the positions are numbered by correspondence with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO:2. In one embodiment, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK. In another embodiment, the alpha-amylase variant is derived from a parent alpha-amylase with the amino acid sequence at least 75% (preferably 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%) identical to a member of the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14. In a preferred embodiment, the positions are selected from the group consisting of: 1, 2, 3, 4, 5, 7, 16, 17, 18, 19, 22, 25, 26, 28, 29, 30, 32, 35, 36, 37, 57, 60, 70, 71, 72, 73, 75, 78, 82, 83, 87, 90, 91, 93, 94, 95, 103, 104, 105, 108, 112, 114, 115, 116, 118, 121, 123, 125, 126, 128, 129, 130, 131, 132, 134, 135, 136, 138, 140, 142, 144, 147, 149, 150, 152, 154, 156, 158, 159, 160, 161, 162, 164, 165, 166, 167, 168, 169, 171, 172, 174, 175, 176, 177, 178, 179, 182, 183, 185, 186, 189, 190, 191, 192, 193, 195, 197, 199, 202, 207, 214, 217, 221, 228, 234, 237, 238, 243, 246, 250, 254, 255, 257, 264, 266, 267, 268, 269, 270, 272, 273, 275, 279, 283, 284, 298, 301, 303, 305, 306, 310, 311, 318, 319, 320, 322, 323, 336, 337, 338, 339, 340, 344, 359, 374, 375, 376, 377, 379, 381, 382, 393, 394, 399, 401, 407, 408, 419, 433, 436, 438, 447, 451, 453, 459, 465, 479, 475, and 483, and wherein the positions are numbered by correspondence with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO:2. In yet another embodiment, the positions are selected from the group consisting of: 1, 2, 3, 4, 5, 7, 16, 17, 18, 19, 22, 25, 26, 28, 29, 30, 32, 35, 36, 37, 57, 60, 70, 71, 72, 73, 75, 78, 82, 83, 90, 91, 93, 94, 95, 103, 104, 105, 108, 112, 114, 115, 116, 118, 121, 123, 125, 126, 128, 129, 130, 131, 132, 134, 135, 136, 138, 140, 142, 144, 147, 149, 150, 152, 154, 156, 158, 159, 160, 161, 162, 164, 165, 166, 167, 168, 169, 171, 172, 174, 175, 176, 177, 178, 179, 185, 186, 189, 190, 191, 192, 193, 195, 197, 199, 202, 207, 214, 217, 221, 228, 234, 237, 238, 246, 250, 254, 255, 257, 264, 266, 267, 268, 269, 270, 273, 275, 279, 283, 284, 298, 301, 303, 305, 306, 310, 311, 318, 319, 322, 323, 336, 337, 338, 339, 340, 344, 374, 375, 376, 377, 379, 381, 382, 393, 394, 399, 401, 407, 408, 419, 433, 436, 438, 447, 451, 453, 459, 465, 479, 475, and 483, and wherein the positions

are numbered by correspondence with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO:2. In a preferred embodiment, the alpha-amylase variant comprises a tyrosine at position 58 and an alanine at position 236, and wherein the positions are numbered by correspondence with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO:2. In another embodiment, the alpha-amylase variant comprises a glutamine at position 243 and a lysine at position 475, and wherein the positions are numbered by correspondence with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO:2.

[0028] In some embodiments, the variant is a mature form having amylase activity and comprising a substitution at from one to eight (*e.g.*, 1, 2, 3, 4, 5, 6, 7, or 8) positions selected from the group consisting of: 182, 183, 305, 320, 379, 407, 419 and 475, and wherein the positions are numbered by correspondence with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO:2. In another embodiment, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK. In another embodiment, the alpha-amylase variant is derived from a parent alpha-amylase with the amino acid sequence at least 75% (preferably 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%) identical to any member of the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14. In a preferred embodiment, the substitution comprises from one to eight of the group consisting of: X182N, X183N, X305Q, X320F, X379A, X407D, X419S and X475T. In a subset of these embodiments, the substitution comprises from one to eight (*e.g.*, 1, 2, 3, 4, 5, 6, 7, or 8) of the group consisting of: T182N, G183N, Y305Q, Q320F, P379A, Q407D, T419S and G475T.

[0029] In some embodiments, the variant is a mature form having amylase activity and comprising a substitution at from one to seven (*e.g.*, 1, 2, 3, 4, 5, 6, or 7) positions selected from the group consisting of: 160, 182, 183, 189, 305, 379, and 475, and wherein the positions are numbered by correspondence with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO:2. In another embodiment, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK. In a preferred embodiment, the alpha-amylase variant is derived from a parent alpha-amylase with the amino acid sequence at least 75% (preferably 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%) identical to any member of the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14. In another preferred embodiment, the substitution comprises from one to seven of the group consisting of: X160E, X182G, X183N, X189P, X305G, X379E, and X475T. In a subset of these embodiments, the substitution comprises from one to seven (*e.g.*, 1, 2, 3, 4, 5, 6, or 7) of the group consisting of: Y160E, T182G, G183N, E189P, Y305G, P379E, and G475T.

[0030] In some embodiments, the variant is a mature form having amylase activity and comprising a substitution at from one to eight (*e.g.*, 1, 2, 3, 4, 5, 6, 7, or 8) positions selected from the group consisting of: 125, 182, 214, 279, 305, 319, 320, and 475, and wherein the positions are numbered by correspondence with

the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO:2. In another embodiment, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK. In a preferred embodiment, the alpha-amylase variant is derived from a parent alpha-amylase with the amino acid sequence at least 75% (preferably 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%) identical to any member of the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14. In another preferred embodiment, the substitution comprises from one to eight of the group consisting of: X125A, X182A, X214Q, X279N, X305R, X319T, X320N, and X475R. In a subset of these embodiments, the substitution comprises from one to eight (*e.g.*, 1, 2, 3, 4, 5, 6, 7, or 8) of the group consisting of: S125A, T182A, T214Q, T279N, Y305R, D319T, Q320N, and G475R.

[0031] In some embodiments, the variant is a mature form having amylase activity and comprising a substitution at from one to eight (*e.g.*, 1, 2, 3, 4, 5, 6, 7, or 8) positions selected from the group consisting of: 7, 182, 298, 376, 379, 407, 419, and 453, and wherein the positions are numbered by correspondence with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO:2. In another embodiment, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK. In a preferred embodiment, the alpha-amylase variant is derived from a parent alpha-amylase with the amino acid sequence at least 75% (preferably 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%) identical to any member of the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14. In another preferred embodiment, the substitution comprises from one to eight of the group consisting of: X7H, X182W, X298Q, X376R, X379K, X407W, X419S, and X453W. In a subset of these embodiments, the substitution comprises from one to eight (*e.g.*, 1, 2, 3, 4, 5, 6, 7, or 8) of the group consisting of: E7H, T182W, T298Q, Y376R, P379K, Q407W, T419S, and L453W.

[0032] In some embodiments, the variant is a mature form having amylase activity and comprising a substitution at from one to four (*e.g.*, 1, 2, 3, or 4) positions selected from the group consisting of: 128, 178, 182 and 185, and the alpha-amylase variant comprises a serine or a glutamine at position 243, and wherein the positions are numbered by correspondence with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO:2. In one embodiment, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK. In a preferred embodiment, the alpha-amylase variant is derived from a parent alpha-amylase with the amino acid sequence at least 75% (preferably 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%) identical to any member of the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14. In yet another embodiment, the substitution comprises from one to four (*e.g.*, 1, 2, 3, or 4) of the group consisting of: N128C, K178L, T182G, and A185D.

[0033] In some embodiments, the variant is a mature form having amylase activity and comprising a substitution at from one to nine (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8 or 9) positions selected from the group consisting of: 125, 182, 183, 189, 279, 305, 319, 379 and 475, and the alpha-amylase variant comprises a glutamine, a phenylalanine or an asparagine at position 320, and wherein the positions are numbered by correspondence with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO:2. In one embodiment, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK. In a preferred embodiment, the alpha-amylase variant is derived from a parent alpha-amylase with the amino acid sequence at least 75% (preferably 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%) identical to any member of the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14. In another embodiment, the alpha-amylase variant comprises: a serine or alanine at position 125; a threonine, an asparagine, a glycine or an alanine at position 182; a glycine or an asparagine at position 183; a glutamic acid or a proline at position 189; a threonine or an asparagine at position 279; a tyrosine, a glutamine, a glycine or an arginine at position 305; an aspartic acid or a threonine at position 319; a proline or an alanine at position 379; and a glycine, threonine or an arginine at position 475; and wherein the positions are numbered by correspondence with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO:2.

[0034] In some embodiments, the variant is a mature form having amylase activity and comprising a substitution at from one to eleven (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11) positions selected from the group consisting of: 125, 128, 178, 182, 183, 189, 279, 305, 319, 379 and 475, and the alpha-amylase variant comprises a serine or a glutamine at position 243, and a glutamine, a phenylalanine or an asparagine at position 320, and wherein the positions are numbered by correspondence with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO:2. In one embodiment, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK. In a preferred embodiment, the alpha-amylase variant is derived from a parent alpha-amylase with the amino acid sequence at least 75% (preferably 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%) identical to any member of the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14. In another embodiment, the alpha-amylase variant comprises: a serine or alanine at position 125; an asparagine or a cysteine at position 128; a lysine or a leucine at position 178; a threonine, an asparagine, a glycine or an alanine at position 182; a glycine or an asparagine at position 183; a glutamic acid or a proline at position 189; a threonine or an asparagine at position 279; a tyrosine, a glutamine, a glycine or an arginine at position 305; an aspartic acid or a threonine at position 319; a proline or an alanine at position 379; and a glycine, threonine or an arginine at position 475; and wherein the positions are numbered by correspondence with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO:2. In another preferred embodiment, the substitution is selected from the group consisting of: N128C, T131I,

T134P, Q138E, Y160L, T165I, T165V, K178L, T182A, T182C, T182D, T182M, T182F, T182N, T182G, T182P, T182Q, A185D, A185E, E189P, S243D, S243E and S243Q.

[0035] The presently described amino acid mutations were made and tested using an exemplary alpha-amylase as a starting point, *i.e.*, a “backbone;” however, it will be appreciated that equivalent amino acid mutations can be made in related alpha-amylases, where they are expected to produce a similar effect and yield similar advantages. Other exemplary alpha-amylases for use as a backbone include but are not limited to those identified, herein.

[0036] The disclosure further provides an isolated nucleic acid encoding an alpha-amylase variant of any of the preceding paragraphs. In one embodiment, an expression vector comprising the isolated nucleic acid in operable combination with a promoter is included. In another embodiment, a host cell comprising the expression vector is included. Another embodiment provides a method for producing an alpha-amylase variant, comprising: transforming a host cell with an expression vector comprising a nucleic acid encoding the alpha-amylase variant; and cultivating the transformed host cell under conditions suitable for the production of the alpha-amylase variant. Another embodiment further comprises the step of harvesting the produced alpha-amylase variant. Yet another embodiment includes as the host cell a *Bacillus* species, and in yet another embodiment the *Bacillus* species is *B. subtilis*.

[0037] In some embodiments, the cleaning composition comprises an alpha-amylase, a protease, at least one additional enzyme, which may be an additional amylase or additional protease. In one embodiment, the additional enzyme is a neutral metalloprotease, an aspartyl protease, a lipase, a cutinase, a carbohydrase, a cellulase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, an oxidase, a perhydrolase, a pectate lyase, and/or a peroxidase. In another embodiment, the cleaning composition is a laundry detergent, and in yet another embodiment, the cleaning composition is a dishwashing detergent. In another embodiment, the cleaning composition is a laundry and/or dish detergent with a bleaching agent. In another embodiment, the cleaning composition is a pretreatment for fabrics, *e.g.*, for application prior to washing. In another embodiment, the cleaning composition is a laundry detergent or dishwashing detergent additive. The disclosure further provides a method of cleaning, comprising the step of contacting a surface and/or an article comprising a fabric with a cleaning composition comprising the alpha-amylase variant. In one embodiment, the method is a dishwashing method, comprising the steps of: providing i) the dishwashing composition, and ii) dishware in need of cleaning; and contacting the dishware with the dishwashing composition under conditions effective to provide cleaning of the dishware. In another embodiment, the method is a fabric cleaning method, comprising the steps of: providing i) the fabric cleaning composition, and ii) laundry in need of cleaning; and contacting the laundry with the fabric cleaning composition under conditions effective to provide cleaning of the laundry. In another embodiment, the method involves removing material from the yarns in woven fabrics, as in the case of textile desizing.

[0038] These and other aspects and embodiments of the compositions and methods will be apparent in view of the description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] Figure 1 provides an alignment of the mature form of various reference amylases set forth as SEQ ID NOS: 2 and 5-14 respectively. Sequences were aligned using the MUSCLE 3.7 multiple sequence alignment algorithm (Edgar, *Nucleic Acids Research*, 32:1792-1797, 2004).

5 [0040] Figure 2 provides a map of the pHPLT vector containing the *B. licheniformis* LAT promoter (Plat) and additional elements from pUB110 (McKenzie *et al.*, *Plasmid*, 15: 93-103, 1986) including a replicase gene (reppUB), a neomycin/kanamycin resistance gene (neo) and a bleomycin resistance marker (bleo).

[0041] Figure 3 provides a map of the pHPLT-BASE plasmid.

[0042] Figure 4 provides a map of the pHPLT-ACE-S243Q plasmid.

10 [0043] Figure 5 provides a graph showing the wash performance of multiple alpha-amylases in a laundry detergent application. The enzymes tested were as follows: W9 (BASE-S125A-N128C-K178L-T182G-S243Q-T279N-D319T-Q320N-G475R); W10 (BASE-N128C-K178L-T182G-S243Q-Y305R-D319T-G475R; W11 (BASE-S125A-N128C-K178L-T182G-S243Q-Y305R-G475R; and ACE-S243Q-G475K.

15 [0044] Figure 6 provides a graph showing the wash performance of multiple alpha-amylases in a laundry detergent application. The enzymes tested were as follows: BASE-X8C, BASE-W10EK, ACE-QK and control (benchmark commercial enzyme).

[0045] Figure 7A provides a graph showing the synergy between ACE alpha-amylase and the BPN^Y217L subtilisin protease in laundry applications. Figure 7B provides a graph showing the synergy between the ACE-S243Q-G475K (ACE-QK) alpha-amylase and the BPN^Y217L subtilisin protease in a laundry
20 applications.

[0046] Figure 8 provides a graph showing the synergy between BASE-X8C (W11-T131I-T165I) alpha-amylase and the BPN^Y217L subtilisin protease in laundry applications.

[0047] Figure 9 provides a graph showing the synergy between BASE W10EK (BASE- N128C-K178L-T182G-S243E-Y305R-D319T-G475K) alpha-amylase and the BPN^Y217L subtilisin protease in laundry
25 applications.

[0048] Figure 10 provides a graph showing the synergy between ACE-S243Q-G475K (ACE-QK) alpha-amylase and the BPN^Y217L subtilisin protease in laundry applications.

DETAILED DESCRIPTION

30 [0049] Described are compositions and methods involving a *Bacillus* alpha-amylase and a protease. The compositions and methods are especially effective for cold water cleaning applications, such as dishwashing (including automatic dishwashing) and laundry. In some case, the compositions and methods involve a variant alpha-amylase. These and other features of the cleaning compositions and methods, and variants, are described in detail.

35 1. **Definitions and Nomenclature for α -Amylase Variants**

[0050] 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. Singleton, *et al.*, DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY, 2D ED., John Wiley and Sons, New York (1994) and Hale & Markham, THE HARPER COLLINS DICTIONARY OF BIOLOGY, Harper Perennial, NY (1991) provide one of skill with general dictionaries of many of the terms used herein.

[0051] Some aspects of the compositions and methods rely on routine techniques and methods used in the field of genetic engineering and molecular biology. The following resources include descriptions of general methodology useful in accordance with the present compositions and methods: Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL (2nd Ed., 1989); Kreigler, GENE TRANSFER AND EXPRESSION; A LABORATORY MANUAL (1990) and Ausubel *et al.*, Eds. CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (1994). These general references provide definitions and methods known to those in the art. However, it is not intended that the present compositions and methods be limited to any particular techniques, protocols, and reagents described, as these may vary. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present compositions and methods, the preferred methods and materials are described.

[0052] When describing proteins and genes that encode them, the name of the gene is generally italicized and not capitalized, while the name of the protein is generally not italicized and the first letter is capitalized.

[0053] The singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an enzyme" includes a plurality of such enzymes and reference to "the formulation" includes reference to one or more formulations and equivalents thereof known to those skilled in the art, and so forth.

[0054] All patents and publications referred to herein, including all sequences disclosed within such patents and publications are expressly incorporated by reference.

1.1 Definitions

[0055] The following terms are defined for clarity.

[0056] As used herein the term "starch" refers to any material comprised of the complex polysaccharide carbohydrates of plants, comprised of amylose and amylopectin with the formula $(C_6H_{10}O_5)_x$, wherein X can be any number. In particular, the term refers to any plant-based material including but not limited to grains, grasses, tubers and roots and more specifically wheat, barley, corn, rye, rice, sorghum, brans, cassava, millet, potato, sweet potato, and tapioca.

[0057] As used herein, an "amylase" refers to an enzyme capable of catalyzing the degradation of starch. Generally, α -amylases (EC 3.2.1.1; α -D-(1 $\rightarrow$ 4)-glucan glucanohydrolase) are endo-acting enzymes that cleave α -D-(1 $\rightarrow$ 4) O-glycosidic linkages within the starch molecule in a random fashion. In contrast, the exo-acting amylolytic enzymes, such as β -amylases (EC 3.2.1.2; α -D-(1 $\rightarrow$ 4)-glucan maltohydrolase), and some product-specific amylases like maltogenic α -amylase (EC 3.2.1.133), cleave the starch molecule from the non-reducing end of the substrate. β -amylases, α -glucosidases (EC 3.2.1.20; α -D-glucoside glucohydrolase),

glucoamylases (EC 3.2.1.3; α -D-(1 $\rightarrow$ 4)-glucan glucohydrolase), and product-specific amylases can produce malto-oligosaccharides of a specific length from starch. As used herein, amylases include any/all amylases, including glucoamylases, α -amylases, β -amylases and wild-type α -amylases, such as those of *Bacillus* sp., e.g., *B. licheniformis* and *B. subtilis*, while α -amylases include the aforementioned subset of these enzymes.

5 [0058] As used herein, “*Bacillus* sp. strain TS-23 α -amylase,” and similar phrases, refer to an α -amylase derived from *Bacillus* sp. strain TS-23. The gene encoding the α -amylase can be the wild-type gene or a codon optimized polynucleotide that encodes the α -amylase. The mature α -amylase of *Bacillus* sp. strain TS-23 is (amino to carboxy orientation) (SEQ ID NO: 1):

10	NTAPINETMM QYFEWDL PND GTLWTKVKNE AANLSSLGIT ALWLPPAYKG	50
	TSQSDVGYGV YDLYDLGEFN QKGTIRTKYG TKTQYIQAIQ AAKAAGMQVY	100
	ADVVFNHKAG ADGTEFVDAV EVDPSNRNQE TSGTYQIQAW TKFDFPGRGN	150
	TYSSFKWRWY HFDGTDWDES RKLNRITYKFR STGKAWDWEV DTENGNYDYL	200
	MFADLDMDHP EVVTELKNWG TWYVNTTNID GFRLDVAKHI KYSFFPDWLT	250
	YVRNQGTGNL FAVGEFWSYD VNKLHNYITK TNGSMSLFDA PLHNNFYTAS	300
15	KSSGYFDMRY LLNNTLMKDQ PSLAVTLVDN HDTQPGQSLQ SWVEPWFKPL	350
	AYAFILTRQE GYPCVFYGDY YGIPKYNIPG LKSKIDPLLI ARRDYAYGTQ	400
	RDYIDHQDII GWTREGIDTK PNSGLAALIT DPGGGSKWMY VGKKHAGKVF	450
	YDLTGNRSDT VTINADGWGE FKVNGGSVSI WVAKTSNVTF TVNNATTTSG	500
	QNVYVVANIP ELGNWNTANA IKMNPSSYPT WKATIALPQG KAIEFKFIKK	550
20	DQAGNVIWES TSNRTYTVPF SSTGSYTASW NVP	583

[0059] As used herein, “ α -amylase variants,” and similar phrases, refer to variants/mutants of a reference α -amylase, which includes an amino acid substitution, insertion, and/or deletion with respect to the parent (wild-type; reference) amino acid sequence of the reference α -amylase. The term “variant” is used interchangeably with the term “mutant.” The variant α -amylase may include mutations in the signal sequence with respect to parent signal sequence. In addition, the variant α -amylase can be in the form of a fusion protein containing a heterologous α -amylase signal sequence, such as from *B. licheniformis* (LAT).

[0060] As used herein, the phrases “parent *Bacillus* sp. strain TS-23 α -amylase,” “wild-type *Bacillus* sp. strain TS-23 α -amylase,” “reference *Bacillus* sp. strain TS-23 α -amylase,” and similar phrases, refer to the polypeptide of *Bacillus* sp. strain TS-23. The term may be abbreviated “parent enzyme,” “wild-type enzyme,” “parent polypeptide,” reference polypeptide,” or the like, for convenience. The parent *Bacillus* sp. strain TS-23 α -amylase may include mutations in the signal sequence of the parent polypeptide. In addition, the parent *Bacillus* sp. strain TS-23 α -amylase can be in the form of a fusion protein containing a heterologous α -amylase signal sequence, such as from *B. licheniformis* (LAT).

[0061] As used herein, a “parent nucleic acid/polynucleotide,” “wild-type nucleic acid/polynucleotide,” or “reference nucleic acid/polynucleotide,” refers to a nucleic acid sequence encoding a parent polypeptide, and a nucleic acid complementary thereto.

[0062] As used herein, a “variant nucleic acid/polynucleotide” refers to a nucleic acid sequence encoding a variant polypeptide or a nucleic acid complementary thereto, or a polynucleotide sequence having at least one base substitution, insertion, or deletion with respect to a parent polynucleotide sequence or a nucleic acid complementary thereto. Where specified such nucleic acids may include those having a specified degree of

homology to a reference sequence, or that are capable of hybridizing to a reference sequence, for example, under stringent conditions [*e.g.*, 50°C and 0.2X SSC (1X SSC = 0.15 M NaCl, 0.015 M Na₃ citrate, pH 7.0)] or highly stringent conditions [*e.g.*, 65°C and 0.1X SSC (1X SSC = 0.15 M NaCl, 0.015 M Na₃ citrate, pH 7.0)]. A variant nucleic acid may be optimized to reflect preferred codon usage for a specified host

5 organisms, such as the methylotrophic yeasts (*e.g.*, *Pichia*, *Hansenula*, etc) or filamentous fungi (*e.g.*, *Trichoderma* (*e.g.*, *T. reesei*), etc) or other expression hosts (*e.g.*, *Bacillus*, *Streptomyces*, etc.).

[0063] As used herein, the term “recombinant,” when used in reference to a subject cell, nucleic acid, protein or vector, indicates that the subject has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, 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.

10 [0064] As used herein, the terms “recovered,” “isolated,” and “separated,” refer to a compound, protein, cell, nucleic acid or amino acid that is removed from at least one component with which it is naturally associated and found in nature.

[0065] As used herein, the term “purified” refers to material (*e.g.*, an isolated polypeptide or polynucleotide) that is in a relatively pure state, *e.g.*, at least about 90% pure, at least about 95% pure, at least about 98% pure, or even at least about 99% pure.

20 [0066] As used herein, the terms, “thermostable” and “thermostability” refer to the ability of an enzyme to retain activity after exposure to an elevated temperature. The thermostability of an enzyme, such as an α -amylase enzymes, is measured by its half-life ($t_{1/2}$) given in minutes, hours, or days, during which half the enzyme activity is lost under defined conditions. The half-life may be calculated by measuring residual α -amylase activity following exposure to (*i.e.*, challenge by) an elevated temperature.

25 [0067] As used herein, a “pH range” refers to the range of pH values under which an enzyme exhibits catalytic activity.

[0068] As used herein, the terms “pH stable” and “pH stability” relate to the ability of an enzyme to retain activity over a wide range of pH values for a predetermined period of time (*e.g.*, 15 min., 30 min., 1 hour, and the like).

30 [0069] As used herein, the term “amino acid sequence” is synonymous with the terms “polypeptide,” “protein,” and “peptide,” and are used interchangeably. Where such amino acid sequence exhibit activity, they may be referred to as an “enzyme.” The conventional one-letter or three-letter code for amino acid residues are used herein.

35 [0070] As used herein, the term “nucleic acid” encompasses DNA, RNA, heteroduplexes, and synthetic molecules capable of encoding a polypeptide. Nucleic acids may be single stranded or double stranded, and may be 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 which encode a particular amino

acid sequence. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively.

[0071] As used herein, the term "homologue" refers to an amino acid or nucleotide sequence having a certain degree of identity to a reference amino acid or nucleotide sequences, or another specified common structural or functional feature. A homologous sequence is taken to include an amino acid sequence that is at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or even 99% identical to the subject sequence, using conventional sequence alignment tools (*e.g.*, Clustal, BLAST, and the like). Typically, homologues will include the same active site residues as the subject amino acid sequence, unless otherwise specified.

[0072] As used herein, "hybridization" refers to the process by which one strand of nucleic acid base pairs with a complementary strand, as occurs during blot hybridization techniques and PCR techniques.

[0073] As used herein, a "synthetic" molecule is produced by *in vitro* chemical or enzymatic synthesis rather than by an organism.

[0074] As used herein, the terms "transformed," "stably transformed," and "transgenic," used with reference to a cell means that the cell has a non-native (*e.g.*, heterologous) nucleic acid sequence integrated into its genome or carried as an episomal plasmid that is maintained through multiple generations.

[0075] The term "introduced" in the context of inserting a nucleic acid sequence into a cell, means "transfection", "transformation" or "transduction," as known in the art.

[0076] As used herein, the terms "host strain" or "host cell" refer to an organism into which an expression vector, phage, virus, or other DNA construct including a polynucleotide encoding a polypeptide of interest (*e.g.*, a variant α -amylase) has been introduced. Exemplary host strains are bacterial cells. The term "host cell" includes protoplasts created from cells, such as those of a *Bacillus* sp.

[0077] As used herein, the term "heterologous" with reference to a polynucleotide or protein refers to a polynucleotide or protein that does not naturally occur in a host cell.

[0078] As used herein, the term, "endogenous" with reference to a polynucleotide or protein refers to a polynucleotide or protein that occurs naturally in the host cell.

[0079] 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 includes both transcription and translation.

[0080] As used herein, a "selective marker" or "selectable marker" refers to a gene capable of being expressed in a host to facilitate selection of host cells carrying the gene. Examples of selectable markers include but are not limited to antimicrobials (*e.g.*, hygromycin, bleomycin, or neomycin) and/or genes that confer a metabolic advantage, such as a nutritional advantage on the host cell.

[0081] As used herein, "culturing" refers to growing a population of microbial cells under suitable conditions in a liquid or solid medium. Culturing includes fermentative bioconversion of a starch substrate containing granular starch to an end-product (typically in a vessel or reactor).

[0082] As used herein, "fermentation" is the enzymatic breakdown of organic substances by microorganisms to produce simpler organic compounds. While fermentation generally occurs under anaerobic

conditions it is not intended that the term be solely limited to strict anaerobic conditions, as fermentation also occurs in the presence of oxygen.

[0083] As used herein, a “gene” refers to a DNA segment that is involved in producing a polypeptide, and includes coding regions, regions preceding and following the coding regions, and, intervening sequences (introns) between individual coding segments (exons).

[0084] As used herein, a “vector” refers to a polynucleotide sequence designed to introduce nucleic acids into one or more cell types. Vectors include cloning vectors, expression vectors, shuttle vectors, plasmids, phage particles, cassettes and the like.

[0085] As used herein, an “expression vector” refers to a DNA construct comprising a DNA sequence encoding a polypeptide of interest, which is operably linked to a suitable control sequence capable of effecting expression of the DNA in a suitable host. Such control sequences may include a promoter to effect transcription, an optional operator sequence to control transcription, a sequence encoding suitable ribosome binding sites on the mRNA, enhancers and sequences which control termination of transcription and translation.

[0086] As used herein, a “promoter” is a regulatory sequence that is involved in binding RNA polymerase to initiate transcription of a gene. The promoter may be an inducible promoter or a constitutive promoter. An exemplary promoter is the *Bacillus licheniformis* α -amylase (AmyL) promoter.

[0087] As used herein, the term “operably linked” means that specified components are in a relationship (including but not limited to juxtaposition) permitting them to function in an intended manner. For example, a regulatory sequence is operably linked to a coding sequence such that expression of the coding sequence is under control of the regulatory sequences.

[0088] As used herein, the term, “under transcriptional control” means that transcription of a polynucleotide sequence, usually a DNA sequence, depends on its being operably linked to an element which contributes to the initiation of, or promotes transcription.

[0089] As used herein, the term, “under translational control” means that translation of a polynucleotide sequence, usually an RNA sequence, into a polypeptide depends on its being operably linked to an element which contributes to the initiation of, or promotes translation.

[0090] As used herein, a “signal sequence” is a sequence of amino acids attached to the N-terminal portion of a protein, which facilitates the secretion of the protein outside the cell. The mature form of an extracellular protein lacks the signal sequence, which is cleaved off during the secretion process.

[0091] As used herein, “biologically active” refer to a sequence having a specified biological activity, such as an enzymatic activity. In the case of the present amylases, the activity is α -amylase activity.

[0092] As used herein, “water hardness” is a measure of the minerals (e.g., calcium and magnesium) present in water.

[0093] As used herein, the term, “cold water” refers to water at a temperature below about 25°C. Where specified, cold water refers to water at a temperature below about 20°C. Exemplary cold water ranges are from about 15°C to about 25°C and from about 15°C to about 20°C.

[0094] As used herein, the term “performance index (PI)” refers to the ratio of performance of a variant to a parent or reference amylase. Measures of performance (i.e., properties) include thermal stability, cleaning ability, expression levels, and the like, and will be apparent from context.

[0095] As used herein, mutations that improve performance are known as “up mutations,” and have a PI > 1 for a specified property. “Neutral mutations” have a PI > 0.5 for a specified property. “Non-deleterious mutations” have a PI > 0.05 for a specified property. “Deleterious mutations” have a PI ≤ 0.05 for a specified property. “Combinable mutations” have a PI ≥ 0.5 for at least one property, and >0.05 for all properties. Combinable mutations can be present together in the same variant to produce an enzyme having at least one beneficial property.

[0096] As used herein, the term “measure of activity” refers to a measure of enzymatic activity as described here. Such measures of activity include cleaning performance at pH 8, cleaning performance at pH 10, cleaning performance at 16°C, cleaning performance at 32°C, and activity using a synthetic substrate.

[0097] As used herein, the term “measure of stability” refers to a measure of enzymatic stability as described here. Such measures of stability include stability in detergents and thermostability.

[0098] As used herein, the term “co-formulation” means that subject ingredients, such as enzymes, are present together in the same liquid, semi-solid, or dry composition.

[0099] As used herein, “saccharification” refers to the enzymatic conversion of starch to glucose.

[0100] As used herein, “gelatinization” refers to solubilization of a starch molecule by cooking to form a viscous suspension.

[0101] As used herein, “liquefaction” refers to the stage in starch conversion in which gelatinized starch is hydrolyzed to give low molecular weight soluble dextrans.

[0102] As used herein, the term “primary liquefaction” refers to a step of liquefaction when the slurry's temperature is raised to or near its gelatinization temperature. Subsequent to the raising of the temperature, the slurry is sent through a heat exchanger or jet to temperatures from 200-300°F, e.g., 220-235°F.

Subsequent to application to a heat exchange or jet temperature, the slurry is held for a period of 3-10 minutes at that temperature. This step of holding the slurry at 200-300°F is primary liquefaction.

[0103] As used herein, the term “secondary liquefaction” refers the liquefaction step subsequent to primary liquefaction (heating to 200-300°F), when the slurry is allowed to cool to atmospheric temperature. This cooling step can be 30 minutes to 180 minutes (3 hours), e.g. 90 minutes to 120 minutes (2 hours).

[0104] As used herein, the term “minutes of secondary liquefaction” refers to the time that has elapsed from the start of secondary liquefaction, to the time that the DE is measured.

[0105] As used herein, the term “degree of polymerization (DP)” refers to the number (n) of anhydroglucopyranose units in a given saccharide. Examples of DP1 are the monosaccharides, such as glucose and fructose. Examples of DP2 are the disaccharides, such as maltose and sucrose. A DP>3 denotes polymers with a degree of polymerization of greater than 3.

[00106] As used herein with respect to starch conversion, the terms “end-product” or “desired end-product” refer to specified carbon-source-derived molecules, which are enzymatically converted from a starch substrate.

5 [00107] As used herein, the term “dry solids content (ds)” refers to the total solids in a slurry, expressed in % dry weight.

[00108] As used herein, the term “slurry” refers to an aqueous mixture containing insoluble solids.

[00109] As used herein, the term, “residual starch” refers to the remaining starch (soluble or insoluble) in a composition after fermentation or enzymatic hydrolysis of a starch containing substrate.

10 [00110] As used herein “a recycling step” refers to the recycling of mash components, which may include residual starch, enzymes and/or microorganisms to ferment substrates comprising starch.

[00111] As used herein, the term “mash” refers to an aqueous mixture including a fermentable carbon source (e.g., carbohydrate), which may be used to produce a fermented product, such as an alcohol. The terms “beer” and “mash” may be used interchangeability.

15 [00112] As used herein, the term “stillage” refers to a mixture of non-fermented solids and water, which represents the residue following removal of alcohol from a fermented mash.

[00113] As used herein, the terms “distillers dried grain (DDG)” and “distillers dried grain with solubles (DDGS)” refer to a useful by-product of grain fermentation.

20 [00114] As used herein “ethanologenic microorganism” refers to a microorganism with the ability to convert a sugar or oligosaccharide to ethanol. The ethanologenic microorganisms are ethanologenic by virtue of their ability to express one or more enzymes that individually or together convert sugar to ethanol.

[00115] As used herein the term “ethanol producer” or ethanol producing microorganism” refers to any organism or cell that is capable of producing ethanol from a hexose or pentose. Generally, ethanol-producing cells contain an alcohol dehydrogenase and a pyruvate decarboxylase. Examples of ethanol producing microorganisms include fungal microorganisms such as yeast. A preferred yeast includes strains of
25 *Sacchromyces*, particularly, *S. cerevisiae*.

[00116] As used herein with respect to amylase enzymes and their substrates, the term “contacting” refers to the placing of the enzyme in sufficiently close proximity to the substrate to enable the enzyme to convert the substrate to an end-product. Contacting may include mixing.

30 [00117] As used herein, the term “derived from” means “originated from,” “based on,” “obtained from,” “obtainable from,” or “isolated from,” depending on context.

[00118] As used herein, the term “enzymatic conversion” generally refers to the modification of a substrate (e.g., starch) by enzyme action (e.g., amylase).

35 [00119] As used herein, the term “disintegration” refers to the hydrolysis of polysaccharides in a biofilm matrix connecting and binding together individual microbial cells in the biofilm, whereby the microbial cells can be released and removed from the biofilm.

[00120] As used herein, a “swatch” is a piece of material, such as a fabric, to which a stain may be applied for evaluating the cleaing efficiency of a composition.

[00121] As used herein the term "specific activity" refers to the number of moles of substrate converted to product by an enzyme preparation per unit time under specific conditions. Specific activity is expressed as units (U)/mg of protein.

5 [00122] As used herein, the term "yield" refers to the amount of end-product produced by a process, *e.g.*, expressed in concentration, volume, amount, or a percentage of starting material.

[00123] As used herein, "ATCC" refers to American Type Culture Collection located at Manassas, Va. 20108 (ATCC).

10 [00124] As used herein, "NRRL" refers to the Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research (and previously known as USDA Northern Regional Research Laboratory), Peoria, IL.

[00125] Numeric ranges are inclusive of the numbers defining the range.

[00126] Generally, headings are descriptive and are not intended as limitations.

1.2 Nomenclature

15 [00127] In the present description and claims, the conventional one-letter and three-letter codes for amino acid residues are used. For ease of reference, α -amylase variants of the present compositions and methods are described by use of the following nomenclature:

Original amino acid(s); position(s); substituted amino acid(s).

20 [00128] According to this nomenclature, for instance the substitution of serine by an alanine in position 242 is shown as: Ser242Ala or S242A. A deletion of alanine in position 30 is shown as: Ala30* or A30* or Δ A30. An insertion of an additional amino acid residue, such as lysine, is shown as: Ala30AlaLys or A30AK.

[00129] A deletion of a consecutive stretch of amino acid residues, such as amino acid residues 30-33, is indicated as (30-33)* or Δ (A30-N33) or Δ 30-33. A deletion of two consecutive amino acids, such as amino acid residues R180-S181, is indicated as Δ RS or Δ 180-181.

25 [00130] Where a specific α -amylase contains a "deletion" in comparison with other α -amylases and an insertion is made in such a position this is indicated as: *36Asp or *36D for insertion of an aspartic acid in position 36.

30 [00131] Multiple mutations are separated by plus or minus signs: Ala30Asp+Glu34Ser or A30N+E34S, Ala30Asp-Glu34Ser or A30N-E34S, representing mutations in positions 30 and 34 substituting alanine and glutamic acid for asparagine and serine, respectively.

[00132] When one or more alternative amino acid residues may be substituted for a residue in a given position it is indicated as: A30N,E or A30N or A30E.

35 [00133] Furthermore, when a position suitable for modification is identified herein without any specific modification being suggested, it is to be understood that any amino acid residue may be substituted for the amino acid residue present in the position. Thus, for instance, when a modification of an alanine in position 30

is mentioned, but not specified, it is to be understood that the alanine may be deleted or substituted for any other amino acid, *i.e.*, any one of: R, N, D, A, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V.

[00134] Further, "A30X" means any one of the following substitutions: A30R, A30N, A30D, A30C, A30Q, A30E, A30G, A30H, A30I, A30L, A30K, A30M, A30F, A30P, A30S, A30T, A30W, A30Y, or A30 V; or in short: A30R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V.

[00135] The following nomenclature is used to indicated an amino acid residue at a position of an unspecified parent amylase, where the position is numbered by correspondence with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO: 2 : "X30N" or "X30N,V" in the case where for instance one of N or V is present in the variant amylase in position 30, while one of the twenty standard amino acids is present in the parent amylase (*e.g.*, wild type or variant enzyme)

1.3 Characteristics of Amino Acid Residues

[00136] Charged amino acids include: Asp, Glu, Arg, Lys, and His. Negatively charged amino acids (with the most negative residue first) are Asp and Glu. Positively charged amino acids (with the most positive residue first) are Arg, Lys, and His.

[00137] Neutral amino acids include: Gly, Ala, Val, Leu, Ie, Phe, Tyr, Trp, Met, Cys, Asn, Gln, Ser, Thr, and Pro.

[00138] Hydrophobic amino acid residues (with the most hydrophobic residue listed last) include: Gly, Ala, Val, Pro, Met, Leu, Ie, Tyr, Phe, and Trp.

[00139] Hydrophilic amino acids (with the most hydrophilic residue listed last) include: Thr, Ser, Cys, Gln, and Asn.

1.4 Homology (Identity)

[00140] A polynucleotide or a polypeptide having a certain percent (*e.g.*, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or even 99%) of sequence identity with another sequence means that, when aligned, that percentage of bases or amino acid residues are the same in comparing the two sequences. This alignment and the percent homology or identity can be determined using any suitable software program known in the art, for example those described in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel *et al.* (eds) 1987, Supplement 30, section 7.7.18). Preferred programs include the Vector NTI Advance™ 9.0 (Invitrogen Corp. Carlsbad, CA), GCG Pileup program, FASTA (Pearson *et al.* (1988) *Proc. Natl. Acad. Sci USA* 85:2444–2448), and BLAST (BLAST Manual, Altschul *et al.*, Natl Cent. Biotechnol. Inf., Natl Lib. Med. (NCIB NLM NIH), Bethesda, Md., and Altschul *et al.*, (1997) *NAR* 25:3389–3402). Another preferred alignment program is ALIGN Plus (Scientific and Educational Software, PA), preferably using default parameters. Another sequence software program that finds use is the TFASTA Data Searching Program available in the Sequence Software Package Version 6.0 (Genetics Computer Group, University of Wisconsin, Madison, WI).

[00141] The homology may be determined as the degree of identity between the two sequences indicating a derivation of the first sequence from the second. The homology may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package (described above). Thus, GAP GCG v8 may be used with the default scoring matrix for identity and the following default
 5 parameters: gap creation penalty of 5.0 and gap extension penalty of 0.3, respectively for nucleic acid sequence comparison, and gap creation penalty of 3.0 and gap extension penalty of 0.1, respectively, for protein sequence comparison. GAP uses the method of Needleman and Wunsch, (1970), J.Mol. Biol. 48:443-453, to make alignments and to calculate the identity.

[00142] A structural alignment between BASE (SEQ ID NO: 2) or a BASE variant and, e.g., another α -
 10 amylase may be used to identify equivalent/corresponding positions in other α -amylases having a high degree of homology, e.g., about 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or even 99%, with AmyTS23. One method of obtaining such a structural alignment is to use the Pile Up programme from the GCG package using default values of gap penalties, i.e., a gap creation penalty of 3.0 and gap extension penalty of 0.1. Other structural alignment methods include the
 15 hydrophobic cluster analysis (Gaboriaud *et al.*, (1987), FEBS LETTERS 224, pp. 149-155) and reverse threading (Huber and Torda, PROTEIN SCIENCE Vol. 7, No. 1 pp. 142-149 (1998)).

[00143] An exemplary alignment of the mature form of various reference amylases is provided as Figure 1. The reference amylases include: a truncated *Bacillus sp.* TS-23 alpha-amylase referred to herein as AmyTS23t or BASE, SEQ ID NO: 2 (GENBANK Accession No. AAA63900); *B. licheniformis* alpha-
 20 amylase referred to herein as AmyL or LAT, SEQ ID NO: 5 (GENBANK Accession No. AAA22240); *Geobacillus* (formerly *Bacillus*) *stearothermophilus* alpha-amylase AmyS, SEQ ID NO: 6 (GENBANK Accession No. AAA22241), *B. amyloliquefaciens* alpha-amylase BACAM, SEQ ID NO: 7 (GENBANK Accession No. AAA22191); *Bacillus sp.* #707 alpha-amylase referred to herein as AmyG6 or Amy#707, SEQ ID NO: 8 (GENBANK Accession No. AAA22231); *B. megaterium* alpha-amylase AmyG5 or AmyBm, SEQ
 25 ID NO: 9 (GENBANK Accession No. AAK00598); *Bacillus sp.* alpha-amylase ALBA, SEQ ID NO: 10 (GENBANK Accession No. CAL48155 and WO 2006/037484); *B. halmapalus* amylase AmyBh, SEQ ID NO: 11 (GENBANK Accession No. AAE00432 and US Patent No. 5,856,164); *Bacillus sp.* amylase AA560, SEQ ID NO: 12 (GENBANK Accession No. CAC16486 and WO 2000/060060); *Bacillus sp.* KSM-AP1378 alpha-amylase AmyKSM1378, SEQ ID NO: 13 (GENBANK Accession No. CAD35985 and EP No. 1199356
 30 A); and *Bacillus sp.* pHSP-K38 amylase AmyK38, SEQ ID NO: 14 (GENBANK Accession No. CAJ00040 and WO 2005/045045). Sequences were aligned using the MUSCLE 3.7 multiple sequence alignment algorithm (Edgar, Nucleic Acids Research, 32:1792-1797, 2004). A matrix showing the percent identity of alpha-amylases of the sequence alignment of Figure 1 is provided in Table 1.

Table 1. α -Amylase Percent Identity Matrix*

Percent_ID	2	5	6	7	8	9	10	11	12	13	14
2	100.0	64.6	88.0	65.2	67.6	66.7	68.2	68.6	67.6	69.5	59.4
5	64.6	100.0	65.5	80.9	68.2	74.2	68.0	70.7	68.4	69.4	62.7
6	88.0	65.5	100.0	65.2	66.5	65.5	67.5	67.3	66.3	67.9	59.0
7	65.2	80.9	65.2	100.0	66.9	71.9	66.7	68.6	66.9	67.1	60.3
8	67.6	68.2	66.5	66.9	100.0	69.4	93.2	86.2	95.5	86.4	64.2
9	66.7	74.2	65.5	71.9	69.4	100.0	70.4	71.1	69.6	70.0	61.5
10	68.2	68.0	67.5	66.7	93.2	70.4	100.0	87.5	95.1	87.7	65.0
11	68.6	70.7	67.3	68.6	86.2	71.1	87.5	100.0	86.8	86.6	66.9
12	67.6	68.4	66.3	66.9	95.5	69.6	95.1	86.8	100.0	86.0	64.4
13	69.5	69.4	67.9	67.1	86.4	70.0	87.7	86.6	86.0	100.0	67.1
14	59.4	62.7	59.0	60.3	64.2	61.5	65.0	66.9	64.4	67.1	100.0

*Numbers in the top row and left column correspond to the SEQ ID NOS of the aligned sequences of Figure 1.

1.5 Hybridisation

[00144] The oligonucleotide probe used in the characterization of AmyTS23, above, may suitably be prepared on the basis of the full or partial nucleotide or amino acid sequence of the α -amylase in question.

5 [00145] Suitable conditions for testing hybridization involve pre-soaking in 5X SSC and prehybridizing for 1 hour at 40°C in a solution of 20% formamide, 5X Denhardt's solution, 50 mM sodium phosphate, pH 6.8, and 50 mg of denatured sonicated calf thymus DNA, followed by hybridization in the same solution supplemented with 100 mM ATP for 18 hours at 40°C, followed by three times washing of the filter in 2X SSC, 0.2% SDS at 40°C for 30 minutes (low stringency), preferred at 50°C (medium stringency), more
10 preferably at 65°C (high stringency), even more preferably at 75°C (very high stringency). More details about the hybridization method can be found in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989.

[00146] In the present context, "derived from" is intended not only to indicate an α -amylase produced or producible by a strain of the organism in question, but also an α -amylase encoded by a DNA sequence
15 isolated from such strain and produced in a host organism transformed with such a DNA sequence. Finally, the term is intended to indicate an α -amylase, which is encoded by a DNA sequence of synthetic and/or cDNA origin and which has the identifying characteristics of the α -amylase in question. The term is also intended to indicate that the parent α -amylase may be a variant of a naturally occurring α -amylase, *i.e.*, a variant, which is the result of a modification (insertion, substitution, deletion) of one or more amino acid residues of the
20 naturally occurring α -amylase.

[00147] One skilled in the art will recognize that sequences encompassed by the present compositions and methods are also defined by the ability to hybridize under stringent hybridization conditions with the exemplified *base* sequence (*e.g.*, SEQ ID NO: 4). A nucleic acid is hybridizable to another nucleic acid sequence when a single stranded form of the nucleic acid can anneal to the other nucleic acid under
25 appropriate conditions of temperature and solution ionic strength. Hybridization and washing conditions are well known in the art (See, *e.g.*, Sambrook (1989) *supra*, particularly chapters 9 and 11). In some embodiments, stringent conditions correspond to a T_m of 65°C and 0.1×SSC, 0.1% SDS.

1.6 Parent α -Amylases

[00148] According to the present disclosure any α -amylase, may be used as the parent (*i.e.*, backbone) α -
30 amylase. In a preferred embodiment the parent α -amylase is BASE (AmyTS23t) having the amino acid sequence shown in SEQ ID NO: 2.

1.7 Altered Properties

[00149] The following section describes the relationship between mutations, which are present in the variant amylases described herein, and desirable alterations in properties (relative to those of a parent α -amylase),

which may result therefrom. The variants encompassed by the present compositions and methods are described in detail throughout the specification, and merely summarized in the following paragraphs.

[00150] As described, above, as aspect of the compositions and methods relate to α -amylases derived or derivable from a *Bacillus* *sp* strain α -amylase, including variants/mutants having altered properties with respect to parent amylases. Parent amylases are the above-mentioned parent α -amylase and hybrid or chimeric amylases that include at least a portion of an α -amylase, such as amino acid sequences of the mature polypeptide.

[00151] While the BASE α -amylase (SEQ ID NO: 2) is used as a starting point for discussing variant amylases, it will be appreciated that other *Bacillus* α -amylases having a high degree of homology to the BASE α -amylase may serve as a parental amylase without defeating the scope of the compositions and methods. This is particularly true of other naturally-occurring *Bacillus* α -amylases that include only minor sequence differences in comparison to the BASE α -amylase, not including the substitutions, deletions, or insertions, that are the subject of the present disclosure.

[00152] In the first aspect of the present compositions and methods, a variant of a parent *Bacillus* *sp.* α -amylase is provided. In some embodiments, the alpha-amylase variant is a mature form having amylase activity and comprising a substitution at one or more (preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) positions selected from the group consisting of:

1, 2, 3, 4, 5, 7, 15, 16, 17, 18, 19, 22, 25, 26, 28, 29, 30, 32, 35, 36, 37, 50, 51, 52, 53, 54, 55, 56, 57, 59, 60, 70, 71, 72, 73, 75, 78, 82, 83, 87, 90, 91, 93, 94, 95, 103, 104, 105, 107, 108, 110, 112, 113, 114, 115, 116, 118, 121, 123, 125, 126, 127, 128, 129, 130, 131, 132, 134, 135, 136, 138, 140, 142, 144, 147, 149, 150, 152, 154, 156, 158, 159, 160, 161, 162, 164, 165, 166, 167, 168, 169, 170, 171, 172, 174, 175, 176, 177, 178, 179, 182, 183, 185, 186, 188, 189, 190, 191, 192, 193, 195, 197, 199, 200, 201, 202, 203, 207, 210, 214, 217, 221, 228, 234, 237, 238, 239, 240, 243, 246, 250, 254, 255, 257, 264, 266, 267, 268, 269, 270, 272, 273, 275, 279, 283, 284, 298, 301, 303, 305, 306, 310, 311, 314, 318, 319, 320, 322, 323, 336, 337, 338, 339, 340, 344, 359, 374, 375, 376, 377, 379, 381, 382, 393, 394, 399, 401, 407, 408, 419, 433, 436, 438, 444, 447, 448, 451, 453, 459, 465, 479, 475, 483, and 484. Unless otherwise indicated, the positions are numbered by correspondence

(*e.g.*, same position in an alignment of alpha-amylase sequences such as that provided in Figure 1) with the amino acid sequence of a reference alpha-amylase set forth as SEQ ID NO: 2 (BASE). In some preferred embodiments, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK. In other preferred embodiments, the alpha-amylase variant is derived from a parent alpha-amylase with the amino acid sequence at least 75% (preferably 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%) identical to any member of the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14. In some embodiments, the alpha-amylase variant comprises a tyrosine at position 58 and an alanine at position 236. In some embodiments, the alpha-amylase variant comprises a glutamine at position 243 and a lysine at position 475.

[00153] Also provided is an isolated alpha-amylase variant, wherein the variant is a mature form having amylase activity and comprising a substitution at from one to eight (*e.g.*, 1, 2, 3, 4, 5, 6, 7, or 8) positions selected from the group consisting of: 182, 183, 305, 320, 379, 407, 419 and 475. In some preferred embodiments, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK, and/or the substitution comprises from one to eight (*e.g.*, 1, 2, 3, 4, 5, 6, 7, or 8) of the group consisting of: T182N, G183N, Y305Q, Q320F, P379A, Q407D, T419S and G475T (*e.g.*, variants of BASE combinatorial library 1).

[00154] Moreover, the present disclosure provides an isolated alpha-amylase variant, wherein the variant is a mature form having amylase activity and comprising a substitution at from one to seven (*e.g.*, 1, 2, 3, 4, 5, 6, or 7) positions selected from the group consisting of: 160, 182, 183, 189, 305, 379, and 475. In some preferred embodiments, the the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK, and/or the substitution comprises from one to seven (*e.g.*, 1, 2, 3, 4, 5, 6, or 7) of the group consisting of: Y160E, T182G, G183N, E189P, Y305G, P379E, and G475T (*e.g.*, variants of BASE combinatorial library 2).

[00155] The present disclosure provides an isolated alpha-amylase variant, wherein the variant is a mature form having amylase activity and comprising a substitution at from one to eight (*e.g.*, 1, 2, 3, 4, 5, 6, 7, or 8) positions selected from the group consisting of: 125, 182, 214, 279, 305, 319, 320, and 475. In some preferred embodiments, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK, and/or the substitution comprises from one to eight (*e.g.*, 1, 2, 3, 4, 5, 6, 7, or 8) of the group consisting of: S125A, T182A, T214Q, T279N, Y305R, D319T, Q320N, and G475R (*e.g.*, variants of BASE combinatorial library 3).

[00156] In addition, the present disclosure provides an isolated alpha-amylase variant, wherein the variant is a mature form having amylase activity and comprising a substitution at from one to eight (*e.g.*, 1, 2, 3, 4, 5, 6, 7, or 8) positions selected from the group consisting of: 7, 182, 298, 376, 379, 407, 419, and 453. In some preferred embodiments, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK, and/or the substitution comprises from one to eight (*e.g.*, 1, 2, 3, 4, 5, 6, 7, or 8) of the group consisting of: E7H, T182W, T298Q, Y376R, P379K, Q407W, T419S, and L453W (*e.g.*, variants of BASE combinatorial library 4).

[00157] The present disclosure provides an an isolated alpha-amylase variant, wherein the variant is a mature form having amylase activity and comprising a substitution at from one to four (*e.g.*, 1, 2, 3, or 4) positions selected from the group consisting of: 128, 178, 182 and 185, and the alpha-amylase variant comprises a serine or a glutamine at position 243. In some preferred embodiments, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK, and/or the substitution comprises from one to four (*e.g.*, 1, 2, 3, or 4) of the group consisting of: N128C, K178L, T182G, and A185D (*e.g.*, BASE-S1 to S32 variants).

[00158] In still further embodiments, the present disclosure provides an isolated alpha-amylase variant, wherein the variant is a mature form having amylase activity and comprising a substitution at from one to nine

(*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8 or 9) positions selected from the group consisting of: 125, 182, 183, 189, 279, 305, 319, 379 and 475, and the alpha-amylase variant comprises a glutamine, a phenylalanine or an asparagine at position 320. In some preferred embodiments, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK. In some preferred
 5 embodiments, the alpha-amylase variant comprises: a serine or alanine at position 125; a threonine, an asparagine, a glycine or an alanine at position 182; a glycine or an asparagine at position 183; a glutamic acid or a proline at position 189; a threonine or an asparagine at position 279; a tyrosine, a glutamine, a glycine or an arginine at position 305; an aspartic acid or a threonine at position 319; a proline or an alanine at position 379; and a glycine, threonine or an arginine at position 475; (*e.g.*, BASE-P1 to P12 variants).

10 **[00159]** The present disclosure also provides an isolated alpha-amylase variant, wherein the variant is a mature form having amylase activity and comprising a substitution at from one to eleven (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11) positions selected from the group consisting of: 125, 128, 178, 182, 183, 189, 279, 305, 319, 379 and 475, and the alpha-amylase variant comprises a serine or a glutamine at position 243, and a
 15 glutamine, a phenylalanine or an asparagine at position 320. In some preferred embodiments, the alpha-amylase variant is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK. In some preferred embodiments, the alpha-amylase variant comprises: a serine or alanine at position 125; an asparagine or a cysteine at position 128; a lysine or a leucine at position 178; a threonine, an asparagine, a glycine or an alanine at position 182; a glycine or an asparagine at position 183; a glutamic acid or a proline at position 189; a threonine or an asparagine at position 279; a tyrosine, a
 20 glutamine, a glycine or an arginine at position 305; an aspartic acid or a threonine at position 319; a proline or an alanine at position 379; and a glycine, threonine or an arginine at position 475 (*e.g.*, BASE-W1 to W13 variants).

Numerous exemplary alpha-amylase variants are disclosed for use in the claimed compositions and methods. The following alpha-amylase variants are exemplary alpha-amylase variants: BASE SEL variants, ACE-Q
 25 SEL variants, BASE combinatorial library 1 variants, BASE combinatorial library 2 variants, BASE combinatorial library 3 variants, BASE combinatorial library 4 variants, BASE-S1 to S32 combinatorial variants, BASE-P1 to P12 combinatorial variants, BASE-W1 to W13 combinatorial variants, and ACE-QK variant. The alpha-amylase variants of the present disclosure, however, are not limited to the exemplary variants, and include variants of other *Bacillus* sp. parent alpha-amylases having substitutions at
 30 corresponding positions. Based on the alignment and other data provided herein, it will also be appreciated that corresponding substitutions can be made in other alpha-amylase polypeptides, *i.e.*, in other “backbones,” and that the resulting amylase variants are expected to possess similar properties to those exemplified.

1.7.1 Stability

[00160] In the context of the variants described herein, mutations (including amino acid substitutions and deletions) of importance with respect to achieving altered stability (*i.e.*, higher or lower), in particular improved stability, at especially high temperatures (*i.e.*, 70-120°C) and/or extreme pH (*i.e.*, low or high pH, *i.e.*, pH 4-6 or pH 8-11, respectively), in particular at free (*i.e.*, unbound, therefore in solution) calcium concentrations below 60 ppm, include any of the mutations described herein. The stability may be determined as described in the "Methods" section below.

1.7.2 Ca²⁺ Stability

[00161] Altered Ca²⁺ stability means the stability of the enzyme under Ca²⁺ depletion has been improved, *i.e.*, higher or lower stability. In the context of the presently described variants, mutations (including amino acid substitutions and deletions) of importance with respect to achieving altered Ca²⁺ stability, in particular improved Ca²⁺ stability, *i.e.*, higher or lower stability, at especially high pH (*i.e.*, pH 8-10.5) include any of the mutations described herein.

1.7.3 Specific Activity

[00162] In a further aspect, important mutations (including amino acid substitutions and deletions) with respect to obtaining variants exhibiting altered specific activity, in particular increased or decreased specific activity, especially at temperatures from 10-60°C, preferably 20-50°C, especially 30-40°C, include any of the mutations described herein. The specific activity may be determined as described in the "Methods" section below.

1.7.4 Oxidation Stability

[00163] The described variants may have altered oxidation stability, in particular higher oxidation stability, in comparison to the parent α -amylase. Increased oxidation stability is advantageous in, *e.g.*, detergent compositions and decreased oxidation stability may be advantageous in composition for starch liquefaction. Oxidation stability may be determined as described in the "Methods" section below.

1.7.5 Altered pH Profile

[00164] Important positions and mutations with respect to obtaining variants with altered pH profile, in particular improved activity at especially high pH (*i.e.*, pH 8-10.5) or low pH (*i.e.*, pH 4-6) include mutations of amino residues located close to the active site residues. Preferred mutations are the ones described herein. Suitable assays are described in the "Methods" section below.

1.7.6 Wash Performance

[00165] Important positions and mutations with respect to obtaining variants with improved wash performance at especially high pH (*i.e.*, pH 8.5-11) include the specific mutations described herein. The wash performance may be tested as described below in the "Methods" section.

2. Methods for Preparing α -Amylase Variants

5 [00166] One aspect of the present compositions and methods is a method for preparing the present α -amylase variants having specified substitutions, deletions, transversions, insertions, and combinations, thereof. These variants may have posses advantageous features, such as , increased pH stability, increased temperature stability, reduced requirements for Ca^{2+} , increased specific activity, increased dishwashing or washing performance, increased solubility, increased storage stability, or combinations thereof.

10 [00167] Several methods for introducing mutations into genes and expressing mutated polypeptides encoded by these genes are known in the art. After a brief discussion of the cloning of α -amylase-encoding DNA sequences, methods for generating mutations at specific sites within the α -amylase-encoding sequence will be discussed.

2.1 Cloning a DNA Sequence Encoding an α -Amylase

15 [00168] DNA sequences encoding a parent α -amylase may be isolated from any cell or microorganism producing the α -amylase in question, using various methods well known in the art. First, a genomic DNA and/or cDNA library may be constructed using chromosomal DNA or messenger RNA from the organism that produces the α -amylase to be studied. Then, if the amino acid sequence of the α -amylase is known, homologous, labeled oligonucleotide probes may be synthesized and used to identify α -amylase-encoding
20 clones from a genomic library prepared from the organism in question. Alternatively, a labeled oligonucleotide probe containing sequences homologous to a known α -amylase gene could be used as a probe to identify α -amylase-encoding clones, using hybridization and washing conditions of lower stringency.

[00169] Yet another method for identifying α -amylase-encoding clones involves inserting fragments of genomic DNA into an expression vector, such as a plasmid, transforming α -amylase-negative bacteria with
25 the resulting genomic DNA library, and then plating the transformed bacteria onto agar containing a substrate for α -amylase, thereby allowing clones expressing the α -amylase to be identified.

[00170] Alternatively, the DNA sequence encoding the enzyme may be prepared synthetically by established standard methods, *e.g.* the phosphoamidite method described by S. L. Beaucage and M. H. Caruthers (1981) or the method described by Matthes *et al.* (1984). In the phosphoamidite method,
30 oligonucleotides are synthesized, *e.g.* in an automatic DNA synthesizer, purified, annealed, ligated and cloned in appropriate vectors.

[00171] Finally, the DNA sequence may be of mixed genomic and synthetic origin, mixed synthetic and cDNA origin or mixed genomic and cDNA origin, prepared by ligating fragments of synthetic, genomic or cDNA origin (as appropriate, the fragments corresponding to various parts of the entire DNA sequence), in

accordance with standard techniques. The DNA sequence may also be prepared by polymerase chain reaction (PCR) using specific primers, for instance as described in U.S. Pat. No. 4,683,202 or R. K. Saiki *et al.* (1988).

2.2 Site-directed Mutagenesis

[00172] Once an α -amylase-encoding DNA sequence has been isolated, and desirable sites for mutation identified, mutations may be introduced using synthetic oligonucleotides. These oligonucleotides contain nucleotide sequences flanking the desired mutation sites; mutant nucleotides are inserted during oligonucleotide synthesis. In a specific method, a single-stranded gap of DNA, bridging the α -amylase-encoding sequence, is created in a vector carrying the α -amylase gene. Then the synthetic nucleotide, bearing the desired mutation, is annealed to a homologous portion of the single-stranded DNA. The remaining gap is then filled in with DNA polymerase I (Klenow fragment) and the construct is ligated using T4 ligase. A specific example of this method is described in Morinaga *et al.* (1984). U.S. Pat. No. 4,760,025 discloses the introduction of oligonucleotides encoding multiple mutations by performing minor alterations of the cassette. However, an even greater variety of mutations can be introduced at any one time by the Morinaga method, because a multitude of oligonucleotides, of various lengths, can be introduced.

[00173] Another method of introducing mutations into α -amylase-encoding DNA sequences is described in Nelson and Long (1989). It involves the 3-step generation of a PCR fragment containing the desired mutation introduced by using a chemically synthesized DNA strand as one of the primers in the PCR reactions. From the PCR-generated fragment, a DNA fragment carrying the mutation may be isolated by cleavage with restriction endonucleases and reinserted into an expression plasmid.

[00174] Alternative methods for providing variants include gene shuffling, *e.g.*, as described in WO 95/22625 (from Affymax Technologies N.V.) or in WO 96/00343 (from Novo Nordisk A/S), or other corresponding techniques resulting in a hybrid enzyme comprising the mutation(s), *e.g.*, substitution(s) and/or deletion(s), in question.

2.3 Expression of α -Amylase Variants

[00175] A DNA sequence encoding an α -amylase variant produced by methods described above, or by any alternative methods known in the art, can be used to express a variant α -amylase (*i.e.*, an enzyme) using an expression vector, which typically includes control sequences, such as a promoter, operator, ribosome binding site, translation initiation signal, and, optionally, a repressor gene or various activator genes.

[00176] A recombinant expression vector carrying DNA sequences encoding an α -amylase variant may be any vector, which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, *i.e.*, a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a plasmid, a bacteriophage or an extrachromosomal element, minichromosome or an artificial chromosome. Alternatively, the vector may be one which, when introduced

into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

[00177] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence.

The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an α -amylase variant of the present compositions and methods, especially in a bacterial host, are the promoter of the *lac* operon of *E. coli*, the *Streptomyces coelicolor* agarase gene *dagA* promoters, the promoters of the *Bacillus licheniformis* α -amylase gene (*amyL*), the promoters of the *Geobacillus stearothermophilus* maltogenic amylase gene (*amyM*), the promoters of the *Bacillus amyloliquefaciens* α -amylase (*amyQ*), the promoters of the *Bacillus subtilis* *xylA* and *xylB* genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding *A. oryzae* TAKA amylase, *Rhizomucor miehei* aspartic proteinase, *A. niger* neutral α -amylase, *A. niger* acid stable α -amylase, *A. niger* glucoamylase, *Rhizomucor miehei* lipase, *A. oryzae* alkaline protease, *A. oryzae* triose phosphate isomerase or *A. nidulans* acetamidase.

[00178] The expression vector may also comprise a suitable transcription terminator and, in eukaryotes, polyadenylation sequences operably connected to the DNA sequence encoding the α -amylase variant of the present compositions and methods. Termination and polyadenylation sequences may suitably be derived from the same sources as the promoter.

[00179] The vector may further comprise a DNA sequence enabling the vector to replicate in the host cell in question. Examples of such sequences are the origins of replication of plasmids pUC19, pACYC177, pUB110, pE194, pAMB1 and pIJ702.

[00180] The vector may also comprise a selectable marker, *e.g.* a gene the product of which complements a defect in the host cell, such as the *dal* genes from *B. subtilis* or *B. licheniformis*, or one which confers antibiotic resistance such as ampicillin, kanamycin, neomycin or tetracyclin resistance. Furthermore, the vector may comprise *Aspergillus* selection markers such as *amdS*, *argB*, *niaD* and *sC*, a marker giving rise to hygromycin resistance, or the selection may be accomplished by co-transformation, *e.g.*, as described in WO 91/17243.

[00181] While intracellular expression may be advantageous in some respects, *e.g.*, when using certain bacteria as host cells, it is generally preferred that the expression is extracellular. In general, the *Bacillus* α -amylases mentioned herein comprise a preregion or signal sequence permitting secretion of the expressed protease into the culture medium. If desirable, this preregion may be replaced by a different preregion or signal sequence, conveniently accomplished by substitution of the DNA sequences encoding the respective preregions.

[00182] The procedures used to ligate the DNA construct encoding an α -amylase variant, the promoter, terminator and other elements, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (*cf.*, for instance, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989).

[00183] The cell, either comprising a DNA construct or an expression vector, is advantageously used as a host cell in the recombinant production of an α -amylase variant. The cell may be transformed with the DNA construct of the present compositions and methods encoding the variant, conveniently by integrating the DNA construct (in one or more copies) in the host chromosome. This integration is generally considered to be an advantage as the DNA sequence is more likely to be stably maintained in the cell. Integration of the DNA constructs into the host chromosome may be performed according to conventional methods, *e.g.*, by homologous or heterologous recombination. Alternatively, the cell may be transformed with an expression vector as described above in connection with the different types of host cells. The cell may be a cell of a higher organism such as a mammal or an insect, but is preferably a microbial cell, *e.g.*, a bacterial or a fungal (including yeast) cell.

[00184] Examples of suitable bacteria are Gram-positive bacteria such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus brevis*, *Geobacillus stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus lautus*, *Bacillus megaterium*, *Bacillus thuringiensis*, or *Streptomyces lividans* or *Streptomyces murinus*, or gram-negative bacteria such as *E. coli*.

The transformation of the bacteria may, for instance, be effected by protoplast transformation or by using competent cells in a manner known per se.

[00185] The yeast organism may favorably be selected from a species of *Saccharomyces* or *Schizosaccharomyces*, *e.g.* *Saccharomyces cerevisiae*. The filamentous fungus may advantageously belong to a species of *Aspergillus*, *e.g.*, *Aspergillus oryzae* or *Aspergillus niger*. Fungal cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a manner known per se. A suitable procedure for transformation of *Aspergillus* host cells is described in EP 238 023.

[00186] The medium used to cultivate the cells may be any conventional medium suitable for growing the host cell in question and obtaining expression of the α -amylase variant. Suitable media are available from commercial suppliers or may be prepared according to published recipes (*e.g.*, as described in catalogues of the American Type Culture Collection).

[00187] The α -amylase variant secreted from the host cells may conveniently be recovered from the culture medium by well-known procedures, including separating the cells from the medium by centrifugation or filtration, and precipitating proteinaceous components of the medium by means of a salt such as ammonium sulfate, followed by the use of chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

3. Detergent Compositions

[00188] One aspect of the present compositions and methods involves a detergent composition, or a detergent additive, comprising an α -amylase and a protease. The detergent composition may be formulated as a hand or machine laundry detergent composition, a laundry additive composition suitable for

pretreatment of stained fabrics, a rinse added fabric softener composition, a detergent composition for general household hard surface cleaning, or a hand or machine dishwashing composition.

[00189] Suitable proteases include those of animal, vegetable or microbial origin. Microbial origin is preferred. Chemically modified or protein engineered mutants are included. The protease may be a serine protease or a metallo protease, preferably an alkaline microbial protease or a trypsin-like protease or a chymotrypsin-like protease. Examples of alkaline proteases are subtilisins, especially those derived from *Bacillus*, e.g., subtilisin *lentus*, subtilisin *amyloliquefaciens*, subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279). Examples of trypsin-like proteases are trypsin (e.g., of porcine or bovine origin) and the *Fusarium* protease described in WO 89/06270 and WO 94/25583.

[00190] Examples of useful proteases also include but are not limited to the variants described in U.S. Patent Nos. RE 34,606, 5,801,039, 5,340,735, 5,500,364, 5,855,625, 5,955,340, 5,700,676, 6,312,936, and 6,482,628, U.S. Pat. Publ. No. 2008/0090747, and International Patent Pub. Nos. WO98/23732, WO99/20770, WO 92/19729, WO 98/20115, WO 98/20116, WO 98/34946, WO95/23221, WO 92/21760, and WO 89/06270, especially variants with substitutions in one or more of the following positions: 27, 36, 57, 76, 87, 97, 101, 104, 120, 123, 167, 170, 194, 206, 218, 222, 224, 235 and 274.

[00191] Exemplary commercially available protease enzymes include ALCALASE®, SAVINASE®, PRIMASE®, DURALASE®, ESPERASE®, DURAZYM™, POLARZYME®, OVOZYME®, LIQUANASE®, NEUTRASE®, RELASE®, and KANNASE® (from Novozymes A/S), MAXATASE®, MAXACAL, MAXAPEM®, PROPERASE®, PURAFECT®, PURAFECT OXP®, FN2®, FN3® and FN4®, OPTICLEAN®, OPTIMASE®, PURAMAX™, EXCELLASE™, and PURAFAST™ (from Genencor), and BLAP™ (from Henkel Kommanditgesellschaft auf Aktien, Duesseldorf, Germany).

[00192] In some further embodiments, metalloproteases find use in the present invention, including but not limited to the neutral metalloprotease described in WO 07/044993.

[00193] In addition to the an alpha-amylase and a protease the detergent composition or additive may comprise one or more other enzymes such as, a lipase, a peroxidase, another amylolytic enzyme, e.g., another α -amylase, glucoamylase, maltogenic amylase, CGTase and/or a cellulose, mannanase (such as MANNASTAR™ from Danisco U.S.A., Inc., Genencor Division), pectinase, pectin lyase, cutinase, and/or laccase. In general the properties of the chosen enzyme(s) should be compatible with the selected detergent, (i.e., pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, etc.), and the enzyme(s) should be present in effective amounts.

[00194] *Lipases*: Suitable lipases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful lipases include but are not limited to lipases from *Humicola* (synonym *Thermomyces*), e.g., from *H. lanuginosa* (*T. lanuginosus*) as described in EP 258 068 and EP 305 216 or from *H. insolens* as described in WO 96/13580, a *Pseudomonas* lipase, e.g., from *P. alcaligenes* or *P. pseudoalcaligenes* (EP 218 272), *P. cepacia* (EP 331 376), *P. stutzeri* (GB 1,372,034), *P. fluorescens*, *Pseudomonas* sp. strain SD 705 (WO 95/06720 and WO 96/27002), *P. wisconsinensis* (WO

96/12012), a *Bacillus* lipase, e.g., from *B. subtilis* (Dartois *et al.* (1993), Biochemica et Biophysica Acta, 1131, 253-360), *B. stearotheophilus* (JP 64/744992) or *B. pumilus* (WO 91/16422). Additional exemplary lipase variants contemplated for use in the formulations include those described in WO 92/05249, WO 94/01541, EP 407 225, EP 260 105, WO 95/35381, WO 96/00292, WO 95/30744, WO 94/25578, WO 95/14783, WO 95/22615, WO 97/04079 and WO 97/07202. Commercially available lipase enzymes include LIPOLASE™ and LIPOLASE ULTRA™ (Novozymes A/S).

[00195] *Polyesterases*: suitable polyesterases can be included in composition. Suitable polyesterases include for example those described in WO 01/34899 and WO 01/14629.

[00196] *Amylases*: One or more additional amylases (in addition to the variant amylase(s) described herein) may also be included. Suitable amylases (α and/or β) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, α -amylases obtained from *Bacillus*, e.g., a special strain of *B. licheniformis*, described in more detail in GB 1,296,839. Examples of useful α -amylases are the variants described in WO 94/18314, WO 96/39528, WO 94/02597, WO 94/18314, WO 96/23873, and WO 97/43424, especially the variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 181, 188, 190, 197, 202, 208, 209, 243, 264, 304, 305, 391, 408, and 444. Commercially available α -amylases are DURAMYL™, LIQUEZYME™, TERMAMYL™, NATALASE™, STAINZYME™ PLUS, STAINZYME™ ULTRA, FUNGAMYL™ and BAN™ (Novozymes A/S), RAPIDASE™ and PURASTAR™ (from Genencor).

[00197] *Cellulases*: Cellulases may be added to the compositions. Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include but are not limited to cellulases from the genera *Bacillus*, *Pseudomonas*, *Trichoderma*, *Humicola*, *Fusarium*, *Thielavia*, *Acremonium*, e.g., the fungal cellulases produced from *Humicola insolens*, *Myceliophthora thermophila* and *Fusarium oxysporum* disclosed in U.S. Pat. No. 4,435,307, U.S. Pat. No. 5,648,263, U.S. Pat. No. 5,691,178, U.S. Pat. No. 5,776,757 and WO 89/09259. Exemplary *Trichoderma reesei* cellulases are disclosed in U.S. Pat. No. 4,689,297, U.S. Pat. No. 5,814,501, U.S. Pat. No. 5,324,649, WO 92/06221 and WO 92/06165. Exemplary *Bacillus* cellulases are disclosed in U.S. Pat. No. 6,562,612. Commercially available cellulases include CELLUZYME®, and CAREZYME® (Novozymes A/S), CLAZINASE®, and PURADAX HA® (Genencor International Inc.), and KAC-500(B)® (Kao Corporation).

[00198] *Peroxidases/Oxidases*: Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinus*, e.g., from *C. cinereus*, and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257. Commercially available peroxidases include GUARDZYME® (Novozymes A/S).

[00199] The detergent enzyme(s) may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of these enzymes. A detergent additive of the present compositions and methods, i.e., a separate additive or a combined additive,

can be formulated, *e.g.*, granulate, a liquid, a slurry, etc. Preferred detergent additive formulations are granulates, in particular non-dusting granulates, liquids, in particular stabilized liquids, or slurries.

[00200] Non-dusting granulates may be produced, *e.g.*, as disclosed in U.S. Pat. Nos. 4,106,991 and 4,661,452 and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molar weights of 1000 to 20000; ethoxylated nonyl-phenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

[00201] Generally, the detergent composition may be in any convenient form, *e.g.*, a bar, a tablet, a powder, a granule, a paste, or a liquid. A liquid detergent may be aqueous, typically containing up to about 70% water, and 0% to about 30% organic solvent. Compact detergent gels contained for example about 30% water or less.

[00202] Persil, TIDE® 2X coldwater, and Ariel detergents are exemplary detergents used herein to test exemplary alpha-amylase variants. The present alpha-amylase variants are not restricted to the exemplary compositions, as they are contemplated to be functional in the presence of a broad range of commonly used cleaning compositions.

[00203] The detergent composition comprises one or more surfactants, which may be non-ionic including semi-polar and/or anionic and/or cationic and/or zwitterionic. The surfactants are typically present at a level of from 0.1% to 60% by weight. Exemplary alpha-amylase variants were tested in detergent compositions containing anionic and nonionic surfactants. The alpha-amylase variants described herein are contemplated to be active in compositions containing other surfactants that are commonly used in detergents.

[00204] When included therein the detergent will usually contain from about 1% to about 40% of an anionic surfactant such as linear alkylbenzenesulfonate, α -olefinsulfonate, alkyl sulfate (fatty alcohol sulfate), alcohol ethoxysulfate, secondary alkanesulfonate, α -sulfo fatty acid methyl ester, alkyl- or alkenylsuccinic acid or soap.

[00205] When included therein the detergent will usually contain from about 0.2% to about 40% of a non-ionic surfactant such as alcohol ethoxylate, nonyl-phenol ethoxylate, alkylpolyglycoside, alkyltrimethylamine-oxide, ethoxylated fatty acid monoethanol-amide, fatty acid monoethanolamide, polyhydroxy alkyl fatty acid amide, or N-acyl N-alkyl derivatives of glucosamine ("glucamides").

[00206] The detergent may contain 0-65% of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, carbonate, citrate, nitrilotriacetic acid, ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid, alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (*e.g.* SKS-6 from Hoechst).

[00207] The detergent may comprise one or more polymers. Examples are carboxymethylcellulose, poly(vinyl-pyrrolidone), poly (ethylene glycol), poly(vinyl alcohol), poly(vinylpyridine-N-oxide), poly(vinylimidazole), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid co-polymers.

5 [00208] The detergent may contain a bleaching system that may comprise a H₂O₂ source, such as perborate or percarbonate, which may be combined with a peracid-forming bleach activator (*e.g.*, tetraacetythylenediamine or nonanoyloxybenzenesulfonate). Alternatively, the bleaching system may comprise peroxyacids (*e.g.* the amide, imide, or sulfone type peroxyacids). The bleaching system can also be an enzymatic bleaching system. See, for example, WO 05/056782.

10 [00209] The enzyme(s) of the detergent composition of the present compositions and methods may be stabilized using conventional stabilizing agents, *e.g.*, a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative, *e.g.*, an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid, and the composition may be formulated as described in, *e.g.*, WO 92/19709 and WO 92/19708.

15 [00210] The detergent may also contain other conventional detergent ingredients such as *e.g.* fabric conditioners including clays, foam boosters, suds suppressors, anti-corrosion agents, soil-suspending agents, anti-soil re-deposition agents, dyes, bactericides, optical brighteners, hydrotropes, tarnish inhibitors, or perfumes.

[00211] The present α -amylases may be added in an amount corresponding to about 0.01 to about 100 mg of enzyme protein per liter of wash liquor, for example about 0.05 to about 5.0 mg of enzyme protein per liter of wash liquor, or about 0.1 to about 1.0 mg of enzyme protein per liter of wash liquor.

20 [00212] The present α -amylases may additionally be incorporated in the detergent formulations disclosed in WO 97/07202, which is hereby incorporated as reference.

4. Compositions and Use

25 [00213] The present α -amylase/protease cleaning compositions may be used in methods relating to detergent compositions and cleaning, in particular laundry detergent compositions, dishwashing detergent compositions, hard surface cleaning compositions, and the like.

4.1 Laundry Detergent Compositions and Use

[00214] One embodiment, the compositions and methods are laundry detergent compositions and method of use, thereof. The detergent composition may be in the form of a non-dusting granulate, a stabilized liquid, or a protected enzyme. The dry formulations may be in the form of a granulate or a microgranulate. Non-dusting granulates may be produced, *e.g.* as disclosed in U.S. Patent Nos. 4,106,991 and 4,661,452 and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molar weights of 1,000 to 20,000; ethoxylated
35 nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol

contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in, for example, GB Patent No. 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Other enzyme stabilizers are well known in the art. Protected enzymes may be prepared according to the method disclosed in for example EP Appln. No. 238,216. Polyols have long been recognized as stabilizers of proteins as well as improving solubility of proteins. See, e.g., J. K. Kaushik *et al. J. Biol. Chem.* 278: 26458-65 (2003) and the references cited therein; and Monica Conti *et al. J. Chromatography* 757: 237-245 (1997).

[00215] The composition may comprise one or more of the present amylases and proteases as the major enzymatic components. Alternatively, the composition may comprise multiple other enzymatic activities, such as an aminopeptidase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, α -galactosidase, β -galactosidase, glucoamylase, α -glucosidase, β -glucosidase, haloperoxidase, invertase, laccase, lipase, mannosidase, oxidase, pectinolytic enzyme, peptidoglutaminase, peroxidase, phytase, polyphenoloxidase, ribonuclease, transglutaminase, or xylanase, as well as other enzymes discussed below. The additional enzyme(s) may be producible by means of a microorganism belonging to the genera *Aspergillus*, *Trichoderma*, *Humicola* (e.g., *H. insolens*), and *Fusarium*. Exemplary members of the *Aspergillus* genus include *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus niger*, or *Aspergillus oryzae*. Exemplary members of the genus *Fusarium* include *Fusarium bactridioides*, *Fusarium cerealis*, *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium heterosporum*, *Fusarium negundinis*, *Fusarium oxysporum*, *Fusarium reticulatum*, *Fusarium roseum*, *Fusarium sambucinum*, *Fusarium sarcochroum*, *Fusarium sulphureum*, *Fusarium torulosum*, *Fusarium trichothecioides*, and *Fusarium venenatum*.

[00216] The detergent composition may be in any useful form, e.g., powders, granules, pastes, or liquids. A liquid detergent may be aqueous, typically containing up to about 70% of water, and 0% to about 30% of organic solvent. It can also be in the form of a compact gel type containing only about 30% water. Enzymes may be used in any detergent composition compatible with the stability of the enzyme. Enzymes can be protected against generally deleterious components by known forms of encapsulation as for example by granulation or sequestration in hydro gels. Enzymes and specifically α -amylases are not limited to laundry and dishwashing applications, but can also be used in surface cleaners, ethanol production from starch or biomass.

[00217] The detergent composition comprises one or more surfactants, each of which may be anionic, nonionic, cationic, or zwitterionic. The detergent will usually contain 0% to about 50% of anionic surfactant, such as linear alkylbenzenesulfonate (LAS); α -olefinsulfonate (AOS); alkyl sulfate (fatty alcohol sulfate) (AS); alcohol ethoxysulfate (AEOS or AES); secondary alkanesulfonates (SAS); α -sulfo fatty acid methyl esters; alkyl- or alkenylsuccinic acid; or soap. The composition may also contain 0% to about 40% of nonionic surfactant such as alcohol ethoxylate (AEO or AE), carboxylated alcohol ethoxylates, nonylphenol

ethoxylate, alkylpolyglycoside, alkyldimethylamineoxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, or polyhydroxy alkyl fatty acid amide (as described for example in WO 92/06154).

[00218] The detergent composition may additionally comprise one or more other enzymes, such as lipase, cutinase, cellulase, peroxidase, and/or laccase in any combination. See *supra*.

5 [00219] The detergent may optionally contain about 1% to about 65% of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTMPA), alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (*e.g.* SKS-6 from Hoechst). The detergent may also be unbuilt, *i.e.*, essentially free of detergent builder.

10 [00220] The detergent may optionally comprise one or more polymers. Examples include carboxymethylcellulose (CMC), poly(vinylpyrrolidone) (PVP), polyethyleneglycol (PEG), poly(vinyl alcohol) (PVA), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

[00221] The detergent may optionally contain a bleaching system, which may comprise a H₂O₂ source such as perborate or percarbonate, which may be combined with a peracid-forming bleach activator such as tetraacetythylenediamine (TAED) or nonanoyloxybenzenesulfonate (NOBS). Alternatively, the bleaching system may comprise peroxy acids of *e.g.* the amide, imide, or sulfone type. The bleaching system can also be an enzymatic bleaching system, where a perhydrolase activates peroxide, as described in for example WO 2005/056783.

20 [00222] The enzymes of the detergent composition may be stabilized using conventional stabilizing agents, *e.g.*, a polyol such as propylene glycol or glycerol; a sugar or sugar alcohol; lactic acid; boric acid or a boric acid derivative such as, *e.g.*, an aromatic borate ester; and the composition may be formulated as described in, *e.g.*, WO 92/19709 and WO 92/19708.

[00223] The detergent may also contain other conventional detergent ingredients such as, *e.g.*, fabric conditioners including clays, foam boosters, suds suppressors, anti-corrosion agents, soil-suspending agents, anti-soil redeposition agents, dyes, bactericides, optical brighteners, or perfume.

[00224] The pH (measured in aqueous solution at use concentration) is usually neutral or alkaline, *e.g.*, pH about 7.0 to about 11.0.

30 [00225] Particular forms of detergent compositions comprising a the α -amylase variants can be formulated to include:

[00226] 1) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising linear alkylbenzenesulfonate (calculated as acid) about 7% to about 12%; alcohol ethoxysulfate (*e.g.*, C₁₂₋₁₈ alcohol, 1-2 ethylene oxide (EO)) or alkyl sulfate (*e.g.*, C₁₆₋₁₈) about 1% to about 4%; alcohol ethoxylate (*e.g.*, C₁₄₋₁₅ alcohol, 7 EO) about 5% to about 9%; sodium carbonate (*e.g.*, Na₂CO₃) about 14% to about 20%; soluble silicate (*e.g.*, Na₂O, 2SiO₂) about 2 to about 6%; zeolite (*e.g.*, NaAlSiO₄) about 15% to about 22%; sodium sulfate (*e.g.*, Na₂SO₄) 0% to about 6%; sodium citrate/citric acid (*e.g.*, C₆H₅Na₃O₇/C₆H₈O₇) about 0% to about 15%; sodium perborate (*e.g.*, NaBO₃H₂O) about 11% to about 18%;

TAED about 2% to about 6%; carboxymethylcellulose (CMC) and 0% to about 2%; polymers (*e.g.*, maleic/acrylic acid, copolymer, PVP, PEG) 0-3%; enzymes (calculated as pure enzyme) 0.0001-0.1% protein; and minor ingredients (*e.g.*, suds suppressors, perfumes, optical brightener, photobleach) 0-5%.

[00227] 2) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L

comprising linear alkylbenzenesulfonate (calculated as acid) about 6% to about 11%; alcohol ethoxysulfate (*e.g.*, C₁₂₋₁₈ alcohol, 1-2 EO) or alkyl sulfate (*e.g.*, C₁₆₋₁₈) about 1% to about 3%; alcohol ethoxylate (*e.g.*, C₁₄₋₁₅ alcohol, 7 EO) about 5% to about 9%; sodium carbonate (*e.g.*, Na₂CO₃) about 15% to about 21%; soluble silicate (*e.g.*, Na₂O, 2SiO₂) about 1% to about 4%; zeolite (*e.g.*, NaAlSiO₄) about 24% to about 34%; sodium sulfate (*e.g.*, Na₂SO₄) about 4% to about 10%; sodium citrate/citric acid (*e.g.*, C₆H₅Na₃O₇/ C₆H₈O₇) 0% to about 15%; carboxymethylcellulose (CMC) 0% to about 2%; polymers (*e.g.*, maleic/acrylic acid copolymer, PVP, PEG) 1-6%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; minor ingredients (*e.g.*, suds suppressors, perfume) 0-5%.

[00228] 3) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L

comprising linear alkylbenzenesulfonate (calculated as acid) about 5% to about 9%; alcohol ethoxylate (*e.g.*, C₁₂₋₁₅ alcohol, 7 EO) about 7% to about 14%; Soap as fatty acid (*e.g.*, C₁₆₋₂₂ fatty acid) about 1 to about 3%; sodium carbonate (as Na₂CO₃) about 10% to about 17%; soluble silicate (*e.g.*, Na₂O, 2SiO₂) about 3% to about 9%; zeolite (as NaAlSiO₄) about 23% to about 33%; sodium sulfate (*e.g.*, Na₂SO₄) 0% to about 4%; sodium perborate (*e.g.*, NaBO₃H₂O) about 8% to about 16%; TAED about 2% to about 8%; phosphonate (*e.g.*, EDTMPA) 0% to about 1%; carboxymethylcellulose (CMC) 0% to about 2%; polymers (*e.g.*, maleic/acrylic acid copolymer, PVP, PEG) 0-3%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; minor ingredients (*e.g.*, suds suppressors, perfume, optical brightener) 0-5%.

[00229] 4) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L

comprising linear alkylbenzenesulfonate (calculated as acid) about 8% to about 12%; alcohol ethoxylate (*e.g.*, C₁₂₋₁₅ alcohol, 7 EO) about 10% to about 25%; sodium carbonate (as Na₂CO₃) about 14% to about 22%; soluble silicate (*e.g.*, Na₂O, 2SiO₂) about 1% to about 5%; zeolite (*e.g.*, NaAlSiO₄) about 25% to about 35%; sodium sulfate (*e.g.*, Na₂SO₄) 0% to about 10%; carboxymethylcellulose (CMC) 0% to about 2%; polymers (*e.g.*, maleic/acrylic acid copolymer, PVP, PEG) 1-3%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, suds suppressors, perfume) 0-5%.

[00230] 5) An aqueous liquid detergent composition comprising linear alkylbenzenesulfonate (calculated as

acid) about 15% to about 21%; alcohol ethoxylate (*e.g.*, C₁₂₋₁₅ alcohol, 7 EO or C₁₂₋₁₅ alcohol, 5 EO) about 12% to about 18%; soap as fatty acid (*e.g.*, oleic acid) about 3% to about 13%; alkenylsuccinic acid (C₁₂₋₁₄) 0% to about 13%; aminoethanol about 8% to about 18%; citric acid about 2% to about 8%; phosphonate 0% to about 3%; polymers (*e.g.*, PVP, PEG) 0% to about 3%; borate (*e.g.*, B₄O₇) 0% to about 2%; ethanol 0% to about 3%; propylene glycol about 8% to about 14%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, dispersants, suds suppressors, perfume, optical brightener) 0-5%.

[00231] 6) An aqueous structured liquid detergent composition comprising linear alkylbenzenesulfonate

(calculated as acid) about 15% to about 21%; alcohol ethoxylate (*e.g.*, C₁₂₋₁₅ alcohol, 7 EO, or C₁₂₋₁₅ alcohol, 5

EO) 3-9%; soap as fatty acid (*e.g.*, oleic acid) about 3% to about 10%; zeolite (as NaA1SiO₄) about 14% to about 22%; potassium citrate about 9% to about 18%; borate (*e.g.*, B₄O₇) 0% to about 2%; carboxymethylcellulose (CMC) 0% to about 2%; polymers (*e.g.*, PEG, PVP) 0% to about 3%; anchoring polymers such as, *e.g.*, lauryl methacrylate/acrylic acid copolymer; molar ratio 25:1, MW 3800) 0% to about 3%; glycerol 0% to about 5%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, dispersants, suds suppressors, perfume, optical brighteners) 0-5%.

[00232] 7) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising fatty alcohol sulfate about 5% to about 10%; ethoxylated fatty acid monoethanolamide about 3% to about 9%; soap as fatty acid 0-3%; sodium carbonate (*e.g.*, Na₂CO₃) about 5% to about 10%; Soluble silicate (*e.g.*, Na₂O, 2SiO₂) about 1% to about 4%; zeolite (*e.g.*, NaA1SiO₄) about 20% to about 40%; Sodium sulfate (*e.g.*, Na₂SO₄) about 2% to about 8%; sodium perborate (*e.g.*, NaBO₃H₂O) about 12% to about 18%; TAED about 2% to about 7%; polymers (*e.g.*, maleic/acrylic acid copolymer, PEG) about 1% to about 5%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, optical brightener, suds suppressors, perfume) 0-5%.

[00233] 8) A detergent composition formulated as a granulate comprising linear alkylbenzenesulfonate (calculated as acid) about 8% to about 14%; ethoxylated fatty acid monoethanolamide about 5% to about 11%; soap as fatty acid 0% to about 3%; sodium carbonate (*e.g.*, Na₂CO₃) about 4% to about 10%; soluble silicate (Na₂O, 2SiO₂) about 1% to about 4%; zeolite (*e.g.*, NaA1SiO₄) about 30% to about 50%; sodium sulfate (*e.g.*, Na₂SO₄) about 3% to about 11%; sodium citrate (*e.g.*, C₆H₅Na₃O₇) about 5% to about 12%; polymers (*e.g.*, PVP, maleic/acrylic acid copolymer, PEG) about 1% to about 5%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, suds suppressors, perfume) 0-5%.

[00234] 9) A detergent composition formulated as a granulate comprising linear alkylbenzenesulfonate (calculated as acid) about 6% to about 12%; nonionic surfactant about 1% to about 4%; soap as fatty acid about 2% to about 6%; sodium carbonate (*e.g.*, Na₂CO₃) about 14% to about 22%; zeolite (*e.g.*, NaA1SiO₄) about 18% to about 32%; sodium sulfate (*e.g.*, Na₂SO₄) about 5% to about 20%; sodium citrate (*e.g.*, C₆H₅Na₃O₇) about 3% to about 8%; sodium perborate (*e.g.*, NaBO₃H₂O) about 4% to about 9%; bleach activator (*e.g.*, NOBS or TAED) about 1% to about 5%; carboxymethylcellulose (CMC) 0% to about 2%; polymers (*e.g.*, polycarboxylate or PEG) about 1% to about 5%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, optical brightener, perfume) 0-5%.

[00235] 10) An aqueous liquid detergent composition comprising linear alkylbenzenesulfonate (calculated as acid) about 15% to about 23%; alcohol ethoxysulfate (*e.g.*, C₁₂₋₁₅ alcohol, 2-3 EO) about 8% to about 15%; alcohol ethoxylate (*e.g.*, C₁₂₋₁₅ alcohol, 7 EO, or C₁₂₋₁₅ alcohol, 5 EO) about 3% to about 9%; soap as fatty acid (*e.g.*, lauric acid) 0% to about 3%; aminoethanol about 1% to about 5%; sodium citrate about 5% to about 10%; hydrotrope (*e.g.*, sodium toluenesulfonate) about 2% to about 6%; borate (*e.g.*, B₄O₇) 0% to about 2%; carboxymethylcellulose 0% to about 1%; ethanol about 1% to about 3%; propylene glycol about 2% to about 5%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, polymers, dispersants, perfume, optical brighteners) 0-5%.

[00236] 11) An aqueous liquid detergent composition comprising linear alkylbenzenesulfonate (calculated as acid) about 20% to about 32%; alcohol ethoxylate (*e.g.*, C₁₂₋₁₅ alcohol, 7 EO, or C₁₂₋₁₅ alcohol, 5 EO) 6-12%; aminoethanol about 2% to about 6%; citric acid about 8% to about 14%; borate (*e.g.*, B₄O₇) about 1% to about 3%; polymer (*e.g.*, maleic/acrylic acid copolymer, anchoring polymer such as, *e.g.*, lauryl methacrylate/acrylic acid copolymer) 0% to about 3%; glycerol about 3% to about 8%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, hydrotropes, dispersants, perfume, optical brighteners) 0-5%.

[00237] 12) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising anionic surfactant (linear alkylbenzenesulfonate, alkyl sulfate, α -olefinsulfonate, α -sulfo fatty acid methyl esters, alkanesulfonates, soap) about 25% to about 40%; nonionic surfactant (*e.g.*, alcohol ethoxylate) about 1% to about 10%; sodium carbonate (*e.g.*, Na₂CO₃) about 8% to about 25%; soluble silicates (*e.g.*, Na₂O, 2SiO₂) about 5% to about 15%; sodium sulfate (*e.g.*, Na₂SO₄) 0% to about 5%; zeolite (NaAlSiO₄) about 15% to about 28%; sodium perborate (*e.g.*, NaBO₃·4H₂O) 0% to about 20%; bleach activator (TAED or NOBS) about 0% to about 5%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; minor ingredients (*e.g.*, perfume, optical brighteners) 0-3%.

[00238] 13) Detergent compositions as described in compositions 1)-12) *supra*, wherein all or part of the linear alkylbenzenesulfonate is replaced by (C₁₂-C₁₈) alkyl sulfate.

[00239] 14) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising (C₁₂-C₁₈) alkyl sulfate about 9% to about 15%; alcohol ethoxylate about 3% to about 6%; polyhydroxy alkyl fatty acid amide about 1% to about 5%; zeolite (*e.g.*, NaAlSiO₄) about 10% to about 20%; layered disilicate (*e.g.*, SK56 from Hoechst) about 10% to about 20%; sodium carbonate (*e.g.*, Na₂CO₃) about 3% to about 12%; soluble silicate (*e.g.*, Na₂O, 2SiO₂) 0% to about 6%; sodium citrate about 4% to about 8%; sodium percarbonate about 13% to about 22%; TAED about 3% to about 8%; polymers (*e.g.*, polycarboxylates and PVP) 0% to about 5%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, optical brightener, photobleach, perfume, suds suppressors) 0-5%.

[00240] 15) A detergent composition formulated as a granulate having a bulk density of at least 600 g/L comprising (C₁₂-C₁₈) alkyl sulfate about 4% to about 8%; alcohol ethoxylate about 11% to about 15%; soap about 1% to about 4%; zeolite MAP or zeolite A about 35% to about 45%; sodium carbonate (as Na₂CO₃) about 2% to about 8%; soluble silicate (*e.g.*, Na₂O, 2SiO₂) 0% to about 4%; sodium percarbonate about 13% to about 22%; TAED 1-8%; carboxymethylcellulose (CMC) 0% to about 3%; polymers (*e.g.*, polycarboxylates and PVP) 0% to about 3%; enzymes (calculated as pure enzyme protein) 0.0001-0.1%; and minor ingredients (*e.g.*, optical brightener, phosphonate, perfume) 0-3%.

[00241] 16) Detergent formulations as described in 1)-15) *supra*, which contain a stabilized or encapsulated peracid, either as an additional component or as a substitute for already specified bleach systems.

[00242] 17) Detergent compositions as described *supra* in 1), 3), 7), 9), and 12), wherein perborate is replaced by percarbonate.

[00243] 18) Detergent compositions as described supra in 1), 3), 7), 9), 12), 14), and 15), which additionally contain a manganese catalyst. The manganese catalyst for example is one of the compounds described in Hage *et al.*, *Nature* 369: 637-639 (1994).

[00244] 19) Detergent composition formulated as a non-aqueous detergent liquid comprising a liquid nonionic surfactant such as, *e.g.*, linear alkoxylated primary alcohol, a builder system (*e.g.*, phosphate), an enzyme(s), and alkali. The detergent may also comprise anionic surfactant and/or a bleach system.

[00245] One or more of the present α -amylases may be incorporated in concentrations conventionally employed in detergents. It is at present contemplated that, in the detergent composition, an α -amylase or variant thereof, may be added in an amount corresponding to 0.00001-1.0 mg (calculated as pure enzyme protein) of enzyme per liter of wash liquor.

[00246] In another embodiment, a 2,6- β -D-fructan hydrolase can be incorporated in detergent compositions and used for removal/cleaning of biofilm present on household and/or industrial textile/laundry.

[00247] The detergent composition may for example be formulated as a hand or machine laundry detergent composition, including a laundry additive composition suitable for pre-treatment of stained fabrics and a rinse added fabric softener composition, or be formulated as a detergent composition for use in general household hard surface cleaning operations, or be formulated for manual or machine laundry operations.

[00248] In a specific aspect, the detergent composition can further comprise 2,6- β -D-fructan hydrolase, one or more additional α -amylases in addition to one or more of the present α -amylase variants, and one or more other cleaning enzymes, such as a protease, a lipase, a cutinase, a carbohydrase, a cellulase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, an oxidase, a laccase, and/or a peroxidase, and/or combinations thereof.

[00249] In general the properties of the chosen enzyme(s) should be compatible with the selected detergent, (*e.g.*, pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, *etc.*), and the enzyme(s) should be present in effective amounts. In some embodiments, the amylase and protease are co-formulated (*i.e.*, formulated together in the same liquid, granule, or the like. Alternatively, the amylase and protease are formulated as separate liquids or granules to be later combined into a cleaning composition.

4.2 Dishwash Detergent Compositions

[00250] In another embodiment, dishwashing detergent compositions are provided, including the following:

[00251] 1) POWDER AUTOMATIC DISHWASHING COMPOSITION

Nonionic surfactant	0.4-2.5%
Sodium metasilicate	0-20%
Sodium disilicate	3-20%
Sodium triphosphate	20-40%
Sodium carbonate	0-20%
Sodium perborate	2-9%
Tetraacetyl ethylene diamine (TAED)	1-4%
Sodium sulphate	5-33%
Enzymes	0.0001-0.1%

[00252] 2) POWDER AUTOMATIC DISHWASHING COMPOSITION

	Nonionic surfactant	1-2%
	(e.g. alcohol ethoxylate)	
	Sodium disilicate	2-30%
5	Sodium carbonate	10-50%
	Sodium phosphonate	0-5%
	Trisodium citrate dihydrate	9-30%
	Nitrilotrisodium acetate (NTA)	0-20%
	Sodium perborate monohydrate	5-10%
10	Tetraacetyl ethylene diamine (TAED)	1-2%
	Polyacrylate polymer	6-25%
	(e.g. maleic acid/acrylic acid copolymer)	
	Enzymes	0.0001-0.1%
	Perfume	0.1-0.5%
15	Water	5-10 %

[00253] 3) POWDER AUTOMATIC DISHWASHING COMPOSITION

	Nonionic surfactant	0.5-2.0%
	Sodium disilicate	25-40%
20	Sodium citrate	30-55%
	Sodium carbonate	0-29%
	Sodium bicarbonate	0-20%
	Sodium perborate monohydrate	0-15%
	Tetraacetyl ethylene diamine (TAED)	0-6%
25	Maleic acid/acrylic acid copolymer	0-5%
	Clay	1-3%
	Polyamino acids	0-20%
	Sodium polyacrylate	0-8%
	Enzymes	0.0001-0.1%
30		

[00254] 4) POWDER AUTOMATIC DISHWASHING COMPOSITION

	Nonionic surfactant	1-2%
	Zeolite MAP	15-42%
	Sodium disilicate	30-34%
35	Sodium citrate	0-12%
	Sodium carbonate	0-20%
	Sodium perborate monohydrate	7-15%
	Tetraacetyl ethylene	0-3%
	diamine (TAED) Polymer	0-4%
40	Maleic acid/acrylic acid copolymer	0-5%
	Organic phosphonate	0-4%
	Clay	1-2%
	Enzymes	0.0001-0.1%
45	Sodium sulphate	Balance

[00255] 5) POWDER AUTOMATIC DISHWASHING COMPOSITION

	Nonionic surfactant	1-7%
	Sodium disilicate	18-30%
	Trisodium citrate	10-24%
50	Sodium carbonate	12-20%
	Monopersulphate	15-21%
	(2 KHSO ₅ .KHSO ₄ .K ₂ SO ₄)	
	Bleach stabilizer	0.1-2%

	Maleic acid/acrylic acid copolymer	0-6%
	Diethylene triamine pentaacetate, pentasodium salt	0-2.5%
	Enzymes	0.0001-0.1%
5	Sodium sulphate, water	Balance

[00256] 6) POWDER AND LIQUID DISHWASHING COMPOSITION WITH CLEANING SURFACTANT SYSTEM

	Nonionic surfactant	0-1.5%
10	Octadecyl dimethylamine N-oxide dihydrate	0-5%
	80:20 wt. C18/C16 blend of octadecyl dimethylamine N-oxide dihydrate and hexadecyldimethyl amine N-oxide dihydrate	0-4%
15	70:30 wt. C18/C16 blend of octadecyl bis (hydroxyethyl)amine N-oxide anhydrous and hexadecyl bis (hydroxyethyl)amine N-oxide anhydrous	0-5%
	C ₁₃ -C ₁₅ alkyl ethoxysulfate with an average degree of ethoxylation of 3	0-10%
20	C ₁₂ -C ₁₅ alkyl ethoxysulfate with an average degree of ethoxylation of 3	0-5%
	C ₁₃ -C ₁₅ ethoxylated alcohol with an average degree of ethoxylation of 12	0-5%
25	A blend of C ₁₂ -C ₁₅ ethoxylated alcohols with an average degree of ethoxylation of 9	0-6.5%
	A blend of C ₁₃ -C ₁₅ ethoxylated alcohols with an average degree of ethoxylation of 30	0-4%
	Sodium disilicate	0-33%
	Sodium tripolyphosphate	0-46%
30	Sodium citrate	0-28%
	Citric acid	0-29%
	Sodium carbonate	0-20%
	Sodium perborate monohydrate	0-11.5%
	Tetraacetyl ethylene diamine (TAED)	0-4%
35	Maleic acid/acrylic acid copolymer	0-7.5%
	Sodium sulphate	0-12.5%
	Enzymes	0.0001-0.1%

[00257] 7) NON-AQUEOUS LIQUID AUTOMATIC DISHWASHING COMPOSITION

40	Liquid nonionic surfactant (<i>e.g.</i> alcohol ethoxylates)	2.0-10.0%
	Alkali metal silicate	3.0-15.0%
	Alkali metal phosphate	20.0-40.0%
	Liquid carrier selected from higher glycols, polyglycols, polyoxides, glycolethers	25.0-45.0%
45	Stabilizer (<i>e.g.</i> a partial ester of phosphoric acid and a C ₁₆ -C ₁₈ alkanol)	0.5-7.0%
	Foam suppressor (<i>e.g.</i> silicone)	0-1.5%
	Enzymes	0.0001-0.1%

[00258] 8) NON-AQUEOUS LIQUID DISHWASHING COMPOSITION

	Liquid nonionic surfactant (<i>e.g.</i> alcohol ethoxylates)	2.0-10.0%
	Sodium silicate	3.0-15.0%
	Alkali metal carbonate	7.0-20.0%

	Sodium citrate	0.0-1.5%
	Stabilizing system (<i>e.g.</i> mixtures of finely divided silicone and low molecular weight dialkyl polyglycol ethers)	0.5-7.0%
	Low molecule weight polyacrylate polymer	5.0-15.0%
5	Clay gel thickener (<i>e.g.</i> bentonite)	0.0-10.0%
	Hydroxypropyl cellulose polymer	0.0-0.6%
	Enzymes 0.0001-0.1%	
	Liquid carrier selected from higher lycols, polyglycols, polyoxides and glycol ethers	Balance

10

[00259] 9) THIXOTROPIC LIQUID AUTOMATIC DISHWASHING COMPOSITION

	C ₁₂ -C ₁₄ fatty acid	0-0.5%
	Block co-polymer surfactant	1.5-15.0%
	Sodium citrate	0-12%
15	Sodium tripolyphosphate	0-15%
	Sodium carbonate	0-8%
	Aluminium tristearate	0-0.1%
	Sodium cumene sulphonate	0-1.7%
	Polyacrylate thickener	1.32-2.5%
20	Sodium polyacrylate	2.4-6.0%
	Boric acid	0-4.0%
	Sodium formate	0-0.45%
	Calcium formate	0-0.2%
	Sodium n-decyldiphenyl oxide disulphonate	0-4.0%
25	Monoethanol amine (MEA)	0-1.86%
	Sodium hydroxide (50%)	1.9-9.3%
	1,2-Propanediol	0-9.4%
	Enzymes 0.0001-0.1%	
	Suds suppressor, dye, perfumes, water	Balance

30

[00260] 10) LIQUID AUTOMATIC DISHWASHING COMPOSITION

	Alcohol ethoxylate	0-20%
	Fatty acid ester sulphonate	0-30%
	Sodium dodecyl sulphate	0-20%
35	Alkyl polyglycoside	0-21%
	Oleic acid	0-10%
	Sodium disilicate monohydrate	18-33%
	Sodium citrate dihydrate	18-33%
	Sodium stearate	0-2.5%
40	Sodium perborate monohydrate	0-13%
	Tetraacetyl ethylene diamine (TAED)	0-8%
	Maleic acid/acrylic acid copolymer	4-8%
	Enzymes 0.0001-0.1%	
	Water	Balance

45

[00261] 11) LIQUID AUTOMATIC DISHWASHING COMPOSITION CONTAINING PROTECTED BLEACH PARTICLES

	Sodium silicate	5-10%
	Tetrapotassium pyrophosphate	15-25%
50	Sodium triphosphate	0-2%
	Potassium carbonate	4-8%
	Protected bleach particles, <i>e.g.</i> chlorine	5-10%
	Polymeric thickener	0.7-1.5%

Potassium hydroxide	0-2%
Enzymes	0.0001-0.1%
Water	Balance

5 [00262] 11) Automatic dishwashing compositions as described in 1), 2), 3), 4), 6) and 10), wherein perborate is replaced by percarbonate.

[00263] 12) Automatic dishwashing compositions as described in 1)-6) which additionally contain a manganese catalyst. The manganese catalyst may, *e.g.*, be one of the compounds described in Hage *et al.*, *Nature* 369: 637-639 (1994).

10 5. Methods

5.1 Filter Screening Assays

[00264] The assays discussed below may be used in the screening of the present α -amylases to identify variants having altered stability at high or low pH and/or under Ca^{2+} depleted conditions compared to a parent or reference α -amylase.

15 5.2 High pH Filter Assay

[00265] *Bacillus* libraries are plated on a sandwich of cellulose acetate (OE 67, Schleicher & Schuell, Dassel, Germany) and nitrocellulose filters (Protran-Ba 85, Schleicher & Schuell, Dassel, Germany) on TY agar plates with 10 $\mu\text{g/ml}$ kanamycin at 37°C for at least 21 hours. The cellulose acetate layer is located on the TY agar plate.

20 [00266] Each filter sandwich is specifically marked with a needle after plating, but before incubation in order to be able to localize positive variants on the filter and the nitrocellulose filter with bound variants is transferred to a container with glycine-NaOH buffer, pH 8.6-10.6 and incubated at room temperature (can be altered from 10-60°C) for 15 min. The cellulose acetate filters with colonies are stored on the TY-plates at room temperature until use. After incubation, residual activity is detected on plates containing 1% agarose, 25 0.2% starch in glycine-NaOH buffer, pH 8.6-10.6. The assay plates with nitrocellulose filters are marked the same way as the filter sandwich and incubated for 2 hours at room temperature. After removal of the filters the assay plates are stained with 10% Lugol solution. Starch degrading variants are detected as white spots on dark blue background and then identified on the storage plates. Positive variants are rescreened twice under the same conditions as the first screen.

30 5.3 Low Calcium Filter Assay

[00267] *Bacillus* libraries are plated on a sandwich of cellulose acetate (OE 67, Schleicher & Schuell, Dassel, Germany) and nitrocellulose filters (Protran-Ba 85, Schleicher & Schuell, Dassel, Germany) on TY agar plates with a relevant antibiotic, *e.g.*, kanamycin or neomycin, at 37°C for at least 21 hours. The cellulose-acetate layer is located on the TY agar plate.

[00268] Each filter sandwich is specifically marked with a needle after plating, but before incubation in order to be able to localize positive variants on the filter and the nitrocellulose filter with bound variants is transferred to a container with carbonate/bicarbonate buffer pH 8.5-10 and with different EDTA concentrations (0.001 mM-100 mM). The filters are incubated at room temperature for 1 hour. The cellulose acetate filters with colonies are stored on the TY-plates at room temperature until use. After incubation, residual activity is detected on plates containing 1% agarose, 0.2% starch in carbonate/bicarbonate buffer pH 8.5-10. The assay plates with nitrocellulose filters are marked the same way as the filter sandwich and incubated for 2 hours at room temperature. After removal of the filters the assay plates are stained with 10% Lugol solution. Starch degrading variants are detected as white spots on dark blue background and then identified on the storage plates. Positive variants are rescreened twice under the same conditions as the first screen.

5.4 Low pH Filter Assay

[00269] *Bacillus* libraries are plated on a sandwich of cellulose acetate (OE 67, Schleicher & Schuell, Dassel, Germany) and nitrocellulose filters (Protran-Ba 85, Schleicher & Schuell, Dassel Germany) on TY agar plates with 10 micro g/ml neomycin at 37°C for at least 21 hours. The cellulose acetate layer is located on the TY agar plate.

[00270] Each filter sandwich is specifically marked with a needle after plating, but before incubation in order to be able to localize positive variants on the filter, and the nitrocellulose filter with bound variants is transferred to a container with citrate buffer, pH 4.5 and incubated at 80°C for 20 minutes (when screening for variants in the wild type backbone) or 85°C for 60 minutes (when screening for variants of the parent α -amylase). The cellulose acetate filters with colonies are stored on the TY-plates at room temperature until use. After incubation, residual activity is detected on assay plates containing 1% agarose, 0.2% starch in citrate buffer, pH 6.0. The assay plates with nitrocellulose filters are marked the same way as the filter sandwich and incubated for 2 hours at 50°C. After removal of the filters the assay plates are stained with 10% Lugol solution. Starch degrading variants are detected as white spots on dark blue background and then identified on the storage plates. Positive variants are re-screened twice under the same conditions as the first screen.

5.5 Secondary Screening

[00271] Positive transformants after rescreening are picked from the storage plate and tested in a secondary plate assay. Positive transformants are grown for 22 hours at 37°C in 5 ml LB+neomycin. The *Bacillus* culture of each positive transformant and as a control a clone expressing the corresponding backbone are incubated in citrate buffer, pH 4.5 at 90°C and samples are taken at 0, 10, 20, 30, 40, 60 and 80 minutes. A 3 micro liter sample is spotted on an assay plate. The assay plate is stained with 10% Lugol solution. Improved variants are seen as variants with higher residual activity (detected as halos on the assay plate) than the backbone. The improved variants are determined by nucleotide sequencing.

5.6 Stability Assay of Unpurified Variants

[00272] The stability of the variants may be assayed as follows: *Bacillus* cultures expressing the variants to be analyzed are grown for 21 hours at 37°C in 10 ml LB+neomycin. 800 micro liter culture is mixed with 200 µL citrate buffer, pH 4.5. A number of 70 µL aliquots corresponding to the number of sample time points are made in PCR tubes and incubated at 70°C or 90°C for various time points (typically 5, 10, 15, 20, 25 and 30 minutes) in a PCR machine. The 0 min sample is not incubated at high temperature. Activity in the sample is measured by transferring 20 µL to 200 µL of the α-amylase PNP-G₇ substrate MPR3 ((Boehringer Mannheim Cat. no. 1660730) as described below under "Assays for Alpha-amylase Activity". Results are plotted as percentage activity (relative to the 0 time point) versus time, or stated as percentage residual activity after incubation for a certain period of time.

5.7 Fermentation and Purification of α-Amylase Variants

[00273] A *B. subtilis* strain harboring the relevant expression plasmid may be fermented and purified as follows: The strain is streaked on a LB-agar plate with 10 µg/ml kanamycin from -80°C stock, and grown overnight at 37°C. The colonies are transferred to 100 ml PS-1 media supplemented with 10 µg/ml neomycin in a 500 ml shaking flask.

Composition of PS-1 medium:

Pearl sugar	100 g/l
Soy Bean Meal	40 g/l
Na ₂ HPO ₄ , 12 H ₂ O	10 g/l
Pluronic TM PE 6100	0.1 g/l
CaCO ₃	5 g/l

[00274] The culture is shaken at 37°C at 270 rpm for 5 days. Cells and cell debris are removed from the fermentation broth by centrifugation at 4500 rpm in 20-25 minutes. Afterwards the supernatant is filtered to obtain a completely clear solution. The filtrate is concentrated and washed on a UF-filter (10,000 MW cut off membrane) and the buffer is changed to 20 mM Acetate pH 5.5. The UF-filtrate is applied on a S-sepharose F.F. and elution is carried out by step elution with 0.2M NaCl in the same buffer. The eluate is dialysed against 10 mM Tris, pH 9.0 and applied on a Q-sepharose F.F. and eluted with a linear gradient from 0-0.3 M NaCl over 6 column volumes. The fractions that contain the activity (measured by the Phadebas assay) are pooled, pH was adjusted to pH 7.5 and remaining color was removed by a treatment with 0.5% W/vol. active charcoal in 5 minutes.

5.8 Specific Activity Determination

[00275] The specific activity is determined using the PHADEBAS® assay (Pharmacia) as activity/mg enzyme. The manufacturer's instructions are followed (see also below under "Assay for Alpha-amylase Activity").

5.9 Determination of Isoelectric Point

[00276] The pI is determined by isoelectric focusing (ex: Pharmacia, Ampholine, pH 3.5-9.3).

5.10 Accelerated Stability Assay

[00277] In 50 ml Propylene tubes, 10 ml of detergent of interest was added. Appropriate dilutions were made so that 180 ppm of each α -amylase was measured with a pipette into separate tubes containing the detergent. The detergent with each α -amylase was vortex for 30 sec and then placed on a RotaMix (ATR RKVS Model) for 10 minutes. 100 μ L of the detergent with the mutant enzyme were measured with a pipette and diluted 1:651. The initial activity of the mutants was assayed using Blocked P-Nitro-Phenyl-Maltoheptaose (Blocked PBNPG7) substrate on a Konelab, Model 20XT. The detergent samples were then incubated in a constant temperature incubator set at 37°C. Samples were removed at 1, 2, 4, 7 and 17 days and the enzyme activity assayed.

5.11 Assays for α -Amylase Activity

5.11.1 Phadebas Assay

[00278] α -Amylase activity is determined by a method employing PHADEBAS® tablets as substrate. Phadebas tablets (PHADEBAS® Amylase Test, supplied by Pharmacia Diagnostic) contain a cross-linked insoluble blue-colored starch polymer, which has been mixed with bovine serum albumin and a buffer substance and tabletted.

[00279] For every single measurement one tablet is suspended in a tube containing 5 ml 50 mM Britton-Robinson buffer (50 mM acetic acid, 50 mM phosphoric add, 50 mM boric acid, 0.1 mM CaCl_2 , pH adjusted to the value of interest with NaOH). The test is performed in a water bath at the temperature of interest. The α -amylase to be tested is diluted in x ml of 50 mM Britton-Robinson buffer. 1 ml of this α -amylase solution is added to the 5 ml 50 mM Britton-Robinson buffer. The starch is hydrolyzed by the α -amylase giving soluble blue fragments. The absorbance of the resulting blue solution, measured spectrophotometrically at 620 nm, is a function of the α -amylase activity.

[00280] It is important that the measured 620 nm absorbance after 10 or 15 minutes of incubation (testing time) is in the range of 0.2 to 2.0 absorbance units at 620 nm. In this absorbance range there is linearity between activity and absorbance (Lambert-Beer law). The dilution of the enzyme must therefore be adjusted to fit this criterion. Under a specified set of conditions (temp., pH, reaction time, buffer conditions) 1 mg of a given α -amylase will hydrolyze a certain amount of substrate and a blue color will be produced. The color intensity is measured at 620 nm. The measured absorbance is directly proportional to the specific activity (activity/mg of pure α -amylase protein) of the α -amylase in question under the given set of conditions.

5.11.2 Alternative Method

[00281] α -Amylase activity is determined by a method employing p-nitrophenyl- α -D-maltoheptaoside (PNP-G₇) substrate, which is a blocked oligosaccharide that can be cleaved by an endo-amylase. Following the cleavage, the α -glucosidase included in the kit digests the substrate to liberate a free PNP molecule which has a yellow color and thus can be measured by visible spectrophotometry at $\lambda=405$ nm (400-420 nm). Kits containing PNP-G₇ substrate and α -glucosidase are manufactured by Boehringer-Mannheim (cat. No.1054635).

[00282] To prepare the reagent solution 10 ml of substrate/buffer solution is added to 50 ml enzyme/buffer solution as recommended by the manufacturer. The assay is performed by transferring a 20 μ L sample to a 96 well microtitre plate and incubating at 25°C. 200 μ L reagent solution pre-equilibrated to 25°C is added. The solution is mixed and pre-incubated 1 minute and absorption is measured every 30 seconds over 4 minutes at OD 405 nm in an ELISA reader.

[00283] The slope of the time dependent absorption-curve is directly proportional to the activity of the α -amylase in question under the given set of conditions.

5.12 Determination of enzyme Performance in Detergent Compositions

5.12.1 US Conditions

[00284] A Terg-o-tometer (United States Testing, Hoboken, N.J.) was used to simulate washing conditions typical in the U.S. A dose efficiency curve (DEC) was conducted at 20°C using standard detergents such as Liquid AATCC 2003 Without Optical Brightener and/or Powder AATCC 1993 (American Association of Textile Chemists and Colorists). A corresponding DEC of a comparative α -amylase was then conducted to compare the stain removal performance of the inventive mutant enzyme. This process was repeated at 40°C. Typically, 4 swatches of CS-28 Rice Starch stain (CFT of Holland) were placed in a steel container of the Terg-o-tometer, which was filled with 1 Liter of DI water and 1.5 g of Liquid AATCC. When Powder AATCC was used, 1.5g of the detergent powder was weighed out on an analytical balance (Model PM4800, Mettler Instrument Corp., Highstown, N.J. 08520 and added to the Terg-o-tometer. Two replicates were run at the same time. Unless otherwise stated, the tests were carried out for 12 minutes and rinsed for 3 minutes. After washing, the swatches were air-dried and the reflectance of the test swatches was measured with a Chroma Meter Model CR-410 manufactured by Konica Minolta. The data collected were treated with appropriate statistical analysis.

5.12.2 European Conditions

[00285] A Launder-O-meter (Atlas Company, Atlanta, Georgia) was used to simulate washing conditions typical in Europe. A dose efficiency curve (DEC) of the mutant enzyme of interest was conducted at 40°C using standard European testing detergents, IEC A and IEC A with Bleach (TAED-Tetra-Acetyl-ethylene-diamine acetate) and Sodium Perborate. A corresponding DEC curve of a comparative mutant enzyme was then conducted to compare the stain removal performance of the inventive mutant enzyme. This process was

repeated at higher wash temperature if desirable. Typically, 4 swatches of EMPA 161, Maize starch (EMPA, Switzerland) were placed in a steel container with 250 ml of DI water containing 6.8g/L of the IEC A detergent or 8.0g/L of the IEC A with Bleach detergent. Two replicates were run at the same time. Unless otherwise stated the tests were carried out for 45 minutes and rinsed for 5 minutes. After washing, the swatches were air-dried and the reflectance of the test swatches was measured with a Chroma Meter Model CR-410. The data collected were treated with appropriate statistical analysis.

5.12.3 Microswatch Method of Assessing Detergent Compositions

[00286] Numerous α -amylase and protease cleaning assays are known in the art. Exemplary cleaning assays involve swatches, which are pieces of material, such as a fabric, to which a stain may be applied. The material can be, for example, fabrics made of cotton, polyester or mixtures of natural and synthetic fibers. The swatch can further be paper, such as filter paper or nitrocellulose, or a piece of a hard material such as ceramic, metal, or glass. For amylases, the stain is starch based, but can include blood, milk, ink, grass, tea, wine, spinach, gravy, chocolate, egg, cheese, clay, pigment, oil, or mixtures of these compounds.

[00287] Some assays may utilize a smaller portion of a larger swatch that has been cut with a single-hole punch device, or has been cut with a custom manufactured 96-hole punch device, where the pattern of the multi-hole punch is matched to standard 96-well microtiter plates, or the section has been otherwise removed from the swatch. The swatch can be of textile, paper, metal, or other suitable material. The smaller swatch can have the stain affixed either before or after it is placed into the well of a 24-, 48- or 96-well microtiter plate. Smaller swatches can also be made by applying a stain to a small piece of material. For example, a smaller swatch can be a stained piece of fabric 5/8" or 0.25" in diameter. The custom manufactured punch is designed in such a manner that it delivers 96 swatches simultaneously to all wells of a 96-well plate. The device allows delivery of more than one swatch per well by simply loading the same 96-well plate multiple times. Multi-hole punch devices can be conceived of to deliver simultaneously swatches to any format plate, including but not limited to 24-well, 48-well, and 96-well plates. In another conceivable method, the soiled test platform can be a bead made of either metal, plastic, glass, ceramic, or other suitable material that is coated with the soil substrate for use in testing cleaning compositions for materials other than textiles. The one or more coated beads are then placed into wells of 96-, 48-, or 24- well plates or larger formats, containing suitable buffer and enzyme. In this case, supernatant can be examined for released soil either by direct absorbance measurement or after a secondary color development reaction. Analysis of the released soil might also be taken by mass spectral analysis. A further microscreening assay can be to deliver and secure a swatch, for example an indigo dyed denim, to a well of a multi-well plate, and add particles such as sand or larger particles such as for example garnet sieved to include particle 6 to 8, or 9 gauge, and agitate the plate so as to cause abrasion of the swatch by the added particles. This assay has found use in the assessment of cellulases in stone washing applications. The effectiveness of the enzyme can be judged by either color release (*e.g.*, released indigo is dissolved in dimethylsulfoxide and absorbance at A_{600} nm is measured) to the reaction buffer or by reflectance measurements of the abraded swatch.

[00288] When, for example, untreated BMI (blood/milk/ink) swatches are washed in detergent without bleach, a large portion of the ink is released even without the help of a protease. Adding a protease leads to a small increase in ink release, which can be hard to quantify over the large background. One aspect provides a treatment protocol that allows one to control the degree of fixation of a stain. As a result, it is possible to produce swatches that, for example, release varying amounts of stain when washed in the absence of the enzyme being tested. The use of fixed swatches leads to a dramatic improvement of the signal-to-noise ratio in the wash assays. Furthermore, by varying the degree of fixation, one can generate stains that give optimum results under the various cleaning conditions.

[00289] Swatches having stains of known "strength" on various types of material are commercially available (EMPA, St. Gallen, Switzerland; wfk--Testgewebe GmbH, Krefeld Germany; or Center for Test Materials, Vlaardingen, The Netherlands) and/or can be made by the practitioner (Morris and Prato, *Textile Research Journal* 52(4): 280-286 (1982)). Other test swatches include but are not limited to blood/milk/ink (BMI) stain(s) on a cotton-containing fabric, a spinach stain on a cotton-containing fabric, or grass on a cotton-containing fabric, and chocolate/milk/soot on a cotton-containing fabric.

[00290] A BMI stain can be fixed to cotton with 0.0003% to 0.3% hydrogen peroxide. Other combinations include grass or spinach fixed with 0.001% to 1% glutaraldehyde, gelatin and Coomassie Brilliant Blue stain fixed with 0.001% to 1% glutaraldehyde, or chocolate, milk and soot fixed with 0.001% to 1% glutaraldehyde.

[00291] The swatch can also be agitated during incubation with the enzyme and/or detergent formulation. Wash performance data is dependent on the orientation of the swatches in the wells (horizontal versus vertical), particularly in the 96-well plate. This would indicate that mixing was insufficient during the incubation period. Although there are a number of ways to ensure sufficient agitation during incubation, a plate holder in which the microtiter plate is sandwiched between two plates of aluminum can be constructed. This can be as simple as placing, for example, an adhesive plate sealer over the wells then clamping the two aluminum plates to the 96-well plate with any type of appropriate, commercially available clamps. It can then be mounted in a commercial incubator shaker. Setting the shaker to about 400 rpm results in very efficient mixing, while leakage or cross-contamination is efficiently prevented by the holder.

[00292] Trinitrobenzenesulfonic acid (TNBS) can be used to quantify the concentration of amino groups in the wash liquor. This can serve as a measure of the amount of protein that was removed from the swatch (see e.g., Cayot and Tainturier, *Anal. Biochem.* 249: 184-200 (1997)). However, if a detergent or an enzyme sample leads to the formation of unusually small peptide fragments (for example, from the presence of peptidases in the sample), then one will obtain a larger TNBS signal, i.e., more "noise".

[00293] Another means of measuring wash performance of blood/milk/ink or other stain that is based on ink release. Proteolysis of protein on the swatches leads to the release of ink particles that can be quantified by measuring the absorbance of the wash liquor. The absorbance can be measured at any wavelength between 350 and 800 nm. The wavelength is measured at 410 nm or 620 nm. The wash liquor can also be examined to determine the wash performance on stains containing grass, spinach, gelatin or Coomassie Brilliant Blue stain. Exemplary wavelengths for these stains include and 670 nm for spinach or grass and 620 nm for gelatin

or Coomassie Brilliant Blue. For example, an aliquot of the wash liquor (typically 100 to 150 μ L from a 96-well microplate, for example) is removed and placed in a cuvette or multiwell microplate. This is then placed in a spectrophotometer and the absorbance is read at an appropriate wavelength.

[00294] The system can also be used to determine an enhanced enzyme and/or detergent composition for dishwashing, for example, using a blood/milk/ink stain on a suitable substrate such as cloth, plastic or ceramic.

[00295] In one aspect, the a BMI stain is fixed to cotton by applying 0.3% hydrogen peroxide to the BMI/cotton swatch for 30 minutes at 25°C or by applying 0.03% hydrogen peroxide to the BMI/cotton swatch for 30 minutes at 60°C. Smaller swatches of approximately 0.25" are cut from the BMI/cotton swatch and placed in the wells of a 96-well microtiter plate. Into each well, a known mixture of a detergent composition and an enzyme such as a variant protein is placed. After placing an adhesive plate sealer onto the top of the microtiter plate, the microtiter plate is clamped to an aluminum plate and agitated on an orbital shaker at approximately 250 rpm for about 10 to 60 minutes. At the end of this time, the supernatants are transferred to wells in a new microtiter plate and the absorbance of the ink at 620 nm is measured. This can be similarly tested with spinach stains or grass stains fixed to cotton by applying 0.01% glutaraldehyde to the spinach/cotton swatch or grass/cotton swatch for 30 minutes at 25°C. The same can be performed with chocolate, milk, and/or soot stains. Additional blood/milk/ink assays and conditions are provided in U.S. Patent No. 7,122,334 (Genencor International, Inc.).

5.13 Determination of LAS Sensitivity

[00296] The variant is incubated with different concentrations of LAS (linear alkyl benzene sulphonate; Nansa 1169/P) for 10 minutes at 40°C. The residual activity is determined using the Phadebas® assay method or the alternative method employing the PNP-G₇ substrate. LAS is diluted in 0.1 M phosphate buffer pH 7.5. The following concentrations are used: 500 ppm, 250 ppm, 100 ppm, 50 ppm, 25 ppm, and 10 ppm or no LAS.

[00297] The variant is diluted in the different LAS buffers to concentration of 0.01-5 mg/l in a total volume of 10 ml and incubated for 10 minutes in a temperature controlled water bath. The incubation is stopped by transferring a small aliquot into cold assay buffer. It is important that during activity measurement the LAS concentration is below 1 ppm, in order not to affect the activity measurement.

[00298] Then the residual activity is determined in duplicate using the above mentioned PHADEBAS® assay or alternative method. The activity is measured after subtraction of the blank. The activity with no LAS is 100%.

[00299] The present application is organized into a number of sections for ease of reading; however, the reader will appreciate that statements made in one section may apply to other sections. In this manner, the headings used for different sections of the disclosure should not be construed as limiting.

[00300] In order to further illustrate the present compositions and methods and advantages thereof, the following specific examples are given with the understanding that they are being offered to illustrate the present compositions and methods and should not be construed in any way as limiting its scope.

EXPERIMENTAL

[00301] The present disclosure is described in further detail in the following examples, which are not in any way intended to limit the scope of the disclosure as claimed.

[00302] The following abbreviations apply throughout the disclosure: AE (alcohol ethoxylate); AEO (alcohol ethoxylate); AEOS (alcohol ethoxysulfate); AES (alcohol ethoxysulfate); AFAU (acid fungal α -amylase units); AGU (glucoamylase activity unit); AOS (α -olefinsulfonate); AS (alcohol sulfate); BAA (*Bacillus amyloliquefaciens* α -amylase); BLA (*Bacillus licheniformis* or LAT); BPNPG7 (p-nitrophenyl maltoheptaoside); BSA (bovine serum albumin); cDNA (complementary DNA); CMC (carboxymethylcellulose); DNA (deoxyribonucleic acid); DP3 (degree of polymerization with three subunits); DPn (degree of polymerization with n subunits); DTMPA (diethyltriaminepentaacetic acid); EC (enzyme commission for enzyme classification); EDTA (ethylenediaminetetraacetic acid); EO (ethylene oxide); F&HC (fabric and household care); FAU (fungal amylase unit); GA (glucoamylase); gpg (grains per gallon); HFCS (high fructose corn syrup); HFSS (high fructose starch based syrup); IPTG (isopropyl β -D-1-thiogalactopyranoside); LAS (linear alkylbenzenesulfonate); LOM (Launder-O-meter); LU (Liquiphon unit); MTP (microtiter plate); MW (molecular weight); MWU (modified Wohlgemuth unit); NOBS (nonanoyloxybenzenesulfonate); NTA (nitrilotriacetic acid); PCR (polymerase chain reaction); PEG (polyethyleneglycol); PI (performance index); PVA (poly(vinyl alcohol); PVP (poly(vinylpyrrolidone)); RNA (ribonucleic acid); SAS (secondary alkane sulfonates); SEL (site evaluation library); TAED (tetraacetylenediamine); TCA (trichloroacetic acid); TSB (tryptic soy broth); UFC (ultrafiltration concentrate); °C (degrees Centigrade); H₂O (water); dH₂O or DI (deionized water); dIH₂O (deionized water, Milli-Q filtration); ETOH (ethanol); eq (equivalents); N (normal); DS (dry solids); g or gm (grams); μ g (micrograms); mg (milligrams); kg (kilograms); μ L and μ l (microliters); mL and ml (milliliters); mm (millimeters); μ m (micrometer); M (molar); mM (millimolar); μ M (micromolar); U (units); sec (seconds); min(s) (minute/minutes); hr(s) (hour/hours); DO (dissolved oxygen); WT% (weight percent); W/V (weight to volume); W/W (weight to weight); V/V (volume to volume); GENEART (GENEART GmbH, Regensburg, Germany); and Genencor (Danisco US Inc, Genencor Division, Palo Alto, CA).

EXAMPLE 1**Assays**

[00303] In the following examples, various assays were used as set forth below for ease in reading. Any deviations from the protocols provided below are indicated. In these experiments, a spectrophotometer was used to measure the absorbance of the products formed after the completion of the reactions.

A. Protein Content Assay

[00304] This assay was performed using filtered culture supernatant from microtiter plates (MTPs) grown 3 days at 37°C with shaking at 300 rpm and 80% humidity. A fresh 96-well flat bottom MTP containing 50µl supernatant per well was used for the protein assay by a High Performance Liquid Chromatography method. Supernatants were diluted three fold into 10 mM potassium phosphate buffer pH 7.25 containing 5% acetonitrile and 10% sodium chloride and 10µl of each diluted sample was analyzed. An Agilent 1100 (Hewlett Packard) HPLC equipped with a Swift™ RP-all PN 68-1030-041 (Teledyne Isco, Inc.) column was used. The solvent system consisted of 0.1% trifluoroacetic acid in aqueous phase and 0.07% trifluoroacetic acid in acetonitrile. Absorbance was read at 222 nm and protein concentration of samples was determined based on a standard curve of purified BASE (AmyTS23t) protein.

B. Ceralpha Amylase Assay

[00305] The principle of this α -amylase assay is based on the hydrolysis of a defined oligosaccharide (BPNPG7) in the presence of excess levels of a thermostable α -glucosidase to glucose and free p-nitrophenol. The absorbance at 400 nm is measured and this relates directly to the level of active amylase in the sample analysed.

[00306] The equipment used was a Biomek FX Robot (Beckman Coulter); a SpectraMAX MTP Reader (type 340-Molecular Devices) and iEMS incubator/shaker (Thermo/Labsystems). In this assay system, the reagent and solutions used were:

- 1) p-nitrophenyl maltoheptaoside (BPNPG7) substrate (Megazyme HR kit);
- 2) 50 mM MOPS, 50 mM NaCl, 0.1 mM CaCl₂, 0.005% TWEEN® 80 buffer, pH 7.15; and
- 3) 200 mM Boric acid / NaOH buffer, pH 10.2 (STOP buffer).

[00307] A vial containing 54.5 mg BPNPG7 substrate was dissolved in 10 ml of milliQ water. The amylase samples (fermentation supernatant) were diluted in MOPS buffer. The assay was performed by adding 25µl of diluted amylase solution into the wells of a MTP followed by the addition of 25µl 5.45 mg/ml BPNPG7 substrate solution. The solutions were mixed and the microtiter plate was sealed with a plate seal and placed in an incubator/shaker (iEMS- Thermo/Labsystems) for 30 minutes at 25°C and 900 rpm. The reaction was terminated by adding 50µl STOP buffer and the absorbance was read at wavelength 400 nm in an MTP-Reader. A non-enzyme control was used to correct for background absorbance values.

C. CS-28 Rice Starch Microswatch Assay

[00308] The principle of this α -amylase assay is based on the liberation of an orange-dye due to the hydrolysis of rice starch incorporated in a microswatch. The absorbance at 488 nm is measured and this relates to the level of amylase activity in the sample analysed, at the desired conditions (pH, temperature, and buffer).

[00309] The equipment used was a Biomek FX Robot (Beckman Coulter), a SpectraMAX MTP Reader (type 340-Molecular Devices) and iEMS incubator/shaker (Thermo/Labsystems). In this assay system the reagent and solutions used were:

- 1) CS-28 Microswatches (rice starch, colored);
- 2) 25mM HEPES, 2mM CaCl₂, 0.005% TWEEN 80 buffer, pH 8.0;
- 3) 25mM CAPS, 2mM CaCl₂, 0.005% TWEEN 80 buffer, pH 10.0; and
- 4) 10mM NaCl, 0.1mM CaCl₂, 0.005% TWEEN 80 (Dilution buffer).

5 [00310] CS-28 Microswatches of ¼" circular diameter were delivered by the Center for Testmaterials (CFT, Vlaardingen, The Netherlands). Two microswatches were placed in each well of a 96-well microtiter plate vertically to expose the whole surface area (*e.g.*, not flat on the bottom of the well). The amylase samples (fermentation supernatant) were tested at appropriate concentrations in several conditions, pre-diluted in 10 mM NaCl, 0.1 mM CaCl₂, 0.005% TWEEN®80 solution:

- 10 1) pH 8 (25mM HEPES buffer) and 16°C; final amylase conc. in assay < 0.025 µg/ml;
- 2) pH 8 (25mM HEPES buffer) and 32°C; final amylase conc. in assay < 0.012 µg/ml;
- 3) pH 10 (25mM CAPS buffer) and 32°C; final amylase conc. in assay < 0.025 µg/ml; and
- 4) pH 10 (25mM CAPS buffer) and 50°C; final amylase conc. in assay < 0.012 µg/ml.

[00311] The incubator/shaker was set at the desired temperature, 16°C (cold storage chamber or refrigerator), 32°C or 50°C. The microswatches were placed into the wells of a 96-well MTP. The culture supernatant samples were diluted in 10 mM NaCl, 0.1mM CaCl₂, 0.005% TWEEN® 80 to 20X the desired final concentration. 190 µl of either HEPES or CAPS buffer was added to each well of a microswatch-MTP and subsequently 10 µl of enzyme solution was added to each well resulting in a total volume of 200 µl/well. The MTP was sealed with a plate seal and placed in the incubator/shaker and incubated for 60 minutes at 1150 rpm at the desired temperature (16°, 32° or 50°C). Following incubation under the appropriate conditions, 100 µl of solution from each well was transferred to a new MTP, and the absorbance at 488 nm was measured using a MTP-spectrophotometer. Controls containing two microswatches and buffer but no enzyme were included for background subtraction.

[00312] The obtained absorbance value was corrected for the blank value (obtained after incubation of microswatches in the absence of enzyme), and the resulting absorbance is a measure of hydrolytic activity. For each sample (variants) the performance index (PI) was calculated. The performance index compares the performance of the variant (actual value) and the reference enzyme (theoretical value) at the same protein concentration. The theoretical values can be calculated, using the parameters of the Langmuir equation of the reference enzyme. A PI that is greater than 1 (PI>1) identifies a better variant (as compared to the reference or standard enzyme [*e.g.*, wild-type]), while a PI of 1 (PI=1) identifies a variant that performs the same as the standard, and a PI that is less than 1 (PI<1) identifies a variant that performs worse than the standard. Thus, the PI identifies winners, as well as variants that are less desirable for use under certain circumstances.

D. Thermostability Assay – Determination of Initial and Residual Activities

35 [00313] The thermostability of the amylase variant in relation to a reference amylase was determined by incubating the amylase samples under defined conditions in MOPS buffer, pH 7.15. The temperature of the

incubation was chosen such that approximately 70% of the initial reference amylase activity was lost. The initial and residual amylase activities were determined using the Ceralpha method.

[00314] The equipment used was a Biomek FX Robot (Beckman Coulter); a SpectraMAX MTP Reader (type 340-Molecular Devices) and iEMS incubator/shaker (Thermo/Labsystems). In this assay system, the reagent solutions used were:

- 1) p-nitrophenyl maltoheptaoside (BPNPG7) substrate (Megazyme HR kit);
- 2) 10 mM NaCl, 10 mM CaCl₂, 0.005% TWEEN® 80 buffer (Dilution buffer);
- 3) 50 mM MOPS, 50 mM NaCl, 0.1 mM CaCl₂, 0.005% TWEEN®80 buffer, pH 7.15;
- 4) 200 mM Boric acid / NaOH buffer, pH 10.2 (STOP buffer); and
- 5) amylase culture supernatants, containing 50-150 µg/ml protein.

[00315] A “master dilution” plate was prepared by diluting the culture supernatant 20X in 10 mM NaCl, 0.1 mM CaCl₂, 0.005% TWEEN®80 buffer, followed by a 42X dilution step in MOPS buffer. From the master dilution 25 µl was used to determine the initial amylase activity and 100µl was used for heat incubation. The 100 µl sample was put in a MTP (Greiner 655.101) that was sealed with aluminum tape and incubated at 65.5°C for 60 minutes with agitation at 900 rpm in an iEMS incubator. After incubation the MTP was cooled on ice water before determining the residual amylase activity. To determine the initial (t₀₀) and residual (t₆₀) activity, a 25 µl sample was transferred into a new MTP, containing 25 µl BPNPG7 solution per well and incubated at 25°C for 30 minutes. The Ceralpha amylase assay was performed as described above in Section B.

[00316] The ratio of the residual and initial amylase activities was used to calculate thermostability as follows: Thermostability = [t₆₀ value] / [t₀₀ value]. For each variant the performance index, which compares the thermostability of the variant to the reference (standard) enzyme, was also calculated. A performance index (PI) that is greater than 1 (PI>1) identifies a better variant (as compared to the reference or standard [e.g., wild-type or backbone]), while a PI of 1 (PI=1) identifies a variant that is as stable as the standard, and a PI that is less than 1 (PI<1) identifies a variant that is less stable than the standard. Thus, the PI identifies winners, as well as variants that are less stable for use under certain circumstances.

E. Thermostability Assay – Determination of T₅₀ values

[00317] The thermostability assay described in Section D above, can only rank variants that lose activity under given conditions. Variants that are 100% stable at the given condition cannot be distinguished from one another. Thus, determination of the T₅₀ value, the incubation temperature by which 50% of the initial activity is lost, is a more suitable assay to rank variants with significantly increased thermostability.

[00318] The equipment used was a Biomek FX Robot (Beckman Coulter); a SpectraMAX MTP Reader (type 340-Molecular Devices) and Eppendorf Mastercycler. Culture supernatant containing the amylase variant was diluted 1000X in MOPS buffer and the initial amylase activity was determined using the Ceralpha amylase assay described above in Section B. Using the diluted amylase sample, PCR plates were prepared containing 100µl/well. The plates were incubated for 60 min on a Eppendorf Mastercycler on temperature

gradient spanning 60°C-80°C (single site mutants) or 60°C-100°C (combinatorial mutants). After incubation the MTPs were cooled down to 4°C before determining the residual amylase activity.

[00319] The ratio of the residual and initial amylase activities was plotted against the incubation temperature, and the data was fitted using the following equation: $y=a_0+a_1/(1+(x/a_2)^{a_3})$. Subsequently, the T_{50} -value for each amylase variant was calculated (*e.g.*, temperature at which the residual activity is 50%). Thus the T_{50} value is a measure for the thermostability of the variant, and can rank the variants in relation to the reference amylase and to one another.

F. 10% Detergent Stability Assay

[00320] The stability of the reference amylase and variants thereof was measured after incubation under defined conditions in the presence of 10% detergent (commercial detergent; heat inactivated), and the initial and residual amylase activities were determined using the Ceralpha amylase assay.

[00321] The equipment used was a Biomek FX Robot (Beckman Coulter); a SpectraMAX MTP Reader (type 340-Molecular Devices) and iEMS incubator/shaker (Thermo/Labsystems). In this assay system, the reagent solutions used were:

- 1) p-nitrophenyl maltoheptaoside (BPNPG7) substrate (Megazyme HR kit);
- 2) liquid detergent (HDL commercial product, enzyme– inactivated, 2 hrs at 95°C);
- 3) 10.5% detergent in 25 mM HEPES buffer, pH 8.0;
- 4) 50 mM MOPS, 50 mM NaCl, 0.1 mM CaCl₂, 0.005% TWEEN®80 buffer, pH 7.15;
- 5) 200 mM Boric acid / NaOH buffer, pH 10.2 (STOP buffer); and
- 6) amylase culture supernatants containing 50-150 µg/ml protein.

[00322] Briefly, 95 µl of a 10.5% detergent solution was transferred to a microtiter plate (MTP) and mixed with 5 µl of culture supernatant. A 3 µl aliquot was removed for determination of the initial amylase activity. The MTP was incubated in an iEMS incubator at 40° (or 50°C in the case of BASE combinatorial variants with higher stability) for 30 minutes with 900 rpm agitation. After incubation the residual amylase activity was measured using 3 µl of the detergent-enzyme mixture. Initial (t_0) and residual (t_{30}) amylase activity: 3 µl 'detergent-enzyme' mix was diluted in 122 µl MOPS buffer and subsequently 25 µl was used to determine the amylase activity using the Ceralpha amylase assay described above.

[00323] The ratio of the residual and initial amylase activities was used to calculate the '10% Detergent' Stability as follows: $\text{Stability} = [t_{30} \text{ value}] / [t_0 \text{ value}]$. For each variant the performance index was also calculated. The performance index compares the 10% Detergent stability of the amylase variants to a reference amylase. A performance index (PI) that is greater than 1 ($PI > 1$) identifies a more stable variant (as compared to the reference or standard [*e.g.*, wild-type]), while a PI of 1 ($PI = 1$) identifies a variant that is as stable as the standard, and a PI that is less than 1 ($PI < 1$) identifies a variant that is less stable than the standard. Thus, the PI identifies winners, as well as variants that are less stable for use under certain circumstances.

G. 100% Detergent Stability Assay – Temperature Gradient Curve

[00324] The HDL Detergent stability of BASE-backbone and BASE-variants were measured after incubation under defined conditions in the presence of 100% detergent (commercial detergent; enzyme-inactivated), and the initial and residual amylase activity were determined using the Ceralpha amylase assay.

5 [00325] The equipment used was a Biomek FX Robot (Beckman Coulter); a SpectraMAX MTP Reader (type 340-Molecular Devices); Eppendorf PCR Mastercycler and iEMS incubator/shaker (Thermo/Labsystems). In this assay system, the reagent solutions used were:

- 1) p-nitrophenyl maltoheptaoside (BPNPG7) substrate (Megazyme HR kit);
- 2) Liquid Detergent (HDL commercial product – inactivated by heating at 95°C for 2 hrs);
- 10 3) 50 mM MOPS, 50mM NaCl, 0.1mM CaCl₂, 0.005% TWEEN®80 buffer, pH 7.15;
- 4) 200 mM Boric acid / NaOH buffer, pH 10.2 (STOP buffer); and
- 5) amylase culture supernatants containing 50-150 µg/ml protein.

[00326] Amylase culture supernatants were diluted 20X in HDL Detergent and mixed thoroughly. The initial amylase activity was determined using the Ceralpha amylase assay described above in Section B.

15 Using the diluted amylase-HDL sample, PCR plates were prepared containing 100 µl/well. The plates were incubated for 30 min on an Eppendorf Master cycler on a temperature gradient spanning 30°C-70°C. After incubation the MTPs were cooled down to 4°C before determining the residual amylase activity as described above.

[00327] To calculate the HDL T₅₀ value, the ratio of the residual and initial amylase activities was plotted
20 against the incubation temperature, and the data was fitted using the following equation:

$$y = a_0 + a_1 / (1 + (x/a_2)^{a_3})$$
 Subsequently, the T₅₀-value for each BASE-variant was calculated (*e.g.*, temperature at which the residual activity is 50%). Thus the T₅₀-value is a measure of the thermostability of the variant, and can rank the variants with regards to the reference amylase as well as to each other.

25 H. AAPF Protease

[00328] In order to determine the protease activity of the subtilisin proteases of the present disclosure, the hydrolysis of N-succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenyl-p-nitroanilide (AAPF) was measured. The reagent solutions used were:

- 1) 100 mM Tris/HCl, pH 8.6, containing 0.005% TWEEN®-80 (Tris dilution buffer);
- 30 2) 100 mM Tris buffer, pH 8.6, containing 10 mM CaCl₂ and 0.005% TWEEN®-80 (Tris/Ca buffer); and
- 3) 160 mM suc-AAPF-pNA in DMSO (suc-AAPF-pNA stock solution) (Sigma: S-7388).

[00329] To prepare a suc-AAPF-pNA working solution, 1 ml AAPF stock was added to 100 ml Tris/Ca buffer and mixed well for at least 10 seconds. The assay was performed by adding 10 µl of diluted protease solution to each well, immediately followed by the addition of 190 µl 1 mg/ml AAPF-working solution. The
35 solutions were mixed for 5 sec., and the absorbance change in kinetic mode (20 readings in 5 minutes) was read at 410 nm in an MTP reader, at 25°C. The protease activity was expressed as AU (activity = Δ OD•min⁻¹ ml⁻¹).

EXAMPLE 2**Generation of *B. subtilis* Strains Expressing BASE (AmyTS23t) and Variants Thereof**

[00330] In this example, the construction of *Bacillus subtilis* strains expressing BASE (a truncated form of *Bacillus sp.* TS-23 alpha-amylase or AmyTS23t) and variants thereof are described. BASE, the mature form of a truncated amylase originated from the TS-23 alpha-amylase (AmyTS23) of an alkaliphilic and thermophilic *Bacillus sp.* strain TS-23 (Lin *et al.*, J Appl Microbiol, 82:325-334, 1997).

[00331] The amino acid sequence of the mature form of AmyTS23 is set forth as SEQ ID NO:1:

NTAPINETMMQYFEWDLPNLWTKVKNEANLSSLGITALWLPPAYKGTSSQ
SDVGYGVYDLYDLGEFNQKGTIRTKYGTQYIQAIQAAKAAGMQVYADVFN
HKAGADGTEFVDAVEVDPNQRNQETSGTYQIQAWTKFDFPGRGNTYSSFKWRW
YHFDGTDWDESRKLNRIYKFRSTGKAWDWEVDTEGNYDYLMFADLDMDHPEV
VTELKNWGTWYVNTTNIDGFRDLDAVKHIKYSFFPDWLTIVRNQTKGNLFAVGE
FWSYDVNKLHNYITKTNGSMSLFDAPLHNNFYTASKSSGYFDMRYLLNNTLMK
DQPSLAVTLVDNHDTPQGQSLQSWVEPWFKPLAYAFILTRQEGYPCVFYGDYY
GIPKYNIPGLKSKIDPLLIARRDYAYGTQRDYIDHQDIIGWTREGIDTKPNSG
LAALITDGPGGSKWMYVGGKKHAGKVFDLTGNRSDTVTINADGWGEFKVNGGS
VSIWVAKTSNVTFITVNNATTTSGQNVYVANIPELGNWNTANAIKMNPSSYPT
WKATIALPQGKAIEFKFIKKDQAGNVIWESTSNRTYTVPFSSSTGSYTASWNVP.

[00332] A codon-modified nucleic acid sequence encoding the mature form of AmyTS23 is set forth as SEQ ID NO:3:

aatacggcgccgatcaacgaaacgatgatgcagatattttgaatgggatctgccgaatg
atggaacgctgtggacgaaagtcaaaaacgaagcggcggaatcttagcagcctgggaat
cacagcactttggcttcgccggcatataaaggaacgagccaaagcgatgtcggctat
ggcgtctatgatctgatgacctgggcgaatttaacaaaaaggcacgatccggacga
aatatggcagcaaaacacagatatccaagcgatccaggcagcaaaagcagcaggcat
gcaagtctatgccgacgtcgtctttaatcataaagcgggagcggatggcacagaattt
gtcgatgccgtcgaagttgatccgagcaacagaaaccaagaaacgagcggcacgtatc
aaatccaagcgtggacgaaatttgattttccgggcagaggcaatacgtatagcagctt
taaatggcgtggtatcattttgacggcacggattgggatgaaagcagaaaactgaac
cggatctataaatttcgggacacgggcaaagcatgggattgggaagtcgatacggaaa
acggcaactatgactatctgatgtttgccgatctggatctggatcatccggaagtcgt
cacggaactgaaaaattggggcacgtggtatggttaatacgacgaacatcgatggctt
agactggatgccgtcaaacatatcaaatatagctttttccggactggctgacgtatg
tcagaaaccagacgggcaaaaacctttttgccgtcggcgaattttggagctatgacgt
caacaaacttcataactatatcacgaaaacgaacggcagcatgagcctttttgatgcc
ccgcttcataacaactttttatacggcgagcaaaagctcaggctattttgatatgagat
atctgctgaacaacacgctgatgaaagatcaaccgagcctggcagtcacactggtcga
taaccatgatacacaaccgggccaagccttcaaagctgggtcgaaccgtggttttaa
ccgctggcgtatgcctttatcctgacgagacaagaagggtatccttgcgtctttatg
gcgactattatggcatcccgaaatataaatatcccgggcctgaaaagcaaaatcgatcc
gctgctgatcgccagacgggattatgcctatggcacacagcgggattatatcgaccat
caggacatcatcggctggacaagagaaggcatcgatacgaaccgaatagcggactgg
cagcactgattacagatggaccgggcggaagcaaatggatgtatgtcggcaaaaaaca
tgccggcaaaagtcttttatgatctgacgggcaacagaagcgatacgggtcacgatcaat
gctgatggctggggagaatttaaagtcaatggcggcagcgtttcaatctgggtcgcca
aaacgagcaatgtcacgtttacgggtcaacaatgccacgacaacgagcggccaaatgt
ctatgtcgtcgccaatatcccggaactgggcaattggaatacggcgaaacgcaatcaaa
atgaacccgagcagctatccgacatggaaagcgacaatcgctctgccgcaaggaaaag
cgatcgaatttaaatatcaaaaaagaccaggcgggcaatgttatttgggaaagcac
gagcaatagaacgtatacgggtcccgtttagcagcacaggaagctatacagcgagctgg
aatgttccgtga.

[00333] BASE was created by deleting both the first 90 bp of the 5'-sequence region encoding the signal peptide and 297 bp of the 3'-sequence encoding the carboxyl-terminal end of the enzyme, yielding a truncated alpha-amylase. The amino acid sequence of the mature form of BASE (AmyTS23t) is set forth as SEQ ID NO:2:

5 NTAPINETMMQYFEWDLPN DGLWTKVKNEAANLSSLGITALWLPPAYKGT SQ
SDVGYGVYDLYDLGEF NQKGTIRTKYGTQTQYIQAIQA AKAAGMQVYADV VFN
HKAGADGTEFVDAVEVD P SNRNQETSGTYQIQAWTKFDFPGRGNTYSSFKWRW
YHFDGTDWDESRKLNRIYKFRSTGKAWDWEVD TENGNYDYLMFADLDMDHPEV
10 VTELKNWGTWYVNTTNIDGFR L DAVKHIKYSFFPDWLT YVRNQTGKNLFAVGE
FWSYDVNKLHNYITKTNGS MS LFDAPLHNNFYTASKSSGYFDMRYLLNNTLMK
DQPSLAVTLVDNHD T QPGQSLQSWVEPWF KPLAYAFILTRQEGYPCVFYGDYY
GIPKYNIPGLKSKIDPLLIARRDYAYGTQRDYIDHQDIIGWTREGIDTKPNSG
LAALITDGP GGSKWMYVGKKHAGKV FYDLTGNRSDTVTINADGWGEFKVNGGS
VSIWVAK.

15 [00334] A synthetic DNA fragment (0723013) produced by GENEART and containing a codon-modified BASE gene for expression in *B. subtilis* served as template DNA (SEQ ID NO: 4) for the construction of *Bacillus subtilis* strains expressing BASE and variants thereof. To express BASE, the BASE DNA fragment was cloned into the pHPLT vector (Solingen *et al.*, Extremophiles 5:333-341, 2001) by GENEART using the unique *Pst*I and *Hpa*I restriction sites. The pHPLT expression vector contains the *B. licheniformis* LAT
20 promoter (Plat) and additional elements from pUB110 (McKenzie *et al.*, Plasmid, 15: 93-103, 1986) including a replicase gene (reppUB), a neomycin/kanamycin resistance gene (neo) and a bleomycin resistance marker (bleo).

[00335] The coding region for the LAT signal peptide is set forth as SEQ ID NO: 15:
atgaaacaacaaaacggctttacgcccgattgctgacgtgtatttgcgctcatcttctgtgcctcattctgcagcttcagca.

25 [00336] The amino acid sequence of the LAT signal peptide is set forth as SEQ ID NO: 16:
MKQQKRLYARLLTLLFALIFLLPHSAASA.

[00337] A map of the pHPLT expression vector is shown in Figure 2, while a map of the pHPLT vector containing the BASE gene is shown in Figure 3. The codon-modified nucleic acid sequence produced by GENEART, encoding the mature form of BASE (AmyTS23t), is set forth as SEQ ID NO: 4, with the N001 N-
30 terminal codon and the K484 C-terminal codon shown in bold:

1 tctgcagct tcagca**aac** accgcgccg attaacgaa accatgatg cagtatttc
gaatgggat ctgccgaac
73 gatggcacc ctgtggacc aaagtga aaacgaagcg gcgaacctg agcagcctg
ggcattacc gcgctgtg
35 145 ctgccgccg gcatataaa ggcaccagc cagagcgat gtgggctat ggcgtgtat
gatctgtac gatctggc
217 gaatttaac cagaaaggc accattcgt accaaatat ggcaccaa acccagtat
attcaggcg atccaggcg
289 gcgaaagcg gcgggtatg caggtgtat gcggatgtg gtgtttaac cataaagcg
40 ggtgcggat ggcaccgaa
361 ttgtgtgat gcggtgaa gtggatccg agcaaccgt aaccaggaa accagcggc
acctatcag attcaggcg
433 tggaccaa tttgatttt cccggccgt ggcaacacc tatagcagc tttaaattg
cgctggtat cattttgat

505 ggcaccgat tgggatgaa agccgtaaa ctgaaccgc atctataaa tttcgtagc
 accggcaaa gcgtgggat
 577 tgggaagtg gataccgaa aacggcaac tatgattac ctgatgttc gcagacctg
 gatatggat catccgaa
 5 649 gtggtgacc gaactgaaa aactggggc acctggtat gtgaacacc accaacatt
 gatggcttt cgtctggat
 721 gcggtgaaa cacatcaaa tacagcttt tttccggat tggctgacc tatgtgcgt
 aaccagacc ggcaaaaac
 793 ctgtttgcg gtgggcgaa ttttggagc tatgatgtg aacaaactg cacaactac
 10 atcaccaaa accaacggc
 865 agcatgagc ctgtttgat gcgccgtg cataacaac ttttatacc gcgagcaaa
 agcagcggc tattttgat
 937 atgcgttat ctgctgaac aacaccctg atgaaagat cagccgagc ctggccgtg
 accctgggtg gataaccat
 15 1009 gataccag ccggggccag agcctgcaa agctgggtg gaaccgtgg tttaaaccg
 ctggcctac gcgtttatt
 1081 ctgaccgt caagagggc tatccgtgc gttttttat ggcgattat tacggcatc
 ccgaaatat aacattccg
 1153 ggctgaaa agcaaaatt gatccgctg ctgattgcg cgtcgtgat tatgcgtat
 20 ggcaccag cgtgattat
 1225 attgatcac caggatatt attggctgg acccgtgaa ggcattgat accaaaccg
 aacagcggc ctggccgcg
 1297 ctgattacc gatggcccg ggtggcagc aaatggatg tatgtgggc aaaaaacat
 gcgggcaaa gtgttttat
 25 1369 gatctgacc ggcaaccgt agcgatacc gtgaccatt aacgcggat ggctggggg
 gagttttaa gtgaacggc
 1441 ggcagcgtg agcatttgg gtggcgaaa taagttaac aga.

[00338] GENEART transformed a *B. subtilis* strain (genotype: $\Delta aprE$, $\Delta nprE$, Δepr , $\Delta ispA$, Δbpr) and ($degU^{Hy32}$, $oppA$, $\Delta spoIIE3501$, $amyE::xylRPxylAcomK-ermC$), using the pHPLT-BASE vector DNA.

30 Transformation of *B. subtilis* was performed as known in the art (WO 02/14490). The *B. subtilis* transformants were selected on agar plates containing Heart infusion agar (Difco, Catalog No. 244400) and 10 mg/L neomycin sulfate (Sigma, Catalog No. N-1876; contains 732 μ g neomycin per mg). Selective growth of *B. subtilis* transformants harboring the pHPLT-BASE GENEART vector was performed in shake flasks containing MBD medium (a MOPS based defined medium), 5 mM $CaCl_2$ and 10 mg/L neomycin. MBD

35 medium was made essentially as known in the art (Neidhardt *et al.*, J Bacteriol, 119: 736-747, 1974), except that NH_4Cl_2 , $FeSO_4$, and $CaCl_2$ were omitted from the base medium, 3 mM K_2HPO_4 was used, and the base medium was supplemented with 60 mM urea, 75 g/L glucose, and 1 % soytone. The micronutrients were made up as a 100X stock solution containing in one liter, 400 mg $FeSO_4 \cdot 7H_2O$, 100 mg $MnSO_4 \cdot H_2O$, 100 mg $ZnSO_4 \cdot 7H_2O$, 50 mg $CuCl_2 \cdot 2H_2O$, 100 mg $CoCl_2 \cdot 6H_2O$, 100 mg $NaMoO_4 \cdot 2H_2O$, 100 mg $Na_2B_4O_7 \cdot 10H_2O$, 10

40 ml of 1M $CaCl_2$, and 10 ml of 0.5 M sodium citrate. Growth resulted in the production of secreted BASE amylase with starch hydrolyzing activity.

EXAMPLE 3

Generation of BASE (AmyTS23t) Site Evaluation Libraries

[00339] Site evaluation library (SEL) production was performed by GENEART using a proprietary process (WO 2004/059556A3). Methods and devices for optimizing a nucleotide sequence for the purpose of expression of a protein by PCR, and the manufacture of DNA molecules utilized technology owned by or licensed to GENEART (European Patent Nos. 0 200 362 and 0 201 184; and US Patent Nos. 4,683,195, 4,683,202 and 6,472,184). The construction of BASE SELs described in this example was performed by GENEART using their technology platform for gene optimization, gene synthesis and library generation under proprietary GENEART know how and/or intellectual property. The sequential permutation approach of GENEART, to produce SELs, is described in general on the company's web site.

[00340] The pHPLT-BASE plasmid DNA served as template to produce the SELs. BASE SELs were produced by GENEART at positions (Table 3-1) pre-selected by the inventors. The corresponding DNA codons were each substituted with codons for at least 16 (out of a possible 19) different amino acids. The codon mutagenized pHPLT-BASE mixes were used to transform competent *B. subtilis* cells (genotype: *ΔaprE*, *AnprE*, *oppA*, *AspoIIE*, *degUHy32*, *ΔamyE::[xylR,pxylA-comK]*) as known in the art (WO 2002/014490) to generate the BASE SELs. Transformation mixes were plated on HI-agar plates (Heart Infusion agar) containing 10 mg/L neomycin sulfate. For each library, single colonies were picked and grown in TSB (tryptone and soy based broth) liquid medium with 10 mg/ml neomycin selection for subsequent DNA isolation and gene sequence analysis. Sequence analysis data revealed a maximum of 19 BASE mature variants per library. The BASE variants that identified in the BASE SELs are listed in Table 3-2. To generate BASE and BASE variant enzyme samples for biochemical characterization, selective growth of the BASE SEL variants was performed in 96 well MTPs at 37°C for 68 hours in MBD medium.

Table 3-1 BASE (AmyTS23t) Site Evaluation Library Positions

N001	G057	F116	H161	Y197	Y269	P374
T002	Y058	D118	F162	Y199	D270	K375
A003	G059	E121	G164	L200	N272	Y376
P004	V060	D123	T165	M201	K273	N377
I005	N070	S125	D166	F202	H275	P379
E007	Q071	N126	W167	A203	T279	L381
W015	K072	R127	D168	M207	G283	K382
D016	G073	N128	E169	P210	S284	R393
L017	I075	Q129	S170	T214	T298	D394
P018	K078	E130	R171	K217	K301	T399
N019	K082	T131	K172	T221	S303	R401
T022	T083	S132	N174	N228	Y305	Q407
T025	Q087	T134	R175	L234	F306	D408
K026	Q090	Y135	I176	A236	Y310	T419
K028	A091	Q136	Y177	V237	L311	P433
N029	K093	Q138	K178	K238	N314	S436
E030	A094	W140	F179	H239	K318	W438
A032	A095	K142	T182	Q243	D319	K444
S035	V103	D144	G183	I240	Q320	G447
S036	V104	G147	A185	P246	S322	K448
L037	F105	G149	W186	T250	L323	Y451

Table 3-1 BASE (AmyTS23t) Site Evaluation Library Positions

G050	H107	N150	W188	N254	G336	L453
T051	K108	Y152	E189	Q255	Q337	D459
S052	G110	S154	V190	G257	S338	A465
Q053	D112	K156	D191	G264	L339	E470
S054	G113	R158	T192	F266	Q340	G475
D055	T114	W159	E193	W267	E344	A483
V056	E115	Y160	G195	S268	Q359	K484

Table 3-2 BASE Variants of the SELs

Pos.	Amino Acid Substitutions Generated in the BASE Amylase Background																		
N001	A	C	D	E	F	G	H	I	K	L	M	P	Q	R	S	T	V	W	Y
T002	A	D	E	F	H	I	K	L	M	N	P	Q	R	S	Y	-	-	-	-
A003	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
P004	A	C	D	E	G	H	I	K	L	M	N	Q	R	S	V	Y	-	-	-
I005	C	D	E	F	G	H	K	L	M	N	R	S	T	V	W	Y	-	-	-
E007	A	C	D	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
W015	A	C	D	E	F	G	H	I	K	L	M	P	Q	R	S	T	V	Y	-
D016	A	C	E	F	G	H	I	K	L	M	N	P	Q	R	T	V	W	Y	-
L017	A	C	D	E	F	G	H	I	K	M	N	P	Q	R	S	T	V	W	Y
P018	A	C	D	E	F	G	H	I	K	L	M	N	Q	R	S	T	V	Y	-
N019	A	C	D	E	F	G	H	I	K	L	M	P	Q	R	S	T	V	W	Y
T022	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	S	V	W	Y	-
T025	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	S	V	W	Y	-
K026	A	C	D	E	F	G	H	I	L	M	N	P	Q	R	S	T	V	W	Y
K028	A	C	D	E	G	H	I	L	M	N	P	Q	R	S	T	V	W	Y	-
N029	A	C	D	E	G	H	I	K	L	M	P	Q	R	S	T	V	W	Y	-
E030	A	C	D	G	H	I	K	L	N	P	Q	R	S	T	V	W	Y	-	-
A032	E	F	G	H	K	L	M	N	P	Q	R	S	T	V	W	Y	-	-	-
S035	A	C	D	E	F	G	H	K	L	N	P	Q	R	T	V	W	Y	-	-
S036	A	C	D	E	F	G	I	K	L	M	N	P	Q	R	T	V	W	Y	-
L037	C	D	E	F	G	H	I	K	M	N	P	Q	R	S	T	V	W	Y	-
G050	A	C	D	E	F	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
T051	A	C	D	E	F	G	H	K	M	N	P	Q	R	S	V	W	Y	-	-
S052	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	T	V	W	Y
Q053	A	C	D	E	F	G	H	I	K	L	M	N	P	R	S	T	V	W	-
S054	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	T	V	W	Y
D055	A	C	E	F	G	H	I	K	M	N	P	R	S	T	V	W	Y	-	-
V056	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	W	Y
G057	A	C	D	E	F	I	L	M	N	P	Q	R	S	T	W	Y	-	-	-
Y058	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W
G059	A	C	D	E	F	I	K	L	M	N	P	Q	R	S	T	V	W	Y	-
V060	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	W	Y
N070	A	C	D	E	F	G	H	I	K	L	M	P	Q	R	S	T	V	W	Y
Q071	A	C	D	E	F	G	H	I	K	L	M	N	P	R	S	T	V	W	Y
K072	A	C	D	E	G	I	L	M	P	Q	R	S	T	V	W	Y	-	-	-
G073	A	C	D	E	F	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
I075	A	C	E	F	G	H	K	L	M	N	P	Q	R	S	T	V	W	Y	-
K078	A	C	D	E	F	G	H	I	L	M	N	P	Q	R	S	T	V	W	Y
K082	A	C	D	E	F	G	H	I	L	M	N	P	Q	S	T	V	W	Y	-

Table 3-2 BASE Variants of the SELs																			
Pos.	Amino Acid Substitutions Generated in the BASE Amylase Background																		
T083	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	V	W	Y
Q087	A	C	D	E	F	G	H	I	K	L	M	N	P	R	S	T	V	W	Y
Q090	A	C	D	E	F	G	H	I	K	L	M	N	P	R	S	T	V	W	Y
A091	C	D	E	F	G	H	I	K	L	M	N	Q	R	S	T	V	W	Y	-
K093	A	C	D	E	F	G	I	M	N	Q	R	S	T	V	W	Y	-	-	-
A094	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A095	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
V103	A	C	D	E	F	G	H	I	K	L	M	N	P	R	S	W	Y	-	-
V104	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	W	Y
F105	C	D	E	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	-
H107	A	C	D	E	F	G	I	K	L	M	N	P	Q	R	S	T	V	W	Y
K108	A	C	E	F	G	I	L	M	N	R	S	V	W	Y	-	-	-	-	-
G110	A	C	D	E	F	H	I	K	L	M	P	Q	R	S	V	W	Y	-	-
D112	A	C	E	F	G	H	K	L	M	N	P	R	S	T	V	W	Y	-	-
G113	A	C	D	E	F	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
T114	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	V	W	Y
E115	A	C	D	G	I	K	L	M	N	Q	R	S	T	V	W	Y	-	-	-
F116	A	C	D	E	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
D118	A	C	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
E121	A	C	D	F	G	H	I	K	L	M	N	P	Q	S	T	V	Y	-	-
D123	A	C	E	F	G	H	I	K	L	M	P	Q	R	S	T	V	W	Y	-
S125	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	T	V	W	Y
N126	A	C	D	E	G	H	I	K	L	M	Q	R	S	T	V	W	Y	-	-
R127	A	C	E	F	G	I	K	L	M	N	P	Q	T	V	-	-	-	-	-
N128	A	C	D	E	F	G	H	K	L	M	P	Q	R	S	T	V	W	Y	-
Q129	A	C	D	E	F	G	H	I	K	L	M	N	P	R	S	T	V	W	-
E130	A	C	D	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
T131	A	C	D	E	F	G	H	I	K	M	N	P	Q	R	S	V	W	Y	-
S132	A	C	D	E	F	G	H	I	K	L	M	N	Q	R	T	V	W	Y	-
T134	A	C	D	E	G	H	I	K	L	M	N	P	Q	R	S	W	Y	-	-
Y135	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W
Q136	A	C	D	E	F	G	H	I	K	L	M	N	P	R	S	T	V	W	Y
Q138	A	C	D	E	G	H	K	L	M	N	P	R	S	T	V	W	Y	-	-
W140	A	C	D	E	F	G	H	I	K	M	N	P	Q	R	S	T	V	Y	-
K142	A	C	D	E	F	G	H	I	L	M	N	P	Q	R	S	T	V	W	Y
D144	A	C	E	F	G	H	I	K	L	M	N	P	R	S	T	V	W	Y	-
G147	A	C	D	E	F	H	K	L	M	N	P	Q	R	S	T	V	W	Y	-
G149	A	C	D	E	F	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
N150	A	C	D	E	F	G	H	I	K	L	M	P	Q	S	T	V	W	-	-
Y152	A	D	E	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	-	-
S154	A	C	D	E	F	G	H	I	K	L	M	N	Q	R	T	V	W	Y	-
K156	A	C	D	E	F	G	H	I	L	M	N	P	Q	R	V	W	Y	-	-
R158	A	C	D	E	G	H	I	K	L	M	Q	S	T	V	W	Y	-	-	-
W159	A	D	E	F	H	I	K	L	M	N	P	Q	R	S	T	V	-	-	-
Y160	A	D	E	F	I	K	L	M	N	P	Q	R	S	T	V	W	-	-	-
H161	A	C	D	E	F	G	I	K	L	M	N	Q	R	S	T	V	Y	-	-
F162	A	C	D	E	G	H	I	K	M	N	Q	S	T	W	Y	-	-	-	-
G164	A	C	N	S	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T165	A	C	D	E	F	G	H	I	L	M	N	P	Q	R	S	V	W	Y	-
D166	A	C	F	G	H	K	L	M	N	P	Q	R	S	T	V	W	-	-	-
W167	A	C	D	E	F	G	I	K	L	N	P	Q	R	S	T	V	Y	-	-

Table 3-2 BASE Variants of the SELs																			
Pos.	Amino Acid Substitutions Generated in the BASE Amylase Background																		
D168	A	C	E	F	H	K	L	M	N	P	Q	R	S	T	V	W	Y	-	-
E169	A	C	D	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
S170	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	T	V	W	Y
R171	A	C	D	E	F	G	H	K	L	M	N	P	Q	S	T	W	Y	-	-
K172	A	C	D	E	F	G	H	I	L	N	P	Q	R	S	T	V	W	Y	-
N174	A	C	D	E	F	H	I	L	M	Q	R	S	T	V	W	Y	-	-	-
R175	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	S	T	V	W	Y
I176	A	C	D	E	F	G	H	K	L	M	N	P	Q	R	S	T	V	W	Y
Y177	A	C	E	F	G	H	I	L	M	N	Q	R	S	V	W	-	-	-	-
K178	A	C	E	F	G	H	I	L	M	N	P	Q	R	S	T	V	W	Y	-
F179	A	C	E	G	H	I	K	L	M	N	P	T	V	W	Y	-	-	-	-
T182	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	V	W	Y	-
G183	A	C	E	F	I	K	L	M	N	P	Q	R	S	T	V	W	-	-	-
A185	C	D	E	F	G	I	K	L	M	N	Q	R	S	T	V	W	Y	-	-
W186	D	F	G	H	I	K	L	M	N	Q	R	S	T	V	Y	-	-	-	-
W188	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	-
E189	C	D	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	-
V190	A	C	D	E	F	G	H	L	M	N	P	Q	S	T	W	Y	-	-	-
D191	A	C	F	G	H	I	L	M	N	P	Q	S	T	V	W	Y	-	-	-
T192	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	V	W	Y
E193	A	C	D	F	I	K	L	M	N	P	Q	R	S	T	V	W	Y	-	-
G195	A	C	D	F	H	I	L	M	N	P	Q	R	S	T	V	W	Y	-	-
Y197	A	C	D	E	F	G	H	K	M	N	P	Q	R	S	T	V	W	-	-
Y199	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W
L200	A	C	D	E	F	G	H	K	M	N	Q	R	T	V	Y	-	-	-	-
M201	A	C	D	E	F	G	H	I	K	L	N	P	Q	R	S	T	V	W	Y
F202	A	C	D	E	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A203	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
M207	A	C	E	F	H	I	K	L	N	Q	S	T	V	W	Y	-	-	-	-
P210	C	D	E	F	G	H	I	K	L	M	N	Q	R	S	T	V	W	-	-
T214	A	C	D	E	F	G	I	K	L	P	Q	R	S	V	W	Y	-	-	-
K217	A	C	D	E	G	F	G	H	L	M	N	P	Q	R	S	T	V	W	Y
T221	A	C	D	E	F	G	H	K	M	N	P	Q	R	S	V	W	Y	-	-
N228	A	C	D	E	F	G	H	I	L	M	P	Q	R	S	T	V	W	Y	-
L234	A	C	D	G	H	I	M	N	P	Q	S	T	V	W	Y	-	-	-	-
A236	C	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	-
V237	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	W	Y
K238	A	C	D	E	F	G	H	I	M	N	Q	R	S	T	V	W	Y	-	-
H239	A	C	D	F	G	I	K	L	M	N	Q	R	S	T	V	W	Y	-	-
I240	A	C	E	F	G	H	K	L	M	N	Q	S	T	W	Y	-	-	-	-
S243	A	C	D	E	F	G	H	I	K	L	M	P	Q	R	T	V	-	-	-
P246	A	C	D	E	F	G	H	I	K	L	M	N	Q	R	S	T	V	W	Y
T250	A	C	D	E	F	G	H	I	K	L	P	Q	R	W	Y	-	-	-	-
N254	A	C	D	E	F	G	H	I	K	L	M	P	Q	R	S	T	V	W	Y
Q255	A	C	D	E	F	I	K	L	M	N	P	R	S	T	V	W	Y	-	-
G257	C	D	E	F	H	I	K	L	M	N	P	R	S	T	V	W	Y	-	-
N259	A	C	D	E	G	H	K	P	Q	R	S	T	Y	-	-	-	-	-	-
G264	D	E	F	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	-	-
F266	A	C	D	E	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
W267	F	I	M	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S268	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	T	V	W	Y

Table 3-2 BASE Variants of the SELs																			
Pos.	Amino Acid Substitutions Generated in the BASE Amylase Background																		
Y269	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W
D270	A	C	E	G	H	K	N	R	S	T	-	-	-	-	-	-	-	-	-
N272	A	C	D	E	F	H	I	K	L	M	P	Q	R	S	T	V	W	Y	-
K273	A	D	E	F	G	H	I	L	M	N	Q	R	T	V	Y	-	-	-	-
H275	A	C	D	E	F	G	I	K	L	M	N	Q	R	S	T	V	W	Y	-
T279	A	C	D	E	H	I	K	L	M	N	Q	R	S	V	W	Y	-	-	-
G283	A	C	D	E	F	H	K	M	N	P	Q	R	S	T	V	Y	-	-	-
S284	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	T	V	W	Y	-
T298	C	D	E	F	G	H	I	K	L	M	N	Q	R	S	V	W	Y	-	-
K301	C	D	E	F	G	H	I	L	M	N	Q	R	S	T	V	W	Y	-	-
S303	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	T	V	Y	-
Y305	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W
F306	A	C	D	E	G	H	I	K	L	M	N	Q	R	S	T	V	W	-	-
Y310	A	C	D	E	F	GH	I	K	L	M	N	P	Q	R	S	T	V	W	-
L311	C	F	G	I	M	Q	S	T	V	-	-	-	-	-	-	-	-	-	-
N314	A	C	D	E	F	G	H	I	K	L	M	P	Q	R	S	T	V	W	Y
K318	A	C	D	E	G	H	I	L	M	N	P	Q	R	S	T	Y	-	-	-
D319	A	C	E	F	H	I	K	M	N	P	Q	R	S	T	V	W	Y	-	-
Q320	A	C	D	E	F	G	H	I	K	L	M	N	R	S	T	V	W	Y	-
S322	A	D	E	F	G	H	I	L	M	N	P	Q	R	T	V	W	Y	-	-
L323	A	C	D	E	F	G	H	K	M	N	P	Q	R	S	T	V	W	-	-
G336	A	C	E	F	H	I	K	M	N	P	Q	R	S	T	W	Y	-	-	-
Q337	A	D	E	F	G	H	L	M	N	P	R	S	T	V	W	Y	-	-	-
S338	A	C	D	E	F	G	H	K	L	M	N	P	Q	R	T	V	Y	-	-
L339	A	C	D	E	F	G	H	I	K	M	N	Q	R	S	T	V	W	Y	-
Q340	A	C	D	E	F	G	H	I	K	L	M	N	P	R	S	T	V	W	Y
E344	A	C	D	F	G	H	K	L	M	N	P	Q	R	S	T	V	-	-	-
Q359	A	C	D	E	F	G	I	M	N	S	T	V	W	Y	-	-	-	-	-
P374	A	D	E	F	H	I	K	L	M	N	Q	R	S	T	V	W	Y	-	-
K375	A	C	D	E	F	G	H	I	L	M	N	P	Q	R	S	T	V	W	Y
Y376	A	C	D	E	F	G	H	I	K	M	N	P	Q	R	S	T	V	W	-
N377	A	C	D	E	F	G	H	K	L	M	P	Q	R	S	T	V	W	Y	-
P379	A	C	E	F	G	H	I	K	L	M	Q	R	T	V	W	Y	-	-	-
L381	A	C	D	E	F	G	H	I	K	M	N	P	Q	R	S	T	V	W	Y
K382	A	C	D	E	F	G	H	I	L	M	N	P	Q	R	S	T	V	W	Y
R393	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	T	V	W	-	-
D394	A	C	E	F	G	H	K	L	M	N	Q	R	S	T	V	W	-	-	-
T399	A	C	D	E	F	G	H	I	K	L	M	P	Q	R	S	W	Y	-	-
R401	A	C	D	E	G	H	I	K	L	M	N	Q	S	T	W	Y	-	-	-
Q407	A	C	D	E	F	G	I	K	M	N	P	R	S	T	V	W	-	-	-
D408	A	C	F	G	H	I	K	M	N	Q	R	S	T	-	-	-	-	-	-
T419	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	V	W	Y
P433	A	C	D	E	H	I	K	L	M	N	Q	R	S	T	V	W	-	-	-
S436	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	T	V	W	Y
W438	C	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	Y	-	-	-
K444	C	D	F	G	H	I	L	M	N	P	Q	R	S	T	V	W	Y	-	-
G447	A	C	D	F	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	-
K448	A	C	D	E	F	G	H	L	N	P	Q	R	S	T	V	W	-	-	-
Y451	A	C	D	E	F	G	K	L	M	N	P	Q	R	S	T	V	-	-	-
L453	A	C	D	E	F	G	H	I	K	M	N	P	Q	R	S	T	V	W	Y
D459	A	C	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y

Table 3-2 BASE Variants of the SELs																			
Pos.	Amino Acid Substitutions Generated in the BASE Amylase Background																		
A465	C	F	G	H	I	K	L	M	N	P	Q	S	T	V	W	Y	-	-	-
E470	A	C	D	F	G	H	I	K	L	N	P	Q	R	S	T	W	Y	-	-
G475	A	C	D	E	F	H	I	K	L	N	P	Q	R	S	T	V	W	-	-
G476	A	C	D	E	H	I	K	L	M	N	P	Q	T	V	W	Y	-	-	-
A483	C	D	E	F	G	H	I	K	M	N	P	Q	R	S	T	V	W	Y	-
K484	A	C	D	E	F	G	H	I	L	M	N	-	-	-	-	-	-	-	-

EXAMPLE 4

Generation of BASE (AmyTS23t) Combinatorial Libraries

[00341] Synthetic BASE combinatorial libraries contain a mix of synthetic BASE genes in which two or more selected codons of the mature sequence are replaced by specific DNA sequences. Four synthetic BASE combinatorial libraries were produced by GENEART under contract to Genencor, using GENEART's technology platform for gene optimization, gene synthesis and library generation under proprietary GENEART know how and/or intellectual property. The advanced mutagenesis approach of GENEART to produce combinatorial libraries is described in general on the company's web site.

[00342] Tables 4-1 to 4-4 list the substitutions that could be present in members of the synthetic BASE combinatorial libraries (numbered according to the BASE mature amino acid sequence of SEQ ID NO: 2). In each library the targeted BASE positions have an equal chance to remain wild type (wt) or to be substituted with the specific amino acid listed in Tables 4-1 to 4-4. The BASE combinatorial libraries were produced by cloning the mutagenized BASE genes in the pHPLT vector to create variants of pHPLT-BASE plasmid DNA, and subsequently transforming *B. subtilis* cells. Transformation mixes were plated on HI-agar plates (Heart Infusion agar) containing 10 mg/L neomycin sulfate and 0.5 % RBB-starch (Sigma-Aldrich Product No. S7629, Potato starch covalently linked with Remazol Brilliant Blue R). For each library, clear zone producing single colonies were picked and grown in TSB (tryptone and soy based broth) liquid medium containing 10 mg/ml neomycin. To generate BASE combinatorial variant enzyme samples for biochemical characterization, selective growth of the BASE combinatorial libraries members was performed in 96 well MTPs at 37°C for 68 hours in MBD medium.

Table 4-1 BASE (AmyTS23t) Combinatorial Library 1		
Targeted Position	Wild Type Residue	Substitution
182	T	N
183	G	N
305	Y	Q
320	Q	F
379	P	A
407	Q	D
419	T	S
475	G	T

Table 4-2 BASE (AmyTS23t) Combinatorial Library 2
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Targeted Position	Wild Type Residue	Substitution
160	Y	E
182	T	G
183	G	N
189	E	P
305	Y	G
379	P	E
475	G	T

Table 4-3 BASE (AmyTS23t) Combinatorial Library 3

Targeted Position	Wild Type Residue	Substitution
125	S	A
182	T	A
214	T	Q
279	T	N
305	Y	R
319	D	T
320	Q	N
475	G	R

Table 4-4 BASE (AmyTS23t) Combinatorial Library 4

Targeted Position	Wild Type Residue	Substitution
7	E	H
182	T	W
298	T	Q
376	Y	R
379	P	K
407	Q	W
419	T	S
453	L	W

EXAMPLE 5**5 Generation of BASE Combinatorial Variants**

[00343] In this example, the construction of *Bacillus subtilis* strains expressing BASE combinatorial variants is described. To express BASE combinatorial variants, BASE variant DNA fragments were cloned in the pHPLT vector, by using the unique *Pst*I and *Hind*III restriction sites, and subsequently introduced into a *Bacillus subtilis* strain. The BASE DNA variant fragments were constructed as described below. For each BASE combinatorial variant listed in Table 5-1 (S1 to S32), PCR reactions were performed using the primers listed in Table 5-2 and Table 5-3.

[00344] For the PCR reactions described below, final concentrations of 0.2 μ M DNA primers and 0.1 - 10 ng of plasmid DNA template were used. Table 5-2 lists the specific pDNA template and primer pairs used to construct each of the variants. In addition, all PCR reactions were completed in a volume of 50 μ L, using Finnzymes (Finnzymes OY, Espoo, Finland) Phusion High-Fidelity DNA Polymerase (Catalog No. F-530L). All PCR reaction mixes contained 10 μ L of 5X Phusion HF buffer, 1 μ L of 10 mM dNTP mixture, 0.75 μ L of Phusion DNA polymerase (2 units/ μ L), 1 μ L of 100% DMSO and de-ionised, autoclaved water for a total volume of 50 μ L. The PCR programs were run using a MJ Research PTC-200 Peltier thermal cycler (MJ

Research, Waltham, MA) as follows: 30 sec at 98°C, 25X (10 sec at 98°C, 20 sec at 55°C, 25 sec at 72°C), and finally 5 min at 72°C.

[00345] For BASE combinatorial variants S1 to S16, the amplified DNA fragments generated by PCR 1 and 2 were fused by a third PCR. A 0.5 µL aliquot of the amplified DNA fragments of both PCR1 and PCR 2 were added to the third reaction mixture containing primers *Pst*I-FW and *Hind*III-RV. The amplified linear 1.5 kb DNA fragments were purified (Qiagen® Qiaquick PCR purification kit, Catalog No. 28106) and digested with *Pst*I and *Hind*III restriction enzymes. Subsequently, the BASE combinatorial variant DNA fragments S1 to S16 and pHPLT pDNA (50 ng/µL digested with *Pst*I and *Hind*III restriction enzymes) were purified (Qiagen® Qiaquick PCR purification kit, Catalog No. 28106) and ligated. The reaction conditions were as follows: 4 µL of purified, *Pst*I and *Hpa*I digested BASE variant fragment, 2 µL of purified, *Pst*I and *Hind*III digested pHPLT DNA fragment, 8 µL T4 DNA Ligase buffer (Invitrogen® Catalog No. 46300-018), 25 µL de-ionised, autoclaved water and 1 µL T4 DNA Ligase, 1 unit/µL (Invitrogen® Catalog No. 15224-017). The ligation reaction was performed for 16-20 hours at 20°C.

[00346] To transform the ligation reaction mix directly into *B. subtilis* cells, the ligated pHPLT-BASE variant DNA was amplified using the TempliPhi kit (Amersham Catalog No. 25-6400). For this purpose 1 µL of the ligation reaction mix was mixed with 5 µL of sample buffer from the TempliPhi kit and heated for 3 minutes at 95°C to denature the DNA. The reaction mixture was placed on ice to cool for 2 minutes and then spun down briefly. Next, 5 µL of reaction buffer and 0.2 µL of phi29 polymerase from the TempliPhi kit were added, and the reactions were incubated at 30°C in an MJ Research PCR machine for 4 hours. The phi29 enzyme was heat inactivated by incubation at 65°C for 10 min.

[00347] For introducing of the BASE variants into *B. subtilis*, 0.1 µL of the TempliPhi amplification reaction product was mixed with 500 µL of competent *B. subtilis* cells [(genotype: *AaprE*, *AnprE*, *oppA*, *AspoII*E, *degUHy32*, *AamyE::(xylR,pxylA-comK)*] followed by vigorous shaking at 37°C for 1 hour and 100 µL and 500 µL was plated on Heart infusion agar (Difco, Cat.no 244400) plates containing 10 mg/L neomycin sulfate and 0.5 % RBB-starch. For each variant, clearing zone producing single colonies were picked and grown in TSB (tryptone and soy based broth) liquid medium with 10 mg/L neomycin selection for subsequent plasmid DNA isolation and gene sequence analysis. The identities of the BASE combinatorial variants were determined by sequence analysis. The pHPLT-BASE S1 to S16 plasmid DNA served as template DNA to construct BASE combinatorial variants S17 to S32 as described herein. To generate BASE combinatorial variant enzyme samples for biochemical characterization, the BASE combinatorial variants were grown in 96 well MTPs at 37°C for 68 hours in MBD medium.

Table 5-1 BASE Combinatorial Variants S1 - S32*

Variants	N128	K178	T182	A185	S243
BASE-S1	-	-	G	D	-
BASE-S3	-	L	G	D	-
BASE-S4	-	L	G	-	-
BASE-S8	-	L	-	D	-

Table 5-1 BASE Combinatorial Variants S1 - S32*					
Variants	N128	K178	T182	A185	S243
BASE-S9	-	-	G	D	Q
BASE-S10	-	-	G	-	Q
BASE-S11	-	L	G	D	Q
BASE-S12	-	L	G	-	Q
BASE-S14	-	-	-	D	Q
BASE-S15	-	L	-	-	Q
BASE-S16	-	L	-	D	Q
BASE-S17	C	-	G	D	-
BASE-S18	C	-	G	-	-
BASE-S19	C	L	G	D	-
BASE-S20	C	L	G	-	-
BASE-S21	C	-	-	D	-
BASE-S23	C	L	-	-	-
BASE-S24	C	L	-	D	-
BASE-S25	C	-	G	D	Q
BASE-S26	C	-	G	-	Q
BASE-S27	C	L	G	D	Q
BASE-S28	C	L	G	-	Q
BASE-S29	C	-	-	-	Q
BASE-S31	C	L	-	-	Q
BASE-S32	C	L	-	D	Q

*minus sign = wild type residue

Table 5-2 Plasmid DNA Templates and Primer Pairs			
Variants	Plasmid DNA Template	PCR1	PCR2
BASE-S1	pHPLT-BASE	KGA/D-FW+HindIII-Rv	KGA/D-RV+PstI-Fw
BASE-S3	pHPLT-BASE	LGA/D-FW+HindIII-Rv	LGA/D-RV+PstI-Fw
BASE-S4	pHPLT-BASE	LGA/D-FW+HindIII-Rv	LGA/D-RV+PstI-Fw
BASE-S8	pHPLT-BASE	LTA/D-FW+HindIII-Rv	LTA/D-RV+PstI-Fw
BASE-S9	pHPLT-BASE-S243Q	KGA/D-FW+HindIII-Rv	KGA/D-RV+PstI-Fw
BASE-S10	pHPLT-BASE-S243Q	KGA/D-FW+HindIII-Rv	KGA/D-RV+PstI-Fw
BASE-S11	pHPLT-BASE-S243Q	LGA/D-FW+HindIII-Rv	LGA/D-RV+PstI-Fw
BASE-S12	pHPLT-BASE-S243Q	LGA/D-FW+HindIII-Rv	LGA/D-RV+PstI-Fw
BASE-S14	pHPLT-BASE-S243Q	KTA/D-FW+HindIII-Rv	KTA/D-RV+PstI-Fw
BASE-S15	pHPLT-BASE-S243Q	LTA/D-FW+HindIII-Rv	LTA/D-RV+PstI-Fw
BASE-S16	pHPLT-BASE-S243Q	LTA/D-FW+HindIII-Rv	LTA/D-RV+PstI-Fw
BASE-S17	pHPLT-BASE-S1	128-FW+HindIII-Rv	128-RV+PstI-Fw
BASE-S18	pHPLT-BASE-T182G	128-FW+HindIII-Rv	128-RV+PstI-Fw
BASE-S19	pHPLT-BASE-S3	128-FW+HindIII-Rv	128-RV+PstI-Fw
BASE-S20	pHPLT-BASE-S4	128-FW+HindIII-Rv	128-RV+PstI-Fw
BASE-S21	pHPLT-BASE-A185D	128-FW+HindIII-Rv	128-RV+PstI-Fw
BASE-S23	pHPLT-BASE-K178L	128-FW+HindIII-Rv	128-RV+PstI-Fw
BASE-S24	pHPLT-BASE-S8	128-FW+HindIII-Rv	128-RV+PstI-Fw
BASE-S25	pHPLT-BASE-S9	128-FW+HindIII-Rv	128-RV+PstI-Fw
BASE-S26	pHPLT-BASE-S10	128-FW+HindIII-Rv	128-RV+PstI-Fw
BASE-S27	pHPLT-BASE-S11	128-FW+HindIII-Rv	128-RV+PstI-Fw
BASE-S28	pHPLT-BASE-S12	128-FW+HindIII-Rv	128-RV+PstI-Fw
BASE-S29	pHPLT-BASE-S243Q	128-FW+HindIII-Rv	128-RV+PstI-Fw
BASE-S31	pHPLT-BASE-S15	128-FW+HindIII-Rv	128-RV+PstI-Fw
BASE-S32	pHPLT-BASE-S16	128-FW+HindIII-Rv	128-RV+PstI-Fw

Table 5-3 Primers Used to Construct BASE Combinatorial Variants S1 - S32*		
Primer Name	Alternate Name	Primer Sequence (SEQ ID NO)
N128C_FW	128-FW	GATCCGAGCAACCGTTGCCAGGAAACCAGCGGC (SEQ ID NO:17)
N128C_RV	128-RV	GCCGCTGGTTTCCTGGCAACGGTTGCTCGGATC (SEQ ID NO:18)
KGA/D_FW	KGA/D-FW	CCGCATCTATAAAATTTTCGTAGCGGAGGCAAAGMYTGGGATTGGG (SEQ ID NO:19)
KGA/D_RV	KGA/D-RV	CCCAATCCCARKCTTTGCCTCCGCTACGAAATTTATAGATGCGG (SEQ ID NO:20)
LGA/D_FW	LGA/D-FW	CCGCATCTATTTATTTTCGTAGCGGAGGCAAAGMYTGGGATTGGG (SEQ ID NO:21)
LGA/D_RV	LGA/D-RV	CCCAATCCCARKCTTTGCCTCCGCTACGAAATAAATAGATGCGG (SEQ ID NO:22)
KTA/D_FW	KTA/D-FW	CCGCATCTATAAAATTTTCGTAGCACCGGCAAAGMYTGGGATTGGG (SEQ ID NO:23)
KTA/D_RV	KTA/D-RV	CCCAATCCCARKCTTTGCCGGTGCTACGAAATTTATAGATGCGG (SEQ ID NO:24)
LTA/D_FW	LTA/D-FW	CCGCATCTATTTATTTTCGTAGCACCGGCAAAGMYTGGGATTGGG (SEQ ID NO:25)
LTA/D_RV	LTA/D-RV	CCCAATCCCARKCTTTGCCGGTGCTACGAAATAAATAGATGCGG (SEQ ID NO:26)
BASE-PstI-FW	PstI-Fw	GCTGCCTCATTCTGCAGCTTCAGCA (SEQ ID NO:27)
BASE-HindIII-RV	HindIII-Rv	CTGTTTTATCCTTTACCTTGTCTC (SEQ ID NO:28)

*M = DNA A or C; Y = DNA C or T; R = DNA G or A; and K = DNA G or T.

5 **[00348]** BASE combinatorial variants W1 to W13 were constructed by making use of BASE combinatorial variants P1 to P12. The P1-P12 variants of Table 5-4 were selected from the BASE combinatorial libraries 1-4 described in Example 4. For each BASE combinatorial variant listed in Table 5-5 (W1 to W32), PCR reactions were performed using the primers listed in Table 5-6. All PCR reaction conditions were similar to the protocol used for generating BASE combinatorial variants S1 to S32, set forth above.

Table 5-4 Amino Acid Substitutions of BASE Variants P1 - P12*										
Variant	S125	T182	G183	E189	T279	Y305	D319	Q320	P379	G475
BASE-P1 (9)	-	-	-	-	-	Q	-	-	A	-
BASE-P2 (3)	-	N	-	-	-	Q	-	F	A	-
BASE-P3 (17)	-	N	-	-	-	-	-	-	A	T
BASE-P4 (18)	-	N	N	-	-	Q	-	F	-	T
BASE-P5 (29)	-	G	-	-	-	G	-	-	-	-

Table 5-4 Amino Acid Substitutions of BASE Variants P1 - P12*										
Variant	S125	T182	G183	E189	T279	Y305	D319	Q320	P379	G475
BASE-P6 (23)	-	G	-	P	-	G	-	-	-	-
BASE-P7 (33)	-	G	-	-	-	-	-	-	-	T
BASE-P8 (41)	A	-	-	-	N	-	T	N	-	R
BASE-P9 (51)	-	-	-	-	-	R	T	-	-	R
BASE-P10 (45)	A	-	-	-	-	R	-	-	-	R
BASE-P11 (59)	-	A	-	-	N	R	-	N	-	-
BASE-P12 (62)	-	-	-	-	-	R	T	N	-	-

*BASE Performance Variant No. is shown in parenthesis. BASE P1 - P12 variants have wild type residues at positions Y160, T214, Q407 and T149.

Table 5-5 Amino Acid Substitutions of BASE Variants W1 - W13													
Variant	S125	N128	K178	T182	G183	E189	S243	T279	Y305	D319	Q320	P379	G475
W1	-	C	L	G	-	-	Q	-	Q	-	-	A	-
W2	-	C	L	G	-	-	Q	-	Q	-	F	A	-
W3	-	C	-	N	-	-	Q	-	Q	-	F	A	-
W4	-	C	L	G	-	-	Q	-	-	-	-	A	T
W5	-	C	L	G	N	-	Q	-	Q	-	F	-	T
W6	-	C	L	G	-	-	Q	-	G	-	-	-	-
W7	-	C	L	G	-	P	Q	-	G	-	-	-	-
W8	-	C	L	G	-	-	Q	-	-	-	-	-	T
W9	A	C	L	G	-	-	Q	N	-	T	N	-	R
W10	-	C	L	G	-	-	Q	-	R	T	-	-	R
W11	A	C	L	G	-	-	Q	-	R	-	-	-	R
W12	-	C	L	A	-	-	Q	N	R	-	N	-	-
W13	-	C	L	G	-	-	Q	-	R	T	N	-	-

Table 5-6 Primers Used to Construct BASE Combinatorial Variants W1 - W13	
Primer Name (Alternate)	Primer Sequence (SEQ ID NO)

Table 5-6 Primers Used to Construct BASE Combinatorial Variants W1 - W13	
Primer Name (Alternate)	Primer Sequence (SEQ ID NO)
BASE-N128C-FW (Pr1)	GGATCCGAGCAACCGTTGCCAGGAAACCAGCGGC (SEQ ID NO: 29)
BASE-N128C-RV (Pr2)	GCCGCTGGTTTCCTGGCAACGGTTGCTCGGATCC (SEQ ID NO: 30)
BASE-S125A-N128C-FW (Pr3)	GGATCCGGCCAACCGTTGCCAGGAAACCAGCGGC (SEQ ID NO: 31)
BASE-S125A-N128C-RV (Pr4)	GCCGCTGGTTTCCTGGCAACGGTTGGCCGGATCC (SEQ ID NO: 32)
BASE-K178L-T182G-FW (Pr5)	GAACCGCATCTATCTATTTTCGTAGCGGCGGCAAAGCGTGGGAT (SEQ ID NO: 33)
BASE-K178L-T182G-RV (Pr6)	ATCCACGCTTTGCCGCGCTACGAAATAGATAGATGCGGTTC (SEQ ID NO: 34)
BASE-K178L-T182G-G183N-FW (Pr7)	GAACCGCATCTATCTATTTTCGTAGCGGCAACAAAGCGTGGGAT (SEQ ID NO: 35)
BASE-K178L-T182G-G183N-RV (Pr8)	ATCCACGCTTTGTTGCCGCTACGAAATAGATAGATGCGGTTC (SEQ ID NO: 36)
BASE-K178L-T182A-FW (Pr9)	GAACCGCATCTATCTATTTTCGTAGCGCGGCAAAGCGTGGGAT (SEQ ID NO: 37)
BASE-K178L-T182A-RV (Pr10)	ATCCACGCTTTGCCGCGCTACGAAATAGATAGATGCGGTTC (SEQ ID NO: 38)
BASE-S243Q-FW (Pr11)	GGTGAAACACATCAAATACCAATTTTTTCCGGATTGGCTG (SEQ ID NO: 39)
BASE-S243Q-RV (Pr12)	CAGCCAATCCGGAATAAATTGGTATTTGATGTGTTTCACC (SEQ ID NO: 40)
BASE-T182G-FW (Pr13)	GAACCGCATCTATAAATTTTCGTAGCGGCGGCAAAGCGTGGGAT (SEQ ID NO: 41)
BASE-T182G-RV (Pr14)	ATCCACGCTTTGCCGCGCTACGAAATTTATAGATGCGGTTC (SEQ ID NO: 42)
BASE-T182G-G182N-FW (Pr15)	GAACCGCATCTATAAATTTTCGTAGCGGCAACAAAGCGTGGGAT (SEQ ID NO: 43)
BASE-T182G-G182N-RV (Pr16)	ATCCACGCTTTGTTGCCGCTACGAAATTTATAGATGCGGTTC (SEQ ID NO: 44)
BASE-T182A-FW (Pr17)	GAACCGCATCTATAAATTTTCGTAGCGCGGCAAAGCGTGGGAT (SEQ ID NO: 45)
BASE-T182A-RV (Pr18)	ATCCACGCTTTGCCGCGCTACGAAATTTATAGATGCGGTTC (SEQ ID NO: 46)
BASE-PstI-FW (Pr19)	GCTGCCTCATCTGCAGCTTCAGCA (SEQ ID NO: 47)
BASE-HindIII-RV (Pr20)	GCTGTTTTATCCTTTACCTTGCTCTC (SEQ ID NO: 48)

[00349] The PCR scheme to construct BASE combinatorial variants W1 to W13 is shown in Tables 5-7 and 5-8. Variant production begins with five PCR reactions (series A to E), and continues with two fusion PCR reactions (series F and G). All PCR fragments were purified using Qiagen® Qiaquick PCR purification kit (Catalog No. 28106). As described for construction of variants S1 to S32, fusion DNA fragments of PCR G1 to G13 were digested with *Pst*I and *Hind*III and ligated to *Pst*I and *Hind*III-digested pHPLT vector DNA. Subsequently, a phi29 polymerase amplified ligation mixture was introduced into *B.subtilis*. For each variant, clear zone producing single colonies were picked and grown in TSB (tryptone and soy based broth) liquid

medium containing 10 mg/L neomycin for subsequent plasmid DNA isolation and gene sequence analysis. Identity of the BASE combinatorial variants was confirmed by sequence analysis. To generate enzyme samples of combinatorial variants W1 to W13 for biochemical characterization, selective growth of the BASE combinatorial variants was performed in 96 well MTPs at 37°C for 68 hours in MBD medium.

Table 5-7 PCR Reactions For Construction of BASE Combinatorial Variants W1 - W13						
BASE Variants	Template DNA	PCR A1 to A13	PCR B1 to B12	PCR C13	PCR D1 to D12	PCR E1 to E13
BASE-W1	BASE-P1	Pr19+Pr2	Pr1+Pr6	-	Pr5+Pr12	Pr11+Pr20
BASE-W2	BASE-P2	Pr19+Pr2	Pr1+Pr6	-	Pr5+Pr12	Pr11+Pr20
BASE-W3	BASE-P3	Pr19+Pr2	Pr1+Pr6	-	Pr5+Pr12	Pr11+Pr20
BASE-W4	BASE-P4	Pr19+Pr2	Pr1+Pr8	-	Pr7+Pr12	Pr11+Pr20
BASE-W5	BASE-P5	Pr19+Pr2	Pr1+Pr6	-	Pr5+Pr12	Pr11+Pr20
BASE-W6	BASE-P6	Pr19+Pr2	Pr1+Pr6	-	Pr5+Pr12	Pr11+Pr20
BASE-W7	BASE-P7	Pr19+Pr2	Pr1+Pr6	-	Pr5+Pr12	Pr11+Pr20
BASE-W8	BASE-P8	Pr19+Pr4	Pr3+Pr6	-	Pr5+Pr12	Pr11+Pr20
BASE-W9	BASE-P9	Pr19+Pr2	Pr1+Pr6	-	Pr5+Pr12	Pr11+Pr20
BASE-W10	BASE-P10	Pr19+Pr4	Pr3+Pr6	-	Pr5+Pr12	Pr11+Pr20
BASE-W11	BASE-P11	Pr19+Pr2	Pr1+Pr10	-	Pr9+Pr12	Pr11+Pr20
BASE-W12	BASE-P12	Pr19+Pr2	Pr1+Pr6	-	Pr5+Pr12	Pr11+Pr20
BASE-W13	BASE-P2	Pr19+Pr2	-	Pr1+Pr2	-	Pr11+Pr20
	Size	424 bp	196 bp	382 bp	229 bp	826 bp

Table 5-8 Series 1 (F) and Series 2 (G) Fusion Reactions For BASE Variants W1 - W13				
BASE Variants	Template DNA	PCR F1 to F12	Template DNA	PCR G1 to G12
BASE-W1	B1+D1	Pr1+Pr6	A1+F1+E1	Pr19+Pr20
BASE-W2	B2+D2	Pr1+Pr6	A2+F2+E2	Pr19+Pr20
BASE-W3	B3+D3	Pr1+Pr6	A3+F3+E3	Pr19+Pr20
BASE-W4	B4+D4	Pr1+Pr8	A4+F4+E4	Pr19+Pr20
BASE-W5	B5+D5	Pr1+Pr6	A5+F5+E5	Pr19+Pr20
BASE-W6	B6+D6	Pr1+Pr6	A6+F6+E6	Pr19+Pr20
BASE-W7	B7+D7	Pr1+Pr6	A7+F7+E7	Pr19+Pr20
BASE-W8	B8+D8	Pr3+Pr6	A8+F8+E8	Pr19+Pr20
BASE-W9	B9+D9	Pr1+Pr6	A9+F9+E9	Pr19+Pr20
BASE-W10	B10+D10	Pr3+Pr6	A10+F10+E10	Pr19+Pr20
BASE-W11	B11+D11	Pr1+Pr10	A11+F11+E11	Pr19+Pr20
BASE-W12	B12+D12	Pr1+Pr6	A12+F12+E12	Pr19+Pr20
BASE-W13	-	-	A13+F13+E13	Pr19+Pr20
	size	382 bp	Size	382 bp

EXAMPLE 6

5 Generation of ACE (AmyTS23tΔRS) Site Evaluation Libraries

[00350] In this example, the construction of *Bacillus subtilis* strains expressing the BASE variants: BASE-ΔR180-ΔS181 (also known as AmyTS23tΔRS or ACE); and BASE-ΔR180-ΔS181-S243Q (also known as AmyTS23tΔRS-S243Q, named ACE-S243Q or ACE-Q) are described. In addition, the generation of ACE-Q site evaluation libraries (SELs) is described.

- 10 **[00351]** Synthetic DNA fragment 056426 (produced by Geneart, Regensburg, Germany) containing a DNA codon-modified BASE gene served as template DNA. This BASE DNA fragment was cloned into the pHPLT vector (Solingen *et al.*, *Extremophiles*, 5:333-341, 2001) using the unique *Pst*I and *Hpa*I restriction

sites. The pHPLT expression vector contains the *B. licheniformis* LAT promoter (Plat) followed by *Pst*I and *Hpa*I restriction sites for cloning BASE, and additional elements from pUB110 (McKenzie *et al.*, *Plasmid*, 15: 93-103, 1986) including a replicase gene (reppUB), a neomycin/kanamycin resistance gene (neo) and a bleomycin resistance marker (bleo). A map of the pHPLT-BASE plasmid is shown in Figure 3. BASE DNA with codons 180 (CGG) and 181 (AGC) deleted (BASE-ΔR180-ΔS181, also named ACE) was generated using pHPLT-BASE as template DNA and the DNA primers listed in Table 6-1. The DNA primers were synthesized and desalted by Sigma (Sigma-Aldrich Chemie B.V., Zwijndrecht, The Netherlands).

[00352] Two PCR reactions were performed using pHPLT-BASE template DNA using primer pairs TS-delRS-FW/pHPLT-HpaI-RV and TS-delRS-RV/pHPLT-PstI-FW. In order to fuse the two PCR generated fragments, 1 μl unpurified PCR mix from both reactions was added to a third PCR reaction in which primers pHPLT-PstI-FW and pHPLT-HpaI-RV were added. The amplified linear 1.5 kb DNA fragment was purified (using Qiagen® Qiaquick PCR purification kit Cat. no. 28106) and digested with *Pst*I and *Hpa*I restriction enzymes.

[00353] For all the PCR reactions described, final concentrations of 0.2 μM DNA primer were used, and 0.1 - 10 ng of DNA template was used. In addition, all PCR reactions were completed in a volume of 50 μl, using Finnzymes (Finnzymes OY, Espoo, Finland) Phusion High-Fidelity DNA Polymerase (Cat. no. F-530L). Also, all PCR reaction mixes contained 10 μL of 5X Phusion HF buffer, 1 μL of 10 mM dNTP mixture, 0.75 μL of Phusion DNA polymerase (2 units/μL), 1 μL of 100% DMSO and de-ionised, autoclaved water to give a final volume of 50 μL. The PCR programs, using a MJ Research PTC-200 Peltier thermal cycler (MJ Research, Waltham, MA) were performed as described by Finnzymes (30 sec at 98°C, 30X[10 sec at 98°C, 20 sec at 55°C, 22 sec/kb at 72°C] with a final step of 5 min at 72°C).

[00354] Subsequently, the BASE-ΔR180-ΔS181 DNA fragment and pHPLT plasmid DNA (50 ng/μL, digested with *Pst*I and *Hpa*I restriction enzymes) were both purified (using Qiagen® Qiaquick PCR purification kit Cat. no. 28106) and then ligated at the *Pst*I and *Hpa*I ends using the following reaction conditions: 4 μL of purified, *Pst*I and *Hpa*I digested BASE-ΔR180-ΔS181 DNA fragment, 2 μL of purified, *Pst*I and *Hpa*I digested pHPLT DNA fragment, 8 μL T4 DNA Ligase buffer (Invitrogen® Cat. no. 46300-018), 25 μl di-ionised, autoclaved water and 1 μL T4 DNA Ligase, 1 unit/μL (Invitrogen® Cat. no. 15224-017). The ligation reaction was performed for 16-20 hours at 20°C.

[00355] The ligation mixture was transformed into a *B. subtilis* strain (genotype: Δ*aprE*, Δ*nprE*, Δ*epi*, Δ*ispA*, Δ*bpr*) and (Δ*degUH32*, Δ*oppA*, Δ*spoIIE3501*, Δ*amyE::xylRPxylAcomK-ermC*, (Δ*avpr*, Δ*awprA*, Δ*ampr-ybfJ*, Δ*nprB*)) as described in WO 02/14490, incorporated herein by reference for the teaching of bacterial transformation. The *B. subtilis* transformants were selected on agar plates containing Heart infusion agar (Difco, Cat. no 244400) and 10 mg/L neomycin. Selective growth of *B. subtilis* transformants harboring the pHPLT-BASE-ΔR180-ΔS181 plasmid was performed in shake flasks containing 25 ml MBD medium (MOPS based defined medium) and 10 mg/L neomycin. This resulted in the production of secreted BASE-ΔR180-ΔS181 amylase with starch hydrolyzing activity. The pHPLT-BASE-ΔR180-ΔS181 plasmid is also referred to herein as pHPLT-ACE.

[00356] For the generation of a *Bacillus subtilis* strain expressing the BASE- Δ R180- Δ S181-S243Q amylase, a similar protocol was used. The first two PCR reactions were performed using primers ACE-S243Q-FW and pHPLT-HpaI-RV, and primers ACE-S243Q -RV and pHPLT-PstI-FW, pHPLT-ACE template DNA. Primer sequences are listed in Table 6-1. The *B. subtilis* transformants harboring the pHPLT-
 5 BASE- Δ R180- Δ S181-S243Q produced and secreted BASE Δ R180- Δ S181-S234Q amylase with starch hydrolyzing activity. A map of the pHPLT-BASE- Δ R180- Δ S181-S243Q plasmid, also termed pHPLT-ACE-S243Q, is shown in Figure 4.

Table 6-1 Primers for Generation of the BASE- Δ R180- Δ S181 (ACE) and ACE-Q Amylases	
Primer name	DNA sequence (SEQ ID NO)
pHPLT-PstI-FW	CTCATTCTGCAGCTTCAGCAAATACGGCG (SEQ ID NO: 49)
pHPLT-HpaI-RV	CTCTGTAACTCATTTGGCGACCCAGATTGAAACG (SEQ ID NO: 50)
TS-delRS-FW	CTATAAATTTACGGGCAAAGCATGGGATTGG (SEQ ID NO: 51)
TS-delRS-RV	TGCTTTGCCCGTAAATTTATAGATCCGGTTCAG (SEQ ID NO: 52)
ACE-S243Q-FW	CAAACATATCAAATATCAATTTTTCGGACTG (SEQ ID NO: 53)
ACE-S243Q-RV	CAGTCCGGAAAAAATTGATATTTGATATGTTTG (SEQ ID NO: 54)

10 [00357] The pHPLT-ACE-S243Q plasmid DNA served as a template for the production of site evaluation libraries (SELs). The amino acid positions selected for the ACE-Q SELs are numbered according to the mature BASE amino acid sequence (SEQ ID NO: 2) and include: R127, Y305, Q320, P379, T419, L453 and G475. The corresponding DNA codons were each replaced with mutated codons encoding a maximum of 20 different amino acids. This pHPLT-ACE-S243Q plasmid contained a unique *Bg*/III restriction site, which was
 15 utilized during SEL construction. Sequences of the primers (commercially synthesized and desalted) used to generate the libraries are listed in Table 6-2.

[00358] To construct ACE-Q SELs, three reactions were performed: two mutagenesis reactions to introduce the mutated codon of interest in the ACE-Q DNA sequence, and a third reaction to fuse the two PCR fragments. The method of mutagenesis was based on the codon-specific mutation approach. In this
 20 method, the creation of all possible mutations in a specific DNA triplet is accomplished using a forward and reverse oligonucleotide primer encoding a specific designed triplet DNA sequence NNS ((A, C, T or G), (A, C, T or G), (C or G)) that corresponds with the sequence of the codon to be mutated and guarantees random incorporation of nucleotides at the codon of interest. The number listed in the primer names of Table 6-2 corresponds with the specific ACE-Q codon position (based on the numbering of the mature BASE amino
 25 acid sequence). Two additional oligonucleotide primers that were used to construct the SEL encode the unique *Bg*/III restriction site and pHPLT DNA sequence flanking the *Bg*/III restriction site.

Table 6-2 Primers for Generation of ACE-Q SEL Variants

Table 6-2 Primers for Generation of ACE-Q SEL Variants	
Primer name	DNA sequence (SEQ ID NO)
ACE-Q-R127-FW	GTTGATCCGAGCAACNNSAACCAAGAAACGAG (SEQ ID NO: 55)
ACE-Q-R127-RV	CTCGTTTCTTGGTTSNNGTTGCTCGGATCAAC (SEQ ID NO: 56)
ACE-Q-Y305-FW	GCAAAAGCTCAGGCNNSTTTGATATGAGATATC (SEQ ID NO: 57)
ACE-Q-Y305-RV	GATATCTCATATCAAASNNGCCTGAGCTTTTGC (SEQ ID NO: 58)
ACE-Q-Q320-FW	CGCTGATGAAAGATNNSCCGAGCCTGGCAGTC (SEQ ID NO: 59)
ACE-Q-Q320-RV	GACTGCCAGGCTCGGSNNATCTTTCATCAGCG (SEQ ID NO: 60)
ACE-Q-P379-FW	CGAAATATAATATCNNSGGCCTGAAAAGC (SEQ ID NO: 61)
ACE-Q-P379-RV	GCTTTTCAGGCCSNNGATATTATATTTTCG (SEQ ID NO: 62)
ACE-Q-T419-FW	GAGAAGGCATCGATNNSAAACCGAATAGCG (SEQ ID NO: 63)
ACE-Q-T419-RV	CGCTATTCGGTTTSNNATCGATGCCTTCTC (SEQ ID NO: 64)
ACE-Q-L453-FW	CAAAGTCTTTTATGATNNSACGGGCAACAGAAGC (SEQ ID NO: 65)
ACE-Q-L453-RV	GCTTCTGTTGCCGTSNNATCATAAAAGACTTTG (SEQ ID NO: 66)
ACE-Q-G475-FW	GAATTTAAAGTCAATNNSGGCAGCGTTTCAATC (SEQ ID NO: 67)
ACE-Q-G475-RV	GATTGAAACGCTGCCSNNATTGACTTTAAATTC (SEQ ID NO: 68)
pHPLT-BglII-FW	GCAATCAGATCTTCCTTCAGGTTATGACC (SEQ ID NO: 69)
pHPLT-BglII-RV	GCATCGAAGATCTGATTGCTTAAGTCTTC (SEQ ID NO: 70)

[00359] Construction of each SEL began with two primary amplification reactions: a first PCR using the pHPLT-BglII-FW primer and a specific ACE-Q reverse mutagenesis primer; and a second PCR using the pHPLT-BglII-RV primer and a specific ACE-Q forward mutagenesis primer. The introduction of the mutations in the mature ACE-Q sequence was performed using Finnzymes Phusion High-Fidelity DNA Polymerase (Finnzymes OY, Espoo, Finland) (Cat. no. F-530L). All reactions were performed according to the protocol supplied by the manufacturer. The PCR conditions for the primary reactions were as follows. For primary PCR 1: the pHPLT-BglII-FW primer and a specific ACE-Q reverse mutagenesis primer – both 1 μ L (10 μ M), and 0.1 - 10 ng of DNA template (pHPLT-ACE-S243Q), 10 μ L of 5X Phusion HF buffer, 1 μ L of 10 mM dNTP mixture, 0.75 μ L of Phusion DNA polymerase (2 units/ μ L), 1 μ L of 100% DMSO and de-ionised, autoclaved water to a total final volume of 50 μ L. For primary PCR 2: the pHPLT-BglII-RV primer and a specific ACE-Q forward mutagenesis primer – both 1 μ L (10 μ M), and 0.1 - 10 ng of DNA template (pHPLT-ACE-S243Q), 10 μ L of 5X Phusion HF buffer, 1 μ L of 10 mM dNTP mixture, 0.75 μ L of Phusion DNA polymerase (2 units/ μ L), 1 μ L of 100% DMSO and de-ionised, autoclaved water to a total final volume of 50 μ L.

[00360] A PTC-200 Peltier thermal cycler (MJ Research, Inc. Waltham, MA) with the following program was used: 30 seconds 98°C, 30X cycles (10 seconds 98°C, 20 seconds 55°C, 1 minute 72°C) and 5 min 72°C. For each SEL primary amplification reaction two DNA fragments of approximately 2-3 kb with an

approximately 30 nucleotide overlap around the ACE-Q codon of interest were produced. In order to fuse the two DNA fragments, 1 µL unpurified PCR mix from both reactions was added to a third amplification reaction to which primers pHPLT-BglIII-FW and pHPLT-BglIII-RV were added. The amplified linear 5.2 kb DNA fragments were purified (using Qiagen® Qiaquick PCR purification kit Cat. no. 28106) and digested with a *Bgl*III restriction enzyme to create cohesive ends on both sides of the fusion fragment. The restriction digest contained 35 µL purified linear DNA fragment, 4 µL React® 3 buffer (Invitrogen, Paisley PA4 9RF, UK) and 1 µL *Bgl*III, 10 units/ml (Invitrogen, Paisley PA4 9RF, UK) as was incubated at 30°C for 1 hour.

[00361] The codon mutagenized pHPLT-ACE-S243Q ligation mixtures were used to transform competent *B. subtilis* cells (genotype: $\Delta aprE$, $\Delta nprE$, $oppA$, $\Delta spoIIE$, $degUHy32$, $\Delta amyE::[xylR,pxylA-comK]$) as described in WO 2002/014490, in order to generate the ACE-Q SELs. Transformation mixes were plated on HI-agar plates (Heart Infusion agar) containing 10 mg/ml neomycin sulfate (Sigma, Catalog No. N-1876; contains 732 µg neomycin per mg). For each library, single colonies were picked and grown in tryptone and soy based broth liquid media under 10 mg/ml neomycin selection for subsequent plasmid DNA isolation and DNA sequence analysis of the ACE-Q gene variants. DNA sequence analysis was performed by BaseClear B.V. (Leiden, The Netherlands). Sequence analysis data revealed a maximum of 18 ACE-Q variants per library. All ACE-Q variants that were identified in the seven ACE-Q SELs are listed in Table 6-3. To generate ACE-Q variant enzyme samples for biochemical characterization selective growth of the ACE-Q SEL members was done in 96 well MTPs at 37°C for 68 hours in MBD medium (MOPS based defined medium).

Table 6-3 ACE-S243Q (ACE-Q) Variants Identified in the Seven ACE-Q SELs

Position	Amino Acid Substitutions Generated in the ACE-Q Amylase Background																	
R127	A	C	D	E	F	G	H	I	K	L	M	P	Q	S	T	V	W	Y
Y305	A	C	D	E	G	H	L	N	P	Q	R	S	T	V	W	-	-	-
Q320	A	C	D	E	F	G	H	I	L	M	N	P	R	S	T	V	W	Y
P379	A	C	E	E	G	H	L	N	Q	R	S	T	V	W	Y	-	-	-
T419	A	C	D	E	F	G	H	K	L	M	N	P	Q	R	S	V	W	Y
L453	A	C	D	F	G	H	I	K	M	N	P	Q	R	S	T	V	W	Y
G475	A	D	E	H	I	K	L	M	N	P	Q	R	S	T	V	W	-	-

EXAMPLE 7

Performance Index Values for BASE Variants

[00362] In this example, results of experiments conducted to determine cleaning performance (CS-28 microswatch assay at pH 10/32°C, pH 10/50°C, pH 8/16°C and pH 8/32°C), detergent stability, thermostability, BPNPG7 amylase activity and HPLC protein concentration (tests of properties of interest) of BASE and variants thereof are described. The results were obtained using the methods of Example 1. As described throughout, functionality of BASE variants was quantified as a performance index (PI), which is the

ratio of performance of a variant to a parent protein. Table 10-1 shows the PI values for numerous BASE variants for the properties tested. The mutations introduced at the various amino acid positions are indicated. Performance indices less than or equal to 0.05 were fixed to 0.05. For every variant with an HPLC protein PI less than or equal to 0.05, all values were fixed at 0.05. Also, for the two stability measures, if the PI of the initial activity in the stability assays was less than or equal to 0.05, the associated stability PI was fixed to 0.05. Table 7-1 provides performance indices of BASE variants having combinable mutations, which are defined herein as mutations in variants with PI values ≥ 0.5 for at least one property, and PI values of >0.05 for all properties.

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
1	N001A	0.81	0.71	2.75	0.26	0.88	0.83	0.83	0.78	0.79
1	N001C	0.75	0.34	2.72	0.11	0.70	1.03	0.75	0.67	0.28
1	N001D	0.92	0.72	2.91	0.28	0.88	0.83	0.80	0.85	0.78
1	N001E	0.93	0.87	3.10	0.29	0.90	0.77	0.82	0.75	0.87
1	N001F	0.68	0.48	2.96	0.19	0.98	0.77	0.69	0.76	0.53
1	N001H	0.67	0.62	2.59	0.33	0.94	0.87	0.77	0.93	0.84
1	N001K	0.64	0.87	2.54	0.37	0.97	1.00	1.21	0.91	1.04
1	N001L	0.81	0.51	2.67	0.20	0.80	0.79	0.80	0.68	0.59
1	N001M	0.91	0.59	3.02	0.23	0.82	0.74	0.73	0.64	0.67
1	N001Q	0.47	0.49	2.66	0.34	0.79	0.94	0.88	0.96	0.88
1	N001R	0.79	1.09	2.63	0.35	0.97	1.20	0.92	0.90	0.96
1	N001S	0.90	0.77	2.72	0.29	0.94	0.88	0.91	0.80	0.84
1	N001T	0.90	0.72	2.87	0.29	0.86	0.82	1.02	0.80	0.81
1	N001Y	0.66	0.46	2.70	0.25	0.89	0.81	0.79	0.80	0.69
2	T002A	0.79	1.06	1.02	0.96	0.90	1.04	0.98	0.73	1.02
2	T002D	0.98	1.01	1.19	1.00	0.90	1.04	0.99	1.00	1.04
2	T002E	0.86	1.14	1.24	0.98	1.03	0.90	1.14	1.02	1.10
2	T002F	0.90	0.54	1.23	0.54	0.80	0.89	1.80	1.01	0.55
2	T002H	0.94	0.97	1.16	0.93	0.99	1.03	0.76	1.01	0.98
2	T002I	0.71	0.63	1.04	0.60	1.11	1.44	0.52	0.95	0.64
2	T002K	0.96	1.06	1.09	1.01	1.00	1.12	0.85	1.00	1.07
2	T002L	0.85	0.50	1.18	0.48	0.90	1.22	0.47	0.88	0.56
2	T002M	0.94	0.65	1.27	0.65	0.93	1.01	0.54	0.88	0.76
2	T002N	0.95	1.16	1.25	1.09	1.04	1.05	1.05	1.03	1.20
2	T002P	1.21	0.39	1.35	0.41	1.52	1.20	0.97	1.41	0.25
2	T002Q	1.00	1.10	1.22	1.07	1.10	1.15	1.05	1.06	0.82
2	T002R	0.92	0.97	0.96	1.01	1.20	1.48	1.06	1.09	0.90
2	T002S	0.92	0.97	1.09	0.94	1.02	1.00	0.86	0.97	0.97
2	T002Y	0.93	0.64	1.21	0.69	0.93	1.07	0.67	0.98	0.68
3	A003C	0.68	0.66	0.94	0.55	1.11	0.99	0.89	1.00	0.31
3	A003D	0.92	1.06	1.10	0.95	0.77	0.75	0.94	0.85	0.76
3	A003E	0.97	1.15	1.20	1.11	0.91	0.82	1.02	0.78	0.94
3	A003F	0.91	0.84	1.10	0.82	0.96	1.05	1.05	0.92	0.58
3	A003G	0.86	0.94	1.30	0.93	0.81	1.02	0.99	0.67	0.74

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
3	A003H	1.06	0.94	1.22	0.95	0.95	0.90	0.90	0.91	0.79
3	A003I	0.81	0.77	0.99	0.75	1.10	1.20	0.88	1.01	0.48
3	A003K	0.83	0.93	1.12	0.86	0.91	1.06	0.87	0.71	0.67
3	A003M	0.89	0.38	1.31	0.40	0.97	1.20	1.13	1.10	0.24
3	A003N	0.94	1.06	1.22	0.97	1.03	1.00	1.12	0.87	0.77
3	A003P	0.97	1.16	1.23	1.16	0.97	0.94	0.82	0.83	0.85
3	A003Q	0.96	1.09	1.20	1.06	1.03	0.97	1.17	0.81	0.84
3	A003R	0.82	1.01	0.96	0.90	1.05	1.19	1.14	0.80	0.67
3	A003S	0.89	1.01	1.02	1.03	1.03	1.23	1.31	0.92	0.66
3	A003T	0.82	1.44	1.13	1.18	0.70	0.98	0.94	0.82	0.94
3	A003V	0.81	0.86	1.25	0.82	0.92	0.77	0.97	0.82	0.69
3	A003W	0.85	0.52	1.19	0.55	0.98	0.96	0.72	0.92	0.42
3	A003Y	0.90	0.77	1.25	0.76	0.95	0.99	0.96	0.91	0.57
4	P004A	0.91	0.78	0.90	0.77	1.03	0.94	0.90	0.90	0.73
4	P004C	0.77	0.41	1.01	0.39	1.13	1.28	0.90	1.18	0.23
4	P004D	0.94	0.82	0.97	0.85	0.92	0.92	1.09	1.02	0.74
4	P004E	0.99	0.81	1.01	0.87	0.91	1.13	0.90	0.89	0.79
4	P004G	1.04	0.69	1.02	0.74	1.00	0.89	0.83	0.97	0.65
4	P004H	0.96	0.92	0.91	0.84	0.99	0.94	0.89	0.89	0.84
4	P004I	0.77	0.65	0.90	0.61	0.95	1.61	0.89	0.83	0.50
4	P004K	0.99	0.90	0.91	0.93	1.01	1.28	1.21	0.89	0.89
4	P004L	0.79	0.54	0.97	0.55	0.96	0.85	0.90	0.88	0.46
4	P004M	0.86	0.61	1.04	0.60	0.97	0.71	0.99	0.92	0.51
4	P004N	0.82	0.69	1.03	0.70	0.86	0.68	0.83	0.85	0.65
4	P004Q	0.95	0.87	0.93	0.90	0.94	1.14	1.05	0.94	0.79
4	P004R	0.92	0.94	0.82	0.89	0.95	1.13	1.02	0.88	0.86
4	P004S	0.92	0.70	0.92	0.72	0.91	0.71	0.94	0.87	0.66
4	P004V	0.89	0.63	1.03	0.67	1.06	0.61	1.00	0.95	0.56
4	P004Y	0.93	0.58	0.97	0.66	1.01	0.86	0.73	0.99	0.52
5	I005C	1.16	0.77	0.85	0.84	0.84	0.78	0.68	0.81	0.85
5	I005D	1.22	0.98	0.74	1.13	0.96	0.97	0.83	0.98	1.12
5	I005E	1.13	1.20	0.79	1.22	0.86	0.95	0.91	0.87	1.46
5	I005F	1.13	0.76	1.00	0.85	0.90	0.97	0.90	0.96	0.90
5	I005G	1.23	0.90	0.95	0.99	0.93	0.97	0.74	0.93	1.02
5	I005H	1.24	1.09	1.03	1.23	0.95	0.93	0.93	1.00	1.28
5	I005K	1.20	1.00	1.06	1.14	0.97	0.96	0.88	0.89	1.13
5	I005L	1.28	0.78	0.92	0.89	0.89	0.81	0.76	0.96	0.96
5	I005M	1.16	1.03	1.05	1.09	0.88	0.84	0.84	0.95	1.11
5	I005N	1.19	1.18	0.94	1.25	0.86	1.09	1.01	1.01	1.23
5	I005R	1.08	0.99	1.05	1.17	1.04	0.97	0.81	1.03	1.11
5	I005S	1.09	1.09	0.81	1.16	0.88	0.76	1.02	0.96	1.17
5	I005T	1.18	1.10	0.91	1.24	0.98	0.94	0.83	0.95	1.28
5	I005V	1.12	1.07	1.01	1.10	1.01	0.72	0.80	0.94	1.16
5	I005W	1.27	0.57	1.00	0.67	0.72	0.89	0.77	0.91	0.73
5	I005Y	1.10	0.90	1.01	1.02	0.93	0.86	0.88	0.97	1.01
7	E007A	0.87	0.92	1.37	0.85	0.89	1.17	0.61	0.87	1.17
7	E007C	0.78	0.58	1.29	0.49	0.98	0.93	0.50	0.82	0.53

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
7	E007D	0.86	0.90	0.96	0.83	1.03	1.05	0.77	1.00	1.02
7	E007F	0.92	0.68	0.96	0.63	0.69	1.28	0.55	0.82	0.78
7	E007G	0.97	0.99	0.92	0.92	0.38	0.67	0.77	0.72	1.07
7	E007H	0.99	0.98	1.07	1.03	0.87	1.60	0.98	0.88	1.33
7	E007I	0.83	0.79	1.02	0.71	0.98	1.14	0.83	1.06	0.93
7	E007K	1.00	1.14	1.10	1.19	1.01	1.21	0.99	1.02	1.52
7	E007L	1.01	0.66	0.99	0.69	0.81	1.06	0.59	0.75	0.97
7	E007M	0.90	0.81	1.41	0.77	0.87	1.04	0.74	0.83	1.02
7	E007N	0.92	0.96	1.14	0.94	0.92	1.18	0.93	0.89	1.18
7	E007P	1.03	0.95	1.51	1.00	1.00	1.07	0.80	1.01	1.19
7	E007Q	1.00	0.98	1.35	0.99	1.07	1.10	1.11	0.98	1.24
7	E007R	0.76	1.08	1.38	1.00	1.19	1.00	1.11	1.00	1.30
7	E007S	0.83	1.01	1.14	0.89	0.79	1.06	0.82	0.90	1.09
7	E007T	0.99	0.90	0.90	0.95	0.97	1.12	0.93	0.99	1.25
7	E007V	0.85	0.79	0.97	0.70	0.92	1.03	0.96	0.72	0.96
7	E007W	0.96	0.52	0.97	0.51	0.57	1.25	0.64	0.79	0.57
7	E007Y	0.96	0.88	0.97	0.88	0.76	1.21	0.84	0.82	1.08
15	W015A	0.60	0.41	1.80	0.24	0.40	0.19	0.44	0.80	1.16
15	W015C	0.93	0.13	0.98	0.11	0.29	0.21	0.51	0.61	0.78
15	W015F	0.89	1.06	0.74	0.83	0.57	0.50	0.90	1.01	0.86
15	W015G	1.70	0.31	0.98	0.28	0.38	0.06	0.66	0.78	0.99
15	W015N	0.61	0.18	0.71	0.08	0.12	0.09	0.51	0.63	1.06
15	W015P	0.95	0.21	0.54	0.18	0.32	0.10	0.71	0.79	1.09
15	W015S	0.81	0.43	1.12	0.35	0.35	0.08	0.46	1.07	0.99
15	W015Y	0.92	0.87	0.86	0.79	0.75	0.35	0.90	1.11	1.01
16	D016A	0.84	0.19	0.56	0.23	0.71	0.67	0.74	0.60	0.46
16	D016C	0.87	0.19	1.03	0.19	0.89	0.18	0.56	0.95	0.30
16	D016E	1.12	0.87	0.93	0.91	0.92	0.61	0.95	0.90	0.82
16	D016F	0.92	0.09	1.26	0.10	0.87	0.53	1.24	1.02	0.18
16	D016G	0.94	0.37	0.51	0.47	0.70	0.81	0.88	0.68	0.69
16	D016H	0.82	0.38	1.03	0.42	0.47	0.86	0.61	0.62	0.63
16	D016I	0.88	0.06	0.43	0.06	0.75	0.23	0.93	0.91	0.22
16	D016K	0.87	0.17	0.52	0.19	0.70	0.49	0.57	0.62	0.71
16	D016L	1.27	0.11	0.53	0.12	0.80	1.12	1.06	1.17	0.15
16	D016M	0.97	0.12	0.66	0.13	0.78	0.60	1.08	0.95	0.26
16	D016N	1.12	0.29	0.82	0.34	0.81	1.29	0.84	0.80	0.29
16	D016P	0.98	0.07	0.23	0.08	0.58	0.22	0.34	0.86	0.14
16	D016Q	0.84	0.26	0.45	0.30	0.75	1.01	0.72	0.69	0.56
16	D016R	0.87	0.13	0.29	0.15	0.60	0.50	0.44	0.45	0.69
16	D016T	0.94	0.23	0.71	0.25	0.74	0.55	1.25	0.73	0.52
16	D016V	0.84	0.06	0.56	0.06	0.67	0.15	0.80	0.96	0.27
16	D016W	1.01	0.21	1.25	0.27	0.64	0.72	0.74	0.98	0.32
16	D016Y	0.89	0.48	1.29	0.51	0.73	1.16	0.61	0.87	0.46
17	L017A	0.85	0.83	0.51	0.80	1.06	1.04	1.04	1.09	1.00
17	L017C	0.98	0.80	0.59	0.83	1.08	0.67	0.99	0.94	0.83
17	L017D	0.98	1.14	0.38	1.08	1.01	0.72	1.00	1.01	0.92
17	L017E	0.97	1.23	0.52	1.16	0.98	0.76	0.94	1.02	0.98

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
17	L017F	1.04	0.98	0.65	0.94	0.90	0.68	0.93	0.94	0.89
17	L017G	1.02	0.73	0.46	0.76	1.00	0.76	0.88	0.92	0.89
17	L017H	1.09	0.72	0.53	0.76	0.91	0.91	1.03	1.02	0.82
17	L017I	0.86	1.04	0.62	1.04	0.95	0.99	0.92	1.00	0.91
17	L017K	0.96	0.95	0.46	1.00	0.94	1.00	0.92	1.00	0.89
17	L017M	0.88	0.61	0.96	0.63	0.78	0.75	0.86	1.02	0.86
17	L017N	1.14	1.07	0.46	1.10	0.92	0.80	1.03	1.04	1.10
17	L017P	1.00	0.49	0.21	0.53	0.85	0.23	0.82	0.98	0.49
17	L017Q	0.80	0.85	0.49	0.78	1.03	0.86	1.19	1.09	1.06
17	L017R	0.75	0.72	0.37	0.71	1.04	1.18	1.09	1.03	0.99
17	L017S	0.92	0.77	0.53	0.80	0.92	0.79	0.91	0.96	0.97
17	L017T	1.04	0.94	0.54	0.99	0.95	0.86	1.04	0.91	0.88
17	L017V	1.02	1.03	0.59	1.06	0.98	0.91	0.88	1.06	0.97
17	L017W	1.07	0.44	0.40	0.51	0.85	0.34	0.85	0.93	0.55
17	L017Y	1.13	1.11	0.77	1.13	0.89	0.91	0.93	1.01	0.94
18	P018A	0.66	0.82	0.57	0.71	0.96	0.85	1.04	1.02	0.91
18	P018C	0.91	0.39	0.72	0.38	1.13	0.59	0.59	1.01	0.36
18	P018D	0.96	1.05	0.70	0.95	1.02	0.81	1.04	1.00	0.98
18	P018E	0.96	1.11	0.73	1.02	0.91	0.76	0.91	0.98	1.12
18	P018F	0.73	0.49	0.59	0.45	1.01	0.87	0.30	0.99	0.64
18	P018G	0.91	0.79	0.57	0.74	1.00	0.94	0.86	1.01	0.99
18	P018H	0.95	0.74	0.43	0.74	0.94	0.91	0.84	1.04	1.02
18	P018I	0.75	0.57	0.55	0.56	0.87	0.92	0.92	1.00	0.77
18	P018K	0.82	0.90	0.50	0.85	0.89	1.01	0.84	1.00	1.06
18	P018L	0.77	0.64	0.53	0.60	0.98	0.85	0.68	0.88	0.72
18	P018M	0.86	0.62	0.60	0.64	0.89	0.84	0.71	0.98	0.77
18	P018N	1.02	0.90	0.59	0.92	0.99	1.02	0.91	1.03	1.02
18	P018Q	0.82	1.07	0.56	0.93	1.01	0.99	1.10	1.01	1.04
18	P018R	0.76	1.01	0.40	0.90	1.10	1.31	0.86	0.90	1.01
18	P018S	0.86	0.79	0.54	0.75	1.09	0.94	0.91	1.09	0.89
18	P018T	0.86	1.14	0.56	0.97	1.08	1.07	1.08	1.12	1.20
18	P018V	0.78	0.69	0.64	0.62	0.89	0.82	0.61	0.97	0.87
18	P018Y	0.80	0.62	0.58	0.60	0.93	0.89	0.66	0.91	0.83
19	N019A	0.81	0.63	0.55	0.69	0.97	1.01	0.76	1.00	0.91
19	N019C	0.94	0.34	0.46	0.38	0.89	0.54	1.12	0.84	0.48
19	N019D	0.87	0.75	0.61	0.79	0.91	0.66	0.99	0.95	0.91
19	N019E	0.95	0.70	0.48	0.69	0.91	0.86	0.78	0.91	0.93
19	N019F	0.93	0.37	0.45	0.43	0.82	0.61	0.92	0.96	0.52
19	N019G	0.93	0.48	0.42	0.57	0.97	0.88	1.10	1.00	0.73
19	N019H	0.91	0.56	0.44	0.65	0.99	0.87	0.98	0.99	0.90
19	N019I	0.80	0.33	0.43	0.40	0.94	0.91	0.94	0.89	0.57
19	N019K	0.95	0.53	0.35	0.66	0.87	0.96	0.96	0.98	0.93
19	N019L	0.79	0.31	0.34	0.35	0.90	0.79	1.04	0.87	0.52
19	N019M	0.91	0.39	0.43	0.48	0.85	0.90	0.86	0.91	0.63
19	N019P	0.91	0.53	0.60	0.64	0.99	0.97	1.00	0.94	0.76
19	N019R	0.95	0.48	0.29	0.62	0.89	1.23	0.76	0.95	0.91
19	N019S	0.82	0.64	0.62	0.66	0.69	0.98	0.93	0.96	0.89

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
19	N019T	1.04	0.52	0.50	0.62	0.91	1.03	0.99	0.90	0.86
19	N019V	1.00	0.40	0.54	0.49	1.03	0.87	0.51	0.92	0.66
19	N019W	0.89	0.30	0.46	0.36	0.89	0.81	0.71	0.83	0.50
19	N019Y	0.96	0.46	0.46	0.57	0.93	0.87	0.91	0.95	0.72
22	T022A	0.84	1.00	0.74	0.96	0.97	0.87	0.95	0.76	1.44
22	T022C	0.97	0.68	1.03	0.71	0.85	0.78	0.90	0.93	0.90
22	T022D	0.97	1.04	0.72	0.98	0.93	0.83	1.08	1.00	1.51
22	T022E	1.08	1.02	0.88	1.08	0.90	0.73	1.00	0.98	1.58
22	T022F	0.98	0.75	0.79	0.80	0.96	0.78	0.91	0.93	1.20
22	T022G	1.15	0.85	0.81	0.96	1.01	0.91	0.93	0.96	1.37
22	T022H	1.13	0.97	0.68	1.06	0.90	1.05	0.99	1.02	1.58
22	T022I	0.95	0.75	0.60	0.85	0.90	0.91	0.95	0.94	1.28
22	T022K	1.05	1.08	0.74	1.06	1.08	1.01	1.17	1.02	1.60
22	T022L	0.81	0.86	0.84	0.80	0.98	0.75	0.93	0.89	1.18
22	T022N	1.03	1.03	0.87	1.02	0.96	1.03	0.96	0.95	1.51
22	T022P	0.91	0.51	0.35	0.56	0.99	0.92	1.06	0.99	1.39
22	T022Q	1.08	0.97	0.87	1.05	0.95	1.05	0.92	0.99	1.55
22	T022R	0.91	1.11	0.61	1.03	1.08	1.26	1.26	1.09	1.34
22	T022S	1.03	0.95	0.91	1.04	0.90	0.87	0.85	1.02	1.40
22	T022V	1.00	0.78	0.66	0.88	2.67	6.35	3.80	3.67	0.99
22	T022Y	0.96	0.74	0.78	0.81	0.96	0.95	0.93	0.94	1.13
25	T025A	0.90	0.96	0.65	0.97	0.85	0.94	0.99	0.81	1.35
25	T025C	1.09	0.73	1.26	0.77	0.74	0.69	0.83	1.67	0.88
25	T025D	1.10	0.88	1.05	1.01	0.78	0.77	0.92	0.41	1.22
25	T025E	0.91	0.88	1.22	0.94	0.77	0.74	0.82	1.26	1.18
25	T025F	0.95	0.82	0.67	0.80	0.81	1.10	0.87	0.93	0.90
25	T025H	1.10	1.07	0.62	1.07	0.89	0.98	1.03	1.07	1.37
25	T025I	0.67	0.61	0.89	0.60	0.80	0.81	0.72	1.14	0.76
25	T025K	1.17	1.06	0.58	1.05	0.78	0.93	0.96	0.97	1.31
25	T025L	0.87	0.91	0.90	0.83	0.87	1.05	0.91	1.63	0.98
25	T025M	0.89	0.79	0.89	0.76	0.88	0.89	0.89	1.43	0.94
25	T025N	1.12	1.06	0.83	1.03	1.03	1.05	1.03	0.80	1.18
25	T025P	1.15	1.15	0.52	1.23	0.89	1.00	1.08	0.69	1.43
25	T025Q	0.95	0.26	0.53	0.25	0.89	0.52	1.04	1.20	1.03
25	T025S	0.90	1.07	0.72	1.00	0.91	0.91	0.98	1.21	1.30
25	T025V	1.01	0.64	0.93	0.71	0.78	0.73	0.82	0.71	1.11
25	T025W	1.00	0.97	0.64	0.97	0.97	1.13	0.85	1.15	1.01
25	T025Y	0.93	0.65	0.72	0.60	0.82	0.81	0.76	1.05	0.67
26	K026A	0.80	0.95	0.67	0.95	0.97	0.76	0.90	0.91	0.82
26	K026C	0.96	0.44	0.63	0.52	0.78	0.39	0.88	0.79	0.46
26	K026D	0.71	0.49	0.56	0.43	0.82	0.65	0.74	0.58	0.43
26	K026E	1.04	1.19	0.67	1.24	0.94	0.65	0.90	0.87	1.09
26	K026F	0.86	0.60	0.67	0.64	0.95	0.63	1.34	0.88	0.54
26	K026G	1.06	1.17	0.59	1.30	1.02	0.75	1.03	1.00	1.06
26	K026H	1.00	0.93	0.59	1.07	0.91	0.66	0.86	0.91	0.93
26	K026I	0.81	0.63	0.69	0.69	0.97	0.55	0.94	0.89	0.60
26	K026L	0.90	0.66	0.70	0.73	0.93	0.55	0.83	0.84	0.59

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
26	K026M	0.78	0.68	0.96	0.73	1.07	0.61	1.01	0.88	0.60
26	K026N	0.96	1.26	0.66	1.29	1.07	0.81	0.97	0.96	1.03
26	K026P	1.07	0.32	0.37	0.33	0.95	0.10	0.56	0.82	0.27
26	K026Q	0.92	0.94	0.80	0.99	1.03	0.73	1.05	1.06	0.84
26	K026R	0.82	0.89	0.79	0.89	0.99	0.90	0.62	0.73	0.82
26	K026S	0.92	1.05	0.67	1.08	0.99	0.69	0.94	0.94	0.87
26	K026T	0.89	0.94	0.73	0.98	0.88	0.80	0.89	0.89	0.93
26	K026V	0.81	0.82	0.72	0.83	0.85	0.59	0.85	0.91	0.68
26	K026W	1.00	0.52	0.65	0.62	1.11	0.67	0.88	0.87	0.49
26	K026Y	0.91	0.74	0.65	0.82	0.92	0.66	0.86	0.85	0.66
28	K028A	0.96	0.89	1.08	0.87	0.98	0.79	1.22	1.00	0.81
28	K028C	0.87	0.52	0.87	0.50	0.93	0.69	0.84	0.93	0.43
28	K028D	1.03	1.28	0.67	1.26	1.02	0.79	1.60	1.03	1.03
28	K028G	1.15	1.20	0.88	1.18	1.09	0.93	1.07	1.09	0.98
28	K028H	1.04	1.18	0.73	1.15	1.07	0.76	1.22	0.88	1.05
28	K028I	0.79	0.59	0.97	0.57	1.10	0.98	0.90	1.11	0.54
28	K028L	0.93	0.55	0.80	0.57	0.97	0.79	0.99	1.05	0.53
28	K028M	1.01	0.63	1.15	0.68	0.73	0.77	0.91	1.04	0.68
28	K028N	1.09	1.22	0.74	1.24	1.16	0.95	1.57	1.13	0.95
28	K028Q	1.08	1.05	1.07	1.06	0.99	0.80	1.47	1.01	0.94
28	K028R	0.88	1.07	0.73	0.96	1.01	1.04	1.12	1.08	0.89
28	K028S	0.99	1.00	0.90	1.01	0.96	0.82	1.32	1.00	0.86
28	K028T	0.99	0.91	1.00	0.89	0.96	0.93	1.28	1.12	0.69
28	K028V	0.88	0.67	1.17	0.65	0.82	0.70	0.84	0.94	0.65
28	K028W	0.89	0.78	0.73	0.74	0.99	0.70	0.70	1.10	0.69
28	K028Y	0.92	0.86	0.82	0.86	1.01	0.77	1.16	1.08	0.77
29	N029A	1.26	0.67	0.73	0.92	0.82	0.83	0.85	0.74	0.88
29	N029D	0.86	0.79	0.96	1.06	0.80	0.80	0.97	0.89	0.97
29	N029E	1.21	0.97	1.15	0.97	0.83	0.67	0.83	0.85	0.85
29	N029G	1.08	1.31	0.74	1.15	0.91	0.94	0.94	1.02	1.01
29	N029H	0.63	0.47	0.81	0.75	0.66	0.62	0.67	0.70	0.91
29	N029I	1.20	0.54	0.69	0.74	0.94	0.84	0.90	0.90	0.57
29	N029K	2.74	0.45	0.64	0.71	0.67	0.68	0.63	0.66	0.93
29	N029L	1.03	0.68	0.78	0.69	0.67	0.59	0.94	0.81	0.63
29	N029M	1.09	0.72	0.98	0.72	0.83	0.86	0.88	0.79	0.62
29	N029P	1.52	1.09	0.54	1.16	0.93	0.88	0.89	0.92	1.12
29	N029Q	1.19	0.91	1.00	0.92	0.87	0.72	0.82	0.86	0.80
29	N029R	1.08	0.97	0.61	0.95	0.90	1.00	0.86	0.96	0.88
29	N029S	1.23	0.91	0.74	0.95	0.84	0.96	0.77	0.90	0.89
29	N029T	1.29	0.84	0.81	0.90	0.88	0.63	0.92	0.94	0.81
29	N029V	0.93	0.53	0.82	0.73	0.78	0.73	0.88	0.86	0.65
29	N029W	1.24	0.52	0.70	0.54	0.79	1.07	1.01	0.75	0.40
29	N029Y	1.98	0.56	0.85	0.82	0.77	0.97	0.75	0.94	0.68
30	E030A	0.79	0.46	0.68	0.49	0.91	0.56	0.89	0.95	0.37
30	E030C	0.92	0.30	0.91	0.35	0.85	0.35	1.12	1.03	0.25
30	E030D	0.93	1.14	0.85	1.07	0.93	1.06	1.29	0.98	0.71
30	E030G	1.21	0.88	0.69	0.98	0.87	0.95	1.00	0.98	0.74

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
30	E030I	0.84	0.33	0.54	0.35	0.87	0.57	0.73	0.89	0.25
30	E030K	1.00	1.11	0.60	1.15	1.02	1.20	1.50	1.04	0.74
30	E030L	0.85	0.31	0.66	0.33	0.79	0.78	1.36	0.81	0.27
30	E030N	1.10	1.09	0.88	1.08	0.89	1.11	1.14	0.99	0.86
30	E030P	1.31	1.08	0.60	1.06	0.86	1.04	1.20	0.89	0.89
30	E030Q	1.04	0.88	0.77	0.93	0.97	1.21	1.12	0.99	0.76
30	E030R	0.93	1.06	0.53	1.05	0.98	1.42	1.50	0.94	0.85
30	E030S	0.82	0.83	0.66	0.80	0.92	1.12	1.18	0.93	0.60
30	E030T	0.94	0.61	0.69	0.64	0.90	1.02	1.04	0.84	0.54
30	E030V	1.05	0.36	0.71	0.41	0.80	0.75	1.02	0.76	0.36
30	E030W	0.83	0.29	0.69	0.28	0.85	0.63	0.81	1.02	0.23
30	E030Y	1.02	0.32	0.75	0.35	0.92	0.29	0.75	0.90	0.26
32	A032E	1.12	0.99	1.13	0.98	0.78	0.94	0.71	0.96	1.14
32	A032F	0.88	0.83	1.10	0.74	0.80	0.97	0.82	1.00	0.90
32	A032G	1.03	1.09	1.06	1.05	0.86	1.16	0.77	0.99	1.27
32	A032H	1.03	0.81	1.06	0.78	0.76	1.00	0.80	1.02	0.95
32	A032K	0.88	1.02	1.13	0.92	1.01	1.11	0.80	1.06	1.13
32	A032L	0.90	0.76	0.97	0.73	0.92	1.00	0.88	0.80	0.83
32	A032M	0.89	0.82	1.14	0.75	0.87	1.16	0.69	0.98	0.83
32	A032N	0.97	1.14	1.08	1.02	0.96	1.17	0.97	1.05	1.25
32	A032P	1.12	1.12	1.18	1.12	0.96	1.19	0.87	1.03	1.42
32	A032Q	0.92	0.71	1.16	0.69	0.98	1.13	0.92	1.00	0.72
32	A032R	0.90	0.89	1.02	0.88	0.93	1.38	0.85	0.98	1.01
32	A032S	0.92	0.95	1.02	0.95	0.84	1.10	0.81	0.99	1.08
32	A032T	0.92	0.83	0.94	0.75	0.95	1.05	0.90	0.98	0.84
32	A032V	1.09	0.77	1.09	0.81	0.84	1.02	0.95	0.87	0.88
32	A032W	0.96	0.77	1.07	0.74	0.98	1.15	0.82	0.95	0.74
32	A032Y	1.03	0.79	1.07	0.77	0.89	1.09	0.87	1.01	0.87
35	S035A	0.74	0.61	2.26	0.58	0.96	0.97	1.09	0.80	0.50
35	S035C	0.74	0.39	0.79	0.38	0.81	0.57	1.09	0.66	0.33
35	S035D	1.02	1.38	0.64	1.29	1.15	1.06	1.14	0.74	1.11
35	S035E	0.99	1.09	0.70	1.01	0.88	0.89	1.18	0.76	1.12
35	S035F	0.66	0.33	0.93	0.44	0.74	0.79	0.83	0.77	0.44
35	S035G	0.99	0.89	0.76	0.91	0.98	0.89	1.01	0.84	0.79
35	S035H	1.01	0.89	0.84	0.90	1.01	0.90	1.05	0.85	0.81
35	S035K	0.92	0.90	0.93	0.90	0.96	0.84	1.11	0.78	0.96
35	S035L	0.80	0.37	0.65	0.36	0.73	0.63	0.96	0.80	0.36
35	S035N	1.01	1.07	0.80	1.00	0.88	0.99	0.71	0.71	0.99
35	S035P	0.82	1.10	0.08	1.75	0.90	0.77	0.84	0.75	1.01
35	S035Q	1.16	0.85	0.73	0.98	0.98	0.95	1.00	0.82	0.87
35	S035R	0.86	0.86	1.11	0.82	0.95	1.06	1.04	0.87	0.80
35	S035T	0.84	0.66	0.81	0.65	1.00	0.79	1.16	0.75	0.74
35	S035V	0.86	0.39	0.98	0.39	0.88	0.62	0.81	0.74	0.38
35	S035W	0.82	0.48	0.84	0.46	0.95	0.75	1.00	0.71	0.47
35	S035Y	0.83	0.61	0.94	0.59	0.80	0.69	0.98	0.80	0.64
36	S036C	1.00	0.60	0.76	0.58	0.82	0.88	0.67	0.78	0.61
36	S036D	1.18	1.16	0.78	1.17	1.04	1.05	1.05	0.94	1.27

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
36	S036E	1.03	1.26	0.96	1.16	0.98	0.95	0.83	0.97	1.43
36	S036F	1.01	0.61	0.86	0.60	0.87	0.73	0.66	0.92	0.68
36	S036G	1.05	0.68	0.89	0.70	0.93	0.94	0.94	0.92	0.73
36	S036I	0.86	0.58	0.89	0.57	1.00	0.86	0.62	0.79	0.64
36	S036K	1.11	0.99	0.95	0.98	0.97	1.10	0.81	0.94	1.09
36	S036L	1.01	0.81	0.95	0.79	0.99	1.18	1.05	0.90	0.74
36	S036M	1.02	0.69	0.96	0.72	1.00	0.98	0.90	0.96	0.78
36	S036N	1.16	1.10	0.91	1.07	0.99	1.03	0.74	1.01	1.18
36	S036P	1.40	1.15	0.83	1.20	1.01	0.98	0.80	0.97	1.29
36	S036Q	1.06	1.07	1.01	1.06	1.08	0.95	0.84	0.95	1.13
36	S036R	0.86	0.45	0.93	0.39	1.02	1.04	0.55	0.95	0.42
36	S036T	1.04	1.11	0.84	1.04	1.10	1.02	0.71	0.98	1.09
36	S036V	1.08	0.86	0.90	0.78	1.03	0.85	0.56	0.89	0.84
36	S036W	0.92	0.49	0.85	0.47	0.88	0.87	0.56	0.73	0.53
36	S036Y	1.08	0.75	0.90	0.76	0.99	0.96	0.60	0.95	0.81
37	L037C	1.19	0.35	0.78	0.40	0.99	1.36	1.12	1.53	0.21
37	L037D	1.30	0.64	0.74	0.69	0.86	1.06	1.33	1.07	0.45
37	L037E	1.23	0.84	0.74	0.82	0.82	0.86	0.95	1.19	0.60
37	L037F	1.24	1.01	0.80	1.02	0.81	1.37	1.18	1.10	0.80
37	L037G	1.44	0.69	0.76	0.78	0.89	1.08	1.10	1.17	0.57
37	L037H	1.14	1.32	0.70	1.17	0.96	0.93	1.05	0.88	1.09
37	L037I	1.10	1.03	0.92	1.03	0.88	1.07	1.06	0.96	0.92
37	L037K	0.91	0.80	0.75	0.66	0.92	1.32	0.97	1.15	0.49
37	L037M	1.15	1.01	1.04	0.97	0.87	0.92	1.21	1.00	0.89
37	L037N	1.39	1.18	0.75	1.14	0.72	1.01	1.04	1.04	0.96
37	L037P	1.29	0.32	0.66	0.30	1.46	2.41	1.34	2.39	0.11
37	L037Q	1.35	1.19	0.76	1.20	0.83	1.01	1.11	1.10	0.97
37	L037R	1.00	1.28	0.71	1.20	0.90	1.21	0.87	1.10	1.05
37	L037S	1.22	1.03	0.79	1.10	0.88	1.05	1.12	0.90	0.94
37	L037T	1.11	1.12	0.82	1.06	0.93	1.06	1.09	1.00	0.88
37	L037V	1.21	1.06	0.92	1.07	0.91	1.10	0.97	1.04	0.88
37	L037W	1.35	0.80	0.92	0.86	0.88	1.02	1.02	1.63	0.65
37	L037Y	1.22	1.03	0.82	1.06	0.91	1.08	1.24	1.01	0.88
50	G050A	0.99	1.50	0.95	1.28	1.16	0.94	0.98	0.89	1.12
50	G050C	1.04	1.03	0.57	0.96	0.84	0.54	0.76	0.78	0.75
50	G050E	0.64	0.25	0.07	0.23	0.07	0.11	0.47	0.77	0.34
50	G050L	0.33	0.15	0.09	0.15	0.28	0.09	0.80	0.89	0.63
50	G050M	0.54	1.19	0.07	0.98	0.47	0.22	0.77	0.90	0.92
50	G050N	0.79	0.14	0.25	0.14	0.36	0.43	0.80	0.83	1.10
50	G050P	1.06	0.94	0.44	0.81	0.99	0.61	0.85	0.91	1.01
50	G050Q	0.56	0.64	0.11	0.59	0.56	0.34	0.76	0.88	0.86
50	G050S	0.97	1.74	0.45	1.51	1.03	0.99	1.09	0.95	1.12
50	G050T	0.88	0.76	0.21	0.67	0.94	0.49	0.51	0.89	0.92
50	G050V	0.64	0.60	0.06	0.55	0.63	0.28	0.77	0.81	0.65
51	T051A	0.62	0.83	1.00	0.81	0.84	0.54	0.71	0.97	1.00
51	T051C	1.20	0.38	1.60	0.32	0.36	0.09	0.63	0.78	0.40
51	T051D	0.78	0.37	0.29	0.29	0.68	0.37	0.85	0.95	0.61

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
51	T051E	1.15	0.60	0.67	0.55	0.61	0.22	0.85	1.05	0.95
51	T051G	0.69	0.29	0.31	0.31	0.85	0.54	0.79	0.97	0.62
51	T051H	0.75	0.11	0.69	0.13	0.75	0.43	0.69	0.79	1.04
51	T051K	0.89	0.35	1.37	0.38	0.70	0.53	0.56	0.70	0.66
51	T051M	1.30	0.81	1.88	0.75	0.79	0.77	0.89	0.86	0.88
51	T051N	0.84	0.44	0.40	0.43	0.90	0.68	0.83	0.97	1.00
51	T051Q	2.19	0.26	1.75	0.27	0.85	0.54	0.84	0.86	0.96
51	T051R	0.79	0.29	1.18	0.28	0.90	0.70	0.63	0.85	1.00
51	T051S	0.70	0.71	0.67	0.72	0.86	0.58	0.69	0.83	0.93
51	T051V	0.96	0.60	1.17	0.69	0.86	0.55	0.82	0.90	0.88
51	T051W	0.58	0.08	0.70	0.07	0.48	0.78	0.58	0.49	0.70
51	T051Y	0.82	0.13	0.90	0.30	0.84	0.75	0.59	0.75	0.83
52	S052A	0.81	1.13	0.62	1.10	0.95	0.91	0.94	0.99	0.93
52	S052C	0.94	0.93	0.67	0.83	0.50	0.35	0.66	0.88	0.63
52	S052D	1.13	1.08	0.79	1.15	0.86	0.45	0.89	0.87	0.99
52	S052E	1.20	1.53	0.72	1.42	0.87	0.50	1.02	0.94	1.01
52	S052F	0.82	0.83	0.84	0.78	0.80	0.77	0.90	0.87	0.86
52	S052G	0.97	0.79	0.62	0.84	0.87	0.82	0.96	0.86	1.01
52	S052H	0.95	1.15	0.76	1.12	0.93	0.94	1.07	0.95	1.15
52	S052I	0.82	1.18	0.42	1.20	0.89	0.61	0.68	0.92	0.88
52	S052K	0.77	1.59	0.53	1.36	1.01	0.96	1.01	0.77	0.97
52	S052L	0.81	0.94	0.35	1.06	0.93	0.49	1.05	1.05	0.80
52	S052M	0.96	1.11	0.61	1.05	0.86	0.85	0.77	0.93	0.65
52	S052N	0.97	0.80	0.74	0.91	0.98	0.87	0.99	0.92	1.00
52	S052P	0.60	0.14	0.12	0.13	0.48	0.17	0.79	0.78	0.74
52	S052Q	0.92	1.08	0.58	1.08	0.91	0.82	0.89	0.91	0.98
52	S052R	0.83	1.47	0.42	1.51	1.08	1.24	0.79	0.98	0.95
52	S052T	0.96	1.24	0.74	1.32	0.98	0.71	1.21	0.92	0.89
52	S052V	0.95	1.27	0.52	1.29	0.94	0.61	0.79	0.81	0.86
52	S052W	0.77	0.45	0.27	0.46	0.96	1.06	0.83	0.78	0.75
52	S052Y	0.88	1.58	0.30	1.67	0.86	0.68	0.95	0.91	0.80
53	Q053A	0.90	0.81	0.50	0.86	1.12	1.17	0.90	1.12	1.17
53	Q053C	0.71	0.46	0.48	0.61	0.77	0.65	0.96	0.85	0.82
53	Q053D	0.90	0.64	0.58	0.88	0.93	0.68	1.06	0.82	1.20
53	Q053E	4.42	0.70	0.48	0.91	0.95	0.71	1.07	0.82	1.13
53	Q053F	2.83	0.57	0.31	0.76	0.95	1.13	1.02	0.89	1.02
53	Q053G	0.76	0.68	0.42	0.91	1.02	1.04	0.82	0.93	1.14
53	Q053K	0.95	0.69	0.24	0.75	0.92	1.13	1.03	0.94	1.25
53	Q053N	0.96	0.89	0.45	0.87	1.06	1.11	0.93	0.99	1.23
53	Q053P	0.43	0.42	0.37	0.79	1.02	1.10	0.73	1.04	1.21
53	Q053R	0.89	0.78	0.21	0.78	0.95	1.60	0.99	0.73	1.14
53	Q053S	6.10	0.56	0.57	0.84	1.04	1.17	0.97	1.03	1.24
53	Q053T	0.90	0.70	0.29	0.73	1.02	1.09	1.20	1.03	1.10
53	Q053V	1.09	0.64	0.51	0.72	1.13	1.27	1.20	0.97	1.08
53	Q053W	0.98	0.63	0.29	0.68	0.99	1.00	1.05	0.98	0.91
53	Q053Y	0.88	0.69	0.31	0.75	1.00	1.03	0.91	0.90	0.98
54	S054A	0.91	0.88	0.89	0.91	0.95	0.88	0.70	0.99	0.88

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
54	S054C	0.95	0.66	1.01	0.60	0.62	0.57	0.90	1.00	0.49
54	S054D	1.00	1.04	1.36	0.92	0.77	0.70	0.80	0.92	0.95
54	S054E	1.14	0.98	1.38	0.92	0.87	0.63	0.87	0.88	0.92
54	S054F	1.10	0.92	0.52	0.92	0.87	0.94	0.91	0.86	0.81
54	S054H	0.91	0.89	0.83	0.87	1.03	0.84	0.91	0.86	0.89
54	S054I	0.91	0.52	0.57	0.54	0.94	0.87	0.72	0.99	0.60
54	S054K	0.79	0.87	0.51	0.91	0.78	0.93	0.88	0.90	0.88
54	S054L	0.84	0.77	0.62	0.71	0.99	0.94	0.86	0.93	0.70
54	S054M	1.07	0.76	0.83	0.75	0.89	0.94	0.65	0.86	0.79
54	S054N	0.91	0.87	0.98	0.85	0.75	0.87	0.81	0.86	1.02
54	S054P	1.06	0.95	0.48	0.98	0.91	0.78	0.82	0.82	0.90
54	S054Q	0.90	1.00	0.90	0.93	0.91	0.87	0.93	0.89	0.91
54	S054R	0.83	0.84	0.45	0.82	0.92	1.10	0.51	0.84	0.92
54	S054T	0.87	0.89	0.71	0.75	0.87	0.87	0.68	0.91	0.79
54	S054V	1.03	0.69	0.75	0.69	0.96	0.79	0.71	0.81	0.72
54	S054W	1.06	0.73	0.94	0.67	0.77	1.09	0.78	0.88	0.72
54	S054Y	0.99	0.85	0.81	0.79	0.93	0.93	0.50	0.88	0.75
55	D055E	1.14	1.18	0.35	1.21	0.84	0.54	0.83	0.85	0.95
55	D055G	0.89	0.08	0.11	0.15	0.48	0.45	0.55	0.66	0.94
55	D055I	0.64	0.25	0.08	0.27	0.33	0.29	0.55	0.67	0.62
55	D055N	0.90	0.32	0.27	0.37	0.98	0.79	0.65	0.74	1.02
55	D055P	0.55	0.09	0.06	0.09	0.38	0.14	0.47	0.70	0.75
55	D055S	0.62	0.07	0.13	0.15	0.59	0.36	0.53	0.74	0.81
55	D055T	0.84	0.19	0.10	0.26	0.61	0.87	0.59	0.70	0.85
56	V056A	0.83	0.98	0.59	0.89	0.92	0.74	0.92	0.86	1.02
56	V056C	0.94	0.58	0.58	0.62	0.71	0.66	0.78	0.79	0.80
56	V056E	1.35	1.71	0.62	1.63	0.60	0.51	0.80	0.86	1.03
56	V056F	0.87	0.12	0.11	0.12	0.06	0.08	0.65	0.52	0.82
56	V056G	0.98	0.29	0.33	0.31	0.69	0.51	0.84	0.61	0.61
56	V056H	1.13	0.68	0.32	0.65	0.70	0.44	0.94	0.82	1.15
56	V056I	0.93	0.39	0.45	0.37	0.84	0.68	0.66	0.86	0.95
56	V056K	0.83	0.11	0.24	0.11	0.70	0.60	0.84	0.83	1.05
56	V056M	1.19	0.43	0.29	0.48	0.76	0.41	1.45	0.84	0.91
56	V056N	1.12	0.82	0.63	0.85	0.91	0.89	1.00	0.90	1.09
56	V056P	1.01	0.90	0.68	0.88	0.75	0.57	0.88	0.91	1.16
56	V056Q	1.08	1.08	0.58	1.08	0.73	0.57	1.10	0.89	1.09
56	V056R	0.62	0.18	0.39	0.26	0.62	0.80	0.95	0.80	1.18
56	V056S	0.79	1.07	0.53	0.99	0.76	0.70	1.16	0.92	0.97
56	V056T	1.03	1.18	0.80	1.30	0.83	0.83	0.75	0.92	1.06
56	V056W	1.18	0.20	0.22	0.20	0.37	0.29	0.49	0.54	0.76
56	V056Y	0.97	0.21	0.18	0.23	0.48	0.13	0.69	0.66	0.64
58	Y058F	0.93	0.61	0.29	0.68	0.91	0.84	0.39	0.50	0.91
58	Y058W	0.64	0.16	0.11	0.16	0.65	0.27	0.34	0.49	0.93
59	G059A	1.00	0.75	1.14	0.75	0.88	0.43	0.68	0.93	0.84
59	G059E	2.22	0.80	0.91	0.74	0.42	0.15	0.55	0.64	0.89
59	G059P	1.20	0.06	0.44	0.07	0.30	0.08	0.38	0.51	0.57
59	G059Q	1.39	0.37	0.72	0.36	0.58	0.44	0.57	0.81	0.92

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
59	G059S	1.11	0.23	0.90	0.23	0.79	0.29	0.67	0.88	0.85
60	V060A	0.96	0.63	2.01	0.60	0.96	1.04	1.01	0.97	0.83
60	V060C	1.10	0.46	1.11	0.49	0.97	1.09	0.71	1.00	0.50
60	V060D	1.04	0.34	0.71	0.38	0.86	1.12	0.66	0.91	0.52
60	V060E	0.89	0.39	1.02	0.40	0.88	1.10	0.84	0.89	0.57
60	V060G	1.73	0.48	0.67	0.58	0.78	0.55	0.89	0.88	0.86
60	V060H	0.92	0.67	0.20	0.72	0.79	0.73	0.76	0.90	0.82
60	V060I	1.11	0.83	1.06	0.81	0.98	0.93	0.70	0.94	0.78
60	V060K	0.81	0.53	0.20	0.56	0.84	0.85	0.65	0.79	0.54
60	V060L	1.18	0.77	0.50	0.78	1.04	0.78	0.79	0.98	0.71
60	V060M	0.97	0.80	0.30	0.87	0.85	0.89	0.84	1.01	0.80
60	V060N	0.92	0.61	0.35	0.60	0.69	0.65	0.96	0.91	0.91
60	V060P	0.65	0.60	0.80	0.61	0.89	0.89	0.89	1.02	1.02
60	V060Q	0.57	0.75	0.60	0.79	0.88	0.89	0.76	0.94	0.92
60	V060R	0.43	0.37	0.19	0.33	0.77	0.78	0.83	0.86	0.36
60	V060S	1.25	0.56	1.37	0.64	0.87	0.94	0.59	0.92	0.86
60	V060T	1.14	0.80	1.25	0.78	0.76	0.90	0.94	0.98	0.91
70	N070A	1.08	0.91	0.65	0.89	1.07	1.03	0.93	0.90	0.69
70	N070C	1.01	0.81	2.09	0.77	0.74	0.60	0.83	0.80	0.62
70	N070D	1.00	1.06	1.41	0.98	0.72	0.72	0.98	0.80	0.73
70	N070E	1.00	0.65	1.15	0.63	0.80	0.72	0.93	0.56	0.48
70	N070F	1.25	0.83	1.40	0.85	0.95	1.03	1.24	0.86	0.58
70	N070G	1.19	0.87	0.64	0.93	0.84	0.95	1.08	0.82	0.79
70	N070H	0.85	0.67	1.03	0.98	0.81	1.01	0.96	0.85	0.79
70	N070I	1.25	0.63	0.57	0.70	0.95	0.97	0.82	0.87	0.57
70	N070K	1.21	0.85	0.61	0.92	0.84	1.54	0.96	0.66	0.77
70	N070L	1.10	0.75	0.98	0.72	0.86	0.73	0.89	0.72	0.64
70	N070M	1.18	0.94	0.64	0.94	0.80	0.89	0.81	0.82	0.70
70	N070P	1.43	0.89	0.96	1.09	0.89	0.91	0.93	0.75	1.01
70	N070Q	1.02	1.10	0.87	1.05	0.90	0.96	0.89	0.88	0.76
70	N070R	1.19	0.89	0.51	0.95	0.91	1.39	1.11	0.85	0.78
70	N070S	1.01	0.91	0.69	0.86	0.91	0.94	1.13	0.85	0.68
70	N070T	1.13	0.74	0.67	0.78	0.82	0.92	1.01	0.91	0.66
70	N070V	1.16	0.71	0.70	0.75	1.01	1.02	1.13	0.89	0.53
70	N070W	1.30	0.96	1.01	0.95	0.83	1.03	0.99	0.76	0.81
70	N070Y	1.33	0.82	1.49	0.87	0.89	0.95	0.88	0.96	0.71
71	Q071A	0.78	1.09	0.56	1.01	0.99	0.93	0.58	1.07	0.65
71	Q071C	0.95	0.79	0.44	0.74	0.92	0.67	1.04	1.08	0.46
71	Q071D	0.89	0.99	0.38	0.92	0.99	0.65	1.17	0.97	0.56
71	Q071E	1.02	1.03	0.31	1.02	0.85	0.65	0.91	1.07	0.60
71	Q071F	0.81	1.10	0.22	0.94	1.06	0.69	0.92	1.10	0.48
71	Q071G	0.89	0.87	0.41	0.79	0.94	0.82	0.94	0.95	0.68
71	Q071H	0.75	0.88	0.32	0.80	0.82	0.67	0.89	0.88	0.47
71	Q071I	0.83	1.06	0.51	0.90	1.01	0.84	0.99	1.12	0.57
71	Q071K	0.77	0.82	0.25	0.75	0.88	0.80	0.90	1.07	0.36
71	Q071L	0.83	0.67	0.25	0.64	0.99	0.79	0.88	1.02	0.43
71	Q071M	0.92	0.86	0.34	0.84	1.03	0.82	1.07	0.96	0.58

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
71	Q071N	0.94	0.93	0.36	0.87	0.86	0.83	1.05	0.89	0.76
71	Q071P	0.71	0.24	0.24	0.22	1.01	0.37	1.20	0.90	0.19
71	Q071R	0.78	0.96	0.22	0.92	1.04	1.11	1.03	1.07	0.46
71	Q071S	0.92	0.78	0.44	0.82	0.97	0.83	0.65	1.00	0.64
71	Q071T	0.86	1.23	0.30	1.18	0.93	0.73	0.94	1.02	0.60
71	Q071V	1.02	1.07	0.42	1.02	1.02	0.83	0.88	1.14	0.62
71	Q071W	0.90	1.18	0.27	0.98	1.05	0.94	0.91	1.01	0.73
71	Q071Y	0.84	0.89	0.24	0.84	0.98	0.55	0.93	1.15	0.55
72	K072A	0.99	1.66	0.33	1.69	0.89	0.56	1.11	1.20	1.01
72	K072C	2.12	0.99	0.18	1.03	0.78	0.40	0.93	0.96	0.51
72	K072D	0.76	1.12	0.21	1.00	0.78	0.28	0.97	0.89	0.68
72	K072E	0.93	1.99	0.38	1.82	0.87	0.42	1.03	0.90	0.97
72	K072G	1.05	1.49	0.29	1.54	0.89	0.55	1.17	1.07	0.91
72	K072I	0.91	1.68	0.22	1.62	0.88	0.57	1.01	0.93	0.75
72	K072L	0.86	1.65	0.19	1.61	0.85	0.70	0.90	0.88	0.80
72	K072M	0.92	1.74	0.25	1.68	0.94	0.79	1.14	0.91	0.90
72	K072P	1.12	1.62	0.31	1.63	0.77	0.41	1.12	0.86	0.89
72	K072Q	1.09	1.87	0.43	1.83	0.98	0.78	1.09	1.04	1.07
72	K072R	0.71	1.46	0.45	1.45	0.89	0.58	1.10	1.08	1.07
72	K072S	1.11	1.47	0.38	1.49	0.98	0.42	0.90	1.07	1.07
72	K072T	0.78	1.43	0.08	1.34	0.81	0.47	1.18	0.97	0.77
72	K072V	0.88	1.91	0.29	1.77	0.83	0.47	1.08	0.97	0.85
72	K072Y	1.01	1.26	0.11	1.23	0.96	0.60	1.19	0.97	0.82
73	G073A	0.84	1.04	0.55	0.91	1.06	0.98	0.74	0.93	0.88
73	G073C	0.87	0.83	0.71	0.70	0.83	0.65	0.81	0.75	0.66
73	G073D	1.02	1.11	0.81	1.03	1.00	0.66	0.88	0.86	0.87
73	G073E	0.99	1.09	0.82	1.01	0.80	0.88	0.81	0.92	0.79
73	G073F	0.82	0.74	0.61	0.58	0.93	0.95	0.76	0.78	0.69
73	G073H	0.93	0.89	0.60	0.84	1.04	0.95	0.91	0.85	0.84
73	G073I	0.84	0.56	0.50	0.51	0.86	0.92	0.48	0.81	0.50
73	G073K	1.00	0.90	0.46	0.92	0.87	1.04	0.78	0.92	0.80
73	G073L	1.00	0.64	0.53	0.64	0.89	0.76	0.86	0.77	0.68
73	G073M	0.91	0.78	0.64	0.70	0.83	1.11	0.89	0.86	0.71
73	G073P	0.72	0.19	0.24	0.17	0.65	0.68	0.84	0.62	0.20
73	G073Q	0.83	1.11	0.66	0.93	1.03	0.89	0.80	0.96	0.81
73	G073R	0.88	0.86	0.39	0.86	0.95	1.17	0.73	0.84	0.89
73	G073S	0.92	0.97	0.58	0.91	1.04	1.07	0.86	0.81	0.81
73	G073V	0.94	0.88	0.50	0.87	1.17	1.06	1.10	1.06	0.53
73	G073Y	0.96	0.68	0.60	0.62	0.93	1.21	0.74	0.83	0.66
75	I075A	0.97	0.90	0.51	0.87	1.01	1.01	0.87	0.89	1.22
75	I075C	0.93	0.72	0.77	0.65	0.97	0.61	0.92	0.86	0.88
75	I075E	0.92	1.08	0.66	0.95	1.05	0.83	0.95	1.04	1.12
75	I075F	0.86	1.24	0.74	1.00	1.05	1.03	0.79	1.00	1.30
75	I075G	0.99	1.00	0.53	0.90	0.92	0.89	0.88	0.91	1.31
75	I075H	1.01	1.31	0.55	1.17	1.02	1.11	0.96	1.03	1.42
75	I075K	0.96	1.10	0.57	0.93	0.97	1.20	1.08	0.95	1.33
75	I075L	0.92	0.90	0.64	0.86	1.09	0.91	0.72	0.96	1.15

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
75	I075M	0.94	1.08	0.65	0.90	0.99	0.99	0.87	0.98	1.14
75	I075N	1.02	1.04	0.60	0.95	1.01	1.06	1.09	0.95	1.20
75	I075P	1.01	0.76	0.43	0.74	1.02	1.79	0.90	0.90	1.10
75	I075Q	0.96	1.05	0.59	1.00	1.04	0.91	0.87	1.02	1.22
75	I075R	0.94	1.11	0.53	0.93	1.09	1.58	0.86	1.15	1.32
75	I075S	0.99	0.89	0.52	0.88	1.07	0.93	0.87	1.02	1.14
75	I075T	0.94	1.02	0.75	0.92	1.05	1.03	0.97	1.08	1.19
75	I075V	0.97	1.01	1.13	0.93	1.00	0.93	1.04	1.06	1.12
75	I075W	1.00	1.13	0.71	1.01	1.01	1.06	0.89	1.06	1.19
75	I075Y	0.99	1.03	0.74	0.93	1.03	0.92	0.95	1.01	1.17
78	K078C	0.79	0.57	0.17	0.51	0.73	0.16	0.82	0.75	0.47
78	K078F	1.21	0.72	0.37	0.62	0.70	0.21	0.72	0.77	0.40
78	K078G	0.82	0.40	0.13	0.40	0.58	0.10	0.79	0.82	0.53
78	K078H	0.86	0.53	0.22	0.53	0.80	0.50	0.83	0.90	0.72
78	K078I	0.76	0.55	0.17	0.49	0.79	0.32	0.70	0.79	0.59
78	K078L	0.87	0.70	0.24	0.61	0.76	0.26	0.92	0.78	0.76
78	K078M	0.97	0.95	0.26	0.89	0.78	1.34	0.89	0.82	0.74
78	K078N	0.83	0.79	0.17	0.72	0.77	0.31	0.99	0.78	0.64
78	K078Q	0.76	0.73	0.14	0.67	0.93	0.54	1.08	0.88	0.66
78	K078S	0.74	0.49	0.17	0.46	0.76	0.23	0.76	0.59	0.56
78	K078T	0.75	0.84	0.13	0.75	0.77	0.69	0.84	0.90	0.72
78	K078Y	0.99	0.92	0.20	0.81	0.77	0.07	0.82	0.67	0.46
82	K082A	0.70	0.91	0.65	0.83	1.03	0.91	1.32	0.99	0.81
82	K082C	0.77	0.68	0.69	0.65	0.99	0.67	1.04	0.86	0.68
82	K082D	0.69	0.86	0.72	0.74	0.89	0.72	1.06	0.80	0.79
82	K082E	0.90	1.11	0.68	0.96	0.90	0.75	0.99	1.00	1.00
82	K082F	0.80	1.03	0.88	0.88	1.04	0.77	1.31	1.00	0.92
82	K082G	0.82	0.84	0.64	0.72	0.86	0.72	1.22	0.86	0.89
82	K082H	0.91	0.95	0.80	0.86	0.81	0.86	1.36	0.86	0.93
82	K082I	0.61	0.99	0.70	0.81	1.03	0.86	1.06	0.96	0.79
82	K082L	0.70	0.86	0.69	0.79	1.02	0.81	1.16	0.89	0.80
82	K082M	0.84	0.80	0.79	0.83	1.12	0.81	0.92	0.97	0.90
82	K082N	0.80	1.32	0.72	1.15	1.26	0.99	1.42	1.05	1.07
82	K082P	0.87	0.33	0.63	0.29	0.59	0.63	0.66	0.66	0.49
82	K082Q	0.91	0.90	0.68	0.87	1.09	1.31	1.25	0.92	0.91
82	K082S	0.73	0.86	0.66	0.80	1.00	0.98	1.09	0.91	0.83
82	K082T	0.76	0.66	0.60	0.63	1.04	0.84	1.08	0.91	0.74
82	K082V	0.71	0.80	0.81	0.73	1.03	0.86	1.41	0.96	0.77
82	K082W	0.75	0.58	0.64	0.50	1.08	0.78	1.03	0.83	0.54
82	K082Y	0.88	0.97	0.83	0.91	1.02	0.79	1.16	0.91	0.95
83	T083A	0.89	0.84	1.07	0.83	1.09	0.82	1.47	1.00	0.81
83	T083C	0.99	0.82	1.13	0.76	0.92	0.74	1.35	1.00	0.74
83	T083D	0.98	0.95	1.92	0.85	1.10	0.78	0.96	0.84	0.89
83	T083E	1.02	0.91	1.99	0.86	0.96	0.80	1.22	0.91	0.89
83	T083F	1.23	1.03	0.80	1.05	1.13	1.05	1.51	0.97	0.91
83	T083G	1.10	0.83	0.96	0.86	1.09	0.84	1.49	1.03	0.87
83	T083H	1.04	1.06	0.97	1.05	1.05	0.95	1.40	0.87	1.05

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
83	T083I	1.13	0.96	0.77	0.96	1.19	1.36	1.06	1.03	0.84
83	T083K	1.28	0.93	0.71	0.97	1.18	0.96	1.22	1.02	1.04
83	T083L	1.05	1.08	0.81	1.03	1.07	0.82	1.29	0.86	1.09
83	T083M	1.16	1.02	0.91	1.06	0.94	0.93	1.46	0.75	0.78
83	T083N	1.24	1.07	1.01	1.06	1.16	0.90	1.09	0.96	1.17
83	T083P	1.22	0.97	0.72	1.04	1.09	0.76	1.32	0.92	1.28
83	T083Q	0.95	1.19	1.25	1.06	1.02	0.78	1.44	0.85	1.15
83	T083R	0.92	1.12	0.67	0.92	1.27	1.27	1.07	0.91	0.94
83	T083S	1.08	1.04	0.90	0.99	1.04	0.97	1.38	0.86	0.99
83	T083V	1.12	0.76	0.90	0.81	1.09	0.99	1.36	1.02	0.72
83	T083W	1.20	1.09	0.85	1.05	1.08	1.13	1.55	0.98	0.96
83	T083Y	1.18	0.93	0.81	1.00	0.97	0.93	1.22	1.00	0.97
87	Q087A	0.70	0.97	0.85	0.91	1.19	1.92	0.80	1.01	0.84
87	Q087C	0.84	0.70	0.97	0.72	0.91	0.18	0.63	0.99	0.74
87	Q087D	0.83	0.89	1.20	0.85	1.06	0.80	0.80	0.85	0.94
87	Q087E	0.97	0.78	1.81	0.83	1.02	1.10	0.77	0.94	0.94
87	Q087F	0.87	1.17	0.68	0.97	1.21	1.47	1.06	1.03	0.91
87	Q087G	0.97	0.65	0.82	0.61	1.02	0.26	0.90	0.95	0.64
87	Q087H	0.92	1.02	0.83	0.93	1.11	1.24	1.21	1.02	0.98
87	Q087I	0.70	0.72	0.65	0.72	1.19	1.00	0.91	1.02	0.73
87	Q087K	0.97	0.91	0.75	0.86	1.15	1.46	0.92	1.09	0.84
87	Q087L	0.86	0.88	0.72	0.86	1.13	1.40	0.86	1.09	0.82
87	Q087M	0.84	0.92	0.86	0.85	1.13	1.26	0.93	0.92	0.92
87	Q087N	1.03	0.98	0.88	0.99	1.16	1.61	1.07	0.91	1.08
87	Q087P	0.95	0.86	0.60	0.85	1.06	1.04	0.93	0.90	1.04
87	Q087R	0.84	0.87	0.62	0.87	1.21	1.72	1.23	1.02	0.96
87	Q087S	0.72	0.98	0.76	0.91	1.04	1.18	0.86	0.88	0.97
87	Q087T	0.91	0.77	0.80	0.80	1.12	0.98	0.84	1.01	0.88
87	Q087W	0.97	0.95	0.71	0.93	1.22	1.37	0.94	1.03	0.94
87	Q087Y	1.00	0.82	0.69	0.90	0.95	0.98	0.89	1.11	0.88
90	Q090A	0.69	1.25	0.86	1.04	0.92	1.10	1.16	0.69	0.82
90	Q090C	0.80	0.81	0.87	0.78	0.76	0.92	1.05	0.83	0.58
90	Q090D	0.91	0.92	0.93	0.89	0.92	0.81	1.02	0.74	0.78
90	Q090E	0.85	0.96	1.15	0.90	0.98	1.19	1.00	0.92	0.75
90	Q090F	0.87	0.83	0.71	0.85	1.13	1.24	1.07	0.94	0.61
90	Q090G	0.93	0.87	0.77	0.91	0.92	0.92	1.06	0.93	0.77
90	Q090H	0.83	1.03	0.79	0.92	0.96	1.04	1.03	0.95	0.79
90	Q090I	0.92	0.88	0.77	0.90	0.93	1.05	0.92	0.94	0.72
90	Q090K	0.82	0.93	0.85	0.89	0.86	0.92	0.89	0.82	0.83
90	Q090L	0.84	0.89	0.78	0.87	0.91	0.94	1.20	0.85	0.80
90	Q090M	1.00	0.85	0.89	0.90	0.89	0.95	0.89	0.97	0.84
90	Q090N	1.01	0.91	0.99	0.96	0.86	1.03	0.85	0.90	0.96
90	Q090P	1.02	0.30	0.63	0.31	0.92	0.77	0.69	0.69	0.36
90	Q090R	0.86	0.99	0.73	0.94	1.08	1.27	0.59	0.71	0.82
90	Q090S	0.77	0.97	0.79	0.94	1.06	1.13	0.86	0.89	0.75
90	Q090T	0.91	0.89	0.84	0.88	1.02	1.27	0.89	0.87	0.79
90	Q090V	0.96	0.83	0.80	0.87	0.90	1.21	1.10	0.86	0.70

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
90	Q090W	0.88	0.91	0.72	0.84	1.03	1.16	1.00	0.99	0.65
90	Q090Y	0.87	0.92	0.72	0.92	0.98	1.15	1.00	1.01	0.72
91	A091C	1.04	0.84	0.78	0.80	0.98	0.81	0.59	0.91	0.81
91	A091D	1.07	1.12	0.68	1.07	0.97	0.75	0.57	0.81	1.22
91	A091E	1.00	1.20	0.73	1.11	1.02	0.88	0.58	0.95	1.12
91	A091F	1.22	1.03	0.66	1.07	1.02	0.85	0.62	0.99	1.03
91	A091G	1.20	1.14	0.69	1.15	0.99	0.87	0.63	0.93	1.15
91	A091H	1.14	1.05	0.62	1.14	0.94	0.87	0.78	0.97	1.21
91	A091I	1.08	1.05	0.62	1.06	1.01	0.84	0.37	1.03	1.04
91	A091K	1.13	1.11	0.67	1.15	1.01	0.87	0.65	0.91	1.34
91	A091L	1.08	1.04	0.57	1.02	1.12	0.88	0.67	1.00	0.98
91	A091M	1.13	1.06	0.66	1.02	0.90	0.84	0.59	0.82	1.07
91	A091N	1.24	1.17	0.64	1.17	0.96	0.84	0.59	0.89	1.30
91	A091Q	1.07	1.02	0.63	1.03	0.84	0.88	0.59	0.94	0.96
91	A091R	0.99	1.16	0.65	1.15	1.07	1.01	0.49	0.91	1.23
91	A091S	1.00	0.29	0.80	0.28	2.28	4.29	0.96	2.11	0.12
91	A091T	1.21	1.02	0.74	1.03	1.11	1.00	0.61	0.98	0.94
91	A091V	1.15	0.94	0.66	0.97	1.02	1.00	0.63	1.06	0.84
91	A091W	1.14	1.13	0.63	1.09	1.00	0.94	0.65	0.84	1.05
91	A091Y	1.19	1.00	0.64	1.02	0.92	1.07	0.67	1.01	0.94
93	K093C	0.79	0.24	0.55	0.22	1.87	1.19	1.35	3.01	0.09
93	K093D	1.06	0.73	0.59	0.71	0.99	1.24	0.91	1.17	0.48
93	K093E	1.05	0.56	0.60	0.59	1.10	0.86	0.79	1.13	0.43
93	K093G	1.00	0.56	0.61	0.56	1.16	0.92	0.86	1.23	0.34
93	K093I	0.90	0.18	0.49	0.20	1.43	0.97	1.05	0.98	0.11
93	K093M	0.91	0.18	0.56	0.17	1.92	1.33	2.36	3.18	0.06
93	K093N	1.04	0.67	0.61	0.66	1.16	1.11	0.85	1.18	0.38
93	K093Q	0.97	0.40	0.89	0.38	1.21	0.96	0.83	1.20	0.22
93	K093R	0.84	0.85	0.59	0.80	1.11	0.98	0.88	0.77	0.65
93	K093S	0.94	0.40	0.52	0.41	1.41	0.59	1.16	1.75	0.22
93	K093T	0.96	0.36	0.58	0.36	1.25	1.09	0.99	1.41	0.23
93	K093V	0.90	0.24	0.54	0.22	1.25	1.31	1.22	1.69	0.11
93	K093W	1.03	0.23	0.55	0.23	1.81	1.59	1.25	2.39	0.08
93	K093Y	1.00	0.35	0.50	0.34	1.34	1.09	0.91	1.85	0.16
94	A094C	0.92	0.79	0.75	0.77	0.95	0.73	0.67	0.87	0.73
94	A094D	0.88	0.65	1.01	0.64	0.93	0.66	0.85	0.83	0.63
94	A094E	0.96	0.92	1.08	0.91	0.94	0.87	1.02	0.88	0.79
94	A094F	1.04	0.98	0.75	0.98	0.99	0.95	0.84	0.89	0.93
94	A094G	1.04	0.68	0.84	0.71	0.90	0.71	0.89	0.82	0.69
94	A094H	1.06	0.88	0.82	0.92	0.92	0.83	0.86	0.93	0.92
94	A094I	0.82	1.02	0.63	0.93	1.10	0.87	0.83	0.74	0.94
94	A094L	0.98	0.93	0.78	0.94	1.06	0.92	0.79	0.78	0.89
94	A094M	1.00	0.06	0.75	0.07	0.19	0.08	0.41	0.55	0.66
94	A094P	1.15	0.24	0.63	0.24	0.72	0.68	0.83	0.66	0.23
94	A094Q	0.86	0.08	1.02	0.07	0.50	0.32	0.58	0.66	0.09
94	A094R	0.85	0.98	0.71	0.94	1.11	1.01	0.73	0.90	0.98
94	A094S	1.02	0.85	0.82	0.90	0.97	0.97	0.95	0.83	0.90

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
94	A094T	0.99	0.89	0.78	0.91	0.96	1.32	0.90	0.83	0.86
94	A094V	1.03	0.82	0.74	0.89	0.96	0.94	1.06	0.90	0.83
94	A094W	1.05	0.89	0.72	0.90	0.95	1.07	0.95	0.91	0.86
94	A094Y	0.92	0.93	0.75	0.88	0.96	0.85	1.03	0.89	0.84
95	A095C	1.02	0.75	0.76	0.78	0.88	0.92	0.86	0.97	0.79
95	A095D	1.02	0.82	0.62	0.80	0.96	0.87	0.77	1.00	0.84
95	A095E	1.13	1.00	0.70	0.98	0.86	0.95	0.86	0.95	1.04
95	A095F	0.78	0.80	0.65	1.05	0.96	1.08	0.91	1.11	1.00
95	A095G	1.17	1.20	0.53	1.25	0.96	0.97	0.86	1.09	1.35
95	A095I	0.98	1.02	0.55	0.97	1.06	0.90	0.57	1.11	1.00
95	A095K	1.12	0.99	0.62	1.06	0.98	0.89	0.94	1.03	1.33
95	A095L	1.02	0.99	0.56	1.04	0.91	0.93	0.68	1.05	1.16
95	A095M	1.08	1.04	0.68	1.03	0.91	0.97	0.79	1.08	1.16
95	A095N	1.06	1.04	0.73	1.03	0.85	0.98	0.94	1.13	1.15
95	A095P	1.16	0.50	0.51	0.52	0.91	0.98	0.65	0.79	0.51
95	A095Q	0.97	1.16	0.60	1.12	1.03	1.04	0.92	1.14	1.08
95	A095R	0.88	1.20	0.54	1.13	0.91	1.09	0.82	1.20	1.27
95	A095S	1.07	0.99	0.67	1.04	0.99	1.03	0.88	1.10	1.10
95	A095T	1.08	0.99	0.66	0.98	1.00	0.93	0.96	1.19	1.06
95	A095V	1.06	0.92	0.80	0.94	0.89	1.05	0.69	1.06	0.98
95	A095W	1.16	0.93	0.63	0.92	0.93	1.09	0.98	1.04	0.90
95	A095Y	1.03	0.91	0.65	0.99	0.92	1.01	0.78	1.11	1.03
103	V103A	0.74	0.88	0.29	0.95	1.14	0.98	1.13	1.00	1.42
103	V103C	0.78	0.80	0.42	0.81	1.00	1.01	0.81	0.99	0.88
103	V103D	0.67	0.13	0.29	0.13	1.18	0.78	0.61	0.78	0.16
103	V103E	0.90	0.36	0.16	0.39	0.82	0.88	0.80	0.81	0.40
103	V103F	0.71	0.78	0.07	0.75	0.99	1.00	0.85	0.81	1.16
103	V103G	0.86	0.99	0.19	1.09	1.10	1.09	1.39	0.99	1.01
103	V103H	0.51	0.49	0.07	0.48	1.02	0.66	0.96	0.78	0.58
103	V103I	0.99	0.87	1.00	0.87	0.98	1.08	1.03	0.95	0.95
103	V103K	0.66	0.31	0.06	0.34	0.88	0.75	0.27	0.73	0.36
103	V103L	0.67	1.08	0.26	0.97	1.00	0.80	1.28	0.97	0.97
103	V103M	0.89	0.84	0.13	0.85	1.03	0.99	1.12	0.86	1.05
103	V103N	0.69	1.22	0.23	1.07	1.06	1.05	1.18	0.94	0.90
103	V103S	0.71	1.08	0.17	1.16	1.28	1.17	1.21	1.10	1.01
104	V104A	0.56	0.10	0.35	0.09	0.46	0.09	0.75	0.54	1.04
104	V104G	1.55	0.16	0.47	0.18	0.63	0.37	0.41	0.27	0.99
104	V104I	1.06	1.17	0.37	1.16	0.89	0.83	0.87	0.84	0.88
104	V104L	0.75	0.22	0.14	0.20	0.51	0.25	0.49	0.39	0.83
104	V104T	0.84	0.32	0.31	0.31	0.87	0.64	0.64	0.63	0.94
105	F105C	0.78	0.87	0.33	0.82	0.86	0.67	1.04	0.92	0.83
105	F105E	0.58	1.00	0.08	0.87	0.98	0.90	1.06	0.96	0.69
105	F105G	0.86	1.01	0.12	0.98	0.97	0.77	1.05	0.87	0.81
105	F105H	1.26	0.96	0.39	0.91	0.92	1.20	0.90	0.84	0.99
105	F105I	0.41	0.86	0.09	0.86	0.82	0.74	0.82	0.93	0.82
105	F105K	0.71	0.79	0.07	0.81	0.77	0.82	1.01	0.88	0.70
105	F105L	0.80	0.91	0.46	0.97	0.96	0.81	1.06	0.94	0.85

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
105	F105M	0.72	0.90	0.25	0.89	0.84	0.86	0.73	0.88	0.89
105	F105N	0.92	0.92	0.12	0.89	0.86	0.84	1.16	0.89	0.94
105	F105Q	0.71	0.92	0.09	0.91	1.02	0.82	0.88	0.91	0.87
105	F105S	0.61	0.89	0.06	0.95	0.95	0.99	0.65	0.93	0.86
105	F105T	0.70	0.82	0.09	0.85	0.92	0.90	0.86	1.00	0.94
105	F105V	0.71	0.65	0.43	0.69	0.87	1.03	0.98	0.91	0.74
105	F105Y	0.62	0.80	0.06	0.85	0.94	0.84	1.04	0.91	0.98
108	K108A	1.32	1.11	0.21	1.21	1.22	1.06	1.86	1.08	0.62
108	K108C	1.80	0.57	0.26	0.65	1.04	0.88	1.24	0.99	0.48
108	K108E	1.78	0.41	0.38	0.42	1.27	0.81	1.67	1.06	0.17
108	K108F	1.44	0.84	0.20	0.83	0.76	0.41	1.38	0.97	0.52
108	K108G	1.46	0.69	0.18	0.83	1.01	0.79	1.15	0.93	0.59
108	K108I	1.77	1.25	0.29	1.33	1.17	0.77	1.22	0.83	0.57
108	K108L	2.05	1.14	0.52	1.19	0.95	0.63	1.61	0.90	0.56
108	K108M	1.64	1.15	0.55	1.20	0.84	0.50	1.50	0.99	0.64
108	K108N	1.56	0.69	0.20	0.75	0.84	0.63	1.03	0.92	0.51
108	K108R	0.97	0.74	0.46	0.83	1.00	0.88	1.12	1.04	0.78
108	K108S	1.56	0.91	0.20	1.04	0.92	0.95	1.31	0.96	0.58
108	K108V	1.93	0.92	0.24	1.08	0.95	0.91	1.46	0.98	0.53
108	K108Y	1.26	0.73	0.11	0.73	0.79	0.57	1.35	0.83	0.51
110	G110A	1.04	1.48	0.53	1.69	1.23	0.83	1.18	1.09	0.88
110	G110C	0.82	0.21	0.28	0.18	0.28	0.06	0.64	0.99	0.35
110	G110D	0.98	0.47	0.18	0.39	1.07	0.71	1.11	0.90	0.55
110	G110E	0.94	0.82	0.26	0.69	1.04	0.31	1.12	1.00	0.97
110	G110F	0.65	0.70	0.14	0.54	0.79	1.02	0.60	0.84	0.71
110	G110H	0.74	0.17	0.18	0.15	0.75	1.08	0.70	0.69	0.99
110	G110I	0.49	0.58	0.12	0.56	0.54	0.06	0.69	1.31	0.52
110	G110K	0.68	0.46	0.17	0.50	1.00	0.26	0.91	1.18	0.86
110	G110L	0.61	0.37	0.11	0.32	0.78	0.22	0.88	0.93	0.71
110	G110M	0.81	0.53	0.19	0.48	1.14	0.66	1.06	1.03	0.78
110	G110P	0.32	0.09	0.08	0.10	0.33	0.08	0.86	1.27	0.76
110	G110Q	0.87	0.92	0.21	0.83	1.15	0.60	1.05	1.15	0.85
110	G110R	0.53	0.76	0.15	1.17	0.73	0.27	0.92	1.12	0.83
110	G110S	0.91	0.97	0.27	0.95	0.95	0.96	0.96	1.01	0.86
110	G110V	0.73	0.64	0.10	0.68	1.00	0.53	0.85	0.96	0.65
110	G110W	0.69	0.34	0.19	0.28	0.59	0.68	0.35	0.67	0.66
110	G110Y	0.70	0.48	0.13	0.44	0.74	0.96	0.65	0.67	0.78
112	D112A	0.56	0.22	0.08	0.25	0.84	0.18	0.92	0.89	0.64
112	D112E	1.28	0.46	0.21	0.49	1.14	1.05	1.13	1.12	1.13
112	D112G	0.93	0.54	0.44	0.53	1.10	0.87	1.18	1.20	1.23
112	D112H	0.71	0.22	0.10	0.25	0.68	0.13	0.66	0.81	0.64
112	D112L	0.65	0.20	0.10	0.20	0.95	0.44	0.92	0.91	0.65
112	D112M	0.69	0.17	0.13	0.20	0.72	0.21	0.81	1.08	0.67
112	D112N	0.80	0.45	0.16	0.41	1.06	1.13	1.17	0.96	1.10
112	D112S	0.68	0.37	0.08	0.40	1.07	0.26	1.00	1.02	0.90
112	D112W	0.77	0.12	0.06	0.13	0.66	0.09	0.53	0.98	0.36
112	D112Y	0.72	0.20	0.19	0.22	0.74	0.07	0.90	1.08	0.51

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
113	G113A	1.08	0.95	0.77	0.94	1.02	1.29	1.07	0.90	0.95
113	G113D	0.75	0.48	1.34	0.95	0.91	0.71	0.94	0.87	0.96
113	G113E	1.41	1.07	1.44	0.95	0.92	0.69	1.03	0.84	0.95
113	G113F	0.97	0.76	0.71	0.86	0.91	0.94	0.95	0.90	0.82
113	G113H	0.86	0.81	0.55	1.07	1.04	0.89	1.09	0.89	1.08
113	G113I	1.04	0.84	0.40	0.79	0.98	1.18	0.76	0.83	0.68
113	G113K	0.82	0.61	0.37	0.93	1.02	1.16	1.03	0.86	0.90
113	G113L	1.02	0.67	0.48	0.77	0.92	0.87	0.79	0.78	0.79
113	G113M	1.25	0.82	0.67	0.89	0.82	0.88	1.01	0.84	0.85
113	G113N	1.08	0.67	0.57	0.99	1.04	0.94	0.94	0.98	1.01
113	G113Q	1.33	0.97	0.76	1.09	1.40	1.09	1.06	0.95	1.03
113	G113R	0.72	0.62	0.30	0.90	0.96	1.16	1.04	0.97	1.01
113	G113S	0.74	0.67	0.66	0.90	0.96	0.92	0.90	0.87	0.96
113	G113T	1.25	0.78	0.57	0.84	0.77	1.00	1.13	0.91	0.83
113	G113V	1.23	0.98	0.51	0.95	0.97	1.04	1.08	0.94	0.78
113	G113W	1.14	0.59	0.58	0.77	0.80	0.94	0.90	0.87	0.79
113	G113Y	1.05	0.45	0.74	0.80	0.93	1.08	0.92	0.90	0.81
114	T114A	0.68	0.61	0.18	0.62	0.88	0.87	0.67	0.98	1.08
114	T114C	0.87	1.23	0.28	1.35	0.79	0.51	0.55	0.99	0.84
114	T114D	0.98	0.49	0.23	0.48	0.84	0.53	0.98	0.98	1.03
114	T114E	0.83	0.61	0.17	0.63	0.81	0.63	0.83	1.01	1.00
114	T114F	0.80	0.56	0.33	0.54	0.82	0.77	1.03	1.05	0.90
114	T114G	0.68	0.96	0.16	0.94	0.86	0.70	1.02	1.01	1.06
114	T114H	0.64	0.71	0.14	0.70	0.87	0.82	0.99	1.02	1.08
114	T114I	0.75	0.91	0.27	0.90	0.77	0.86	0.91	0.99	0.92
114	T114K	0.77	1.14	0.12	1.31	0.81	1.02	1.02	0.94	1.08
114	T114L	0.94	1.48	0.49	1.54	0.83	0.68	0.98	1.02	0.92
114	T114M	0.92	0.90	0.28	0.98	0.90	0.75	0.81	1.02	0.90
114	T114N	0.79	0.46	0.18	0.48	0.86	0.93	0.84	0.96	1.01
114	T114P	0.89	0.45	0.19	0.55	0.78	0.92	0.76	0.90	0.97
114	T114Q	0.82	1.59	0.15	1.71	0.92	0.76	1.08	1.04	0.92
114	T114R	0.80	1.27	0.11	1.52	0.96	0.98	1.02	1.01	1.08
114	T114S	0.74	0.53	0.23	0.54	0.90	0.77	0.80	0.96	1.02
114	T114V	0.72	1.07	0.39	1.04	0.93	0.98	1.08	1.01	0.85
114	T114W	0.90	0.46	0.30	0.46	0.75	0.64	1.05	1.05	0.94
114	T114Y	0.87	0.39	0.37	0.45	0.80	0.82	0.99	0.98	0.87
115	E115C	0.29	0.35	0.15	0.38	1.04	0.89	0.69	1.11	0.69
115	E115D	0.35	0.64	0.09	0.56	0.89	1.17	0.82	1.09	0.97
115	E115Q	0.49	0.89	0.10	0.84	0.99	1.17	1.04	1.06	0.98
116	F116A	0.57	1.23	0.36	1.15	0.86	0.81	0.93	0.89	1.03
116	F116C	0.81	1.00	1.05	0.92	0.97	0.73	0.68	0.95	0.87
116	F116D	0.82	1.10	0.58	0.94	1.03	0.62	0.88	0.98	1.00
116	F116E	0.79	1.14	0.78	0.99	0.98	0.76	0.81	1.01	0.96
116	F116G	0.58	1.26	0.28	1.17	1.05	1.01	0.94	1.07	0.90
116	F116H	0.73	1.25	0.44	1.05	1.06	1.00	0.85	1.03	0.98
116	F116I	0.78	1.01	0.71	1.00	1.04	1.01	0.90	1.07	0.95
116	F116K	0.71	1.00	0.39	0.98	1.09	1.08	1.01	1.04	0.87

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
116	F116L	0.70	1.05	0.87	0.93	1.01	0.93	0.72	0.99	0.98
116	F116M	0.83	0.97	0.88	0.96	1.01	0.67	0.95	1.02	0.99
116	F116N	0.81	1.08	0.52	1.04	1.00	0.63	0.77	1.06	1.09
116	F116P	0.58	1.13	0.40	1.11	1.06	1.00	0.92	0.99	1.02
116	F116Q	0.74	1.12	0.55	1.09	1.01	1.05	1.15	1.05	0.99
116	F116R	1.43	1.08	0.22	1.09	1.21	1.21	0.90	1.05	1.00
116	F116S	0.66	1.15	0.35	1.17	1.04	1.05	0.91	1.01	1.08
116	F116T	0.75	1.01	0.65	1.05	1.03	0.94	0.87	0.97	1.01
116	F116V	0.77	0.99	0.82	0.97	0.97	0.88	0.95	0.99	1.03
116	F116W	0.96	1.07	2.02	1.00	1.11	0.90	0.91	0.96	1.01
116	F116Y	0.77	1.20	0.93	1.00	1.08	0.88	0.84	1.05	0.98
118	D118A	0.60	1.16	0.23	1.02	1.06	1.59	0.47	0.99	0.60
118	D118C	1.05	0.54	2.15	0.46	0.54	0.73	0.62	0.77	0.55
118	D118E	0.66	0.87	0.79	0.86	0.93	1.01	0.94	0.90	0.81
118	D118F	0.76	0.58	0.64	0.54	0.76	1.03	0.64	0.63	0.61
118	D118G	0.54	0.63	0.09	0.61	0.79	1.32	0.60	0.84	0.57
118	D118H	0.55	0.82	0.25	0.81	0.89	1.23	0.68	0.93	0.82
118	D118I	0.38	0.40	0.14	0.41	0.69	1.26	0.60	0.78	0.47
118	D118K	0.71	0.79	0.13	0.80	0.96	1.55	0.84	1.01	0.71
118	D118L	0.73	0.49	0.65	0.51	0.88	1.29	0.41	0.84	0.49
118	D118M	0.72	0.41	0.96	0.43	0.77	0.91	0.57	0.77	0.49
118	D118N	0.62	1.07	0.36	0.96	0.88	1.29	0.79	0.86	0.97
118	D118P	1.05	0.98	0.59	1.01	0.92	1.24	0.89	0.91	0.89
118	D118Q	0.60	0.83	0.37	0.81	0.89	1.10	0.75	0.90	0.83
118	D118S	0.60	0.77	0.26	0.79	0.88	1.10	0.71	0.92	0.86
118	D118T	0.47	0.64	0.18	0.66	0.79	0.99	0.92	0.97	0.62
118	D118V	0.39	0.22	0.14	0.21	0.71	0.92	0.53	0.60	0.26
118	D118W	0.74	0.66	1.23	0.63	0.95	1.38	0.83	0.91	0.60
118	D118Y	0.65	0.41	0.53	0.43	0.82	0.77	0.61	0.81	0.49
121	E121Q	0.51	0.69	0.09	0.77	0.84	1.02	0.90	1.00	0.76
121	E121T	0.82	0.65	0.20	0.70	0.85	0.95	0.86	1.03	0.74
123	D123A	0.71	1.14	0.42	1.07	0.91	1.20	0.97	1.03	1.01
123	D123E	0.73	1.07	0.35	1.04	0.97	0.98	0.84	0.86	0.99
123	D123H	0.61	0.99	0.37	0.98	0.97	1.14	0.72	0.89	0.97
123	D123K	0.57	0.89	0.13	0.93	0.83	1.21	0.71	0.83	0.98
123	D123L	0.82	0.86	0.32	0.88	1.00	0.98	1.04	0.99	0.77
123	D123M	0.62	0.94	0.29	0.91	0.87	1.12	0.90	0.87	0.89
123	D123Q	0.54	0.92	0.12	0.98	0.91	1.17	0.85	0.91	0.95
123	D123R	0.42	0.91	0.07	0.90	0.78	1.89	0.71	0.82	0.90
123	D123S	0.79	0.90	0.59	0.92	0.83	1.28	0.74	0.85	0.90
123	D123W	0.34	0.71	0.06	0.69	0.85	1.16	0.72	0.89	0.73
125	S125A	0.96	0.93	1.19	0.92	1.16	1.05	0.69	1.13	1.03
125	S125C	0.72	0.69	1.34	0.60	1.05	0.63	0.89	1.03	0.70
125	S125D	0.47	0.90	0.61	0.81	1.08	0.84	0.90	0.97	0.93
125	S125E	0.93	1.36	0.74	1.25	1.01	0.73	0.89	1.08	0.95
125	S125F	0.96	1.01	1.52	0.88	1.04	0.96	0.81	1.02	0.96
125	S125G	0.92	0.89	0.78	0.92	1.12	0.98	0.91	1.04	0.99

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
125	S125H	0.91	0.89	1.23	0.93	1.11	1.09	0.79	0.94	0.99
125	S125I	0.56	0.88	0.40	0.91	1.07	0.95	0.72	1.03	0.92
125	S125K	0.93	1.08	1.58	0.89	1.01	1.06	0.76	0.96	1.02
125	S125L	0.84	0.55	1.41	0.50	1.06	0.74	0.75	0.94	0.46
125	S125M	1.01	0.69	1.37	0.77	0.83	0.91	1.05	0.91	0.82
125	S125N	0.88	0.89	1.28	0.86	1.03	0.96	0.96	0.86	0.98
125	S125Q	0.89	0.94	1.11	0.94	0.97	1.07	0.74	1.06	0.95
125	S125R	0.88	1.01	1.23	0.92	0.97	1.32	0.51	0.95	1.05
125	S125T	0.89	0.81	1.43	0.74	1.01	0.91	0.86	0.98	0.72
125	S125V	0.57	0.78	0.56	0.81	1.07	0.86	0.66	0.98	0.82
125	S125W	0.64	0.64	0.44	0.62	1.04	0.86	0.60	1.07	0.57
125	S125Y	0.97	1.23	1.32	1.02	1.09	1.29	0.94	0.96	0.94
126	N126C	0.37	0.86	0.08	0.81	0.77	0.39	0.84	1.19	0.72
126	N126D	1.84	1.04	1.52	0.96	0.81	0.82	0.98	0.94	0.99
126	N126E	0.63	1.10	0.10	1.03	0.97	0.80	0.79	1.02	0.93
128	N128C	3.90	0.70	2.31	0.69	0.94	0.36	1.41	1.56	0.66
128	N128D	0.84	1.05	0.62	0.93	0.99	0.74	0.99	1.04	0.94
128	N128E	0.91	0.98	0.69	0.97	1.07	0.41	1.09	1.42	0.87
128	N128L	0.43	0.89	0.76	0.92	1.15	0.35	1.32	1.30	0.83
128	N128M	0.41	0.89	0.59	0.91	1.05	0.40	1.26	1.25	0.82
128	N128Q	0.18	0.96	0.08	0.97	1.23	0.70	1.09	1.45	0.93
128	N128T	0.25	0.86	0.21	0.89	1.10	0.56	0.85	1.26	0.89
128	N128V	0.15	0.90	0.08	0.91	1.16	0.49	1.27	1.29	0.82
128	N128W	1.43	1.14	0.88	1.00	1.12	1.17	1.14	0.95	0.87
128	N128Y	2.25	0.96	0.95	0.95	1.15	0.55	1.21	1.34	0.82
129	Q129A	0.49	0.58	0.86	0.58	0.68	0.53	1.00	0.76	0.62
129	Q129C	0.98	0.49	2.59	0.47	0.80	0.40	0.99	1.15	0.48
129	Q129D	0.65	0.81	0.38	0.77	0.86	0.68	1.12	0.95	0.84
129	Q129E	0.94	0.52	0.49	0.56	1.02	0.37	0.99	1.12	0.52
129	Q129F	0.75	0.92	0.80	0.85	0.91	0.88	1.04	0.98	0.77
129	Q129H	0.60	0.58	1.05	0.73	0.87	0.69	0.91	0.91	0.84
129	Q129I	1.28	0.91	1.78	0.85	0.91	0.70	0.88	0.94	0.87
129	Q129K	0.79	0.76	1.10	0.79	0.84	0.93	1.01	0.90	0.82
129	Q129L	1.07	0.78	1.48	0.75	1.02	0.93	0.91	1.04	0.71
129	Q129M	0.92	0.80	1.25	0.78	0.97	0.73	1.03	0.97	0.74
129	Q129N	0.88	0.92	0.89	0.90	0.82	0.86	1.01	0.91	1.03
129	Q129R	0.76	0.91	1.18	0.79	0.90	1.13	0.79	1.01	0.67
129	Q129S	0.48	0.56	0.41	0.75	0.97	0.95	0.84	1.05	0.52
129	Q129T	0.76	0.76	0.62	0.78	1.05	0.82	1.04	1.04	0.71
129	Q129V	1.17	0.54	1.94	0.80	0.99	0.91	1.07	1.02	0.67
129	Q129W	0.69	0.78	0.48	0.80	0.89	1.03	0.83	1.02	0.77
130	E130A	1.32	0.70	0.29	0.75	1.05	0.51	0.99	1.19	0.87
130	E130C	1.43	0.70	0.39	0.63	0.94	0.23	1.03	1.31	0.75
130	E130G	0.75	0.79	0.11	0.74	0.99	0.53	0.95	1.04	1.01
130	E130H	0.90	0.75	0.08	0.80	0.94	0.73	0.90	1.05	0.99
130	E130I	0.89	0.89	0.17	0.87	1.07	0.62	1.00	1.06	0.83
130	E130K	1.75	0.73	0.75	0.85	0.96	0.65	0.92	1.15	1.01

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
130	E130M	0.84	0.81	0.09	0.79	1.01	0.44	0.90	1.08	0.99
130	E130N	0.50	0.87	0.09	0.79	0.86	0.64	0.99	1.02	1.02
130	E130Q	0.53	0.93	0.06	0.92	1.01	0.71	1.02	1.12	1.03
130	E130R	0.73	0.86	0.21	0.80	0.80	1.01	0.98	0.88	0.99
130	E130S	0.60	0.74	0.09	0.74	1.03	0.55	1.12	1.03	0.95
130	E130T	0.50	0.94	0.09	0.81	0.99	0.57	0.95	1.09	0.96
130	E130W	0.85	0.70	0.11	0.63	0.90	0.81	1.00	1.10	0.83
130	E130Y	0.66	0.68	0.08	0.68	0.96	0.52	1.13	1.14	0.85
131	T131A	0.40	1.02	0.14	0.96	1.08	0.92	1.06	0.97	1.03
131	T131C	0.66	0.85	0.63	0.82	0.90	0.70	0.84	1.03	0.84
131	T131I	1.68	0.94	2.12	0.98	1.03	0.91	1.04	0.96	0.90
131	T131K	0.50	0.32	0.25	0.32	1.11	1.08	0.69	1.00	0.21
131	T131M	0.89	0.85	0.93	0.93	1.17	1.02	1.01	0.86	0.85
131	T131N	0.38	0.96	0.12	0.95	0.95	0.86	1.08	0.96	0.99
131	T131Q	0.58	0.94	0.26	0.94	0.96	0.86	1.12	0.94	0.90
131	T131R	0.43	1.03	0.21	0.99	1.08	1.22	0.82	0.89	1.04
131	T131S	0.53	0.96	0.15	0.99	1.06	0.87	0.97	0.94	0.94
131	T131V	1.42	0.46	1.23	0.52	1.08	0.95	1.17	1.07	0.40
131	T131W	0.43	1.02	0.08	0.98	1.07	1.08	0.99	0.94	0.84
132	S132T	0.65	0.88	0.29	0.88	0.83	0.88	0.99	0.98	1.01
134	T134A	0.98	1.09	0.80	0.98	1.17	1.30	1.02	1.04	1.09
134	T134C	1.53	0.81	1.17	0.78	0.98	0.74	0.67	1.08	0.88
134	T134D	1.40	0.98	0.59	1.03	0.92	0.82	0.76	1.04	1.14
134	T134E	1.03	0.96	0.76	0.94	1.03	0.74	1.01	1.06	1.03
134	T134G	0.92	0.88	0.29	0.93	1.18	0.64	0.89	1.14	1.08
134	T134H	0.64	0.87	0.14	0.95	1.12	0.74	0.93	1.14	1.20
134	T134I	0.92	0.89	1.05	0.84	1.04	0.88	0.81	0.94	1.04
134	T134K	0.45	1.10	0.12	0.93	1.05	0.92	0.97	0.89	1.25
134	T134L	0.52	0.80	0.25	0.84	1.02	0.81	1.15	0.90	1.01
134	T134M	0.77	0.90	0.57	0.85	1.10	0.78	0.86	0.93	1.01
134	T134N	0.77	1.09	0.26	1.05	1.19	0.78	1.04	1.01	1.22
134	T134P	1.52	1.03	1.86	1.01	0.96	0.78	0.97	0.95	1.37
134	T134Q	0.80	1.07	0.49	1.02	1.17	0.87	1.15	1.07	1.18
134	T134S	0.80	1.02	0.48	0.95	1.13	0.94	0.89	0.98	1.07
134	T134Y	0.16	0.87	0.06	0.83	1.08	0.82	1.02	1.04	0.89
135	Y135C	0.20	0.43	0.13	0.80	1.08	0.73	1.00	0.76	1.07
135	Y135E	0.19	0.57	0.06	1.04	1.03	0.86	1.08	0.95	1.31
135	Y135F	0.35	0.48	0.25	0.96	1.13	0.90	1.05	0.99	1.05
135	Y135H	0.30	0.77	0.07	1.02	1.00	0.90	1.05	0.92	1.44
135	Y135I	0.24	0.43	0.50	0.89	1.18	1.08	0.88	0.98	0.94
135	Y135K	0.29	0.79	0.09	1.05	0.98	0.98	1.06	0.87	1.42
135	Y135L	0.47	0.84	0.34	0.87	1.12	0.90	1.23	1.04	0.94
135	Y135M	0.24	0.45	0.35	0.85	1.07	0.96	1.02	0.92	1.03
135	Y135Q	0.24	0.51	0.12	1.06	1.09	1.01	1.04	0.99	1.41
135	Y135V	0.42	0.67	0.23	0.88	1.09	0.97	0.93	0.98	0.84
136	Q136A	0.75	0.93	0.37	0.99	1.07	1.04	1.09	0.72	1.17
136	Q136C	1.03	0.82	1.05	0.77	0.89	0.60	0.92	0.96	1.00

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
136	Q136D	0.78	1.01	0.24	1.02	0.93	0.73	0.85	0.97	1.25
136	Q136E	1.03	0.96	0.89	0.85	0.79	0.64	1.07	0.95	1.07
136	Q136F	0.58	0.89	0.10	0.83	0.92	0.71	1.10	0.90	1.19
136	Q136G	0.77	1.16	0.30	1.10	1.04	0.90	1.22	0.96	1.38
136	Q136H	0.59	0.96	0.13	0.92	1.10	0.86	1.42	1.02	1.22
136	Q136I	0.84	0.72	0.83	0.69	1.04	0.84	1.22	0.80	0.96
136	Q136K	0.87	1.03	1.10	1.00	0.95	0.96	1.21	0.83	1.42
136	Q136L	0.90	0.57	0.84	0.55	0.90	0.73	0.65	0.88	0.75
136	Q136M	0.92	0.82	0.94	0.77	0.93	0.74	0.95	0.90	1.15
136	Q136N	0.88	1.05	0.44	0.99	0.99	0.78	1.16	0.90	1.46
136	Q136P	0.79	1.14	0.36	1.16	1.01	0.93	1.31	0.90	1.54
136	Q136R	0.68	0.98	0.75	0.93	1.06	1.07	1.00	0.93	1.30
136	Q136S	0.68	1.03	0.51	0.93	1.06	0.95	1.27	0.83	1.07
136	Q136T	0.95	0.91	1.60	0.84	0.97	0.78	1.02	0.96	1.10
136	Q136V	0.97	0.76	1.32	0.73	0.92	0.76	0.92	0.91	0.97
136	Q136W	0.61	0.93	0.12	0.94	1.05	0.80	1.29	1.03	1.59
136	Q136Y	0.55	0.75	0.11	0.76	1.05	0.81	0.95	1.00	1.00
138	Q138A	0.66	1.16	0.62	1.02	1.08	1.31	1.18	0.98	0.89
138	Q138C	0.87	0.87	0.72	0.82	0.97	0.34	0.64	0.89	0.73
138	Q138D	0.85	1.28	0.26	1.15	1.11	0.80	1.36	0.86	1.09
138	Q138E	1.11	1.07	1.54	1.02	1.03	1.05	1.02	0.84	0.98
138	Q138G	0.69	1.04	0.22	1.07	1.04	0.93	1.32	0.91	1.08
138	Q138H	0.74	0.89	0.22	0.94	1.02	1.21	1.00	0.86	1.07
138	Q138K	0.68	1.03	0.34	0.93	1.08	1.01	1.12	0.79	1.07
138	Q138L	0.83	0.89	1.14	0.86	1.11	0.97	0.95	0.91	0.81
138	Q138M	0.84	0.80	0.70	0.78	0.94	1.35	0.81	0.86	0.83
138	Q138N	0.79	1.09	0.24	1.03	1.08	0.81	1.10	0.81	1.05
138	Q138R	0.61	1.07	0.24	1.04	1.09	1.62	1.15	0.92	0.94
138	Q138S	0.70	1.20	0.50	1.04	1.11	1.16	1.17	0.91	0.93
138	Q138T	0.86	0.77	0.32	0.80	1.06	0.72	0.96	0.87	0.85
138	Q138V	0.92	0.75	0.49	0.74	1.01	0.83	2.12	0.91	0.72
138	Q138W	0.81	0.85	0.27	0.90	1.21	1.11	1.08	1.01	0.81
138	Q138Y	0.64	1.06	0.19	1.00	1.12	0.95	0.94	0.95	0.92
140	W140A	0.48	0.18	0.15	0.17	0.13	0.14	0.75	1.19	0.73
140	W140C	0.55	0.21	0.21	0.21	0.08	0.18	0.47	0.91	0.54
140	W140D	0.61	0.12	0.16	0.15	0.11	0.18	1.02	1.08	0.66
140	W140F	0.90	2.47	0.31	2.67	0.89	0.49	1.01	1.17	0.78
140	W140G	0.48	0.12	0.10	0.13	0.12	0.10	0.91	1.01	0.79
140	W140H	0.95	1.74	0.28	1.80	0.57	0.17	0.87	1.19	0.80
140	W140I	0.64	0.15	0.18	0.16	0.12	0.18	0.64	1.09	0.59
140	W140M	0.56	0.39	0.16	0.40	0.18	0.22	0.95	1.32	0.63
140	W140N	0.68	0.24	0.15	0.27	0.25	0.07	1.02	1.24	0.81
140	W140P	0.39	0.23	0.07	0.25	0.21	0.06	1.08	1.21	0.84
140	W140R	0.41	0.75	0.13	0.83	0.66	0.15	0.98	1.37	0.72
140	W140S	0.61	0.20	0.14	0.22	0.21	0.23	1.12	1.38	0.65
140	W140T	0.62	0.11	0.13	0.13	0.17	0.09	0.90	1.30	0.59
140	W140V	0.70	0.14	0.19	0.16	0.16	0.26	0.90	1.16	0.74

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
140	W140Y	0.85	2.17	0.30	2.27	1.01	0.45	1.16	1.17	0.67
142	K142A	1.20	0.89	0.68	0.91	1.02	0.84	0.97	1.00	0.83
142	K142C	1.43	1.02	0.85	0.94	0.93	0.63	1.03	0.97	0.70
142	K142D	0.82	0.87	0.23	0.81	0.65	0.70	0.89	0.95	0.71
142	K142E	1.19	0.91	0.58	0.83	0.93	0.68	0.95	0.98	0.75
142	K142F	1.06	1.11	0.60	1.01	0.98	0.86	1.03	0.84	0.72
142	K142G	0.97	0.92	0.37	0.94	0.94	0.69	0.86	0.90	0.84
142	K142H	1.00	1.10	0.44	1.05	0.97	0.73	0.96	0.99	0.90
142	K142I	1.12	1.13	0.74	0.98	1.01	0.70	0.87	1.04	0.86
142	K142L	1.21	1.04	0.93	0.90	1.05	0.88	1.06	1.05	0.66
142	K142M	1.02	0.99	0.84	0.86	0.97	0.76	0.88	1.03	0.73
142	K142N	0.73	0.98	0.30	0.89	1.01	0.87	0.99	1.02	0.85
142	K142P	1.51	1.60	0.46	1.53	0.85	0.35	1.14	0.89	0.54
142	K142Q	1.22	1.13	0.71	1.09	1.00	0.75	0.80	1.03	0.85
142	K142S	1.15	0.83	0.59	0.81	0.99	1.03	1.22	1.03	0.68
142	K142T	0.91	0.72	0.36	0.71	1.04	0.61	1.06	0.95	0.71
142	K142V	1.18	0.93	0.94	0.91	1.03	0.72	1.09	0.92	0.83
142	K142W	1.22	1.04	0.72	0.96	0.94	0.76	0.98	0.92	0.77
142	K142Y	0.96	0.96	0.74	0.91	1.01	0.98	1.03	1.03	0.69
144	D144A	0.64	0.90	0.09	0.97	0.95	1.11	1.03	1.00	1.13
144	D144C	0.72	0.76	0.56	0.74	0.84	0.69	0.81	0.82	0.82
144	D144E	1.00	0.74	0.57	0.84	0.88	0.94	0.98	0.91	0.90
144	D144F	0.73	1.00	0.17	1.00	0.90	1.10	1.02	0.99	1.02
144	D144G	0.82	0.79	0.11	0.93	0.95	1.15	0.93	0.94	1.05
144	D144H	0.73	0.80	0.13	0.88	0.80	1.09	0.91	0.90	1.11
144	D144I	0.72	0.85	0.19	0.90	0.95	1.11	0.95	1.03	0.98
144	D144K	0.87	0.73	0.13	0.85	0.79	1.50	0.79	0.81	1.13
144	D144L	0.70	0.80	0.18	0.89	0.90	1.04	0.91	0.93	1.08
144	D144M	0.77	0.85	0.18	0.90	0.92	1.07	0.90	0.91	1.12
144	D144N	0.90	0.93	0.28	0.97	0.96	1.09	1.06	1.00	1.15
144	D144P	0.90	0.35	0.12	0.40	0.60	0.86	0.63	0.77	0.59
144	D144R	0.74	0.78	0.09	0.82	0.89	1.37	0.74	0.87	1.02
144	D144S	0.74	0.74	0.15	0.87	0.79	0.94	0.77	1.02	0.99
144	D144T	0.78	0.80	0.26	0.88	0.88	0.97	0.87	0.96	0.95
144	D144V	0.81	0.75	0.23	0.86	0.92	1.03	0.87	0.86	1.05
144	D144W	0.78	0.95	0.25	0.96	0.91	1.20	0.83	1.05	1.26
144	D144Y	0.72	0.80	0.20	0.85	1.16	1.18	0.63	0.94	1.00
147	G147A	0.91	0.97	0.43	0.96	0.98	0.75	0.52	0.89	0.68
147	G147C	0.92	0.75	0.45	0.72	0.79	0.61	0.74	0.54	0.55
147	G147D	1.02	0.97	0.49	0.96	0.95	0.64	0.86	0.77	0.70
147	G147E	1.05	0.84	0.48	0.93	0.90	0.68	0.78	0.68	0.70
147	G147F	0.94	1.00	0.38	0.93	1.01	0.85	0.79	0.88	0.68
147	G147H	0.95	1.01	0.35	0.98	0.85	1.26	0.72	0.90	0.78
147	G147K	0.93	0.85	0.30	0.84	0.98	0.94	0.96	0.85	0.71
147	G147L	0.82	0.93	0.34	0.86	1.09	0.80	0.85	0.77	0.67
147	G147M	0.92	0.92	0.42	0.90	0.90	0.83	0.79	0.79	0.71
147	G147N	0.99	0.93	0.49	0.94	0.94	0.83	0.79	0.83	0.84

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
147	G147P	0.94	0.69	0.33	0.71	0.95	0.85	0.92	0.85	0.57
147	G147Q	0.92	0.72	0.43	0.75	1.04	0.91	0.85	1.08	0.51
147	G147R	0.86	0.93	0.27	0.85	1.05	1.30	0.81	0.87	0.75
147	G147S	1.04	0.78	0.39	0.88	0.91	0.87	0.94	0.81	0.68
147	G147T	0.83	0.96	0.39	0.88	1.04	0.87	0.93	0.81	0.63
147	G147V	0.89	0.86	0.37	0.82	0.91	0.80	0.94	0.86	0.65
147	G147W	1.01	0.90	0.35	0.88	0.99	0.95	1.21	0.89	0.69
147	G147Y	0.88	1.08	0.37	0.96	0.87	0.96	0.81	0.84	0.66
149	G149A	0.90	1.02	0.73	1.02	1.12	1.07	1.03	0.98	0.89
149	G149C	0.85	0.62	0.68	0.56	0.88	0.63	0.70	0.73	0.54
149	G149D	0.96	0.97	0.87	0.92	0.98	0.73	0.90	0.77	0.98
149	G149E	0.97	0.93	0.76	0.94	0.79	0.68	0.87	0.74	0.97
149	G149F	0.93	1.04	0.59	0.98	0.92	0.79	0.96	0.77	0.98
149	G149H	0.94	1.00	0.57	1.03	0.98	0.98	0.85	0.81	1.12
149	G149I	0.85	0.99	0.40	0.96	1.24	1.03	0.96	0.92	0.92
149	G149L	1.08	0.91	0.51	1.04	1.14	0.91	0.84	0.85	0.98
149	G149M	0.99	1.02	0.67	1.03	1.12	0.81	0.79	0.90	1.01
149	G149N	0.96	1.06	0.89	1.03	1.12	0.72	0.85	0.84	1.16
149	G149P	0.79	0.86	0.30	0.90	0.94	0.81	0.76	0.79	1.06
149	G149Q	0.92	1.01	0.63	1.07	0.98	0.84	0.78	0.86	1.07
149	G149R	1.11	0.95	0.49	0.99	1.11	1.18	1.04	0.99	0.95
149	G149S	0.87	0.50	0.63	0.48	0.92	0.58	0.83	0.58	0.50
149	G149V	0.87	1.03	0.45	0.98	1.19	0.95	0.89	0.80	0.90
149	G149W	1.11	1.00	0.47	1.04	1.11	0.90	0.85	0.92	1.03
149	G149Y	0.92	1.19	0.51	1.08	1.04	0.97	0.84	0.90	0.99
150	N150A	0.87	0.94	0.53	0.97	0.93	0.95	0.83	0.84	0.94
150	N150C	0.82	0.91	0.68	0.76	0.83	0.58	0.81	1.01	0.83
150	N150D	0.98	1.04	1.21	0.97	0.95	0.78	1.01	0.92	1.13
150	N150E	1.02	0.84	0.97	0.91	0.85	0.79	1.08	0.96	0.96
150	N150F	1.06	0.93	0.48	0.93	1.03	0.80	1.16	1.07	0.95
150	N150G	0.99	0.92	0.66	0.98	0.98	0.80	1.11	1.02	1.07
150	N150H	0.95	1.04	0.53	1.04	0.91	0.87	0.89	1.07	1.08
150	N150I	0.92	0.79	0.51	0.83	0.95	0.93	0.83	1.02	0.83
150	N150K	0.89	0.96	0.39	0.95	0.92	0.84	0.95	1.02	1.08
150	N150M	1.07	0.81	0.57	0.88	0.91	0.86	0.83	1.14	0.93
150	N150P	0.80	0.48	0.35	0.50	0.88	0.69	0.50	0.80	0.52
150	N150Q	0.88	1.03	0.55	1.04	1.00	0.88	1.00	0.99	1.10
150	N150S	0.95	0.91	0.64	0.96	0.93	0.89	0.90	1.02	1.03
150	N150T	0.96	0.86	0.63	0.93	0.88	0.76	1.10	1.08	0.96
150	N150V	0.99	0.80	0.60	0.83	0.95	0.96	0.83	1.04	0.89
150	N150W	0.93	0.92	0.40	0.88	1.01	0.90	0.98	0.65	1.04
152	Y152A	0.79	0.81	0.28	0.77	0.97	0.95	0.92	0.88	0.68
152	Y152D	0.80	0.58	0.35	0.54	0.87	0.91	0.77	0.91	0.49
152	Y152E	1.00	0.60	0.37	0.58	0.97	0.87	0.84	0.95	0.61
152	Y152G	0.75	0.84	0.29	0.77	0.94	0.94	0.99	1.09	0.65
152	Y152H	1.14	0.97	0.51	0.91	0.89	0.93	0.87	1.02	1.01
152	Y152I	0.94	0.70	0.31	0.67	0.78	1.10	0.89	0.95	0.84

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
152	Y152K	0.96	0.45	0.25	0.43	0.93	0.97	0.69	0.89	0.54
152	Y152L	0.84	0.69	0.31	0.69	0.90	0.98	0.88	1.07	0.86
152	Y152M	0.84	0.44	0.34	0.44	0.81	0.87	0.82	0.93	0.51
152	Y152N	0.92	0.94	0.36	0.84	0.94	0.91	0.86	0.96	0.88
152	Y152P	0.86	0.75	0.33	0.69	0.86	0.80	0.82	0.88	0.75
152	Y152Q	0.79	0.61	0.28	0.58	0.92	0.95	1.49	1.01	0.59
152	Y152R	0.76	0.60	0.18	0.61	1.00	1.20	0.98	1.02	0.66
152	Y152S	0.77	0.85	0.31	0.75	0.88	0.98	0.85	0.98	0.65
152	Y152T	0.83	0.61	0.31	0.56	0.94	1.09	0.82	0.99	0.64
152	Y152V	0.96	0.61	0.33	0.60	0.93	1.03	0.80	1.02	0.68
152	Y152W	0.93	0.88	0.42	0.81	0.96	0.95	0.85	1.05	0.82
154	S154A	0.89	1.20	1.11	1.11	1.11	1.02	1.37	1.18	1.33
154	S154C	1.01	0.91	0.95	0.88	0.86	0.90	0.96	1.08	0.98
154	S154D	1.06	1.03	1.26	0.96	0.93	1.04	1.05	0.89	1.18
154	S154E	1.12	0.95	1.07	0.93	0.99	0.88	1.02	0.99	1.11
154	S154F	1.09	0.97	0.63	1.00	0.92	1.04	1.33	1.11	1.21
154	S154G	0.98	1.08	0.82	1.03	1.02	0.96	1.24	1.08	1.24
154	S154H	0.96	1.04	0.66	1.00	1.05	1.10	1.14	0.99	1.09
154	S154I	0.86	0.94	0.63	0.88	1.05	1.18	1.18	1.09	1.07
154	S154K	0.90	1.02	0.69	0.99	1.02	1.17	1.09	1.10	1.17
154	S154L	0.90	1.07	0.59	1.02	0.98	1.07	1.22	1.11	1.19
154	S154M	1.02	0.94	0.71	0.95	0.94	1.04	0.97	1.03	1.10
154	S154N	1.00	1.28	0.91	1.15	1.00	1.09	1.43	1.04	1.39
154	S154Q	0.95	1.02	0.69	1.02	0.96	1.09	1.10	1.06	1.10
154	S154R	1.01	1.02	0.62	1.05	1.07	1.22	1.06	1.03	1.30
154	S154T	0.99	0.89	0.98	0.86	0.93	1.13	1.32	1.03	1.02
154	S154V	0.94	0.96	0.68	0.92	0.98	0.98	1.24	1.03	1.12
154	S154W	1.04	0.98	0.59	0.97	1.00	1.07	1.16	0.99	1.26
154	S154Y	0.92	0.87	0.60	0.84	0.90	1.06	1.06	1.05	1.06
156	K156A	0.91	0.85	0.28	0.92	1.06	0.95	1.19	0.97	1.04
156	K156C	0.94	0.79	0.29	0.74	0.86	0.70	1.01	0.92	0.83
156	K156D	0.93	0.85	0.20	0.89	1.11	0.86	1.14	0.90	1.01
156	K156E	0.84	0.72	0.21	0.82	0.85	0.77	0.70	0.84	0.88
156	K156F	1.56	0.85	0.56	0.93	0.92	0.68	1.05	0.91	1.12
156	K156G	0.91	0.72	0.29	0.78	0.97	0.63	0.88	0.97	0.99
156	K156H	0.95	0.84	0.36	0.87	0.91	0.83	0.90	0.89	1.07
156	K156I	0.78	0.92	0.26	0.95	0.97	0.91	0.79	0.87	0.99
156	K156L	1.11	0.76	0.49	0.79	0.95	0.67	0.80	0.99	0.94
156	K156M	0.96	0.75	0.32	0.82	0.99	0.70	0.94	0.87	0.97
156	K156N	0.93	0.88	0.29	0.95	1.09	0.68	0.85	1.02	1.10
156	K156P	1.13	0.86	0.39	0.90	0.94	0.72	0.85	0.92	1.06
156	K156Q	0.89	0.89	0.36	0.91	0.89	0.66	0.86	0.99	1.02
156	K156R	0.90	0.91	0.42	0.94	1.00	0.96	1.02	0.95	1.13
156	K156V	0.99	0.67	0.40	0.69	0.89	0.74	1.00	1.36	0.76
156	K156W	1.04	0.89	0.33	0.92	0.92	0.79	1.13	1.03	0.97
156	K156Y	1.35	0.74	0.52	0.79	0.94	0.59	1.09	1.02	0.93
158	R158A	0.95	1.02	0.36	0.97	0.97	0.93	0.81	1.00	0.74

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
158	R158C	1.17	0.85	0.49	0.80	0.89	0.83	0.84	0.89	0.65
158	R158D	0.92	1.12	0.29	1.02	0.91	0.46	1.09	1.06	0.73
158	R158E	0.82	0.81	0.25	0.82	0.89	0.72	0.81	0.92	0.64
158	R158G	1.03	1.06	0.44	1.01	1.05	0.75	0.87	1.03	0.73
158	R158H	0.78	0.98	0.32	0.91	0.83	0.83	0.87	0.95	0.85
158	R158I	0.75	0.73	0.23	0.73	0.89	0.98	0.86	0.78	0.65
158	R158K	0.74	0.88	0.25	0.93	0.86	1.01	0.77	0.93	0.86
158	R158L	1.06	0.88	0.39	0.90	0.82	0.86	0.81	0.87	0.78
158	R158M	0.87	0.75	0.29	0.81	0.85	0.95	0.82	0.90	0.66
158	R158Q	0.79	1.04	0.33	0.94	0.94	0.98	0.92	0.96	0.69
158	R158S	1.06	0.87	0.46	0.91	0.89	0.94	0.91	1.01	0.68
158	R158T	0.82	0.85	0.36	0.82	0.90	0.95	0.62	0.90	0.59
158	R158V	0.89	0.69	0.36	0.68	0.95	1.03	0.87	0.85	0.57
158	R158W	1.30	1.09	0.60	1.04	0.88	0.87	0.91	0.97	0.72
158	R158Y	1.23	1.03	0.70	0.91	0.90	1.01	0.80	0.93	0.67
159	W159D	0.33	0.95	0.14	0.40	0.80	0.18	1.13	1.17	0.76
159	W159F	0.22	1.00	0.16	0.39	1.07	0.30	1.29	1.27	0.80
159	W159H	0.29	1.03	0.17	0.43	0.97	0.19	1.04	1.22	0.95
159	W159N	0.31	0.90	0.09	0.43	1.00	0.25	1.06	1.14	1.04
160	Y160A	1.05	0.95	0.56	0.92	1.01	0.44	1.06	1.17	1.01
160	Y160D	1.77	1.21	1.00	1.06	0.71	0.25	1.56	1.20	1.05
160	Y160E	1.71	1.06	1.27	1.03	0.70	0.18	1.23	1.31	1.04
160	Y160F	1.31	0.99	0.97	1.08	0.88	0.95	0.66	0.94	1.05
160	Y160I	1.92	0.96	1.42	0.90	1.09	0.56	1.07	1.30	0.82
160	Y160K	0.93	0.93	0.39	0.94	0.99	0.36	1.25	1.34	1.19
160	Y160L	1.58	0.87	0.81	0.93	0.95	0.36	1.16	1.34	1.06
160	Y160M	1.18	0.87	0.79	0.89	0.96	0.41	1.03	0.95	1.05
160	Y160N	1.05	1.20	0.57	1.14	0.88	0.25	1.00	1.28	1.22
160	Y160P	1.01	0.94	0.61	1.02	0.91	0.51	1.32	1.12	1.30
160	Y160Q	1.31	1.09	0.80	1.17	0.91	0.34	1.40	1.19	1.19
160	Y160R	1.05	0.75	0.45	0.79	1.00	0.34	1.22	1.30	1.13
160	Y160S	1.10	0.96	0.50	1.00	0.78	0.31	1.07	1.27	1.14
160	Y160T	1.24	0.92	0.74	0.91	0.99	0.32	1.24	1.30	0.95
160	Y160V	1.65	0.85	1.27	0.85	0.96	0.55	0.98	1.19	0.84
160	Y160W	1.45	1.34	0.96	1.30	0.98	1.06	1.04	0.97	1.25
161	H161A	1.15	0.47	0.42	0.46	0.93	0.69	1.04	0.99	0.37
161	H161C	0.86	0.70	0.35	0.70	1.00	0.42	1.10	1.02	0.58
161	H161D	1.52	0.60	0.56	0.59	1.04	0.44	1.14	0.94	0.47
161	H161E	2.81	0.17	0.40	0.18	0.83	0.14	1.05	0.82	0.17
161	H161F	0.55	0.82	0.16	0.85	1.02	1.08	1.29	0.99	0.48
161	H161G	1.24	0.08	0.28	0.09	0.84	0.60	1.48	0.67	0.09
161	H161K	0.40	0.19	0.06	0.20	1.00	0.45	1.10	1.00	0.18
161	H161L	0.89	0.78	0.34	0.80	0.99	1.09	0.91	1.07	0.55
161	H161N	0.48	0.88	0.24	0.83	1.10	0.59	1.06	1.12	0.57
161	H161Q	0.59	0.66	0.16	0.70	1.12	0.74	1.00	0.93	0.44
161	H161S	1.46	0.34	0.54	0.38	1.04	0.75	1.30	0.87	0.25
161	H161T	0.64	0.42	0.16	0.44	1.00	0.51	1.11	1.19	0.32

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
161	H161V	0.26	0.43	0.07	0.45	1.04	0.76	1.35	1.21	0.28
161	H161Y	0.54	0.79	0.21	0.81	1.02	0.95	1.04	0.91	0.55
162	F162K	0.25	0.09	0.07	0.07	0.23	0.31	0.08	0.60	0.18
162	F162W	1.52	0.50	0.25	0.51	0.65	0.52	0.61	0.85	0.96
162	F162Y	1.19	1.03	0.32	1.14	0.88	0.90	1.01	1.03	1.06
165	T165A	0.46	0.75	0.16	0.76	0.86	0.90	0.84	0.85	1.33
165	T165C	0.88	0.56	1.55	0.49	0.70	0.79	0.91	0.77	0.99
165	T165D	0.83	0.28	0.10	0.27	0.16	0.08	0.85	0.99	0.98
165	T165E	0.71	0.79	0.11	0.82	0.28	0.10	0.94	0.94	0.99
165	T165H	0.58	0.48	0.07	0.45	0.66	1.05	0.83	0.74	1.30
165	T165I	2.76	0.28	2.59	0.37	0.63	0.58	0.79	1.12	1.06
165	T165L	0.72	0.45	0.19	0.44	0.67	0.57	0.87	0.88	0.92
165	T165M	0.69	0.86	0.12	0.88	0.89	0.91	1.08	1.06	0.97
165	T165N	0.58	0.21	0.11	0.23	0.44	0.44	0.63	0.77	1.20
165	T165P	1.34	0.10	2.27	0.09	1.02	1.62	1.47	0.98	0.11
165	T165Q	0.64	0.69	0.07	0.73	0.99	0.92	0.78	1.16	1.10
165	T165S	0.50	0.77	0.16	0.79	0.85	0.96	0.99	0.99	1.11
165	T165V	1.61	0.60	2.68	0.56	0.87	1.17	0.81	0.93	1.04
165	T165Y	0.64	0.25	0.09	0.27	0.58	0.27	0.83	0.79	0.96
166	D166N	1.36	0.20	0.49	0.25	0.65	0.56	0.90	0.93	1.24
167	W167A	0.46	0.59	0.08	0.65	1.06	0.25	1.37	1.35	0.83
167	W167C	0.65	0.29	0.29	0.30	0.71	0.27	0.98	1.12	0.77
167	W167E	0.72	0.21	0.15	0.24	0.51	0.17	1.08	1.27	0.84
167	W167F	0.67	0.65	0.61	0.66	1.08	0.79	1.21	1.11	0.92
167	W167I	0.59	0.32	0.19	0.31	0.94	0.60	0.78	1.21	0.82
167	W167K	0.45	0.18	0.11	0.23	1.03	0.66	0.82	0.93	0.81
167	W167L	0.66	0.53	0.26	0.52	1.08	0.55	1.12	1.12	0.87
167	W167N	0.52	0.44	0.09	0.43	1.13	0.31	1.24	1.24	0.83
167	W167R	0.42	0.35	0.18	0.47	1.11	1.02	0.99	1.00	0.88
167	W167S	0.41	0.54	0.09	0.61	0.86	0.29	1.20	1.21	0.89
167	W167T	0.56	0.43	0.19	0.39	1.08	0.67	0.99	1.01	0.83
167	W167V	0.76	0.26	0.23	0.25	1.08	0.73	1.05	1.11	0.75
167	W167Y	0.74	0.50	0.77	0.55	1.03	1.69	0.91	1.00	0.87
168	D168C	1.17	0.73	0.22	0.84	1.06	1.10	0.61	0.97	0.93
168	D168N	0.58	0.66	0.06	0.77	1.32	1.30	0.96	0.84	1.14
169	E169A	0.71	0.98	0.18	1.10	1.07	1.17	0.99	1.02	0.87
169	E169C	0.82	1.21	0.18	1.13	0.78	0.37	0.81	0.95	0.80
169	E169D	0.67	0.89	0.25	0.88	0.99	0.84	0.81	0.85	0.85
169	E169F	0.77	0.95	0.15	1.17	1.10	0.81	1.04	0.99	0.90
169	E169G	0.66	0.72	0.08	0.88	1.01	1.03	1.15	0.86	0.84
169	E169H	0.80	0.60	0.23	0.68	0.95	1.23	1.07	0.77	0.86
169	E169I	0.89	1.57	0.14	1.64	0.86	0.50	0.87	1.07	0.96
169	E169K	0.68	1.42	0.12	1.99	0.97	0.87	1.00	1.00	1.23
169	E169L	1.00	1.66	0.14	1.84	0.87	0.47	1.07	1.07	1.03
169	E169M	0.77	1.07	0.12	1.23	1.02	0.81	0.91	0.87	0.80
169	E169N	0.73	1.01	0.16	1.09	1.01	1.04	1.02	0.96	0.90
169	E169Q	0.85	0.63	0.22	0.83	1.09	1.17	1.04	0.97	0.76

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
169	E169S	0.66	0.95	0.13	1.03	1.04	1.06	0.90	0.96	0.76
169	E169T	0.81	1.14	0.11	1.46	1.01	0.90	0.93	1.07	0.79
169	E169V	0.99	1.78	0.18	1.87	1.03	0.54	0.93	1.03	0.91
169	E169W	0.69	0.79	0.15	0.74	1.06	0.86	0.74	0.90	0.94
169	E169Y	0.83	0.82	0.16	1.08	1.03	0.74	0.99	1.09	0.85
170	S170A	0.74	0.84	0.58	0.90	0.97	0.85	0.97	1.04	1.05
170	S170C	1.07	0.79	1.14	0.80	0.80	0.49	0.94	0.98	0.94
170	S170D	0.82	0.81	0.49	0.80	1.05	0.55	0.96	0.98	0.93
170	S170E	0.80	0.78	0.64	0.81	0.96	0.51	0.83	0.97	0.94
170	S170F	0.41	1.02	0.09	0.98	1.30	1.15	1.11	1.10	0.85
170	S170G	0.74	0.99	0.42	1.06	1.25	0.89	1.07	1.08	1.00
170	S170H	0.75	0.93	0.60	0.91	1.00	0.81	0.82	1.01	1.02
170	S170K	0.87	0.78	0.62	0.84	1.05	0.85	0.91	0.99	1.04
170	S170L	1.25	0.51	1.38	0.53	0.88	0.50	1.07	0.98	0.65
170	S170M	0.88	0.93	1.25	0.94	0.89	0.78	0.94	0.96	1.11
170	S170N	0.77	0.78	0.79	0.79	1.04	0.82	0.99	1.03	1.05
170	S170Q	0.92	1.05	0.83	1.05	1.20	0.88	0.90	1.04	1.12
170	S170R	0.61	0.85	0.45	0.83	1.16	1.25	0.86	1.05	1.00
170	S170T	0.84	0.76	0.91	0.82	1.06	0.84	1.03	0.99	0.94
170	S170V	0.73	0.67	0.53	0.70	1.12	0.58	0.88	0.90	0.96
170	S170W	1.10	0.96	0.92	1.04	1.09	0.89	0.84	1.01	1.16
170	S170Y	0.85	0.95	0.90	0.97	1.15	0.88	1.01	0.94	1.05
171	R171A	0.68	0.93	0.20	0.97	1.00	0.62	0.98	0.98	0.62
171	R171C	0.70	0.94	0.25	0.88	0.79	0.32	0.94	0.89	0.69
171	R171D	0.72	1.00	0.12	1.10	0.77	0.53	0.94	0.91	0.79
171	R171E	0.68	0.90	0.17	0.87	0.78	0.52	1.00	0.80	0.64
171	R171F	0.57	0.88	0.13	0.83	0.80	0.75	0.90	0.83	0.77
171	R171G	0.73	0.99	0.22	0.90	0.95	0.55	1.04	0.98	0.66
171	R171H	0.67	0.85	0.16	0.89	0.87	0.72	0.96	0.92	0.72
171	R171K	0.55	1.01	0.30	0.94	0.89	0.75	0.99	0.99	0.65
171	R171L	0.69	0.96	0.23	0.91	0.99	0.65	1.06	0.93	0.63
171	R171M	0.79	0.87	0.24	0.97	0.82	0.71	0.91	0.95	0.63
171	R171N	0.46	0.82	0.22	0.94	0.84	0.69	1.06	0.88	0.68
171	R171Q	0.76	0.86	0.27	0.91	0.79	0.66	0.83	0.93	0.68
171	R171S	0.75	0.82	0.31	0.87	0.94	0.59	0.88	1.00	0.59
171	R171T	0.70	0.78	0.35	0.72	0.83	0.69	0.98	1.01	0.60
171	R171W	0.65	0.80	0.11	0.82	0.91	0.68	0.81	0.92	0.64
171	R171Y	0.64	0.78	0.12	0.80	0.91	0.51	0.98	0.90	0.63
172	K172A	0.78	1.15	0.40	1.02	1.01	0.79	1.06	0.96	0.64
172	K172C	0.94	0.96	0.62	0.87	0.84	0.28	1.01	0.97	0.55
172	K172D	0.78	1.01	0.21	0.87	0.98	1.03	1.11	0.89	0.55
172	K172E	0.94	0.87	0.33	0.82	0.94	0.58	1.02	0.99	0.56
172	K172F	0.89	1.21	0.29	1.14	1.00	0.74	0.92	0.97	0.74
172	K172G	1.17	0.79	0.69	0.86	1.05	0.83	1.12	1.31	0.40
172	K172H	0.85	1.05	0.61	0.97	1.01	0.89	0.93	1.02	0.63
172	K172I	0.61	0.97	0.12	0.84	0.95	0.78	0.95	0.92	0.68
172	K172L	0.97	0.87	0.30	0.92	1.02	0.74	1.01	0.85	0.67

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
172	K172N	0.86	1.12	0.41	1.02	1.05	0.95	1.12	1.02	0.61
172	K172P	0.84	0.64	0.09	0.47	0.99	0.74	0.96	0.90	0.63
172	K172Q	1.21	0.95	0.83	0.98	0.96	0.79	1.08	0.91	0.65
172	K172R	0.76	1.36	0.68	1.15	1.05	1.14	1.19	0.99	0.66
172	K172S	0.90	0.96	0.43	0.96	1.04	0.55	0.99	1.03	0.62
172	K172T	0.83	0.75	0.22	0.81	1.03	0.69	1.05	0.99	0.57
172	K172V	0.71	0.85	0.16	0.83	0.82	0.74	1.05	0.91	0.63
172	K172W	1.01	1.07	0.28	1.09	0.80	0.93	0.81	0.86	0.74
172	K172Y	0.86	1.03	0.29	0.98	1.02	0.88	0.95	0.94	0.60
174	N174A	0.88	0.78	0.90	0.87	1.14	0.81	0.90	1.03	0.91
174	N174C	1.29	0.35	0.89	0.43	0.87	0.45	0.72	0.97	0.81
174	N174D	0.78	0.69	0.26	0.75	1.07	0.63	0.70	1.00	0.80
174	N174E	1.19	0.77	0.55	0.83	0.74	0.52	0.74	1.01	0.77
174	N174F	0.89	0.52	0.54	0.52	0.95	0.85	0.84	0.76	0.80
174	N174H	0.92	0.74	1.04	0.71	1.05	0.90	0.73	0.81	0.83
174	N174I	0.94	0.58	0.42	0.63	0.87	0.94	0.74	0.74	0.62
174	N174L	0.88	0.40	0.47	0.44	0.58	0.76	0.50	0.57	0.64
174	N174M	1.02	0.47	0.81	0.55	0.91	0.86	0.80	0.73	0.68
174	N174Q	1.01	0.90	0.92	0.91	0.98	0.80	0.86	0.90	0.83
174	N174R	0.84	0.54	1.43	0.67	0.99	0.87	0.88	0.86	0.89
174	N174S	1.06	0.76	1.05	0.86	1.01	0.95	0.85	0.89	0.83
174	N174T	1.01	0.77	0.71	0.79	1.05	0.83	0.86	0.99	0.71
174	N174V	0.91	0.68	0.53	0.70	0.95	0.96	0.90	0.89	0.59
174	N174W	0.90	0.70	0.49	0.67	0.91	1.21	0.59	0.89	0.82
174	N174Y	0.87	0.51	0.57	0.53	0.94	0.85	0.67	0.67	0.74
175	R175A	0.88	0.83	0.63	0.89	1.00	0.39	1.09	1.14	1.01
175	R175C	0.68	0.73	0.23	0.78	1.03	0.47	1.02	1.08	0.89
175	R175E	0.59	0.99	0.10	0.87	0.99	0.66	1.26	0.99	1.11
175	R175F	0.66	0.94	0.11	0.97	1.02	0.79	1.25	0.97	0.94
175	R175G	0.51	0.81	0.26	0.82	0.99	0.35	0.91	0.97	1.08
175	R175H	0.88	0.95	0.31	0.99	1.53	0.65	1.08	1.05	1.15
175	R175I	0.50	0.64	0.07	0.64	0.92	0.77	1.01	0.96	0.99
175	R175K	0.92	0.91	0.79	1.00	0.99	0.78	0.76	1.01	1.06
175	R175L	0.69	0.78	0.17	0.78	0.91	0.64	0.65	0.94	1.09
175	R175M	0.67	0.79	0.11	0.86	1.00	0.73	1.07	1.01	0.98
175	R175N	0.96	1.03	0.42	1.01	1.03	0.69	1.15	1.15	1.13
175	R175P	0.90	0.71	0.34	0.72	1.01	0.57	0.91	1.08	1.12
175	R175S	0.57	1.03	0.22	1.06	1.09	0.47	0.93	1.12	1.17
175	R175T	0.54	0.71	0.13	0.76	0.99	0.64	1.20	1.03	0.93
175	R175V	0.63	0.63	0.15	0.64	0.96	0.66	1.11	0.94	0.83
175	R175W	0.53	0.70	0.09	0.95	1.07	0.55	0.95	0.91	1.28
175	R175Y	0.76	0.95	0.13	0.92	0.97	0.80	0.99	1.06	1.08
176	I176L	0.44	0.68	0.11	0.76	0.87	0.78	0.91	0.94	1.15
176	I176V	0.71	0.85	0.24	0.92	0.91	0.88	1.04	0.92	0.97
177	Y177F	1.22	1.00	1.37	1.12	1.00	0.96	0.90	1.00	1.11
177	Y177L	0.59	0.87	0.15	0.83	1.05	0.92	0.98	1.01	0.66
177	Y177M	0.67	0.44	0.08	0.46	1.09	1.13	1.64	0.93	0.38

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
177	Y177R	0.24	0.71	0.09	0.78	1.15	1.18	1.12	1.05	0.73
177	Y177W	1.12	1.19	0.57	1.27	1.02	0.99	1.10	1.00	0.92
178	K178A	0.96	0.65	0.57	0.63	1.07	0.22	1.35	1.17	0.60
178	K178C	1.15	0.50	0.60	0.53	1.12	0.26	1.73	1.35	0.43
178	K178E	0.12	0.55	0.12	0.57	1.61	0.17	1.93	1.33	0.49
178	K178G	1.66	0.67	0.96	0.68	0.90	0.31	1.53	0.94	0.63
178	K178I	2.39	0.60	0.64	0.68	1.09	1.34	1.63	1.34	0.45
178	K178L	7.94	0.77	3.22	0.82	1.12	0.35	1.60	1.35	0.57
178	K178M	2.13	0.70	1.36	0.73	1.09	0.37	1.94	1.18	0.53
178	K178Q	2.12	0.69	1.18	0.78	1.27	0.30	1.64	1.10	0.61
178	K178R	0.65	0.73	0.69	0.73	1.09	0.85	1.52	1.12	0.69
178	K178V	0.30	0.56	0.09	0.58	1.25	0.35	1.80	1.45	0.46
182	T182A	1.69	0.94	2.01	0.96	1.18	0.64	1.15	1.09	1.08
182	T182C	4.67	0.65	2.66	0.72	0.85	0.23	1.19	1.11	0.70
182	T182D	2.63	0.84	2.15	0.94	0.94	0.68	1.18	1.06	1.03
182	T182E	3.03	0.85	2.46	0.94	1.01	0.72	1.19	0.93	1.02
182	T182F	1.78	0.93	1.39	0.98	0.84	1.21	1.14	0.93	1.08
182	T182G	5.64	0.79	3.12	0.97	0.98	0.26	1.66	1.36	1.14
182	T182H	1.70	1.00	1.34	0.99	0.87	0.92	0.98	0.88	1.16
182	T182I	1.65	0.86	1.46	0.90	0.94	1.02	1.02	1.02	1.02
182	T182K	0.23	0.81	0.38	0.93	0.92	0.91	1.15	0.86	1.16
182	T182L	2.23	0.78	1.88	0.86	0.98	0.78	0.93	0.96	0.96
182	T182M	2.41	0.85	1.98	0.93	0.81	0.73	1.24	1.03	1.02
182	T182N	2.41	1.01	1.97	1.06	1.04	0.62	1.49	1.22	1.19
182	T182P	2.20	0.91	2.30	1.02	1.05	0.73	1.28	1.16	1.19
182	T182Q	1.82	0.97	1.74	1.01	1.10	0.64	1.17	1.01	1.07
182	T182R	1.11	1.03	1.05	0.95	0.97	1.13	1.13	0.97	1.11
182	T182V	1.31	0.79	1.25	0.89	0.70	0.95	1.18	0.90	0.89
182	T182W	1.62	0.91	1.27	0.95	0.76	1.53	0.99	0.86	1.02
182	T182Y	1.65	0.79	1.29	0.93	0.95	1.07	1.04	0.90	1.02
183	G183A	0.80	0.95	0.20	0.99	1.16	0.24	1.05	1.38	0.99
183	G183C	1.47	0.69	0.87	0.74	0.79	0.19	1.08	1.33	0.77
183	G183E	0.93	0.94	0.60	0.97	0.83	0.21	1.16	1.41	0.98
183	G183F	0.97	1.01	0.22	1.00	1.12	0.47	1.02	1.09	0.96
183	G183L	1.13	0.94	0.17	0.95	1.06	0.44	1.01	1.20	1.00
183	G183M	1.01	0.92	0.25	0.91	0.98	0.30	1.02	1.21	1.01
183	G183N	1.13	1.01	0.74	1.04	1.00	0.24	1.21	1.28	1.15
183	G183P	1.80	1.02	0.28	1.07	0.88	0.22	1.35	1.33	1.16
183	G183Q	0.76	0.93	0.13	1.03	1.06	0.22	1.28	1.36	1.08
183	G183S	0.40	0.93	0.16	0.92	1.11	0.31	1.05	1.30	0.98
183	G183T	0.47	0.87	0.11	0.90	1.05	0.21	1.14	1.35	0.94
183	G183W	1.18	0.96	0.26	0.93	1.25	0.44	0.95	1.21	0.96
185	A185C	1.39	0.75	0.83	0.79	0.89	0.32	1.03	1.02	0.74
185	A185D	2.88	0.87	2.60	0.92	0.94	0.38	1.17	1.01	0.81
185	A185E	2.40	0.89	2.18	1.00	0.93	0.31	1.12	1.05	0.81
185	A185G	0.61	0.86	1.06	0.91	0.96	0.68	1.07	0.93	0.89
185	A185K	0.56	0.96	0.09	1.01	1.00	0.77	0.75	0.81	0.93

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
185	A185M	0.55	0.86	0.34	0.97	1.02	0.91	1.05	0.85	0.77
185	A185N	0.67	0.98	1.19	0.99	1.39	0.63	1.04	0.91	0.91
185	A185Q	0.62	1.13	0.86	1.06	1.11	0.75	1.15	1.03	0.86
185	A185R	0.50	0.94	0.06	0.95	1.03	0.59	1.07	0.86	0.89
185	A185S	0.71	0.84	0.97	0.92	1.03	0.59	1.01	0.97	0.77
185	A185T	0.58	0.87	0.61	0.96	1.16	0.54	1.22	1.03	0.76
189	E189K	0.72	0.81	0.52	0.92	0.77	0.86	0.72	0.33	1.23
189	E189P	5.13	0.93	2.56	0.94	0.82	0.81	0.92	1.42	1.50
191	D191C	0.89	0.55	0.84	0.54	1.04	0.66	1.32	1.17	0.56
192	T192C	0.56	0.72	0.33	0.74	0.97	0.58	1.02	0.99	0.78
192	T192D	1.22	1.16	1.65	1.07	0.90	0.66	1.16	1.01	0.90
192	T192E	0.14	0.90	0.21	0.98	0.89	1.02	0.87	1.05	0.80
192	T192I	0.71	0.84	0.19	0.79	1.05	0.86	0.98	1.02	0.81
192	T192N	0.48	1.15	0.62	1.01	1.02	0.82	1.02	0.95	0.98
192	T192S	0.45	0.94	0.28	0.88	1.09	0.89	0.94	0.97	0.81
192	T192V	0.85	0.68	0.22	0.79	0.93	0.89	0.83	1.02	0.79
193	E193C	0.99	0.17	0.56	0.19	0.56	0.12	0.73	1.09	0.99
193	E193D	0.42	0.24	0.39	0.25	0.93	0.40	0.82	1.13	1.27
193	E193Q	0.57	0.19	0.08	0.25	0.94	0.68	0.80	0.89	1.22
193	E193T	0.36	0.09	0.06	0.14	0.78	0.20	0.98	1.12	0.81
195	G195A	1.22	1.02	0.15	1.08	1.00	0.82	0.83	1.05	1.18
195	G195C	2.92	1.04	0.46	0.93	0.99	0.46	1.28	0.98	0.99
195	G195H	1.45	1.21	0.19	1.12	0.94	0.89	1.06	0.95	1.41
195	G195M	0.92	0.97	0.07	0.96	0.87	0.78	1.14	0.96	1.16
195	G195Q	0.71	1.17	0.07	1.12	0.91	0.73	1.12	1.02	1.29
195	G195V	2.70	1.02	0.34	1.03	0.96	0.54	1.14	1.01	1.23
197	Y197A	0.32	0.10	1.13	0.10	0.70	0.31	1.12	1.05	0.97
197	Y197C	0.31	0.08	0.35	0.07	0.47	0.08	0.70	1.01	0.74
197	Y197D	1.14	0.11	1.63	0.11	0.53	0.16	1.12	1.08	0.95
197	Y197F	1.17	0.64	1.08	0.61	0.86	0.86	1.15	0.99	0.91
197	Y197G	0.21	0.06	0.11	0.06	0.51	0.20	0.96	0.99	0.72
197	Y197N	0.51	0.09	1.37	0.08	0.66	0.27	0.90	1.12	0.88
197	Y197S	0.16	0.08	0.58	0.08	0.70	0.29	1.04	1.06	0.78
199	Y199I	0.06	0.09	0.08	0.09	0.19	0.17	0.67	0.58	0.75
200	L200A	0.46	0.08	0.08	0.06	0.15	0.06	0.69	0.83	1.17
200	L200M	0.38	0.11	0.09	0.11	0.40	0.08	0.52	0.63	1.06
200	L200V	0.69	0.10	0.45	0.10	0.38	0.23	0.41	0.37	0.96
201	M201A	0.77	0.14	0.49	0.13	0.51	0.42	0.85	0.81	1.34
201	M201C	1.15	0.18	1.18	0.21	0.58	0.36	0.53	0.68	1.17
201	M201I	0.79	0.32	0.35	0.33	0.65	0.63	0.70	0.72	1.16
201	M201L	0.68	0.30	0.21	0.30	0.79	0.56	0.98	0.74	1.16
201	M201T	0.73	0.15	0.27	0.17	0.29	0.28	0.80	0.76	1.41
201	M201V	0.78	0.08	0.47	0.09	0.34	0.19	0.47	0.67	1.15
202	F202A	0.84	0.36	0.67	0.38	0.69	0.29	1.06	0.88	1.47
202	F202G	0.82	0.15	0.86	0.13	0.31	0.08	0.72	0.93	1.32
202	F202H	1.29	0.97	1.19	0.91	0.89	0.50	0.83	1.02	1.39
202	F202L	1.01	0.22	0.26	0.21	0.34	0.07	0.88	1.01	0.91

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
202	F202M	0.94	0.21	0.39	0.19	0.40	0.18	0.79	0.92	1.27
202	F202N	1.14	0.78	0.41	0.80	0.76	0.51	0.99	0.99	1.55
202	F202S	0.90	0.36	0.31	0.35	0.82	0.29	0.82	0.97	1.34
202	F202Y	1.01	0.81	1.54	0.76	1.08	0.83	1.05	1.08	1.33
203	A203C	0.73	0.12	1.84	0.12	0.73	0.70	0.88	0.89	0.77
203	A203E	1.67	0.40	0.80	0.41	0.29	0.11	0.66	0.87	0.94
203	A203G	0.50	0.83	0.11	0.88	0.80	0.76	1.16	0.83	1.04
203	A203L	0.25	0.06	1.19	0.06	0.34	0.25	0.90	0.89	0.84
203	A203P	0.90	0.06	0.08	0.07	0.76	0.33	0.92	0.89	0.72
203	A203V	0.45	0.17	1.41	0.16	0.63	0.54	0.93	1.12	0.68
207	M207F	0.81	0.95	1.10	0.97	0.97	0.87	0.88	0.89	0.85
207	M207L	0.07	1.30	0.08	1.20	1.18	0.92	1.17	1.05	1.03
207	M207Y	1.40	0.93	0.48	0.92	0.99	0.93	0.91	0.84	0.79
210	P210C	1.77	0.77	1.02	0.75	1.04	0.97	0.84	1.17	0.49
210	P210D	1.46	1.04	1.21	0.96	0.98	0.80	1.06	1.02	0.81
210	P210E	1.69	0.88	1.24	0.96	0.99	0.93	1.09	0.98	0.76
210	P210F	1.16	0.67	0.61	0.73	1.06	1.02	0.70	1.05	0.60
210	P210G	1.37	0.88	0.83	0.87	1.00	0.89	1.23	1.07	0.69
210	P210H	1.19	0.89	0.73	0.91	1.04	1.07	0.98	1.06	0.82
210	P210I	1.02	0.69	0.52	0.71	1.05	1.09	1.10	1.09	0.57
210	P210K	0.80	1.02	0.33	1.09	0.98	1.06	0.85	1.09	0.91
210	P210L	1.34	0.71	0.69	0.73	1.10	0.92	1.06	0.91	0.66
210	P210M	1.47	0.70	0.83	0.74	0.99	0.90	0.80	0.95	0.67
210	P210N	1.26	0.87	0.79	0.99	0.96	0.79	1.26	1.13	0.79
210	P210Q	1.36	0.98	0.80	1.05	0.93	1.12	1.08	1.06	0.82
210	P210R	0.82	1.07	0.28	1.10	0.93	1.13	0.97	0.95	0.99
210	P210S	1.02	0.10	0.69	0.10	0.70	0.59	0.34	0.94	0.11
210	P210T	1.30	0.76	0.68	0.86	1.00	1.18	1.08	1.15	0.64
210	P210V	1.07	0.76	0.63	0.73	0.93	0.88	0.82	1.05	0.58
210	P210W	1.33	0.81	0.64	0.87	0.99	1.10	1.09	1.12	0.74
214	T214A	0.87	0.96	1.24	0.88	1.16	0.94	0.81	0.95	0.95
214	T214C	1.16	0.78	1.17	0.76	1.25	0.79	0.78	0.93	0.75
214	T214D	1.24	0.69	1.56	0.69	0.96	0.81	1.05	0.88	0.90
214	T214E	1.33	0.93	1.78	0.94	1.06	0.72	0.82	0.87	1.15
214	T214F	1.09	0.81	0.81	0.78	1.08	0.95	0.58	0.89	0.89
214	T214G	0.98	0.92	0.88	0.91	1.03	0.92	0.81	0.93	0.98
214	T214I	1.11	0.70	0.85	0.73	1.15	1.22	0.92	1.00	0.69
214	T214K	0.91	0.92	0.87	0.97	1.12	0.96	1.10	0.85	1.04
214	T214L	0.95	0.60	0.96	0.57	1.09	1.02	1.03	0.74	0.60
214	T214P	0.69	1.04	0.59	1.04	0.96	0.90	0.78	0.91	1.06
214	T214Q	1.08	1.02	1.61	1.05	1.12	0.89	0.74	1.00	1.18
214	T214R	0.75	0.76	0.81	0.89	1.10	1.04	0.95	0.90	1.00
214	T214S	1.04	0.85	1.05	0.89	1.15	0.97	0.68	0.95	0.98
214	T214V	0.96	0.78	0.92	0.75	1.03	0.87	0.63	1.07	0.70
214	T214W	1.18	0.91	1.10	0.87	1.11	0.96	0.76	0.97	0.95
214	T214Y	1.09	0.75	0.82	0.77	1.04	0.90	0.75	0.91	0.87
217	K217A	1.25	0.92	0.68	0.95	0.88	0.70	1.11	0.75	1.03

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
217	K217C	1.43	0.67	0.76	0.73	0.86	0.58	1.11	0.89	0.66
217	K217D	1.47	0.72	0.48	0.78	0.84	0.58	1.38	0.88	0.75
217	K217E	1.51	0.76	0.66	0.84	0.93	0.63	1.05	0.82	0.84
217	K217F	1.13	0.89	0.80	0.93	0.83	0.56	1.63	0.84	0.76
217	K217G	1.54	0.69	0.51	0.75	0.96	0.75	1.20	0.95	0.73
217	K217H	1.21	0.79	0.61	0.89	0.90	0.59	1.23	0.84	0.84
217	K217L	1.15	0.75	0.69	0.82	0.81	0.56	1.23	0.85	0.69
217	K217M	1.58	0.29	0.86	0.34	1.28	0.93	1.27	1.38	0.13
217	K217N	1.08	0.93	0.60	0.93	0.96	0.71	1.23	0.98	0.81
217	K217P	2.41	0.32	0.95	0.41	0.88	0.86	1.17	0.79	0.27
217	K217Q	1.23	0.75	0.50	0.88	0.99	0.72	1.13	0.94	0.82
217	K217R	0.50	0.86	0.34	0.92	0.93	0.88	1.27	0.83	1.09
217	K217S	1.09	0.80	0.55	0.79	0.90	0.72	1.48	0.89	0.69
217	K217T	1.05	0.21	0.52	0.23	1.51	0.49	1.56	1.19	0.08
217	K217V	1.12	0.70	0.31	0.77	0.99	0.77	1.25	0.95	0.79
217	K217W	1.12	0.75	0.50	0.85	0.85	0.57	1.03	0.79	0.73
217	K217Y	0.60	0.66	0.61	0.73	0.92	0.61	0.93	0.84	0.68
221	T221A	0.94	0.90	0.66	1.04	1.04	1.07	1.10	1.03	1.04
221	T221C	0.94	0.72	0.61	0.72	0.93	0.94	1.04	0.92	0.64
221	T221D	1.14	0.88	0.70	0.94	0.91	0.96	1.09	0.82	1.08
221	T221E	1.28	0.80	0.91	0.94	0.97	0.82	0.92	0.94	1.02
221	T221F	1.16	0.79	0.73	0.82	0.88	1.02	0.76	0.93	0.85
221	T221G	1.03	0.89	0.60	0.94	0.95	0.80	1.20	0.85	0.96
221	T221H	1.10	0.83	0.65	0.94	0.99	0.89	0.86	0.89	1.06
221	T221K	0.66	0.71	0.68	0.96	0.93	1.01	1.19	1.04	1.06
221	T221M	1.11	0.65	0.74	0.73	0.93	1.46	1.12	0.95	0.81
221	T221N	1.10	0.98	0.64	0.99	1.21	1.05	1.11	1.02	1.09
221	T221P	1.03	0.85	1.04	0.97	0.91	0.82	0.99	0.94	1.13
221	T221Q	1.67	0.69	0.65	0.99	0.94	0.98	0.96	1.04	1.03
221	T221R	0.77	0.66	0.57	1.01	1.06	1.02	1.09	0.97	1.09
221	T221S	0.79	0.76	0.61	0.96	1.04	1.00	1.02	0.86	0.99
221	T221V	1.03	0.66	2.11	0.80	1.04	1.09	1.19	0.98	0.78
221	T221W	1.01	0.88	0.64	0.92	1.04	1.07	1.03	0.96	0.91
221	T221Y	1.12	0.83	0.73	0.86	1.07	1.04	0.95	0.94	0.82
228	N228A	0.67	0.89	0.78	0.91	1.08	1.02	0.99	0.80	0.90
228	N228C	0.87	0.85	0.85	0.79	0.95	0.98	0.95	0.96	0.69
228	N228D	0.94	1.11	1.17	0.98	0.96	0.98	1.07	0.81	0.96
228	N228E	1.06	0.92	0.92	0.92	0.87	0.65	0.74	0.94	0.91
228	N228F	1.06	0.99	0.65	0.99	1.03	1.13	1.08	0.96	0.95
228	N228G	0.97	1.03	1.13	1.00	0.99	1.10	0.71	0.96	0.86
228	N228H	1.05	0.90	0.71	0.96	0.97	0.87	0.90	0.90	1.00
228	N228I	1.03	0.83	0.61	0.82	1.12	1.09	1.00	0.84	0.82
228	N228L	0.92	0.86	0.68	0.89	1.04	0.99	0.94	0.92	0.90
228	N228M	0.98	0.93	0.77	0.99	1.10	0.98	1.01	0.89	0.95
228	N228P	1.10	0.67	0.68	0.75	1.01	0.90	0.92	0.90	1.08
228	N228Q	1.14	0.90	0.83	0.99	0.87	1.09	1.04	0.93	1.01
228	N228R	0.92	0.90	0.64	0.90	0.98	1.11	0.92	0.91	0.96

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
228	N228S	1.01	0.88	0.74	0.94	1.01	1.07	0.85	0.95	0.92
228	N228T	0.94	0.90	0.65	0.88	1.05	1.06	0.92	0.93	0.87
228	N228V	0.89	0.46	0.66	0.50	0.94	0.79	0.92	0.81	0.55
228	N228W	1.02	0.56	0.63	0.58	0.96	1.00	0.59	0.83	0.58
228	N228Y	0.95	0.86	0.61	0.86	1.06	0.99	0.83	0.91	0.83
234	L234A	2.32	0.76	1.23	0.77	1.00	0.69	1.39	1.08	1.45
234	L234C	1.41	0.53	0.86	0.47	0.82	1.42	1.07	0.96	0.91
234	L234D	1.16	0.17	0.48	0.15	0.80	0.50	0.79	0.98	0.30
234	L234G	1.75	0.59	0.65	0.61	0.95	0.89	1.21	0.98	1.12
234	L234H	1.18	0.50	0.38	0.53	0.92	0.74	1.04	0.96	1.11
234	L234I	0.68	0.67	0.37	0.74	1.06	0.82	1.16	1.07	1.44
234	L234M	1.08	0.82	1.10	0.83	1.01	0.97	1.05	0.97	1.34
234	L234N	1.79	0.56	0.80	0.59	0.94	0.77	1.17	0.97	0.94
234	L234P	1.18	0.12	0.36	0.13	0.86	0.43	1.12	0.80	1.17
234	L234Q	1.22	0.57	0.41	0.63	0.88	0.65	1.07	0.94	1.04
234	L234S	1.45	0.58	0.53	0.56	0.91	0.77	1.15	1.00	1.01
234	L234T	1.28	0.59	0.40	0.61	1.05	0.94	1.42	1.17	1.09
234	L234V	0.87	0.74	0.41	0.70	1.03	0.85	1.28	1.16	1.23
234	L234W	0.59	0.49	0.15	0.52	1.06	0.70	1.29	0.96	1.05
234	L234Y	0.61	0.43	0.22	0.48	1.02	1.39	1.20	1.07	1.12
236	A236G	1.06	0.06	1.17	0.06	0.46	0.29	0.47	0.17	0.91
236	A236T	0.47	0.19	0.13	0.18	0.78	0.63	0.32	0.29	0.86
237	V237A	0.76	1.12	0.22	1.04	1.02	0.57	0.90	0.91	1.07
237	V237C	1.02	0.16	0.74	0.16	0.75	0.32	1.06	1.03	0.22
237	V237D	0.33	0.72	0.18	0.75	0.80	0.07	1.14	0.93	0.84
237	V237G	0.98	0.71	0.16	0.68	0.78	0.64	1.39	0.95	1.04
237	V237I	0.89	0.66	0.47	0.75	1.47	0.91	1.01	0.99	0.95
237	V237L	0.44	0.59	0.24	0.66	1.02	0.62	1.80	1.08	0.93
237	V237N	0.92	1.11	0.20	1.15	0.91	0.54	1.24	0.97	1.10
237	V237P	0.47	0.16	0.06	0.15	1.11	0.68	1.48	0.79	1.03
237	V237T	0.90	0.85	0.25	0.89	0.89	0.74	1.23	0.88	0.97
240	I240V	0.68	0.94	0.23	1.04	0.95	0.98	1.10	0.95	1.21
243	S243A	2.52	0.68	1.35	0.71	1.16	0.97	0.90	0.91	0.53
243	S243C	4.02	0.63	0.91	0.68	0.98	0.93	0.94	0.89	0.45
243	S243D	6.78	0.92	2.18	0.90	0.95	0.86	0.82	0.79	0.73
243	S243E	8.88	0.83	2.59	1.00	1.01	0.71	0.86	0.83	0.76
243	S243F	1.52	0.64	0.12	0.71	1.05	1.16	0.98	0.89	0.53
243	S243G	1.02	0.96	0.55	0.97	0.93	0.98	0.87	0.81	0.77
243	S243H	0.89	0.64	0.06	0.81	0.86	1.01	0.87	0.90	0.64
243	S243I	2.19	0.58	0.44	0.60	1.02	1.12	0.98	0.88	0.46
243	S243K	0.41	0.97	0.09	0.93	0.97	1.14	0.78	0.78	0.81
243	S243L	2.01	0.70	0.48	0.62	1.00	1.00	0.90	0.93	0.52
243	S243M	1.95	0.53	0.49	0.63	0.85	0.93	0.81	0.79	0.53
243	S243Q	5.93	0.79	1.93	0.77	1.02	1.06	1.01	1.00	0.69
243	S243T	1.45	0.66	0.77	0.74	0.91	1.18	0.92	0.84	0.61
243	S243V	1.79	0.50	0.40	0.56	0.97	0.95	1.08	0.84	0.41
246	P246A	2.26	1.15	0.80	1.19	0.83	0.99	0.75	0.91	0.88

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
246	P246C	1.41	0.99	0.20	0.99	1.09	0.88	0.78	0.72	0.74
246	P246D	2.41	1.24	0.67	1.23	1.00	0.98	0.76	0.94	0.96
246	P246E	2.78	1.10	1.07	1.15	0.90	0.94	0.82	0.93	0.88
246	P246F	2.61	1.09	0.84	1.06	1.02	0.94	0.67	0.83	0.79
246	P246G	1.06	1.19	0.32	1.20	1.01	0.92	0.61	0.88	0.95
246	P246I	0.69	0.96	0.12	0.97	1.05	0.91	0.76	0.91	0.80
246	P246K	1.34	1.18	0.36	1.25	1.01	0.93	0.71	0.80	1.05
246	P246M	1.41	1.11	0.23	1.06	0.90	0.93	0.68	0.86	0.79
246	P246N	0.63	1.15	0.14	1.19	0.94	0.97	0.79	0.98	0.97
246	P246Q	2.14	1.16	0.54	1.34	1.01	0.97	0.84	1.00	0.92
246	P246R	1.39	1.25	0.36	1.21	1.03	0.90	0.63	0.69	0.99
246	P246S	2.01	1.14	0.62	1.21	0.92	1.09	0.71	0.93	0.93
246	P246T	1.21	1.12	0.40	1.13	1.06	0.99	0.70	0.89	0.93
246	P246V	1.28	1.06	0.73	1.03	0.97	0.90	0.74	0.81	0.83
246	P246W	2.51	1.08	0.75	1.03	0.97	0.94	0.63	0.83	0.80
246	P246Y	2.46	1.10	1.07	1.01	0.95	0.92	0.77	0.86	0.80
250	T250A	0.79	1.00	1.15	0.87	1.08	0.97	0.84	1.00	0.90
250	T250C	1.10	0.81	1.01	0.72	0.90	0.89	0.85	1.03	0.60
250	T250D	1.00	0.94	1.39	0.80	0.86	0.74	0.91	1.08	0.76
250	T250E	1.18	0.84	1.46	0.82	0.90	0.87	0.89	1.05	0.82
250	T250F	0.71	0.72	0.85	0.76	0.90	0.92	0.83	0.98	0.71
250	T250G	0.95	1.04	0.93	0.90	0.86	0.73	0.64	0.89	1.29
250	T250H	0.98	1.03	0.85	0.88	0.82	0.77	0.74	0.91	1.04
250	T250I	1.02	0.88	0.73	0.81	1.01	0.84	0.86	0.97	0.87
250	T250K	0.76	0.94	0.76	0.91	0.95	0.95	0.98	1.06	0.87
250	T250L	0.82	1.05	0.84	0.77	0.81	0.84	1.10	0.95	0.74
250	T250P	0.49	0.81	0.31	0.72	0.94	0.85	0.90	0.95	0.90
250	T250Q	0.94	1.04	0.98	0.87	0.94	0.88	0.89	1.22	0.85
250	T250R	0.64	1.01	0.69	0.92	0.96	0.94	0.89	0.98	0.92
250	T250W	0.99	1.00	0.85	0.82	0.84	1.04	1.17	1.01	0.77
250	T250Y	1.11	0.87	0.87	0.82	0.87	0.92	0.70	1.05	0.86
254	N254A	0.91	0.95	1.04	0.97	0.89	0.84	0.83	1.00	0.69
254	N254C	0.97	0.79	1.03	0.74	0.92	0.74	0.38	1.13	0.44
254	N254D	1.07	0.74	1.11	0.80	0.81	0.73	1.00	1.00	0.57
254	N254E	1.17	0.84	1.42	0.90	0.77	0.71	0.91	0.93	0.67
254	N254F	1.09	0.80	0.93	0.84	0.77	0.75	0.85	0.85	0.66
254	N254G	1.07	0.89	0.91	0.96	0.86	0.98	0.75	0.94	0.73
254	N254H	1.10	0.91	0.84	0.92	0.80	0.89	0.95	0.92	0.70
254	N254I	0.84	0.88	0.92	0.84	0.86	0.93	0.86	1.16	0.15
254	N254K	0.83	1.01	1.10	0.95	0.82	0.94	0.59	0.91	0.72
254	N254L	0.97	0.87	0.89	0.91	0.82	0.76	0.65	0.91	0.76
254	N254M	0.87	0.98	1.03	0.93	0.77	0.82	0.85	0.88	0.79
254	N254P	1.05	0.73	0.53	0.77	0.90	0.87	0.67	1.06	0.61
254	N254Q	1.20	0.88	1.12	0.95	0.89	0.81	0.81	0.97	0.70
254	N254R	0.84	1.00	0.92	0.96	0.92	1.01	0.86	0.96	0.75
254	N254S	0.97	0.72	1.11	0.78	0.80	0.88	0.60	0.98	0.58
254	N254T	1.08	0.87	1.28	0.87	0.92	0.86	0.95	1.01	0.65

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
254	N254V	1.04	0.82	0.96	0.82	0.80	0.88	0.93	1.00	0.46
254	N254W	1.01	0.90	0.71	0.87	0.86	0.87	0.83	0.93	0.68
254	N254Y	1.00	0.77	0.77	0.79	0.86	0.97	0.97	0.95	0.58
255	Q255A	0.77	0.81	1.05	0.82	0.92	0.74	0.74	1.00	0.65
255	Q255C	0.95	0.55	0.90	0.59	1.27	0.13	1.37	1.42	0.23
255	Q255D	1.07	0.75	0.69	0.79	0.88	0.44	0.74	0.86	0.62
255	Q255E	0.67	0.68	1.01	0.84	0.85	0.67	0.72	0.85	0.66
255	Q255F	0.89	0.87	0.72	0.80	0.93	0.77	0.70	0.82	0.64
255	Q255I	0.93	0.94	0.66	0.99	0.94	0.85	0.87	1.05	0.82
255	Q255K	0.83	0.92	0.92	0.88	0.90	0.68	0.69	0.88	0.71
255	Q255L	0.91	0.78	0.72	0.82	0.83	0.59	0.64	0.94	0.61
255	Q255M	1.00	0.71	0.93	0.78	0.93	0.54	0.94	0.92	0.60
255	Q255N	0.85	0.79	0.87	0.78	0.88	0.57	0.92	1.05	0.62
255	Q255P	1.09	0.53	0.77	0.51	0.90	0.57	0.95	1.04	0.39
255	Q255R	0.78	0.99	0.78	0.95	0.99	1.04	0.95	1.04	0.72
255	Q255S	0.92	0.72	1.01	0.81	0.80	0.57	0.83	0.97	0.68
255	Q255T	0.74	0.54	1.03	0.76	0.96	0.63	1.07	1.23	0.46
255	Q255V	0.97	0.71	0.91	0.77	0.89	0.53	0.90	0.96	0.58
255	Q255W	1.02	0.78	0.68	0.77	0.94	0.49	0.86	1.01	0.60
255	Q255Y	0.94	0.78	0.79	0.78	1.00	0.82	0.96	1.20	0.53
257	G257C	1.08	0.55	0.74	0.60	1.16	1.02	1.04	0.91	0.55
257	G257D	1.05	0.78	0.74	0.82	0.89	0.95	0.86	0.93	0.97
257	G257E	1.09	0.59	0.50	0.66	0.96	1.07	0.81	0.93	0.94
257	G257F	1.12	0.59	0.65	0.67	0.92	0.87	1.32	0.94	0.77
257	G257H	0.91	0.82	0.73	0.84	0.92	0.98	0.92	0.93	1.00
257	G257I	0.84	0.50	0.56	0.52	1.03	1.02	0.85	0.94	0.53
257	G257K	1.02	0.81	0.85	0.89	0.98	1.03	0.92	0.89	1.00
257	G257L	0.85	0.67	0.64	0.64	0.91	0.93	0.99	0.94	0.75
257	G257M	0.97	0.75	0.79	0.74	0.94	0.94	0.92	0.84	0.85
257	G257N	1.11	0.86	0.88	0.92	1.00	0.93	0.94	0.97	1.04
257	G257P	0.89	0.78	0.63	0.75	0.92	0.93	0.94	0.96	0.98
257	G257R	0.95	0.86	0.69	0.91	1.10	1.07	0.99	1.09	1.07
257	G257S	0.95	0.80	0.72	0.83	0.95	1.00	0.78	0.88	1.00
257	G257T	1.02	0.81	0.67	0.82	0.92	0.74	0.89	0.88	1.00
257	G257V	1.01	0.61	0.63	0.66	0.91	0.84	0.88	0.83	0.85
257	G257W	0.99	0.63	0.62	0.68	0.94	1.01	0.95	0.95	0.77
257	G257Y	0.91	0.70	0.66	0.69	0.93	0.82	0.72	0.96	0.87
259	N259C	0.72	1.03	0.87	0.83	1.74	2.31	2.93	2.03	0.26
259	N259D	0.80	1.33	0.95	1.28	1.21	1.23	1.10	0.96	1.12
259	N259E	0.88	1.25	0.92	1.17	1.04	1.21	1.14	0.97	0.99
259	N259G	0.81	1.23	0.73	1.15	1.00	1.17	1.07	0.97	0.99
259	N259H	0.84	1.28	0.80	1.31	1.00	1.06	0.83	0.95	1.32
259	N259K	0.78	1.32	0.88	1.19	1.08	1.24	1.00	1.08	1.03
259	N259P	0.99	1.09	0.91	1.23	1.04	1.06	1.05	1.03	1.09
259	N259Q	0.74	1.47	0.83	1.30	0.93	1.03	0.94	0.99	1.30
259	N259R	0.72	1.20	0.82	1.17	0.99	1.29	1.02	1.08	1.07
259	N259S	0.79	1.40	0.79	1.38	0.97	1.10	1.04	0.94	1.21

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
259	N259T	0.89	1.01	0.85	1.12	1.04	1.00	0.87	0.96	1.00
259	N259Y	0.93	1.03	0.77	1.13	0.96	1.03	1.00	0.92	1.08
264	G264D	0.69	0.73	0.17	0.62	0.88	0.53	0.83	0.85	0.88
264	G264N	1.10	0.56	0.29	0.55	1.00	0.50	0.99	0.88	0.72
264	G264P	0.76	0.06	0.26	0.07	1.14	0.58	0.89	0.93	0.48
264	G264R	0.34	0.21	0.06	0.18	0.60	0.39	0.42	0.79	0.25
264	G264S	1.06	0.74	0.56	0.64	0.88	1.11	0.83	0.88	0.91
264	G264T	0.83	0.62	0.22	0.59	0.78	0.63	1.04	0.92	0.74
264	G264V	0.76	0.37	0.16	0.38	0.87	0.46	0.91	0.79	0.55
266	F266Y	0.20	0.95	0.53	0.96	1.05	1.04	1.05	1.14	1.05
267	W267F	1.11	0.32	0.45	0.36	0.42	0.58	0.25	0.51	0.49
268	S268A	0.72	0.71	0.44	0.68	0.96	0.76	1.08	1.05	0.67
268	S268D	0.08	0.39	0.12	0.34	0.80	0.36	1.00	0.78	0.91
268	S268G	0.41	0.29	0.15	0.27	0.93	0.60	1.20	1.14	0.31
268	S268H	0.52	0.35	0.27	0.35	0.93	0.57	1.02	0.85	0.73
268	S268N	0.84	0.22	0.40	0.22	0.69	0.46	0.88	0.94	0.89
268	S268T	0.83	0.89	0.83	0.84	1.08	1.41	1.04	0.95	0.74
268	S268V	0.45	0.20	0.17	0.20	0.71	0.45	0.78	0.83	0.37
269	Y269A	0.63	0.56	0.36	0.40	0.90	0.71	1.11	0.99	0.91
269	Y269C	0.82	0.31	0.32	0.24	0.31	0.19	0.99	1.25	0.41
269	Y269D	0.53	0.64	0.29	0.45	0.81	0.45	0.98	1.06	0.83
269	Y269E	0.56	0.63	0.22	0.48	0.69	0.23	1.13	1.08	0.89
269	Y269F	0.94	0.48	0.94	0.39	0.84	0.81	1.20	0.99	0.76
269	Y269G	0.70	0.87	0.42	0.76	0.96	1.04	1.39	1.04	0.79
269	Y269H	0.74	0.82	0.44	0.69	0.88	0.61	0.92	0.99	0.70
269	Y269I	4.55	0.13	0.26	0.13	0.76	0.75	0.58	0.93	0.77
269	Y269K	0.62	0.23	0.28	0.17	0.71	0.43	0.80	0.94	0.83
269	Y269L	0.38	0.35	0.20	0.23	0.82	0.83	0.95	0.91	0.80
269	Y269M	0.73	0.43	0.41	0.37	0.86	0.67	1.04	0.81	0.68
269	Y269N	0.59	0.55	0.43	0.51	0.80	0.66	1.01	0.98	0.85
269	Y269P	0.73	0.44	0.48	0.35	0.86	0.44	1.47	1.03	0.79
269	Y269Q	0.50	0.34	0.31	0.31	0.86	0.50	0.92	1.05	0.68
269	Y269R	0.92	0.25	0.36	0.25	0.62	0.57	0.66	0.81	0.69
269	Y269S	3.35	0.32	0.36	0.36	0.74	0.56	1.52	0.99	0.83
269	Y269T	0.56	0.20	0.34	0.20	0.74	0.61	0.84	1.01	0.94
269	Y269V	58.10	0.17	0.34	0.15	0.64	0.58	0.46	0.95	0.81
269	Y269W	0.57	0.31	0.52	0.32	0.76	1.16	0.89	0.95	0.64
270	D270A	0.80	0.17	0.59	0.20	1.14	0.91	0.85	1.11	0.18
270	D270C	1.08	0.16	0.73	0.17	2.40	0.49	4.26	3.04	0.08
270	D270E	1.20	0.54	0.87	0.61	1.11	0.56	1.18	1.18	0.70
270	D270G	0.61	0.11	0.37	0.12	1.61	0.06	2.08	1.34	0.09
270	D270H	0.81	0.25	0.60	0.27	0.98	0.39	0.59	0.90	0.30
270	D270K	0.53	0.32	0.42	0.38	0.89	0.82	0.80	0.84	0.46
270	D270N	0.69	0.90	0.75	0.87	0.96	1.18	0.83	1.04	0.92
270	D270R	0.49	0.23	0.31	0.26	0.69	0.67	0.73	0.52	0.33
270	D270S	0.73	0.60	0.57	0.64	1.05	1.03	0.79	1.00	0.64
270	D270T	0.76	0.14	0.69	0.14	1.36	0.14	1.08	0.66	0.13

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
272	N272A	1.01	1.05	0.99	1.01	0.99	0.95	1.11	0.95	1.02
272	N272C	1.16	0.49	1.03	0.50	1.07	0.77	1.28	1.10	0.40
272	N272D	0.94	0.98	0.95	0.92	1.01	0.86	1.17	0.99	0.94
272	N272E	0.86	1.11	1.00	0.99	0.98	0.88	0.93	0.88	1.00
272	N272F	1.06	0.65	0.86	0.64	0.85	0.80	1.01	0.92	0.61
272	N272H	1.15	0.93	0.89	0.97	0.98	0.94	1.02	0.96	0.98
272	N272I	0.80	0.86	0.59	0.85	1.11	0.79	1.21	1.06	0.77
272	N272K	0.90	1.03	1.02	0.98	0.98	0.87	1.12	0.98	1.08
272	N272L	0.99	0.70	0.59	0.64	1.19	0.89	1.29	1.04	0.60
272	N272M	0.96	0.97	0.96	0.87	1.01	0.92	0.97	0.91	0.87
272	N272P	1.27	0.60	0.78	0.57	1.09	0.80	1.15	0.93	0.51
272	N272Q	0.95	1.07	0.98	1.01	1.08	0.98	1.00	0.92	0.99
272	N272R	0.88	0.96	0.87	1.00	0.79	1.10	0.84	0.89	1.04
272	N272S	1.04	0.98	1.07	0.95	0.98	0.90	1.04	0.94	1.00
272	N272T	0.85	0.82	1.03	0.85	0.92	0.83	0.96	0.93	0.90
272	N272V	0.86	0.89	0.71	0.91	0.99	0.89	1.16	0.97	0.86
272	N272W	1.36	0.71	1.14	0.66	0.83	0.92	0.83	0.84	0.73
272	N272Y	1.10	0.70	0.89	0.74	0.94	0.98	1.10	0.90	0.75
273	K273R	0.51	1.33	0.30	1.06	0.96	0.92	0.83	1.03	0.85
275	H275A	0.49	1.26	0.46	1.20	1.13	0.75	1.18	1.22	1.00
275	H275C	0.52	0.45	0.61	0.50	1.31	0.76	1.77	1.12	0.22
275	H275D	0.97	0.93	0.46	1.10	1.14	0.66	1.35	1.07	0.86
275	H275E	0.84	1.16	0.63	1.12	1.13	0.67	1.25	1.12	1.00
275	H275F	1.12	0.72	0.94	0.81	1.00	0.77	1.34	1.08	0.70
275	H275G	0.77	1.11	0.54	1.17	1.29	0.86	1.52	1.16	0.72
275	H275I	0.50	0.94	0.86	1.05	1.06	0.78	0.89	1.26	0.86
275	H275K	0.49	1.09	1.32	1.15	0.99	0.77	1.41	1.19	1.08
275	H275L	0.43	1.19	1.13	1.17	1.30	0.96	1.40	1.15	0.73
275	H275M	0.60	0.69	0.99	0.76	1.04	0.72	1.08	1.03	0.57
275	H275N	0.43	1.44	0.83	1.31	1.14	0.76	1.24	1.17	1.13
275	H275Q	0.62	1.34	0.85	1.28	1.17	0.76	1.19	1.19	1.06
275	H275R	0.67	1.24	0.68	1.28	1.19	0.90	0.97	1.04	1.18
275	H275S	0.42	0.97	0.50	1.08	0.89	0.74	1.24	1.00	0.94
275	H275T	0.36	0.99	0.78	1.05	1.05	0.71	1.16	1.09	0.91
275	H275V	0.41	0.94	0.84	1.05	1.14	0.70	1.36	1.09	0.84
275	H275W	1.22	0.69	0.71	0.78	1.19	0.71	1.27	1.04	0.55
275	H275Y	0.98	0.77	0.73	0.89	1.13	0.69	1.26	1.17	0.77
279	T279A	0.81	0.61	1.15	0.65	1.01	0.88	0.11	1.09	0.66
279	T279D	0.82	0.84	0.31	0.80	1.06	1.18	0.12	1.09	0.87
279	T279E	0.76	1.13	1.17	1.01	1.01	0.74	0.09	1.12	1.04
279	T279H	1.02	0.86	0.98	0.93	1.22	0.97	0.64	1.12	0.93
279	T279K	0.84	1.03	1.10	0.98	1.17	1.21	0.09	1.18	0.93
279	T279L	1.17	0.81	1.13	0.79	1.08	0.83	0.12	1.17	0.75
279	T279N	1.15	1.14	1.22	1.10	1.24	0.92	0.12	1.21	1.18
279	T279Q	0.74	0.72	1.24	1.04	1.10	0.88	1.18	1.14	1.03
279	T279R	0.81	1.00	0.95	1.01	1.09	1.10	0.45	1.13	1.10
279	T279S	0.90	0.94	0.93	0.95	1.03	0.93	0.15	1.09	0.97

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
279	T279Y	1.19	0.74	1.10	0.74	1.01	1.09	0.59	1.08	0.74
283	G283A	0.97	0.99	0.96	0.97	1.02	1.55	1.18	1.12	0.77
283	G283C	1.18	0.39	0.93	0.40	1.29	0.76	1.88	1.44	0.22
283	G283D	0.81	0.74	0.52	0.67	1.11	0.63	1.20	1.13	0.59
283	G283E	1.26	0.93	0.89	0.96	1.08	0.70	1.15	0.99	0.99
283	G283F	1.03	0.90	0.84	0.87	1.04	0.77	1.04	1.06	0.23
283	G283H	1.02	0.96	0.94	0.92	0.95	0.68	1.13	1.04	0.94
283	G283K	0.96	1.11	0.97	1.02	1.02	0.88	1.31	1.03	1.00
283	G283M	1.11	0.84	0.96	0.81	1.14	0.93	1.38	0.99	0.18
283	G283N	1.05	1.01	0.80	1.02	1.12	0.88	1.19	1.05	0.90
283	G283P	0.58	0.25	0.14	0.27	1.21	0.75	1.40	1.32	0.22
283	G283Q	1.08	0.99	0.91	0.99	0.89	0.73	1.03	1.07	0.91
283	G283R	0.89	1.19	1.00	1.07	1.16	0.92	1.13	1.05	0.94
283	G283S	1.13	0.81	0.93	0.84	0.96	0.81	1.03	0.91	0.87
283	G283T	1.12	0.56	0.66	0.62	1.15	0.83	1.17	1.08	0.54
283	G283V	0.97	0.29	0.44	0.30	1.41	0.81	1.16	1.52	0.17
283	G283Y	0.97	0.96	0.91	0.91	1.05	0.77	1.07	1.10	0.64
284	S284C	0.98	0.53	1.38	0.60	0.69	0.96	0.87	0.85	0.48
284	S284D	1.17	0.86	0.62	0.98	0.90	0.84	0.94	0.91	0.96
284	S284E	1.43	0.88	0.87	1.03	0.90	0.74	0.87	0.93	1.01
284	S284F	0.88	0.57	0.51	0.66	0.87	0.88	0.80	0.70	0.72
284	S284G	0.80	0.74	0.45	0.82	0.95	0.75	0.83	0.94	0.90
284	S284H	0.94	0.85	0.61	0.97	0.87	0.90	0.83	0.93	0.91
284	S284I	0.79	0.77	1.10	0.89	0.79	0.89	0.86	0.87	0.90
284	S284K	0.95	0.91	0.55	1.04	0.97	0.94	0.82	0.88	0.99
284	S284L	1.18	0.80	1.03	0.90	0.86	0.80	0.81	0.78	0.83
284	S284M	1.42	0.74	1.33	0.89	0.91	0.75	0.85	0.78	0.92
284	S284N	1.15	0.87	0.60	1.05	1.01	0.95	0.86	0.92	0.99
284	S284P	0.54	0.24	0.15	0.25	0.78	0.57	0.85	0.72	0.24
284	S284Q	1.14	1.03	0.89	1.09	1.07	0.91	0.88	0.92	0.98
284	S284R	0.78	0.92	0.39	1.00	0.93	0.84	0.77	0.98	1.01
284	S284T	0.93	0.86	1.07	1.03	0.92	0.83	0.80	0.86	0.92
284	S284V	1.00	0.73	1.30	0.93	0.92	0.89	0.71	0.84	0.91
284	S284W	1.19	0.67	0.57	0.77	0.88	0.86	0.95	0.87	0.75
284	S284Y	1.09	0.77	0.66	0.87	0.84	0.90	0.89	0.91	0.86
298	T298C	0.84	0.69	0.47	0.68	1.08	0.88	0.89	0.96	0.60
298	T298D	1.01	0.82	0.31	0.83	1.07	0.89	1.15	1.02	1.00
298	T298E	1.00	0.94	0.45	0.91	1.12	0.92	1.27	1.11	0.87
298	T298F	0.97	0.56	0.31	0.54	0.86	1.10	0.84	0.80	0.59
298	T298G	0.97	0.78	0.30	0.78	0.84	1.08	1.16	0.92	0.88
298	T298H	1.09	0.75	0.36	0.88	0.82	1.08	0.80	1.00	1.05
298	T298I	0.95	0.94	0.56	0.89	1.08	1.14	1.17	1.00	0.94
298	T298K	0.89	1.05	0.41	1.04	0.94	0.95	1.07	1.01	1.19
298	T298L	0.99	0.84	0.44	0.84	0.98	1.00	1.11	0.95	0.85
298	T298M	0.90	0.73	0.54	0.73	0.91	0.92	1.03	0.90	0.77
298	T298N	1.01	0.98	0.43	0.95	0.95	1.20	1.20	1.04	1.03
298	T298Q	0.86	1.13	0.47	1.03	1.01	1.14	1.26	1.05	1.08

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
298	T298R	0.91	0.94	0.38	0.97	0.90	1.21	1.04	0.86	1.05
298	T298S	1.11	0.81	0.33	0.91	0.91	1.06	1.13	0.90	0.99
298	T298V	0.95	0.91	0.51	0.91	0.97	1.24	1.18	1.02	0.79
298	T298W	1.05	0.56	0.37	0.62	0.73	1.62	0.77	0.79	0.52
298	T298Y	1.01	0.59	0.30	0.65	0.84	0.80	0.92	0.75	0.73
301	K301C	1.04	0.30	0.75	0.32	0.67	0.49	0.97	0.97	0.44
301	K301D	1.11	0.61	0.70	0.63	0.75	0.54	0.92	1.05	0.85
301	K301E	1.19	0.64	0.80	0.69	0.96	0.78	0.99	0.96	0.92
301	K301F	1.20	0.57	0.77	0.58	0.90	0.83	1.04	1.11	0.70
301	K301G	1.06	0.73	0.79	0.71	0.82	0.66	0.79	1.04	0.87
301	K301H	0.99	0.78	0.87	0.76	0.86	1.28	0.93	1.01	0.95
301	K301I	1.02	0.76	1.02	0.78	1.03	0.94	0.99	1.14	0.89
301	K301L	1.10	0.65	0.78	0.70	0.82	0.94	0.89	0.92	0.87
301	K301M	1.17	0.74	0.85	0.76	0.86	0.86	0.91	1.10	0.87
301	K301N	1.28	0.86	0.82	0.88	0.88	0.91	0.85	1.15	1.03
301	K301Q	1.11	0.85	0.93	0.88	0.86	1.02	0.96	1.09	0.98
301	K301R	0.98	0.89	0.82	0.92	0.85	0.95	0.95	0.98	1.14
301	K301S	1.07	0.83	0.75	0.83	0.86	0.62	0.99	1.05	1.01
301	K301T	1.16	0.80	0.97	0.80	0.90	0.80	1.05	1.18	0.95
301	K301V	1.37	0.54	0.82	0.61	0.83	0.56	1.15	1.10	0.70
301	K301W	1.25	0.55	0.73	0.59	0.85	0.85	0.97	0.92	0.68
301	K301Y	1.12	0.60	0.78	0.61	0.74	0.79	1.08	0.99	0.76
303	S303A	0.82	0.95	1.04	0.93	0.95	0.79	0.84	1.07	0.91
303	S303C	0.89	0.66	1.10	0.65	1.10	0.32	1.17	1.49	0.53
303	S303D	1.00	0.85	1.18	0.96	1.00	0.57	0.91	1.25	0.99
303	S303E	0.95	0.89	1.31	0.90	0.86	1.00	1.05	1.20	0.91
303	S303F	0.98	0.72	1.11	0.73	0.75	0.91	0.91	0.97	0.64
303	S303G	1.04	0.84	1.54	0.90	0.77	0.90	0.84	0.98	0.96
303	S303H	0.81	0.58	1.51	0.86	0.84	0.99	0.95	0.87	0.79
303	S303I	0.92	0.69	0.70	0.75	1.12	0.72	0.81	1.29	0.69
303	S303K	0.95	0.83	1.19	0.92	0.88	0.94	0.53	0.85	0.85
303	S303L	0.96	0.64	0.92	0.73	1.08	0.64	0.73	1.07	0.45
303	S303M	0.93	0.64	1.32	0.65	0.97	0.82	0.78	0.96	0.66
303	S303N	1.06	0.95	1.51	1.01	0.85	0.78	0.93	1.08	1.02
303	S303P	0.97	0.80	0.83	0.87	0.97	0.26	1.44	1.70	0.79
303	S303Q	1.06	0.93	1.42	0.97	1.00	0.77	0.86	1.12	0.86
303	S303R	0.93	0.80	1.01	0.90	0.85	0.90	0.72	0.81	0.93
303	S303T	0.96	0.83	0.85	0.85	0.89	0.77	0.99	1.15	0.82
303	S303V	1.10	0.60	0.90	0.73	0.93	0.69	0.66	1.05	0.65
303	S303Y	0.94	0.87	1.25	0.87	0.93	0.76	0.66	0.87	0.80
305	Y305A	0.85	1.26	1.44	1.17	1.14	0.54	1.52	1.26	1.33
305	Y305C	0.97	0.51	1.11	0.52	1.09	0.18	1.37	1.42	0.42
305	Y305D	0.91	1.20	1.57	1.14	1.16	0.29	1.57	1.33	1.27
305	Y305E	0.96	1.26	1.59	1.21	1.20	0.40	1.76	1.39	1.18
305	Y305F	0.98	1.08	1.07	0.98	1.01	0.84	0.93	0.89	0.94
305	Y305G	0.98	1.28	1.25	1.25	1.22	0.61	1.39	1.30	1.34
305	Y305H	0.85	1.22	1.08	1.11	1.19	0.71	1.43	1.19	1.15

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
305	Y305I	0.88	1.11	0.81	1.02	1.14	0.50	1.22	1.30	1.12
305	Y305K	0.93	1.14	1.38	1.16	1.25	0.66	1.44	1.30	1.30
305	Y305L	1.07	0.94	1.23	1.01	1.14	0.56	1.53	1.30	1.03
305	Y305M	0.95	1.06	1.38	1.03	1.16	0.56	1.36	1.27	1.03
305	Y305N	0.98	1.35	1.61	1.22	1.17	0.63	1.44	1.26	1.22
305	Y305P	1.04	0.88	0.72	0.93	0.91	0.25	1.88	1.53	0.87
305	Y305Q	0.98	1.18	1.43	1.20	1.13	0.58	1.60	1.33	1.22
305	Y305R	0.96	1.23	1.16	1.21	1.35	0.76	1.37	1.33	1.28
305	Y305S	0.97	1.26	1.38	1.17	1.18	0.52	1.60	1.37	1.22
305	Y305T	1.01	1.10	1.32	1.07	1.19	0.45	1.50	1.31	1.19
305	Y305V	1.01	1.01	0.99	0.99	1.16	0.43	1.55	1.23	1.03
305	Y305W	0.97	0.99	0.96	0.93	1.13	0.70	1.15	1.11	0.90
306	F306A	0.52	0.43	0.67	0.86	1.01	0.97	0.79	0.92	0.91
306	F306C	1.05	0.43	0.67	0.40	0.97	0.66	1.98	0.93	0.38
306	F306D	1.03	0.23	0.65	0.22	1.04	0.96	3.32	0.63	0.19
306	F306E	0.91	0.29	0.70	0.31	0.92	0.56	2.22	0.71	0.30
306	F306G	0.77	0.43	0.71	0.60	0.89	0.82	1.44	0.88	0.58
306	F306H	1.19	0.79	0.71	0.81	0.97	1.01	1.23	0.93	0.66
306	F306I	1.06	0.38	0.62	0.41	1.03	0.91	1.49	0.89	0.40
306	F306K	0.91	0.26	0.65	0.43	0.79	0.86	1.47	0.68	0.42
306	F306L	0.96	0.33	0.62	0.44	0.92	0.87	1.30	0.72	0.47
306	F306M	0.92	0.50	0.72	0.72	0.91	0.96	1.27	0.83	0.79
306	F306N	1.21	0.38	0.69	0.38	0.83	0.79	1.83	0.64	0.38
306	F306Q	0.80	0.38	0.69	0.50	1.05	0.79	1.55	0.95	0.47
306	F306R	0.83	0.18	0.59	0.25	0.77	1.20	2.42	0.62	0.21
306	F306S	0.45	0.40	0.65	0.65	0.92	0.89	0.72	0.84	0.71
306	F306T	0.97	0.38	0.67	0.55	0.95	0.84	1.33	0.92	0.57
306	F306V	1.14	0.35	0.69	0.37	0.94	0.79	2.14	0.88	0.34
306	F306W	1.28	0.90	0.66	0.95	0.94	1.11	0.90	0.85	0.93
310	Y310A	0.94	0.93	1.20	0.85	1.09	0.91	1.04	0.95	0.91
310	Y310C	1.04	0.93	1.29	0.96	0.99	0.92	0.84	1.01	0.87
310	Y310D	1.11	1.21	1.41	1.19	1.33	1.10	1.52	1.23	0.66
310	Y310E	0.99	1.09	1.57	1.02	1.07	0.87	1.13	1.12	1.15
310	Y310F	1.10	0.97	1.03	0.94	0.86	1.37	1.03	1.25	0.20
310	Y310G	1.20	1.07	1.10	1.06	0.71	0.93	1.00	1.12	0.33
310	Y310H	1.02	1.12	1.02	1.05	1.13	1.19	1.32	1.10	1.15
310	Y310I	0.93	1.07	0.84	1.06	1.13	1.17	1.44	1.25	0.94
310	Y310K	0.90	1.20	1.25	1.04	0.94	1.05	1.13	1.08	1.06
310	Y310L	0.88	1.08	1.30	0.94	1.07	0.88	0.97	0.96	1.48
310	Y310M	1.03	0.97	1.37	0.91	0.95	0.95	0.92	1.08	1.27
310	Y310N	1.17	1.14	1.47	1.13	0.91	1.03	1.50	1.18	1.19
310	Y310Q	1.10	1.04	1.24	1.09	1.13	1.02	1.32	1.06	1.28
310	Y310R	0.97	1.07	1.06	1.08	0.90	1.33	0.91	0.90	1.12
310	Y310S	1.05	1.02	1.16	1.03	0.97	0.84	0.84	1.10	1.31
310	Y310T	1.06	0.96	1.51	0.96	0.94	1.07	0.96	0.96	1.19
310	Y310V	1.10	0.89	0.93	0.95	1.04	1.12	1.20	1.07	1.16
311	L311C	0.93	0.44	0.68	0.46	0.99	0.75	0.74	0.93	0.39

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
311	L311F	1.00	0.79	0.58	0.81	0.95	0.80	0.81	0.81	0.70
311	L311G	1.04	0.09	0.66	0.10	1.25	0.50	1.18	1.04	0.07
311	L311I	0.70	0.75	1.34	0.96	0.94	0.98	1.00	0.92	0.94
311	L311M	0.93	1.00	0.82	0.92	0.94	0.82	0.80	0.95	0.83
311	L311Q	0.90	0.23	0.67	0.22	1.00	0.49	0.88	0.85	0.20
311	L311S	0.66	0.42	0.61	0.54	1.17	0.89	1.00	1.29	0.41
311	L311T	1.03	0.67	0.78	0.71	1.03	0.90	0.98	0.97	0.64
311	L311V	1.18	0.73	0.95	0.79	1.05	0.80	0.85	0.90	0.76
314	N314A	0.79	1.13	0.68	1.05	0.95	0.89	0.91	1.12	1.21
314	N314C	0.95	0.65	0.75	0.61	1.00	0.53	0.93	1.24	0.75
314	N314D	0.93	1.07	0.94	0.98	0.94	0.72	0.97	1.18	1.22
314	N314E	0.92	1.06	0.85	0.95	1.01	0.66	0.76	1.11	1.34
314	N314F	0.94	0.96	0.74	0.80	0.87	0.94	0.82	1.04	0.99
314	N314G	0.98	1.22	1.25	1.10	0.91	0.91	1.01	1.12	1.27
314	N314H	0.90	1.06	0.78	0.94	0.94	0.87	0.91	1.02	1.32
314	N314I	0.92	0.59	0.64	0.50	0.95	0.85	0.83	1.05	0.57
314	N314K	0.83	0.94	0.74	0.93	0.86	0.78	0.84	0.97	1.18
314	N314L	0.95	0.79	0.67	0.75	1.05	0.83	0.85	1.18	1.01
314	N314M	0.87	0.86	0.76	0.80	0.88	0.80	0.81	1.02	1.09
314	N314P	0.95	0.45	0.57	0.37	0.90	0.74	0.83	1.01	0.36
314	N314Q	0.92	1.00	0.78	0.91	0.87	0.89	0.83	1.16	1.26
314	N314R	0.79	1.00	0.65	0.93	0.84	0.96	0.90	0.86	1.23
314	N314S	0.88	0.98	0.75	0.92	0.92	0.72	0.83	1.14	1.18
314	N314V	0.92	0.82	0.68	0.70	0.88	0.88	0.82	1.00	0.73
314	N314W	0.98	0.84	0.68	0.77	0.96	0.93	0.94	0.92	0.89
314	N314Y	0.94	0.93	0.72	0.85	0.86	0.88	0.90	1.07	1.10
318	K318A	0.83	1.07	0.79	1.00	1.13	1.03	1.00	1.12	1.04
318	K318C	0.87	0.70	0.63	0.58	1.04	0.75	0.85	1.06	0.67
318	K318D	1.05	0.70	0.53	0.69	1.03	0.95	0.96	1.26	0.68
318	K318E	1.14	0.94	0.60	1.02	1.02	1.09	1.02	1.09	0.94
318	K318G	0.96	0.81	0.73	0.84	1.09	0.97	0.84	1.15	0.93
318	K318H	0.97	1.00	0.80	0.92	1.00	0.96	0.76	1.10	1.12
318	K318I	1.13	0.89	0.52	1.00	1.10	0.93	1.01	1.20	1.03
318	K318L	1.18	0.79	0.78	0.83	1.12	0.97	1.14	1.11	0.86
318	K318M	1.05	0.88	0.73	0.87	0.97	0.93	1.02	1.06	0.98
318	K318N	1.09	0.92	0.78	0.94	0.97	0.91	0.69	1.14	1.15
318	K318P	0.84	0.46	0.50	0.47	0.96	0.71	0.98	1.26	0.46
318	K318Q	1.16	0.91	0.71	1.00	1.04	1.00	0.98	1.12	1.06
318	K318R	1.06	0.97	0.89	1.01	1.07	1.17	0.68	1.15	1.15
318	K318S	0.96	1.04	0.70	0.98	1.12	1.05	0.91	1.06	1.06
318	K318T	0.89	0.99	0.52	0.97	1.14	0.79	1.05	1.20	1.00
318	K318Y	1.32	0.81	0.67	0.88	0.96	0.93	1.04	1.24	0.99
319	D319A	0.69	1.01	0.54	1.00	1.04	0.91	1.46	1.20	0.91
319	D319E	0.94	1.13	1.18	1.08	0.95	0.86	1.23	0.99	1.07
319	D319F	0.79	1.01	0.63	0.93	1.01	0.60	1.04	1.03	0.84
319	D319H	0.85	0.99	0.79	1.03	1.11	1.02	1.09	1.04	0.86
319	D319I	0.38	0.87	0.44	0.89	1.20	1.53	1.46	0.87	0.96

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
319	D319K	0.38	0.98	0.30	1.00	1.13	0.65	0.82	1.07	0.95
319	D319M	0.63	0.92	0.64	0.90	0.89	0.60	1.18	1.09	0.95
319	D319N	0.96	0.95	0.91	1.00	1.18	0.94	1.21	1.02	1.05
319	D319P	0.81	0.46	0.55	0.45	1.03	0.33	1.01	0.97	0.33
319	D319Q	0.69	0.97	0.69	1.02	1.03	1.05	1.38	1.00	1.04
319	D319R	0.29	1.16	0.28	1.06	1.03	1.27	1.20	0.85	1.02
319	D319S	0.89	0.95	0.95	0.98	1.14	0.86	1.41	1.04	0.95
319	D319T	0.89	0.90	1.13	0.95	1.17	0.66	1.42	1.09	1.02
319	D319V	0.36	0.94	0.44	0.93	1.37	0.99	1.49	1.30	0.71
319	D319W	0.56	0.90	0.45	0.83	1.00	0.52	1.23	0.90	0.84
319	D319Y	0.78	0.87	0.61	0.93	0.94	0.56	1.40	1.05	0.83
320	Q320A	0.94	0.70	1.15	0.84	1.20	0.77	0.76	0.95	0.72
320	Q320C	0.88	0.50	1.77	0.51	1.33	0.88	1.66	1.62	0.31
320	Q320D	0.93	0.86	0.77	0.95	1.09	0.63	1.27	1.23	0.86
320	Q320E	1.06	0.93	0.95	0.98	1.22	0.68	1.28	1.14	0.81
320	Q320F	1.32	0.78	1.51	0.87	1.12	0.56	1.46	1.21	0.79
320	Q320G	0.87	0.71	0.61	0.79	1.20	0.80	1.26	1.11	0.67
320	Q320H	0.86	0.98	1.20	0.95	1.14	0.70	1.31	1.05	0.86
320	Q320I	0.98	0.71	1.10	0.79	1.23	0.76	0.98	1.24	0.70
320	Q320K	0.96	0.84	1.16	0.97	1.17	0.74	1.25	1.06	0.95
320	Q320L	0.84	0.77	1.05	0.77	1.15	0.67	0.93	1.11	0.66
320	Q320M	0.91	0.87	1.25	0.83	1.06	0.74	1.03	1.12	0.77
320	Q320N	1.08	0.87	1.41	0.97	1.31	0.69	1.35	1.09	0.85
320	Q320R	0.78	0.99	1.06	0.98	1.29	0.83	1.18	1.06	0.92
320	Q320S	0.95	0.88	0.87	0.98	1.17	0.71	1.14	1.08	0.83
320	Q320T	1.12	0.77	1.00	0.89	1.09	0.66	0.96	1.10	0.80
320	Q320V	1.15	0.75	1.26	0.83	1.12	0.66	1.22	1.05	0.74
320	Q320W	1.06	0.80	1.07	0.88	1.03	0.60	2.41	1.09	0.73
320	Q320Y	1.12	0.85	1.61	0.88	1.14	0.56	1.35	1.08	0.80
322	S322A	0.90	1.09	0.97	1.01	1.04	1.06	1.14	1.05	0.99
322	S322D	0.91	1.04	1.27	0.92	1.11	0.87	1.30	1.09	0.80
322	S322E	0.96	1.23	1.22	1.04	0.91	0.78	1.06	0.95	1.04
322	S322F	0.87	0.89	0.87	0.76	0.89	0.92	0.73	0.94	0.70
322	S322G	0.68	0.88	1.08	1.03	0.95	0.83	1.00	0.98	1.00
322	S322H	0.51	0.52	1.24	0.94	1.05	0.72	1.00	0.96	0.96
322	S322I	0.83	0.96	0.71	0.81	1.02	0.94	1.07	1.03	0.80
322	S322L	0.78	0.99	0.90	0.82	1.12	0.85	1.13	1.04	0.74
322	S322M	0.75	1.39	0.97	1.10	1.18	0.83	1.18	1.09	0.91
322	S322N	1.04	1.13	1.34	1.06	1.02	0.88	1.02	1.08	0.96
322	S322P	0.95	0.89	0.87	0.82	0.86	0.83	1.26	1.01	0.74
322	S322Q	0.82	0.83	1.21	1.02	1.07	0.81	1.04	1.06	1.03
322	S322R	0.78	1.25	0.89	1.01	1.06	1.22	1.34	1.07	0.88
322	S322T	0.91	1.04	1.09	0.94	0.97	1.10	1.02	0.79	0.89
322	S322V	1.00	0.88	0.90	0.85	0.93	1.07	1.00	0.85	0.80
322	S322W	0.90	0.91	0.92	0.75	0.95	0.87	0.94	0.87	0.71
322	S322Y	0.97	0.89	0.96	0.87	0.83	1.02	0.93	0.81	0.84
323	L323A	0.66	0.97	0.56	0.98	1.10	0.87	1.13	1.09	0.95

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
323	L323C	0.68	0.48	0.47	0.48	1.10	0.85	0.81	1.04	0.40
323	L323D	0.67	0.66	0.42	0.67	1.00	0.69	0.96	1.17	0.70
323	L323E	0.68	0.86	0.45	0.85	0.82	0.70	1.02	0.98	1.04
323	L323F	0.79	0.98	0.93	0.95	1.01	0.83	1.09	1.13	1.00
323	L323G	0.76	0.87	0.38	0.91	0.96	0.76	1.00	1.09	0.93
323	L323H	0.65	0.81	0.65	1.05	0.97	0.84	0.81	1.20	1.09
323	L323K	0.76	1.15	0.78	1.13	1.01	0.91	1.08	0.97	1.13
323	L323M	1.02	0.81	0.79	0.90	1.00	0.99	0.99	1.01	0.88
323	L323N	0.86	0.98	0.44	1.04	0.98	0.80	1.23	1.20	1.07
323	L323P	0.69	0.39	0.41	0.41	0.94	0.63	0.94	1.09	0.43
323	L323Q	0.84	1.04	0.49	1.06	1.02	0.89	1.06	1.15	1.07
323	L323R	0.77	0.56	0.46	0.58	1.10	1.02	1.12	0.93	0.57
323	L323S	0.73	1.07	0.48	1.03	1.02	0.92	1.21	1.08	0.98
323	L323T	0.86	0.58	0.50	0.60	0.92	0.45	1.05	1.01	0.62
323	L323V	0.91	0.93	0.62	0.86	0.99	0.76	1.00	0.91	0.95
323	L323W	0.67	0.83	0.47	0.77	0.83	0.81	0.99	0.96	0.76
336	G336A	0.77	0.83	0.57	0.74	0.86	0.81	1.18	1.01	0.86
336	G336C	0.93	0.44	0.68	0.42	0.89	0.59	1.01	1.03	0.47
336	G336E	0.95	0.89	0.67	0.84	0.90	0.67	1.16	0.98	0.92
336	G336F	1.00	0.75	0.73	0.73	0.88	0.95	1.26	0.84	0.53
336	G336H	0.88	0.99	0.70	0.90	0.89	1.02	1.02	0.93	0.86
336	G336I	0.97	0.41	0.51	0.39	0.84	0.39	1.13	0.93	0.49
336	G336K	0.95	0.86	0.56	0.90	0.68	0.62	1.33	0.85	0.94
336	G336M	0.91	0.73	0.70	0.72	0.90	0.82	1.38	1.01	0.73
336	G336N	0.87	0.30	0.74	0.28	0.76	0.69	1.14	0.92	0.32
336	G336P	1.22	0.09	0.70	0.08	0.37	0.12	1.28	1.00	0.66
336	G336Q	0.98	0.92	0.64	0.86	0.93	0.71	1.16	0.97	0.93
336	G336R	0.90	0.83	0.49	0.75	0.74	0.87	0.67	0.81	0.88
336	G336S	0.94	0.71	0.60	0.70	0.83	0.68	1.11	0.92	0.91
336	G336T	1.00	0.66	0.57	0.68	0.76	0.62	1.04	0.91	0.90
336	G336W	0.88	0.98	0.60	0.82	0.90	0.99	1.00	0.92	0.66
336	G336Y	1.04	0.99	0.73	0.98	0.95	0.95	1.00	1.02	0.79
337	Q337A	0.97	0.37	1.18	0.38	1.02	1.02	0.65	0.91	0.83
337	Q337E	1.27	0.56	0.55	0.61	0.24	0.17	0.94	0.93	0.99
337	Q337G	1.18	0.46	1.74	0.47	0.91	0.55	0.59	0.78	0.88
337	Q337N	1.20	0.11	0.91	0.12	0.68	0.38	1.01	0.80	0.95
337	Q337S	0.97	0.22	1.18	0.21	0.83	0.52	0.46	0.77	0.72
337	Q337T	1.04	0.06	0.87	0.06	0.56	0.33	0.81	0.77	0.77
338	S338A	1.01	0.54	1.00	0.57	0.93	0.95	0.96	0.97	1.03
338	S338C	1.07	0.06	0.87	0.06	0.31	0.13	0.89	0.82	0.52
338	S338D	1.14	0.14	0.91	0.14	0.75	0.18	1.03	0.91	1.02
338	S338E	1.05	0.06	0.92	0.06	0.50	0.11	1.46	0.74	1.11
338	S338G	1.03	0.27	0.83	0.28	0.75	0.86	0.97	0.96	0.81
338	S338K	0.72	0.06	1.13	0.06	0.59	0.41	0.97	0.82	1.07
338	S338M	1.01	0.09	0.88	0.10	0.63	0.28	0.88	0.67	0.93
338	S338N	1.01	0.08	0.86	0.08	0.66	0.26	1.06	0.88	1.09
338	S338P	0.99	0.15	1.70	0.15	0.74	0.42	0.90	0.70	0.92

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
338	S338Q	1.01	0.14	0.80	0.15	0.62	0.29	0.91	0.89	1.07
338	S338T	1.05	0.08	0.80	0.09	0.47	0.30	0.80	0.64	0.92
338	S338Y	0.96	0.09	0.78	0.09	0.30	0.13	0.74	0.60	0.83
339	L339G	1.07	0.09	0.90	0.10	0.31	0.13	0.35	0.61	1.18
339	L339H	0.65	0.22	0.78	0.26	0.61	0.38	0.34	0.84	1.39
339	L339I	2.08	0.08	0.68	0.15	0.66	0.29	0.78	0.65	1.17
339	L339M	1.16	0.28	1.27	0.30	0.82	0.42	0.66	0.71	1.29
339	L339Q	0.87	0.57	0.78	0.49	0.77	0.56	0.45	1.04	1.36
339	L339V	0.99	0.13	0.76	0.13	0.45	0.22	0.78	0.74	1.26
340	Q340A	0.90	0.67	0.80	0.68	1.01	0.99	0.97	0.97	0.87
340	Q340C	0.97	0.33	0.88	0.33	1.12	0.32	1.18	1.14	0.39
340	Q340D	1.01	0.70	0.81	0.68	0.87	0.33	1.06	0.96	0.99
340	Q340E	1.02	0.98	1.53	0.97	1.02	0.99	1.08	0.96	1.07
340	Q340F	0.89	0.79	0.72	0.67	1.11	1.04	0.98	0.93	0.51
340	Q340G	0.93	0.62	0.74	0.56	0.68	0.18	0.94	0.92	0.91
340	Q340H	1.01	0.74	0.70	0.72	0.87	0.69	0.92	0.92	0.94
340	Q340I	0.98	0.30	0.63	0.27	0.59	0.18	0.95	0.83	0.53
340	Q340K	0.87	0.64	0.56	0.56	0.65	0.31	0.62	0.77	1.09
340	Q340L	0.97	0.42	0.63	0.37	0.47	0.07	1.10	0.92	0.45
340	Q340M	0.99	0.73	0.83	0.67	0.86	0.77	1.02	0.85	0.54
340	Q340N	1.07	0.60	0.80	0.60	0.88	0.40	1.19	0.93	0.91
340	Q340R	0.97	0.53	0.52	0.54	0.57	0.50	0.58	0.76	0.91
340	Q340S	1.01	0.51	0.87	0.54	0.86	0.70	0.94	0.92	1.01
340	Q340T	1.13	0.55	0.81	0.55	0.99	1.07	1.21	0.96	1.10
340	Q340V	1.15	0.58	0.75	0.64	0.89	0.69	0.82	0.79	0.86
340	Q340W	0.90	0.59	0.59	0.52	0.71	0.53	0.54	0.72	0.46
340	Q340Y	1.00	0.86	0.70	0.87	1.05	1.36	1.00	0.85	0.65
344	E344A	0.96	1.01	0.70	1.01	0.82	1.08	0.68	0.69	1.28
344	E344C	0.85	0.83	0.87	0.76	0.91	0.90	1.04	0.99	0.87
344	E344D	0.90	0.92	0.84	0.82	0.87	0.90	0.83	0.93	1.17
344	E344F	0.96	0.70	0.67	0.64	0.73	0.77	0.71	0.77	0.95
344	E344G	0.98	0.73	0.67	0.76	0.87	0.76	0.49	0.78	1.11
344	E344H	0.95	0.80	0.64	0.76	0.80	0.99	0.54	0.94	1.13
344	E344K	0.99	1.08	0.71	1.14	0.63	0.98	0.38	0.51	1.60
344	E344L	0.90	0.90	0.72	0.87	0.81	0.83	0.18	0.60	1.18
344	E344M	0.85	0.70	0.81	0.67	0.59	1.02	0.20	0.59	0.97
344	E344N	1.14	0.75	0.69	0.79	0.78	0.80	0.53	0.83	1.11
344	E344P	1.08	0.54	0.71	0.61	0.63	0.88	0.34	0.60	1.14
344	E344R	0.83	1.14	0.62	1.13	0.67	1.11	0.51	0.56	1.42
344	E344S	0.85	0.78	0.73	0.81	0.77	1.03	0.28	0.61	1.09
344	E344T	0.97	0.75	0.70	0.80	0.86	0.93	0.34	0.69	1.10
344	E344V	1.08	0.71	0.68	0.75	0.65	0.73	0.40	0.62	1.09
359	Q359A	0.92	0.96	1.04	1.02	1.01	0.87	0.81	1.20	1.01
359	Q359C	0.92	0.23	0.76	0.28	1.63	1.13	3.36	1.55	0.12
359	Q359D	1.02	0.92	0.79	0.93	1.01	0.83	1.02	0.94	0.95
359	Q359E	1.06	0.83	0.95	0.93	1.00	0.83	0.81	0.92	0.93
359	Q359F	1.11	0.93	0.75	1.06	0.96	0.93	0.90	0.93	1.00

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
359	Q359G	0.99	0.87	0.78	0.99	0.87	0.82	0.80	0.91	1.01
359	Q359I	0.89	0.74	0.90	0.78	0.95	0.82	0.71	0.93	0.77
359	Q359M	1.00	0.81	0.85	0.85	1.10	0.85	0.84	0.88	0.88
359	Q359N	1.03	1.06	0.81	1.15	1.02	0.86	0.83	0.94	1.19
359	Q359S	0.94	0.93	0.89	1.02	0.93	0.92	0.93	0.88	1.00
359	Q359T	0.99	0.79	0.83	0.92	1.13	0.97	0.79	0.91	0.93
359	Q359V	0.96	0.80	0.82	0.84	1.05	0.81	0.89	0.94	0.87
359	Q359W	0.88	0.99	0.75	1.00	1.08	0.98	0.95	0.95	0.97
359	Q359Y	0.89	0.98	0.74	0.98	1.05	0.90	0.92	0.97	0.95
374	P374A	0.83	0.75	1.39	0.70	0.97	0.84	1.09	0.92	0.70
374	P374D	0.94	0.80	1.25	0.81	1.09	0.81	1.25	0.96	0.68
374	P374E	0.93	0.77	1.77	0.71	0.96	0.86	1.24	0.90	0.73
374	P374F	1.07	0.29	1.27	0.33	1.24	1.03	1.80	1.40	0.23
374	P374H	0.97	0.75	1.25	0.72	0.97	0.96	0.98	0.97	0.67
374	P374I	0.95	0.43	1.81	0.42	1.03	1.11	1.33	1.07	0.45
374	P374K	0.91	0.82	1.25	0.80	0.96	1.08	1.21	0.98	0.70
374	P374L	0.94	0.41	1.60	0.40	1.01	0.90	1.47	0.94	0.39
374	P374M	0.96	0.53	1.67	0.53	0.87	1.06	1.35	0.92	0.49
374	P374N	1.03	0.84	1.30	0.82	1.06	0.95	1.23	0.89	0.72
374	P374Q	0.97	0.67	1.71	0.72	0.99	0.98	1.12	1.03	0.73
374	P374R	0.84	0.76	1.16	0.76	0.99	1.20	1.08	1.00	0.75
374	P374S	0.85	0.69	1.36	0.66	0.92	1.03	1.15	0.84	0.62
374	P374T	0.82	0.64	1.44	0.60	0.92	1.00	0.86	0.88	0.68
374	P374V	0.76	0.50	1.98	0.43	1.01	0.90	1.28	0.90	0.48
374	P374W	0.99	0.35	1.37	0.35	1.02	1.05	1.27	1.06	0.31
374	P374Y	0.98	0.34	1.34	0.33	1.04	0.79	1.23	0.93	0.29
375	K375A	0.98	0.87	0.99	0.79	0.83	0.54	1.30	0.98	0.72
375	K375C	1.03	0.08	0.81	0.07	0.36	0.19	1.47	0.95	0.09
375	K375D	1.26	0.86	0.78	0.84	0.81	1.10	1.11	0.87	0.76
375	K375E	1.11	1.03	0.86	0.97	0.87	0.81	1.27	0.78	0.74
375	K375F	1.05	0.44	0.89	0.38	0.70	0.44	1.06	0.85	0.39
375	K375G	1.29	0.72	0.83	0.74	0.87	0.62	0.98	0.87	0.68
375	K375H	1.07	0.85	0.78	0.81	0.89	0.57	1.07	0.89	0.74
375	K375I	1.04	0.66	0.72	0.61	0.86	0.60	1.26	0.93	0.63
375	K375L	1.03	0.66	0.75	0.58	0.83	0.73	2.17	0.86	0.56
375	K375N	1.16	0.88	0.89	0.82	0.90	0.73	1.14	0.92	0.75
375	K375P	1.22	0.67	0.75	0.65	0.84	0.57	1.20	0.83	0.64
375	K375Q	1.12	0.97	0.80	0.92	0.80	0.71	1.30	0.86	0.88
375	K375R	0.88	0.99	0.79	0.86	0.98	1.14	1.22	0.96	0.80
375	K375S	1.06	0.90	1.03	0.80	0.83	0.64	1.15	0.81	0.68
375	K375T	1.03	1.00	1.08	0.87	0.66	0.65	0.96	0.75	0.70
375	K375V	1.12	0.72	0.89	0.73	0.87	0.61	1.12	0.87	0.68
375	K375W	1.10	0.63	0.72	0.57	0.85	0.55	1.08	0.85	0.54
375	K375Y	0.98	0.40	0.86	0.39	0.72	0.52	1.17	0.93	0.38
376	Y376A	1.32	1.15	0.76	1.36	0.97	0.82	0.76	1.01	1.04
376	Y376C	1.11	0.21	0.64	0.24	2.09	0.57	1.45	1.82	0.09
376	Y376D	1.13	1.18	0.61	1.29	1.00	0.85	0.76	1.04	0.97

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
376	Y376E	1.28	1.34	0.73	1.46	1.01	0.72	0.86	1.10	1.20
376	Y376F	1.20	1.10	0.79	1.16	1.02	0.75	1.02	0.97	0.86
376	Y376G	1.16	1.17	0.69	1.26	1.00	0.82	0.78	0.96	0.96
376	Y376H	0.99	0.71	1.00	0.99	1.07	0.88	0.91	1.04	0.90
376	Y376I	1.08	1.03	0.59	1.12	0.98	0.87	0.77	0.99	0.88
376	Y376K	1.22	1.10	0.70	1.44	0.83	0.79	0.42	0.80	1.25
376	Y376M	1.09	1.19	0.75	1.26	0.96	0.88	0.95	1.11	0.93
376	Y376N	1.13	1.15	0.71	1.22	0.93	0.83	0.81	0.98	1.02
376	Y376P	1.21	0.82	0.51	0.89	0.96	0.64	0.75	0.86	0.65
376	Y376Q	1.12	1.21	0.86	1.34	1.03	0.93	0.91	1.03	1.01
376	Y376R	1.12	1.06	0.91	1.28	0.90	1.78	0.51	0.81	1.16
376	Y376S	1.20	1.09	0.75	1.18	0.96	0.72	0.78	1.00	0.98
376	Y376T	1.13	0.95	0.67	0.99	0.99	0.79	0.93	1.00	0.84
376	Y376V	1.13	0.98	0.69	1.07	0.89	0.80	0.60	1.06	0.88
376	Y376W	1.05	0.84	0.98	0.86	0.98	0.90	0.87	1.06	0.63
377	N377A	0.94	0.76	0.81	0.86	1.01	0.87	0.72	1.09	0.93
377	N377C	0.91	0.26	0.87	0.27	0.66	0.46	0.71	0.74	0.34
377	N377D	0.97	0.91	1.06	0.95	0.96	0.77	0.73	1.12	1.08
377	N377E	1.03	0.83	0.98	0.92	0.95	0.72	0.89	1.17	1.02
377	N377F	0.97	0.62	0.81	0.59	0.93	0.84	0.79	1.05	0.62
377	N377G	1.16	0.73	1.03	0.82	0.98	0.89	0.92	1.13	0.79
377	N377H	1.07	0.78	0.83	0.89	1.02	0.93	0.87	0.90	0.94
377	N377K	0.92	1.02	0.83	1.16	0.93	0.96	1.08	1.07	1.35
377	N377L	0.89	0.67	0.80	0.69	0.89	0.78	0.95	0.98	0.81
377	N377M	0.92	0.66	0.88	0.72	1.00	0.80	0.97	0.96	0.79
377	N377P	1.09	0.43	0.52	0.49	0.82	0.68	0.60	0.88	0.45
377	N377Q	0.94	0.86	0.87	0.92	0.98	0.93	1.10	1.27	0.93
377	N377R	0.79	1.08	0.74	1.02	1.05	1.13	1.01	1.19	1.00
377	N377S	0.88	0.84	0.90	0.91	0.94	0.93	0.98	1.05	0.97
377	N377T	0.98	0.54	0.85	0.65	0.80	0.79	0.85	0.90	0.68
377	N377V	0.89	0.48	0.83	0.53	0.89	0.84	0.93	1.02	0.50
377	N377W	1.04	0.52	0.81	0.52	0.82	0.77	0.86	0.79	0.65
377	N377Y	0.95	0.58	0.84	0.64	0.91	0.91	1.03	1.08	0.71
379	P379A	1.02	1.01	0.97	1.13	1.03	0.90	1.17	1.02	1.15
379	P379C	0.81	0.31	0.85	0.28	0.90	0.60	0.60	0.88	0.35
379	P379E	1.01	1.36	1.17	1.34	0.97	0.84	0.89	1.07	1.27
379	P379F	0.96	0.55	0.83	0.53	0.96	0.62	0.89	0.99	0.51
379	P379G	0.90	0.91	0.95	0.85	1.10	0.83	0.82	1.02	0.80
379	P379H	0.98	1.10	0.82	1.14	1.05	1.03	1.05	1.10	1.10
379	P379I	0.63	1.02	0.18	0.98	1.08	0.84	0.93	1.09	0.90
379	P379K	1.03	1.18	0.93	1.25	1.01	1.00	0.89	1.04	1.24
379	P379L	0.95	0.76	0.89	0.77	1.03	0.98	1.00	0.95	0.76
379	P379M	1.13	0.82	0.95	0.94	1.03	0.90	0.95	0.99	0.89
379	P379Q	0.90	1.22	0.91	1.19	1.04	0.96	0.95	1.05	1.11
379	P379R	0.98	1.23	0.84	1.26	1.13	1.19	0.98	1.01	1.10
379	P379T	1.02	1.08	0.97	1.13	1.01	0.98	1.13	0.87	1.13
379	P379V	1.00	1.14	0.92	1.14	0.96	0.82	1.00	0.95	1.22

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
379	P379W	0.94	0.64	0.80	0.63	0.90	1.10	0.69	0.76	0.64
379	P379Y	0.88	0.88	0.81	0.81	1.00	1.02	0.91	1.12	0.78
381	L381A	0.94	1.09	0.65	1.12	1.02	1.21	1.22	1.04	1.23
381	L381D	1.14	0.85	0.71	0.94	1.02	0.95	0.97	0.89	1.20
381	L381E	0.97	1.06	0.78	1.01	1.03	0.91	1.11	1.31	1.31
381	L381F	0.99	0.84	0.92	0.78	1.32	0.96	0.93	0.86	0.99
381	L381G	1.02	1.00	0.83	1.02	0.97	1.05	1.09	0.97	1.21
381	L381H	0.99	1.00	0.75	1.10	1.02	1.10	1.10	0.98	1.23
381	L381I	0.94	0.85	0.77	0.86	1.05	1.06	1.17	0.93	1.14
381	L381K	1.05	0.66	0.76	0.72	0.89	0.94	0.86	0.96	1.07
381	L381M	0.98	0.99	1.08	0.96	1.00	1.00	1.10	0.97	1.31
381	L381N	0.98	1.16	0.78	1.11	1.00	1.04	0.90	0.94	1.36
381	L381P	1.03	0.43	0.58	0.42	0.75	0.20	0.73	0.62	0.52
381	L381Q	0.99	1.16	0.82	1.18	1.09	1.10	0.97	0.99	1.36
381	L381R	1.04	0.67	0.63	0.71	0.91	1.11	0.91	0.93	0.94
381	L381S	0.89	1.08	0.71	1.03	1.06	1.03	1.06	0.91	1.29
381	L381T	0.99	0.81	0.73	0.82	0.96	1.06	1.10	0.99	1.14
381	L381V	1.18	0.78	0.74	0.88	0.97	0.90	1.18	0.86	1.19
381	L381W	0.97	0.91	0.79	0.87	0.99	0.99	1.03	0.98	0.95
381	L381Y	1.10	0.88	0.86	0.95	0.97	1.01	1.11	0.90	1.35
382	K382A	0.89	0.74	0.78	0.72	1.13	0.68	0.99	0.77	0.69
382	K382D	1.01	0.68	0.86	0.69	1.12	0.67	1.29	0.85	0.60
382	K382E	0.87	0.95	0.86	0.85	0.90	0.76	1.25	0.84	0.78
382	K382F	0.98	0.44	0.90	0.38	0.87	0.47	1.08	0.79	0.41
382	K382G	1.05	0.83	0.86	0.81	1.02	0.77	1.33	0.88	0.79
382	K382H	0.70	0.62	0.92	0.75	1.01	0.69	1.14	0.86	0.75
382	K382L	0.91	0.43	0.77	0.41	0.97	0.42	0.73	0.74	0.41
382	K382M	1.04	0.56	0.94	0.61	1.09	0.78	1.06	0.70	0.54
382	K382N	0.93	0.86	0.95	0.80	1.07	0.75	1.19	0.81	0.79
382	K382P	1.09	0.51	0.84	0.53	1.01	0.51	1.21	0.77	0.62
382	K382Q	0.93	0.88	1.05	0.87	0.99	0.76	1.15	0.86	0.84
382	K382R	0.99	0.74	0.84	0.80	0.98	0.81	1.39	0.91	0.80
382	K382T	0.96	0.59	0.83	0.61	1.01	0.64	1.20	0.87	0.58
382	K382V	1.03	0.46	0.85	0.47	1.01	0.52	1.14	0.76	0.47
382	K382W	0.97	0.39	0.94	0.36	0.98	0.56	0.93	0.84	0.36
382	K382Y	0.98	0.55	0.96	0.53	0.91	0.60	1.04	0.81	0.54
393	R393A	0.97	0.90	0.56	0.93	0.87	1.03	1.10	0.85	0.75
393	R393C	0.93	0.64	0.58	0.63	0.94	0.95	1.00	0.88	0.51
393	R393D	0.95	0.72	0.60	0.71	0.85	0.91	0.99	0.83	0.62
393	R393E	0.91	0.69	0.64	0.63	0.94	0.86	1.05	0.86	0.51
393	R393F	1.06	0.43	0.62	0.46	0.91	0.75	0.96	0.82	0.36
393	R393G	1.04	0.62	0.66	0.66	0.87	0.79	0.91	0.88	0.55
393	R393H	0.93	0.97	0.61	1.03	1.03	1.05	0.81	0.96	0.85
393	R393I	1.03	0.75	0.58	0.77	1.02	1.07	1.03	0.92	0.59
393	R393K	0.89	0.97	0.78	0.98	1.00	0.94	1.11	0.96	0.85
393	R393L	0.93	0.66	0.64	0.67	0.96	0.89	1.26	0.83	0.60
393	R393M	1.22	0.68	0.71	0.76	0.81	0.76	0.94	0.88	0.67

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
393	R393N	1.00	0.97	0.62	1.01	0.92	0.98	1.00	0.87	0.85
393	R393Q	1.04	0.98	0.66	1.05	1.03	1.09	1.03	0.91	0.67
393	R393T	1.17	0.89	0.66	1.00	0.87	1.05	1.09	1.00	0.78
393	R393V	0.99	0.88	0.65	0.86	0.86	0.93	1.07	0.83	0.69
393	R393W	0.99	0.31	0.63	0.31	1.09	0.91	0.92	0.88	0.24
394	D394A	0.91	1.21	0.52	1.27	1.11	1.33	1.25	0.98	1.42
394	D394C	1.06	0.57	0.60	0.66	0.87	1.00	1.20	0.86	0.75
394	D394E	1.03	0.99	0.71	1.07	0.90	1.09	0.94	0.94	1.34
394	D394F	0.98	0.69	0.62	0.77	0.92	1.54	1.01	0.79	1.03
394	D394G	0.86	1.10	0.63	1.03	1.12	1.09	0.93	0.99	1.24
394	D394H	0.67	0.79	0.61	0.99	0.98	1.24	0.99	0.92	1.20
394	D394K	0.90	1.09	0.58	1.13	0.99	1.20	1.18	0.92	1.37
394	D394L	0.93	0.89	0.61	0.91	0.98	1.23	1.16	0.95	0.96
394	D394M	1.01	0.89	0.63	0.99	0.96	1.14	0.96	0.94	1.34
394	D394N	0.58	0.36	0.07	0.33	0.74	0.42	0.96	0.90	0.51
394	D394Q	1.05	1.07	0.65	1.18	1.11	1.20	1.04	0.91	1.37
394	D394R	0.75	0.93	0.52	0.95	1.14	1.30	1.02	1.04	1.10
394	D394S	0.92	1.00	0.57	1.15	1.05	1.22	0.94	0.99	1.40
394	D394T	0.83	1.13	0.61	1.13	1.07	1.20	1.22	0.98	1.47
394	D394V	0.84	0.91	0.66	0.91	0.92	1.17	1.11	0.85	1.23
394	D394W	0.81	0.83	0.61	0.77	0.89	1.50	1.15	0.86	1.14
399	T399A	0.98	1.03	0.72	1.07	0.96	0.85	1.12	1.02	1.15
399	T399C	1.05	0.65	0.77	0.74	0.81	0.59	1.26	0.83	0.88
399	T399E	1.07	1.10	1.16	1.10	0.95	0.75	0.77	0.94	1.21
399	T399F	1.10	0.88	1.05	0.91	1.13	0.98	1.18	0.87	0.85
399	T399G	1.15	1.03	0.93	1.15	0.94	0.77	1.05	0.91	1.21
399	T399H	1.02	0.84	1.00	0.81	1.06	0.81	0.99	1.15	0.67
399	T399I	1.06	0.89	0.67	1.00	1.09	0.77	1.20	0.98	1.01
399	T399K	1.02	1.03	0.82	1.08	0.96	0.81	0.79	0.87	1.19
399	T399L	1.12	0.82	0.92	0.93	0.88	0.83	0.88	1.02	1.05
399	T399M	1.12	0.84	1.02	0.96	0.87	0.74	1.09	0.91	1.09
399	T399P	1.09	1.01	1.22	1.06	0.93	0.83	1.09	0.97	1.20
399	T399Q	1.23	0.94	1.09	1.12	0.97	0.88	0.71	0.98	1.23
399	T399R	0.99	1.07	0.58	1.10	1.05	0.95	0.90	0.95	1.27
399	T399S	0.96	1.05	0.80	1.09	1.02	0.85	1.10	0.89	1.19
399	T399W	1.13	0.82	0.93	0.88	0.96	0.96	0.97	0.95	0.99
399	T399Y	1.08	0.91	1.03	0.95	0.95	1.28	0.68	0.93	1.03
401	R401A	1.11	0.94	0.62	1.06	0.90	0.82	1.00	1.18	0.87
401	R401C	1.05	0.72	0.71	0.84	0.94	0.56	0.96	1.14	0.74
401	R401D	1.04	0.77	0.71	0.86	0.87	0.67	1.00	1.19	0.70
401	R401E	1.10	0.96	0.76	1.10	0.77	0.83	0.99	1.14	0.94
401	R401G	1.25	1.22	0.75	1.31	0.84	0.96	0.96	1.02	1.06
401	R401H	0.94	0.70	1.55	0.96	0.81	0.81	0.71	1.13	0.84
401	R401I	0.89	0.97	0.67	1.04	0.75	0.95	0.82	1.05	0.89
401	R401K	1.05	1.05	0.72	1.10	0.77	0.92	1.00	1.15	0.99
401	R401L	1.28	1.04	0.72	1.21	0.76	0.83	1.07	1.25	1.02
401	R401M	1.24	0.90	0.80	1.04	0.74	0.73	1.02	1.17	0.91

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
401	R401N	1.26	0.90	1.00	1.03	0.90	0.67	0.88	1.17	0.89
401	R401Q	1.16	0.95	0.81	1.14	0.78	0.91	0.98	1.07	0.93
401	R401S	0.99	0.96	0.70	1.04	0.79	0.93	0.90	1.03	0.87
401	R401T	1.04	0.99	0.77	1.10	0.78	0.87	0.95	1.20	0.93
401	R401W	1.05	0.81	1.08	0.81	0.82	0.89	1.02	1.25	0.64
401	R401Y	1.26	0.69	1.22	0.86	0.77	0.89	0.86	1.07	0.69
407	Q407A	0.92	1.03	0.73	1.06	1.05	0.81	1.32	1.07	0.91
407	Q407C	1.09	0.81	0.80	0.90	0.82	0.52	1.21	1.04	0.81
407	Q407D	1.11	0.83	0.80	0.89	1.04	0.65	1.71	1.19	0.81
407	Q407E	1.05	0.93	1.17	0.99	0.76	0.80	1.20	0.89	0.91
407	Q407F	1.07	0.88	0.73	0.87	0.76	1.22	1.01	0.86	0.72
407	Q407G	1.03	0.80	0.74	0.86	0.93	0.95	1.42	1.07	0.72
407	Q407I	0.95	0.89	0.62	0.88	0.88	1.02	0.93	0.96	0.80
407	Q407K	0.98	0.98	0.73	1.01	1.05	0.99	1.54	1.20	0.83
407	Q407M	1.13	0.77	0.80	0.87	0.84	0.90	1.19	0.97	0.75
407	Q407N	1.06	0.97	0.77	0.95	0.90	1.02	1.24	1.05	0.78
407	Q407P	1.06	0.96	1.36	0.98	0.81	0.83	1.32	1.05	0.88
407	Q407R	1.11	0.93	0.63	1.03	0.83	1.06	1.19	0.83	0.85
407	Q407S	1.01	0.77	0.73	0.80	0.88	0.85	0.73	0.94	0.70
407	Q407T	1.06	0.64	0.70	0.71	1.01	1.02	1.25	1.14	0.54
407	Q407V	1.01	0.85	0.75	0.87	0.87	1.12	1.27	1.00	0.67
407	Q407W	1.16	0.78	0.81	0.85	0.80	1.26	1.25	0.92	0.71
408	D408A	0.89	0.16	0.62	0.16	1.04	0.43	1.01	1.86	0.20
408	D408C	0.65	0.16	0.58	0.14	0.90	0.75	0.90	1.68	0.17
408	D408H	0.82	0.44	0.70	0.56	1.15	0.71	1.37	1.32	0.70
408	D408N	1.08	0.80	0.79	0.85	1.08	1.08	1.45	1.10	1.12
408	D408Q	0.97	0.21	0.73	0.22	0.99	0.64	0.94	1.39	0.30
408	D408S	0.78	0.46	0.74	0.40	1.06	0.78	1.09	1.03	0.53
408	D408T	0.99	0.13	0.70	0.14	0.99	0.61	0.78	1.69	0.18
419	T419A	0.99	0.91	0.98	1.04	0.96	0.93	0.99	0.90	1.00
419	T419C	1.06	0.84	1.10	0.98	1.02	1.68	1.03	0.94	0.84
419	T419D	1.06	0.73	1.19	0.81	0.94	0.91	1.04	0.93	0.82
419	T419E	1.20	0.82	1.37	0.99	1.00	0.88	1.04	0.92	0.97
419	T419F	1.06	0.77	0.86	0.81	0.96	0.90	1.06	0.93	0.69
419	T419G	1.09	0.99	0.98	1.09	0.96	0.87	1.01	0.79	0.96
419	T419H	1.10	0.93	0.89	0.99	0.96	0.85	0.89	0.98	0.89
419	T419I	1.05	0.78	0.81	0.88	1.04	1.01	1.26	1.04	0.74
419	T419K	1.01	1.05	0.86	1.06	1.16	0.97	1.03	0.99	0.90
419	T419L	1.12	0.83	0.93	0.91	1.06	1.57	1.02	0.92	0.73
419	T419M	1.13	0.84	0.98	0.93	1.04	0.94	1.14	0.90	0.85
419	T419N	1.13	1.08	0.98	1.10	0.96	0.89	0.91	0.89	1.11
419	T419P	1.08	0.89	0.88	0.98	1.01	1.02	1.19	0.85	0.91
419	T419Q	1.01	1.04	1.00	1.04	0.96	0.86	0.77	0.94	0.98
419	T419R	0.95	0.96	0.78	1.00	1.09	1.12	1.03	0.96	0.88
419	T419S	1.09	1.32	1.03	1.42	1.08	1.19	1.18	0.96	1.20
419	T419V	1.07	0.85	0.96	0.92	1.09	1.09	1.01	0.94	0.76
419	T419W	1.20	0.78	0.83	0.84	0.98	0.90	0.97	0.90	0.91

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
419	T419Y	1.03	0.86	0.85	0.87	0.95	0.98	1.08	0.83	0.82
433	P433A	0.84	1.06	1.07	1.01	1.12	0.70	1.16	1.02	0.89
433	P433D	0.93	1.01	1.13	0.94	1.05	0.81	1.39	1.30	0.83
433	P433E	0.80	1.12	1.32	0.96	0.90	0.40	1.31	1.21	0.88
433	P433H	0.87	0.97	0.80	0.90	1.03	0.69	0.88	1.06	0.76
433	P433I	0.82	1.05	1.18	0.96	1.12	0.57	1.22	1.36	0.82
433	P433K	0.84	1.01	0.95	0.96	1.06	0.68	0.91	1.17	0.92
433	P433L	0.77	1.07	0.99	0.91	1.06	0.52	0.97	1.14	0.85
433	P433M	0.72	0.68	1.13	0.88	1.05	0.55	1.24	1.23	0.83
433	P433N	0.94	1.12	0.92	1.05	0.95	0.70	1.05	1.23	0.88
433	P433Q	0.83	1.12	0.97	1.00	1.05	0.59	1.15	1.24	0.90
433	P433R	0.76	1.08	0.90	0.95	1.06	1.00	0.69	1.00	0.90
433	P433S	0.79	1.01	0.96	0.95	1.19	0.76	1.14	1.14	0.83
433	P433T	0.89	0.99	0.98	0.93	1.10	0.59	1.13	1.29	0.83
433	P433V	0.87	1.04	1.22	0.98	1.16	0.53	1.23	1.42	0.74
433	P433W	0.85	0.89	0.81	0.77	1.04	0.87	0.95	0.95	0.57
436	S436A	0.86	0.86	0.88	0.92	0.97	0.83	1.01	0.96	0.76
436	S436C	0.77	0.85	0.94	0.84	0.87	0.70	0.85	0.83	0.74
436	S436D	0.97	0.81	0.89	0.89	0.87	0.65	0.60	0.90	0.79
436	S436E	1.02	0.86	1.02	0.92	0.82	0.72	0.81	0.83	0.78
436	S436F	1.02	0.88	0.96	0.92	0.95	0.81	0.94	0.89	0.77
436	S436G	0.96	0.94	1.00	0.93	0.89	0.86	1.06	0.88	0.76
436	S436H	0.91	0.26	0.72	0.27	0.99	1.02	0.92	0.93	0.19
436	S436I	0.86	0.90	0.80	0.92	0.99	0.94	1.06	0.82	0.75
436	S436K	0.82	1.32	0.79	1.30	0.90	1.05	0.93	0.93	0.88
436	S436L	0.82	0.79	0.87	0.77	0.91	0.92	1.05	0.97	0.62
436	S436M	0.91	0.85	0.96	0.86	0.79	0.90	0.94	0.98	0.70
436	S436N	0.92	1.02	1.03	0.97	0.93	1.25	1.04	0.90	0.82
436	S436P	0.93	0.96	0.92	0.97	0.87	0.95	1.04	0.92	0.69
436	S436Q	0.98	0.93	1.00	0.95	1.00	0.90	0.72	0.91	0.76
436	S436R	0.76	0.93	0.76	0.90	1.02	1.06	0.86	0.97	0.68
436	S436T	1.02	0.87	1.03	0.91	0.80	0.82	1.06	0.91	0.80
436	S436V	0.94	0.99	0.99	0.97	0.95	0.85	0.87	0.86	0.82
436	S436W	1.00	0.79	0.90	0.84	0.98	0.86	0.88	0.88	0.67
436	S436Y	0.74	0.15	0.91	0.14	0.97	0.79	0.71	0.64	0.13
438	W438C	0.97	1.00	1.14	1.00	1.01	0.35	1.12	1.00	1.01
438	W438F	0.98	1.09	1.01	1.06	0.97	0.61	1.12	1.07	1.03
438	W438G	1.00	1.05	0.83	1.11	0.97	0.51	1.03	1.22	1.12
438	W438H	1.04	1.04	0.84	1.14	0.99	0.57	1.09	1.16	1.17
438	W438I	0.93	0.93	0.99	0.99	1.01	0.59	1.14	1.13	0.97
438	W438K	0.94	0.97	0.97	1.04	0.96	0.50	1.12	1.08	1.09
438	W438L	0.99	0.95	0.91	1.02	0.99	0.38	1.23	1.05	1.02
438	W438M	0.94	1.01	1.02	1.03	0.93	0.52	1.19	1.08	1.11
438	W438N	1.05	1.08	0.95	1.11	1.07	0.54	1.23	1.08	1.12
438	W438P	1.05	1.02	0.88	1.09	1.09	0.61	0.99	1.13	1.12
438	W438Q	4.28	0.80	0.82	1.19	1.14	0.55	1.16	1.13	1.20
438	W438R	0.90	1.09	0.93	1.09	0.94	0.79	1.05	1.26	1.03

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
438	W438S	0.94	1.01	0.93	1.08	0.96	0.57	1.12	1.19	1.13
438	W438T	0.96	1.18	0.97	1.17	1.10	0.64	1.18	1.15	1.17
438	W438V	0.95	1.03	0.96	1.04	0.97	0.45	0.89	1.14	1.04
438	W438Y	1.00	0.96	0.93	1.04	1.06	0.57	1.16	1.17	1.00
444	K444C	1.14	0.89	0.84	0.99	0.90	0.74	0.82	0.98	1.20
444	K444D	1.03	1.03	0.90	1.02	0.94	0.79	0.81	1.14	1.17
444	K444F	1.18	0.95	0.89	1.05	0.92	0.97	0.93	1.04	1.13
444	K444G	1.10	1.01	0.95	1.08	0.96	0.87	0.96	1.01	1.21
444	K444H	0.91	0.88	0.98	0.85	0.99	0.81	0.84	0.97	0.97
444	K444I	1.02	1.05	0.85	1.06	0.96	0.93	0.95	1.09	1.18
444	K444L	1.18	0.82	0.87	0.99	0.93	0.86	0.94	1.03	1.26
444	K444M	1.01	1.01	0.97	1.03	0.86	1.02	1.15	1.07	1.07
444	K444N	1.20	0.98	1.03	1.10	0.89	0.94	0.85	1.02	1.27
444	K444P	1.23	0.92	0.91	1.10	0.90	0.94	0.98	1.01	1.31
444	K444Q	1.14	0.92	1.01	1.08	0.95	1.01	1.02	0.99	1.23
444	K444R	1.05	0.81	1.13	0.92	1.00	1.01	0.92	1.03	1.07
444	K444S	1.16	0.89	0.94	1.01	0.94	0.90	1.04	1.05	1.20
444	K444T	1.10	0.91	0.98	0.99	0.94	0.88	0.74	1.00	1.14
444	K444V	1.17	0.87	0.96	0.99	1.01	0.83	0.97	0.99	1.12
444	K444W	1.13	0.98	0.92	0.99	0.91	0.90	1.13	1.07	1.03
444	K444Y	1.21	0.89	0.90	1.04	0.99	1.03	1.04	1.09	1.08
447	G447A	1.06	0.99	0.75	1.06	1.03	1.11	0.91	1.20	1.01
447	G447C	1.09	0.97	0.87	0.93	0.92	0.79	0.86	0.93	0.98
447	G447D	1.09	1.02	0.85	1.00	0.98	0.81	0.76	0.89	1.08
447	G447F	1.10	0.94	0.87	0.88	0.97	1.00	0.89	0.90	0.95
447	G447H	1.26	0.96	0.83	1.02	1.10	0.96	0.86	1.03	1.01
447	G447I	1.13	0.69	0.67	0.67	1.02	0.86	0.79	1.08	0.67
447	G447K	1.07	0.94	0.77	0.92	1.03	1.12	0.74	0.83	0.98
447	G447L	1.18	0.95	0.79	0.92	1.02	0.94	0.79	0.86	1.03
447	G447M	1.23	0.89	0.86	0.91	0.98	0.93	0.77	0.87	1.03
447	G447N	1.16	1.14	0.95	1.06	1.10	0.99	0.96	0.96	1.17
447	G447P	1.18	0.80	0.73	0.79	0.99	0.85	0.78	0.95	0.88
447	G447Q	0.90	0.73	0.76	1.01	1.15	1.05	0.77	1.05	0.99
447	G447R	0.93	0.59	0.80	0.60	0.94	1.10	0.77	1.04	0.58
447	G447S	1.13	0.77	0.54	0.82	1.05	1.08	0.64	0.93	0.95
447	G447T	1.12	1.01	0.79	0.97	1.02	0.96	0.84	0.85	1.05
447	G447V	1.07	0.46	0.84	0.48	0.68	0.67	0.73	0.83	0.79
447	G447W	1.16	1.03	0.80	0.98	1.01	1.02	0.88	0.97	1.05
447	G447Y	1.10	1.03	0.83	0.98	0.91	0.93	0.84	1.07	1.02
448	K448A	0.92	1.27	0.61	1.23	0.92	1.06	1.13	1.03	1.29
448	K448C	0.90	1.42	0.59	1.33	0.94	1.11	1.10	0.97	1.26
448	K448D	0.98	1.17	0.68	1.14	0.97	1.11	0.96	1.01	1.19
448	K448E	1.15	1.01	0.79	1.12	0.96	1.03	0.99	1.08	1.23
448	K448F	1.10	1.02	0.70	1.04	0.97	0.93	1.00	1.39	1.23
448	K448G	1.14	1.16	0.71	1.29	0.95	0.96	1.01	1.11	1.32
448	K448H	0.98	1.15	0.70	1.13	0.89	0.98	1.05	0.85	1.23
448	K448L	1.03	0.88	0.66	0.90	0.98	1.01	0.72	1.03	0.88

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
448	K448N	1.02	1.32	0.73	1.22	0.95	1.04	1.05	0.96	1.34
448	K448P	0.95	0.24	0.69	0.25	0.87	0.84	0.81	0.98	0.29
448	K448Q	0.97	1.09	0.77	1.09	0.90	0.97	1.05	1.03	1.15
448	K448R	0.85	0.85	0.66	0.90	0.96	1.37	0.93	1.01	0.98
448	K448S	1.05	1.15	0.67	1.14	0.82	1.02	1.01	0.98	1.16
448	K448T	1.12	1.03	0.70	1.11	0.93	0.90	1.21	1.01	1.17
448	K448V	1.08	1.03	0.70	1.05	0.89	0.95	1.14	0.97	1.11
448	K448W	1.03	1.03	0.68	0.99	0.94	0.97	1.35	1.15	0.96
451	Y451A	0.99	1.09	0.63	1.03	0.92	1.03	1.15	1.02	0.92
451	Y451C	1.10	0.94	0.69	1.02	0.85	1.04	1.12	0.95	0.88
451	Y451D	0.97	1.05	0.69	1.00	1.03	0.84	0.99	0.91	1.00
451	Y451E	1.14	1.06	0.76	1.07	0.97	0.90	1.29	0.90	1.06
451	Y451F	1.03	1.07	0.94	0.94	0.94	0.94	1.06	0.90	0.93
451	Y451G	1.08	1.06	0.77	1.04	0.94	0.88	0.87	0.90	1.04
451	Y451K	1.15	0.98	0.71	1.08	1.00	1.00	1.08	0.95	1.06
451	Y451L	1.09	1.04	0.62	1.02	1.00	0.91	1.02	0.90	1.08
451	Y451M	1.14	0.98	0.83	0.96	0.98	0.85	1.23	0.98	0.89
451	Y451N	1.14	1.27	0.75	1.16	1.12	1.04	1.26	0.96	1.04
451	Y451P	1.18	0.22	0.69	0.24	1.01	0.72	1.25	0.95	0.25
451	Y451Q	1.09	1.12	0.77	1.14	1.00	0.94	1.16	0.97	1.10
451	Y451R	0.97	0.98	0.66	1.02	1.10	1.14	1.22	0.99	1.00
451	Y451S	0.99	1.02	0.69	1.04	0.90	0.89	1.24	0.92	1.04
451	Y451T	1.17	0.96	0.75	1.01	0.87	1.07	1.18	0.98	0.98
451	Y451V	1.07	1.07	0.82	1.00	1.06	1.03	1.14	0.84	0.93
453	L453A	1.16	1.11	0.74	1.31	1.11	1.08	0.91	1.15	1.40
453	L453C	0.95	1.20	0.81	1.20	1.14	0.92	1.01	1.13	1.25
453	L453D	0.99	1.01	0.82	1.07	1.04	1.02	0.90	1.00	1.21
453	L453E	0.96	1.23	0.80	1.22	0.92	0.92	0.80	0.93	1.70
453	L453F	1.04	1.11	0.97	1.10	0.93	1.00	0.91	0.98	1.41
453	L453G	1.18	0.94	0.80	1.10	1.18	1.09	0.87	1.07	1.29
453	L453H	0.97	0.88	0.90	0.93	1.04	1.15	0.89	1.02	1.07
453	L453I	0.93	1.04	0.87	1.06	1.14	1.07	1.02	1.15	1.08
453	L453K	0.92	1.44	0.81	1.34	1.16	0.92	0.94	1.00	1.47
453	L453M	1.16	0.19	0.63	0.20	0.28	0.25	0.38	0.28	0.21
453	L453N	1.10	1.49	0.80	1.55	1.03	1.00	1.23	1.21	1.49
453	L453P	1.06	0.40	0.80	0.48	0.94	0.72	0.90	1.14	0.54
453	L453Q	1.00	1.29	0.78	1.34	1.11	1.12	0.95	1.11	1.32
453	L453R	1.00	1.46	0.73	1.43	1.19	1.21	1.12	1.10	1.47
453	L453S	0.97	1.26	0.79	1.28	0.97	0.86	1.00	1.13	1.29
453	L453T	0.89	1.08	0.70	1.12	1.13	1.04	0.95	1.06	1.26
453	L453V	1.06	1.10	0.84	1.14	1.15	1.06	0.95	1.10	1.17
453	L453W	1.17	0.98	0.99	1.03	1.11	1.06	0.71	1.10	1.13
453	L453Y	0.98	1.14	0.91	1.09	1.06	1.11	0.96	1.08	1.16
459	D459A	0.86	0.86	0.63	0.94	1.01	1.07	1.01	0.94	1.01
459	D459C	1.00	1.04	0.65	1.13	1.07	0.82	0.84	0.96	1.18
459	D459E	1.01	1.16	0.75	1.27	1.05	0.88	1.04	0.97	1.40
459	D459F	0.99	0.95	0.73	1.01	0.89	0.95	0.99	0.94	1.12

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNG7 initial	THER STAB Pi	(THER) BPNG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
459	D459G	0.92	1.42	0.70	1.58	1.14	1.08	1.16	0.92	1.57
459	D459H	1.01	1.15	0.71	1.25	1.08	1.10	0.94	1.02	1.31
459	D459I	0.99	1.00	0.58	1.14	1.11	1.00	0.90	0.95	1.18
459	D459K	1.03	1.07	0.63	1.20	0.83	0.98	0.62	0.77	1.38
459	D459L	0.95	0.96	0.59	1.05	1.03	1.00	0.85	0.87	1.12
459	D459M	0.87	1.17	0.70	1.14	0.96	1.01	0.55	0.91	1.30
459	D459N	1.04	1.25	0.72	1.34	1.07	0.97	0.91	0.90	1.51
459	D459P	0.92	1.10	0.72	1.15	1.16	0.95	0.75	0.96	1.31
459	D459Q	1.08	1.09	0.72	1.28	1.03	1.00	0.85	0.87	1.40
459	D459R	0.94	1.29	0.56	1.31	0.90	1.25	0.86	0.67	1.39
459	D459S	0.90	1.20	0.65	1.22	1.02	1.05	0.88	0.92	1.37
459	D459T	1.01	1.08	0.68	1.17	1.11	0.97	0.79	0.96	1.28
459	D459V	1.06	1.10	0.69	1.21	1.07	0.95	0.67	0.87	1.32
459	D459W	0.93	0.92	0.70	0.94	0.90	1.00	0.70	0.72	1.46
459	D459Y	0.95	0.84	0.71	0.87	1.06	0.88	0.89	0.90	1.00
465	A465C	1.00	0.88	1.06	0.84	0.94	0.85	1.19	1.15	0.82
465	A465F	1.10	1.10	0.87	1.01	1.06	1.00	0.89	1.18	1.03
465	A465G	1.19	0.94	0.94	1.05	0.90	1.01	1.11	1.02	1.07
465	A465H	1.24	0.69	0.46	0.66	0.61	0.19	1.20	1.21	0.95
465	A465I	1.08	0.90	0.86	0.92	0.99	0.92	0.95	1.19	0.95
465	A465K	1.07	0.86	0.97	0.88	0.88	0.94	0.98	1.08	0.99
465	A465L	1.16	0.88	0.86	0.94	0.89	0.91	1.07	1.16	0.97
465	A465M	1.17	0.88	1.00	0.93	0.97	0.92	1.08	1.15	1.01
465	A465N	1.07	1.01	1.02	0.99	0.98	0.93	1.04	1.17	1.08
465	A465P	1.11	1.13	0.93	1.17	0.95	0.94	0.92	1.17	1.23
465	A465Q	1.07	0.96	0.92	1.03	0.93	0.92	1.10	1.13	1.07
465	A465S	1.05	0.89	1.02	0.89	1.03	1.13	1.17	1.24	0.82
465	A465T	1.07	0.94	0.96	0.91	0.92	0.95	1.08	1.19	0.98
465	A465V	1.17	0.85	0.91	0.91	0.94	0.96	0.94	1.10	0.98
465	A465W	1.08	0.99	0.86	0.99	1.00	1.09	1.01	1.19	0.97
465	A465Y	1.09	1.01	0.85	1.03	1.10	1.04	1.17	1.09	1.10
470	E470A	0.82	1.02	0.61	1.02	1.10	1.07	0.90	0.92	1.17
470	E470C	1.01	0.87	1.33	0.94	1.04	0.73	0.98	1.00	0.93
470	E470D	0.88	1.02	1.04	0.96	1.05	0.82	0.91	0.89	1.04
470	E470F	1.07	0.91	0.87	0.96	1.07	1.00	1.07	1.02	0.88
470	E470G	1.09	0.88	0.83	0.95	1.07	1.30	0.81	0.83	1.08
470	E470H	1.07	0.96	0.92	1.01	1.05	1.08	0.81	0.85	1.19
470	E470I	0.91	0.96	0.86	1.00	1.12	0.94	1.02	0.93	1.03
470	E470K	1.01	1.10	0.88	1.08	0.97	0.99	0.80	0.65	1.29
470	E470L	1.01	0.87	0.86	0.95	1.03	1.06	0.73	0.67	0.96
470	E470N	1.08	0.97	0.95	0.97	1.02	1.30	0.57	0.84	1.13
470	E470P	0.99	1.05	0.81	1.03	1.04	1.07	0.88	0.80	1.20
470	E470Q	0.97	1.06	0.84	1.03	0.96	1.13	0.87	0.84	1.19
470	E470R	0.87	1.11	0.88	1.04	0.89	1.29	0.86	0.77	1.22
470	E470S	0.97	0.96	0.88	0.98	1.05	0.75	0.75	0.88	1.08
470	E470T	0.95	0.94	0.89	0.98	0.98	0.76	0.99	1.05	1.08
470	E470W	1.06	0.91	0.88	0.96	0.99	1.42	0.85	1.02	1.00

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)

Position	Variant	DET STAB Pi	(DET) BPNPG7 initial	THER STAB Pi	(THER) BPNPG7 initial	CS28 pH10 32 Pi	CS28 pH10 50 Pi	CS28 pH 8 16 Pi	CS28 pH 8 32 Pi	HPLC PROT Pi
470	E470Y	1.05	0.95	0.89	1.02	0.98	1.23	0.82	0.85	1.09
475	G475A	0.89	1.30	1.24	1.11	1.14	0.41	1.48	1.52	1.20
475	G475C	1.04	0.92	1.12	0.88	0.87	0.09	1.90	1.40	0.96
475	G475D	1.00	1.16	1.75	1.09	0.98	0.23	1.39	1.48	1.24
475	G475E	1.01	1.05	1.24	1.00	0.90	0.11	1.76	1.41	1.00
475	G475F	1.04	0.93	0.92	0.89	1.08	0.21	2.02	1.78	0.83
475	G475H	1.02	1.14	0.92	1.13	1.17	0.26	1.96	1.45	1.29
475	G475I	1.03	0.89	0.84	0.86	1.20	0.17	1.77	1.80	0.80
475	G475K	0.95	1.25	0.94	1.12	1.15	0.28	1.93	1.51	1.36
475	G475L	0.95	0.89	0.80	0.82	1.17	0.22	1.97	1.66	0.82
475	G475N	1.04	1.26	1.17	1.13	1.13	0.44	1.92	1.65	1.09
475	G475P	1.02	1.05	1.09	1.03	1.12	0.40	1.53	1.36	1.16
475	G475Q	1.05	1.19	0.97	1.16	1.08	0.22	1.94	1.59	1.22
475	G475R	0.90	1.33	0.92	1.16	1.28	0.41	1.82	1.58	1.35
475	G475S	0.87	1.29	1.19	1.08	1.21	0.32	1.56	1.48	1.20
475	G475T	1.05	1.16	1.12	1.13	1.14	0.24	2.09	1.70	1.22
475	G475V	0.96	1.00	0.87	0.93	1.08	0.15	1.76	1.77	0.89
475	G475W	1.05	0.95	0.87	0.90	1.24	0.37	1.77	1.76	0.77
476	G476A	0.72	1.00	1.19	0.86	1.07	0.33	2.52	1.49	1.03
476	G476C	0.79	0.40	1.42	0.33	0.52	0.24	3.02	2.48	0.31
476	G476D	0.75	1.64	0.84	1.36	1.00	0.34	2.69	1.62	1.32
476	G476H	0.71	1.02	0.99	0.80	0.98	0.24	2.65	1.65	0.85
476	G476I	0.55	0.59	1.04	0.48	0.83	0.19	1.87	2.10	0.53
476	G476K	0.76	0.96	1.32	0.76	1.22	0.35	1.83	1.70	0.82
476	G476L	0.75	0.80	1.05	0.64	0.84	0.26	3.83	2.01	0.56
476	G476M	0.89	0.69	1.35	0.67	0.88	0.28	3.25	1.95	0.68
476	G476N	0.87	1.18	1.09	1.03	1.11	0.22	2.83	1.83	1.17
476	G476P	0.80	1.35	0.91	1.14	1.18	0.39	2.13	1.59	1.21
476	G476Q	0.77	1.22	1.34	1.04	1.08	0.34	2.67	1.64	1.24
476	G476T	0.80	1.21	1.26	1.05	1.08	0.28	2.90	1.76	1.15
476	G476V	0.81	1.01	1.00	0.91	0.93	0.23	2.68	1.68	1.55
476	G476W	0.77	0.28	1.15	0.24	0.86	0.37	2.58	2.33	0.25
476	G476Y	1.05	0.49	1.14	0.45	0.97	0.19	4.48	2.24	0.38
483	A483C	0.91	1.28	0.84	1.04	0.98	0.82	1.34	1.06	1.12
483	A483D	0.97	1.16	0.82	1.05	0.99	0.83	0.97	0.77	1.38
483	A483E	1.03	1.18	0.95	1.03	1.00	0.84	0.89	1.00	1.09
483	A483F	1.11	1.21	0.92	1.09	0.97	0.88	0.86	1.04	1.33
483	A483G	1.17	1.10	1.01	1.11	0.97	0.89	0.89	1.09	1.35
483	A483H	0.93	0.70	0.91	0.62	0.90	0.70	0.88	1.03	0.75
483	A483I	1.04	1.08	0.70	1.04	1.04	0.98	1.02	1.13	1.14
483	A483K	1.08	1.09	0.80	1.03	0.96	0.88	1.05	1.03	1.29
483	A483M	1.12	0.95	0.93	0.90	0.90	0.83	0.97	0.98	1.15
483	A483N	0.96	0.81	0.99	0.79	0.94	0.80	0.76	0.99	1.05
483	A483P	1.20	0.94	1.31	0.93	0.93	0.83	0.98	1.02	1.26
483	A483Q	1.02	1.07	1.05	0.96	0.98	0.84	0.93	1.04	1.20
483	A483R	0.85	1.07	0.73	0.94	0.93	1.04	1.03	1.10	1.02
483	A483S	1.04	0.96	0.98	0.92	0.95	0.83	0.84	1.03	1.33

Table 7-1 Performance Indices of BASE Variants (3,408 alpha-amylases)										
Position	Variant	DET STAB PI	(DET) BPNPG7 initial	THER STAB PI	(THER) BPNPG7 initial	CS28 pH10 32 PI	CS28 pH10 50 PI	CS28 pH 8 16 PI	CS28 pH 8 32 PI	HPLC PROT PI
483	A483T	1.04	1.06	0.90	0.94	0.96	0.88	1.13	1.38	1.10
483	A483V	1.11	1.12	0.86	1.04	0.95	1.03	1.09	1.09	1.28
483	A483W	1.09	1.03	0.86	1.00	0.95	0.87	1.03	1.00	1.71
483	A483Y	1.03	1.13	0.85	1.10	0.96	0.90	0.81	1.17	1.38
484	K484A	0.85	0.95	0.78	0.99	0.77	0.93	1.02	0.92	0.87
484	K484C	0.78	0.94	0.84	0.93	0.78	0.76	0.90	0.87	0.81
484	K484D	0.81	1.13	0.80	1.11	0.80	0.99	1.20	0.90	0.95
484	K484E	0.85	1.07	0.88	1.08	0.83	0.91	1.06	0.81	0.99
484	K484F	0.92	0.77	0.89	0.78	0.85	0.90	0.89	0.89	0.68
484	K484G	0.96	0.96	0.98	1.03	0.92	0.97	1.08	0.93	0.87
484	K484H	0.70	0.91	0.99	0.90	0.91	0.80	0.87	0.87	0.86
484	K484I	0.74	0.90	0.68	0.94	1.01	1.13	1.04	1.12	0.65
484	K484L	0.88	0.72	0.86	0.76	0.77	0.85	0.83	0.96	0.63
484	K484M	0.88	0.82	0.98	0.86	0.86	0.80	0.95	0.86	0.75
484	K484N	0.95	1.07	0.90	1.06	0.94	0.81	1.12	0.87	0.93

[00363] The other 821 mutations of the BASE SEL members present in Table 3-2, but absent in Table 7-1 are less suitable for inclusion in combinatorial variants of a parent alpha-amylase. Likewise residues of naturally occurring alpha-amylases at positions corresponding to SEQ ID NO: 2 that are identical to the 821 non-combinable mutations are contemplated to be present in underperforming naturally occurring alpha-amylases, and thus are candidates for mutagenesis. As such, the present disclosure provides a detailed recipe for producing variant alpha-amylases that have a desired set of properties

EXAMPLE 8

Restrictive and Non-Restrictive Positions in Alpha-Amylases

[00364] Results of experiments conducted to determine wash performance (CS-28 microswatch assay at pH 10/32°C, pH 10/50°C, pH 8/16°C and pH 8/32°C), detergent stability, thermostability, BPNPG7 amylase activity and HPLC protein concentration (tests of properties of interest) of BASE and variants thereof are described. The results were obtained using the methods described in Example 1. As described throughout, functionality of BASE variants was quantified as a performance index (PI), which is the ratio of performance of a variant to a parent or reference amylase. Various terms set forth below are used to describe the mutation: up mutations have a $PI > 1$; neutral mutations have a $PI > 0.5$, non-deleterious mutations have a $PI > 0.05$; deleterious mutations have a $PI \leq 0.05$; combinable mutations are those mutations for which the variant has $PI \geq 0.5$ for at least one property, and >0.05 for all properties. Combinable mutations are mutations that can be combined to deliver proteins with appropriate PIs for one or more desired properties.

[00365] Positions at which mutations occur are classed as follows: non-restrictive positions have $\geq 20\%$ neutral mutations for at least one property; and restrictive positions have $< 20\%$ neutral mutations for activity and stability. Table 8-1 shows the restrictive positions where less than 20% neutral mutations (PI>0.5) for activity and stability were detected. Table 8-2 shows the non-restrictive positions where $\geq 20\%$ neutral mutations (PI>0.5) for at least one property tested were detected. % = percent of variants evaluated that meet definition of neutral mutation.

Table 8-1 Restrictive Positions < 20% Neutral Mutations for Activity and Stability

Position	# mutants	Detergent stability PI > 0.5	Detergent initial PI %>0.5	Thermostability PI > 0.5	Thermostability initial PI > 0.5	CS28 pH10 32°C PI > 0.5	CS28 pH10 50°C PI > 0.5	CS28 pH 8 16°C PI > 0.5	CS28 pH 8 32°C PI > 0.5	HPLC Protein PI > 0.5
58	19	11	5	0	5	11	5	0	5	84
236	18	6	0	6	0	6	6	0	0	100

Table 8-2 Non-restrictive Positions $\geq 20\%$ Neutral Mutations for at least One Property

Position	# mutants	Detergent stability PI > 0.5	Detergent initial PI %>0.5	Thermostability PI > 0.5	Thermostability initial PI > 0.5	CS28 pH10 32°C PI > 0.5	CS28 pH10 50°C PI > 0.5	CS28 pH 8 16°C PI > 0.5	CS28 pH 8 32°C PI > 0.5	HPLC Protein PI > 0.5
1	19	74%	53%	74%	0%	84%	84%	84%	74%	68%
2	15	100%	87%	100%	87%	100%	100%	93%	100%	93%
3	19	95%	89%	95%	89%	95%	95%	95%	95%	74%
4	16	100%	94%	100%	94%	100%	100%	100%	100%	81%
5	16	100%	100%	100%	100%	100%	100%	100%	100%	100%
7	19	100%	100%	100%	95%	95%	100%	100%	100%	100%
15	19	58%	11%	47%	11%	11%	0%	42%	63%	89%
16	18	100%	6%	78%	11%	94%	67%	89%	94%	39%
17	19	100%	89%	58%	100%	100%	89%	100%	100%	95%
18	18	100%	89%	89%	89%	100%	100%	94%	100%	94%
19	19	95%	42%	26%	58%	95%	95%	95%	95%	89%
22	18	94%	94%	89%	94%	94%	94%	94%	94%	94%
25	18	100%	94%	100%	94%	100%	100%	100%	89%	100%
26	19	100%	84%	95%	89%	100%	89%	100%	100%	79%
28	18	89%	89%	89%	89%	89%	94%	89%	89%	83%
29	18	94%	83%	94%	94%	94%	94%	94%	94%	89%
30	17	94%	53%	94%	53%	100%	82%	100%	94%	53%
32	16	100%	100%	100%	100%	100%	100%	100%	100%	100%
35	17	100%	71%	94%	71%	100%	100%	100%	100%	71%
36	18	94%	83%	94%	83%	94%	94%	94%	94%	89%
37	18	100%	89%	100%	89%	100%	100%	100%	100%	78%
50	19	63%	42%	11%	42%	37%	21%	53%	74%	79%
51	17	94%	29%	71%	29%	76%	65%	100%	88%	88%
52	19	100%	89%	68%	89%	95%	79%	100%	100%	100%

Table 8-2 Non-restrictive Positions $\geq$ 20% Neutral Mutations for at least One Property

Position	# mutants	Detergent stability PI > 0.5	Detergent initial PI %>0.5	Thermostability PI > 0.5	Thermostability initial PI > 0.5	CS28 pH10 32°C PI > 0.5	CS28 pH10 50°C PI > 0.5	CS28 pH 8 16°C PI > 0.5	CS28 pH 8 32°C PI > 0.5	HPLC Protein PI > 0.5
53	19	74%	68%	21%	79%	89%	95%	95%	89%	84%
54	19	95%	95%	84%	95%	95%	95%	100%	100%	89%
55	17	71%	6%	0%	6%	35%	24%	41%	82%	100%
56	19	95%	53%	42%	53%	74%	68%	95%	95%	95%
57	16	13%	6%	0%	6%	13%	6%	25%	56%	19%
59	18	28%	11%	22%	11%	17%	0%	22%	50%	89%
60	19	84%	63%	53%	74%	95%	89%	95%	100%	84%
70	19	100%	100%	100%	100%	100%	100%	100%	100%	95%
71	19	100%	95%	11%	95%	100%	95%	100%	100%	63%
72	16	94%	94%	0%	94%	94%	50%	94%	94%	94%
73	19	84%	79%	58%	79%	84%	84%	79%	84%	74%
75	18	100%	100%	94%	100%	100%	100%	100%	100%	100%
78	19	79%	58%	0%	53%	68%	21%	84%	84%	53%
82	18	100%	94%	100%	89%	100%	100%	100%	100%	94%
83	19	100%	100%	100%	100%	100%	100%	100%	100%	100%
87	19	95%	95%	95%	95%	95%	84%	95%	95%	100%
90	19	100%	95%	100%	95%	100%	100%	100%	100%	95%
91	18	100%	94%	100%	94%	100%	100%	89%	100%	94%
93	16	88%	31%	75%	31%	88%	88%	88%	94%	6%
94	19	89%	74%	89%	74%	84%	79%	84%	89%	79%
95	18	100%	94%	100%	100%	100%	100%	100%	100%	100%
103	17	82%	53%	6%	53%	94%	88%	82%	82%	71%
104	19	37%	5%	5%	5%	26%	11%	32%	26%	84%
105	18	78%	94%	0%	94%	94%	89%	94%	100%	94%
107	19	11%	0%	0%	0%	5%	0%	16%	32%	79%
108	14	100%	93%	14%	93%	100%	79%	100%	100%	79%
110	17	88%	53%	6%	47%	88%	59%	94%	100%	94%
112	17	94%	6%	0%	6%	94%	24%	100%	100%	82%
113	19	89%	79%	68%	89%	89%	89%	89%	89%	89%
114	19	100%	74%	0%	79%	100%	100%	100%	100%	100%
115	16	0%	56%	0%	38%	100%	100%	100%	100%	100%
116	19	100%	100%	63%	100%	100%	100%	100%	100%	100%
118	19	79%	68%	42%	68%	95%	95%	84%	95%	68%
121	17	12%	100%	0%	100%	100%	100%	100%	100%	100%
123	17	47%	100%	6%	100%	100%	100%	100%	100%	100%
125	19	89%	100%	84%	100%	100%	100%	100%	100%	95%
126	17	12%	100%	6%	100%	100%	88%	100%	100%	94%
127	14	0%	21%	0%	36%	100%	36%	100%	100%	93%
128	18	28%	100%	39%	100%	100%	67%	100%	100%	100%
129	18	78%	89%	67%	89%	100%	83%	100%	100%	89%
130	19	74%	84%	5%	84%	89%	68%	84%	84%	84%
131	18	33%	89%	22%	94%	100%	100%	100%	100%	89%
132	17	6%	94%	0%	94%	100%	71%	100%	100%	94%
134	17	76%	100%	41%	100%	100%	100%	100%	100%	100%

Table 8-2 Non-restrictive Positions $\geq$ 20% Neutral Mutations for at least One Property										
Position	# mutants	Detergent stability PI > 0.5	Detergent initial PI %>0.5	Thermostability PI > 0.5	Thermostability initial PI > 0.5	CS28 pH10 32°C PI > 0.5	CS28 pH10 50°C PI > 0.5	CS28 pH 8 16°C PI > 0.5	CS28 pH 8 32°C PI > 0.5	HPLC Protein PI > 0.5
135	19	11%	74%	5%	100%	100%	95%	100%	100%	100%
136	19	100%	100%	53%	100%	100%	100%	100%	100%	100%
138	17	94%	100%	29%	100%	100%	94%	100%	100%	100%
140	18	72%	22%	0%	22%	22%	0%	94%	100%	100%
142	18	100%	100%	67%	100%	100%	94%	100%	100%	100%
144	18	100%	94%	11%	94%	100%	100%	100%	100%	100%
147	18	100%	100%	0%	100%	100%	100%	100%	100%	100%
149	19	89%	84%	63%	84%	89%	95%	89%	89%	89%
150	17	94%	88%	71%	94%	94%	94%	88%	94%	94%
152	17	100%	88%	6%	88%	100%	100%	100%	100%	94%
154	18	100%	100%	100%	100%	100%	100%	100%	100%	100%
156	17	100%	100%	12%	100%	100%	100%	100%	100%	100%
158	16	100%	100%	13%	100%	100%	94%	100%	100%	100%
159	16	0%	94%	0%	0%	94%	0%	94%	94%	94%
160	16	100%	100%	81%	100%	100%	31%	100%	100%	100%
161	17	65%	47%	12%	47%	100%	76%	100%	100%	29%
162	15	13%	33%	0%	40%	87%	60%	73%	93%	53%
164	5	0%	0%	0%	0%	40%	0%	60%	80%	60%
165	18	78%	50%	22%	44%	72%	67%	89%	94%	94%
166	16	6%	0%	0%	0%	19%	13%	50%	75%	94%
167	17	53%	29%	12%	29%	82%	47%	88%	88%	82%
168	17	12%	41%	0%	41%	100%	71%	71%	100%	100%
169	19	89%	95%	0%	95%	95%	79%	95%	95%	95%
170	19	84%	95%	68%	95%	95%	89%	100%	95%	95%
171	17	88%	94%	0%	100%	100%	94%	100%	100%	100%
172	18	100%	100%	28%	94%	100%	94%	100%	100%	94%
174	16	100%	81%	75%	88%	100%	94%	100%	100%	100%
175	19	95%	95%	11%	95%	95%	74%	95%	95%	95%
176	19	5%	84%	0%	95%	95%	74%	100%	95%	95%
177	15	40%	80%	13%	80%	100%	100%	100%	100%	80%
178	18	61%	83%	44%	94%	100%	11%	100%	100%	56%
179	15	7%	67%	0%	73%	93%	27%	93%	93%	67%
182	18	94%	100%	94%	100%	100%	89%	100%	100%	100%
183	16	81%	100%	19%	100%	100%	0%	100%	100%	100%
185	17	59%	94%	47%	94%	100%	76%	100%	100%	94%
186	15	0%	13%	0%	13%	47%	13%	80%	93%	20%
188	18	0%	89%	0%	100%	100%	83%	100%	100%	100%
189	18	22%	83%	11%	100%	100%	94%	94%	83%	100%
190	16	0%	44%	0%	44%	50%	38%	88%	100%	44%
191	16	6%	31%	6%	31%	88%	56%	100%	100%	94%
192	19	21%	100%	11%	100%	100%	100%	100%	100%	100%
193	17	12%	0%	6%	0%	88%	12%	82%	94%	94%
195	17	65%	88%	0%	88%	94%	82%	100%	100%	94%
197	17	18%	6%	29%	6%	82%	12%	100%	100%	94%

Table 8-2 Non-restrictive Positions $\geq$ 20% Neutral Mutations for at least One Property

Position	# mutants	Detergent stability PI > 0.5	Detergent initial PI %>0.5	Thermostability PI > 0.5	Thermostability initial PI > 0.5	CS28 pH10 32°C PI > 0.5	CS28 pH10 50°C PI > 0.5	CS28 pH 8 16°C PI > 0.5	CS28 pH 8 32°C PI > 0.5	HPLC Protein PI > 0.5
199	19	0%	0%	0%	0%	16%	0%	58%	79%	63%
200	15	7%	0%	0%	0%	0%	0%	13%	40%	100%
201	19	32%	0%	5%	0%	21%	11%	53%	53%	84%
202	19	53%	16%	21%	16%	26%	11%	63%	79%	100%
203	19	21%	5%	21%	5%	32%	26%	84%	89%	79%
207	15	13%	93%	7%	100%	100%	100%	100%	100%	100%
210	17	100%	94%	88%	94%	100%	100%	94%	100%	88%
214	16	100%	100%	100%	100%	100%	100%	100%	100%	100%
217	18	94%	83%	83%	83%	100%	94%	100%	100%	83%
221	17	100%	100%	100%	100%	100%	100%	100%	100%	100%
228	18	100%	94%	100%	100%	100%	100%	100%	100%	100%
234	15	100%	73%	40%	73%	100%	87%	100%	100%	93%
237	19	32%	53%	5%	47%	84%	47%	84%	84%	74%
238	17	6%	0%	6%	0%	0%	0%	35%	41%	100%
239	17	0%	0%	0%	0%	0%	0%	53%	53%	71%
240	16	6%	75%	0%	88%	94%	44%	100%	100%	88%
243	16	81%	94%	44%	100%	100%	100%	100%	100%	81%
246	19	89%	89%	47%	89%	89%	89%	89%	89%	89%
250	15	93%	100%	93%	100%	100%	100%	100%	100%	100%
254	19	100%	100%	100%	100%	100%	100%	95%	100%	84%
255	17	100%	100%	100%	100%	100%	82%	100%	100%	82%
257	17	100%	94%	94%	100%	100%	100%	100%	100%	100%
259	13	92%	92%	92%	92%	92%	92%	92%	92%	85%
264	17	41%	24%	6%	24%	94%	35%	94%	100%	41%
266	19	0%	53%	5%	53%	95%	84%	84%	100%	74%
267	4	50%	0%	0%	0%	0%	75%	0%	100%	0%
268	19	21%	11%	5%	11%	79%	42%	89%	95%	32%
269	19	95%	32%	11%	16%	95%	68%	95%	100%	95%
270	10	90%	30%	70%	30%	100%	60%	100%	100%	30%
272	18	100%	94%	100%	100%	100%	100%	100%	100%	94%
273	15	7%	100%	0%	93%	100%	100%	100%	100%	67%
275	18	61%	94%	89%	100%	100%	100%	100%	100%	94%
279	16	100%	100%	94%	100%	100%	100%	19%	100%	94%
283	16	100%	81%	88%	81%	100%	100%	100%	100%	69%
284	18	100%	94%	83%	94%	100%	100%	100%	100%	89%
298	17	100%	100%	18%	100%	100%	100%	100%	100%	100%
301	17	100%	94%	100%	94%	100%	94%	100%	100%	94%
303	18	100%	100%	100%	100%	100%	89%	100%	100%	94%
305	19	100%	100%	100%	100%	100%	63%	100%	100%	95%
306	17	94%	12%	100%	41%	100%	100%	100%	100%	41%
310	19	95%	89%	95%	89%	89%	89%	89%	89%	89%
311	9	100%	56%	100%	67%	100%	89%	100%	100%	56%
314	19	95%	89%	95%	84%	95%	95%	95%	95%	89%
318	16	100%	94%	100%	94%	100%	100%	100%	100%	94%

Table 8-2 Non-restrictive Positions $\geq$ 20% Neutral Mutations for at least One Property										
Position	# mutants	Detergent stability PI > 0.5	Detergent initial PI %>0.5	Thermostability PI > 0.5	Thermostability initial PI > 0.5	CS28 pH10 32°C PI > 0.5	CS28 pH10 50°C PI > 0.5	CS28 pH 8 16°C PI > 0.5	CS28 pH 8 32°C PI > 0.5	HPLC Protein PI > 0.5
319	17	76%	88%	71%	88%	100%	88%	100%	100%	88%
320	18	100%	100%	100%	100%	100%	100%	100%	100%	94%
322	17	100%	100%	100%	100%	100%	100%	100%	100%	100%
323	17	100%	88%	41%	88%	100%	94%	100%	100%	88%
336	16	100%	75%	94%	75%	94%	88%	100%	100%	81%
337	16	38%	6%	38%	6%	38%	19%	63%	88%	100%
338	17	71%	6%	82%	6%	59%	12%	100%	94%	100%
339	18	33%	6%	33%	0%	28%	6%	22%	56%	89%
340	19	100%	79%	100%	79%	89%	58%	100%	100%	84%
344	16	100%	94%	94%	94%	94%	94%	44%	100%	94%
359	14	100%	93%	100%	93%	100%	100%	100%	100%	93%
374	17	100%	65%	100%	65%	100%	100%	100%	100%	59%
375	19	95%	79%	95%	79%	89%	84%	95%	95%	79%
376	18	100%	94%	100%	94%	100%	100%	94%	100%	94%
377	18	100%	83%	100%	89%	100%	94%	100%	100%	83%
379	16	100%	94%	94%	94%	100%	100%	100%	100%	94%
381	19	95%	89%	95%	89%	95%	89%	95%	95%	95%
382	19	84%	63%	84%	63%	84%	74%	84%	84%	63%
393	17	94%	82%	94%	82%	94%	94%	94%	94%	82%
394	16	100%	94%	94%	94%	100%	94%	100%	100%	100%
399	17	94%	94%	94%	94%	94%	94%	100%	94%	94%
401	16	100%	100%	100%	100%	100%	100%	100%	100%	100%
407	16	100%	100%	100%	100%	100%	100%	100%	100%	100%
408	13	54%	8%	54%	15%	54%	46%	54%	54%	23%
419	19	100%	100%	100%	100%	100%	100%	100%	100%	100%
433	16	100%	94%	94%	94%	100%	94%	100%	100%	94%
436	19	100%	89%	100%	89%	100%	100%	100%	100%	89%
438	16	100%	100%	100%	100%	100%	75%	100%	100%	100%
444	17	100%	100%	100%	100%	100%	100%	100%	100%	100%
447	18	100%	94%	100%	94%	100%	100%	100%	100%	100%
448	16	100%	94%	100%	94%	100%	100%	100%	100%	94%
451	16	100%	94%	100%	94%	100%	100%	100%	100%	94%
453	19	100%	89%	100%	89%	95%	95%	95%	95%	95%
459	19	100%	100%	100%	100%	100%	100%	100%	100%	100%
465	16	100%	100%	94%	100%	100%	94%	100%	100%	100%
470	17	100%	100%	100%	100%	100%	100%	100%	100%	100%
475	17	100%	100%	100%	100%	100%	0%	100%	100%	100%
476	16	94%	75%	94%	69%	94%	0%	94%	94%	75%
483	18	100%	100%	100%	100%	100%	100%	100%	100%	100%
484	18	100%	100%	100%	100%	100%	100%	100%	100%	100%

[00366] In general, restrictive positions should not be mutated, as there are only few substitutions at these positions that are neutral for any property. When these positions must be changed, they require that the amino

acid replacement be a conservative replacement. For example two mutations at position 58, *i.e.*, A58G and A58T, are combinable, and two mutations at position 236, *i.e.*, Y236F and Y236W, are combinable. Non-restrictive positions are the positions that are most suitable for use in constructing combinatorial libraries, since they have a large number of combinable mutations. Since homologous proteins share the same structure, restrictive positions are restrictive in all homologous amylases, since they are important for the structure and/or function of the protein. As demonstrated in the present disclosure, restrictive positions can only be identified by testing the possible amino acid mutations and measuring the properties of one of the proteins (*e.g.*, alpha-amylase). Note that the two restrictive sites are conserved in the sequence alignment in Figure 1, but that conservation itself is not an indicator that a position is restrictive. For example, Q71, K72, and G73 are all conserved in the sequence alignment, but all three positions have several mutations that are better than the parent for activity, stability, or both. Non-restrictive positions with more than 20% neutral mutations for each property are generally very good choices for combinatorial mutations. There are 115 positions out of the 198 positions tested in alpha-amylase that meet this condition.

EXAMPLE 9

Detergent Stability of ACE-Q Amylase Variants

[00367] The detergent stability of ACE-Q (ACE-S243Q) amylase variants was measured after incubation under defined conditions in the presence of 10% detergent (commercial detergent; inactivated) at 70°C, and the initial and residual amylase activities were determined using the Ceralpha method (BPNPG7) as described in Example 1. The numbering of amino acid residues corresponds to that of the BASE alpha-amylase. The results in Table 9-1 below are shown as a performance index (PI) for each variant in relation to ACE alpha mylase.

Table 9-1 Detergent Stability of ACE-Q Variants with $PI \geq 0.5$

Position	Variant	PI		Position	Variant	PI
127	R127C	0.58		320	Q320Y	3.80
305	Y305A	4.05		379	P379A	4.54
305	Y305C	4.12		379	P379C	4.27
305	Y305D	4.53		379	P379E	3.82
305	Y305E	3.86		379	P379F	3.88
305	Y305G	3.97		379	P379G	4.04
305	Y305H	3.96		379	P379H	3.99
305	Y305L	4.27		379	P379L	3.85
305	Y305N	4.20		379	P379N	3.87
305	Y305P	4.36		379	P379Q	4.17
305	Y305Q	3.95		379	P379R	4.39
305	Y305R	3.92		379	P379S	4.09
305	Y305S	4.01		379	P379T	3.98
305	Y305T	4.12		379	P379V	4.30
305	Y305V	3.92		379	P379W	3.79
305	Y305W	4.18		379	P379Y	4.17
320	Q320A	3.96		419	T419A	3.78
320	Q320C	3.78		419	T419C	3.88
320	Q320D	3.93		419	T419D	3.59
320	Q320E	3.88		419	T419E	4.06
320	Q320F	3.73		419	T419F	3.64
320	Q320G	3.58		419	T419G	3.71
320	Q320H	4.05		419	T419H	3.69
320	Q320I	4.00		419	T419K	3.58
320	Q320L	3.78		419	T419L	3.65
320	Q320M	3.83		419	T419M	3.66
320	Q320N	4.01		419	T419N	3.69
320	Q320P	3.68		419	T419P	3.87
320	Q320R	4.28		419	T419Q	3.93
320	Q320S	3.76		419	T419R	3.78
320	Q320T	3.82		419	T419S	3.64
320	Q320V	4.03		419	T419V	3.68
320	Q320W	3.55		419	T419W	3.92

Position	Variant	PI	Position	Variant	PI
419	T419Y	4.14	475	G475V	3.47
453	L453A	4.18	475	G475W	3.42
453	L453C	4.14			
453	L453D	4.12			
453	L453F	4.41			
453	L453G	4.21			
453	L453H	4.26			
453	L453I	4.20			
453	L453K	4.09			
453	L453M	4.52			
453	L453N	4.08			
453	L453P	4.24			
453	L453Q	4.14			
453	L453R	4.31			
453	L453S	4.09			
453	L453T	4.34			
453	L453V	4.01			
453	L453W	4.03			
453	L453Y	4.05			
475	G475A	3.74			
475	G475D	3.40			
475	G475E	3.36			
475	G475H	3.43			
475	G475I	3.49			
475	G475K	3.41			
475	G475L	3.33			
475	G475M	3.40			
475	G475N	3.40			
475	G475P	3.49			
475	G475Q	3.56			
475	G475R	3.87			
475	G475S	3.42			
475	G475T	3.39			

[00368] The variants shown are the combinable mutations identified in the SEL data. Only variants with a $PI \geq 0.5$ are shown. These mutations can be combined to give a desired combination of stability and activity.

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EXAMPLE 10

Cleaning Performance of ACE-Q variants

[00369] The wash performance of ACE-Q (ACE-S243Q) amylase variants was measured using CS28 microswatches under four different conditions pH 8/16°C, pH 8/32°C, pH 10/32°C and pH 10/50°C as described in Example 1. The numbering of amino acid residues corresponds to that of the BASE alpha-amylase. The results in Tables 10-1 to 10-4 below are shown as a performance index (PI) for each variant in relation to ACE alpha amylase.

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Table 10-1 ACE-Q Variants (118) with $PI \geq 0.5$ for CS-28, pH 8, 16°C Wash Performance

15

Position	Variant	PI		Position	Variant	PI
127	R127A	1.22		320	Q320A	1.29
127	R127C	1.28		320	Q320C	1.28
127	R127D	1.28		320	Q320D	1.27
127	R127E	1.14		320	Q320E	1.25
127	R127F	1.13		320	Q320F	1.28
127	R127G	0.86		320	Q320G	1.53
127	R127H	0.97		320	Q320H	1.29
127	R127I	1.20		320	Q320I	1.16
127	R127K	1.26		320	Q320L	1.32
127	R127L	1.27		320	Q320M	1.28
127	R127M	1.03		320	Q320N	1.28
127	R127P	1.06		320	Q320P	2.36
127	R127Q	1.25		320	Q320R	1.20
127	R127S	1.23		320	Q320S	1.09
127	R127T	1.24		320	Q320T	1.10
127	R127V	1.29		320	Q320V	1.09
127	R127W	1.43		320	Q320W	1.02
127	R127Y	1.01		320	Q320Y	1.27
305	Y305A	1.54		379	P379A	0.96
305	Y305C	1.63		379	P379C	0.90
305	Y305D	1.65		379	P379E	1.00
305	Y305E	1.61		379	P379F	0.99
305	Y305G	1.58		379	P379G	1.00
305	Y305H	1.48		379	P379H	0.97
305	Y305L	1.65		379	P379L	1.05
305	Y305N	1.61		379	P379N	0.98
305	Y305P	1.91		379	P379Q	1.10
305	Y305Q	1.78		379	P379R	0.81
305	Y305R	1.44		379	P379S	1.01
305	Y305S	1.49		379	P379T	1.04
305	Y305T	1.68		379	P379V	1.00
305	Y305V	1.68		379	P379W	0.86
305	Y305W	1.44		379	P379Y	0.95

Position	Variant	PI	Position	Variant	PI
419	T419A	1.21	453	L453V	1.02
419	T419C	1.08	453	L453W	0.88
419	T419D	1.19	453	L453Y	0.89
419	T419E	1.17	475	G475A	1.88
419	T419F	1.27	475	G475D	1.91
419	T419G	1.14	475	G475E	1.92
419	T419H	1.42	475	G475H	1.73
419	T419K	1.13	475	G475I	2.31
419	T419L	1.01	475	G475K	1.93
419	T419M	1.20	475	G475L	2.20
419	T419N	1.13	475	G475M	2.17
419	T419P	1.29	475	G475N	1.86
419	T419Q	1.18	475	G475P	1.78
419	T419R	1.32	475	G475Q	1.99
419	T419S	1.18	475	G475R	1.92
419	T419V	1.23	475	G475S	1.75
419	T419W	1.20	475	G475T	2.06
419	T419Y	1.02	475	G475V	2.10
453	L453A	0.97	475	G475W	2.11
453	L453C	0.93			
453	L453D	0.80			
453	L453F	0.92			
453	L453G	0.85			
453	L453H	1.05			
453	L453I	0.91			
453	L453K	1.02			
453	L453M	0.82			
453	L453N	1.00			
453	L453P	0.76			
453	L453Q	1.02			
453	L453R	0.96			
453	L453S	0.95			
453	L453T	0.95			

Table 10-2 ACE-Q Variants (118) with $PI \geq 0.5$ for CS-28, pH 8, 32°C Wash Performance

Position	Variant	PI		Position	Variant	PI
127	R127A	0.64		320	Q320A	1.12
127	R127C	0.95		320	Q320C	1.18
127	R127D	0.82		320	Q320D	1.21
127	R127E	0.83		320	Q320E	1.17
127	R127F	0.90		320	Q320F	1.23
127	R127G	0.75		320	Q320G	1.32
127	R127H	0.81		320	Q320H	1.13
127	R127I	1.24		320	Q320I	1.18
127	R127K	0.86		320	Q320L	1.15
127	R127L	1.12		320	Q320M	1.23
127	R127M	1.05		320	Q320N	1.32
127	R127P	1.05		320	Q320P	1.64
127	R127Q	1.16		320	Q320R	1.22
127	R127S	0.88		320	Q320S	1.11
127	R127T	1.19		320	Q320T	1.20
127	R127V	1.26		320	Q320V	1.15
127	R127W	1.14		320	Q320W	1.05
127	R127Y	1.11		320	Q320Y	1.15
305	Y305A	1.17		379	P379A	1.11
305	Y305C	1.19		379	P379C	0.89
305	Y305D	1.09		379	P379E	1.05
305	Y305E	1.08		379	P379F	1.09
305	Y305G	1.19		379	P379G	1.08
305	Y305H	1.11		379	P379H	1.05
305	Y305L	1.21		379	P379L	1.03
305	Y305N	1.20		379	P379N	1.05
305	Y305P	1.16		379	P379Q	1.06
305	Y305Q	1.23		379	P379R	1.03
305	Y305R	1.20		379	P379S	1.13
305	Y305S	1.14		379	P379T	1.14
305	Y305T	1.18		379	P379V	1.11
305	Y305V	1.18		379	P379W	0.99
305	Y305W	1.11		379	P379Y	1.07

Position	Variant	PI	Position	Variant	PI
419	T419A	0.79	453	L453V	0.98
419	T419C	0.79	453	L453W	0.95
419	T419D	0.82	453	L453Y	0.99
419	T419E	0.70	475	G475A	1.24
419	T419F	0.81	475	G475D	1.22
419	T419G	0.71	475	G475E	1.12
419	T419H	0.85	475	G475H	1.21
419	T419K	0.78	475	G475I	1.44
419	T419L	0.77	475	G475K	1.41
419	T419M	1.01	475	G475L	1.26
419	T419N	1.01	475	G475M	1.42
419	T419P	1.03	475	G475N	1.25
419	T419Q	0.84	475	G475P	1.26
419	T419R	0.86	475	G475Q	1.25
419	T419S	0.98	475	G475R	1.39
419	T419V	1.01	475	G475S	1.26
419	T419W	1.09	475	G475T	1.26
419	T419Y	1.04	475	G475V	1.30
453	L453A	1.02	475	G475W	1.36
453	L453C	0.96			
453	L453D	0.93			
453	L453F	1.02			
453	L453G	1.01			
453	L453H	1.00			
453	L453I	0.98			
453	L453K	0.99			
453	L453M	0.96			
453	L453N	1.01			
453	L453P	0.96			
453	L453Q	1.07			
453	L453R	1.04			
453	L453S	0.95			
453	L453T	1.05			

Table 10-3 ACE-Q Variants (87) with $PI \geq 0.5$ for CS-28, pH 10, 32°C Wash Performance

Position	Variant	PI	Position	Variant	PI
127	R127A	0.55	320	Q320T	0.79
127	R127C	0.76	320	Q320V	0.60
127	R127F	0.63	320	Q320Y	0.56
127	R127G	0.60	379	P379A	0.91
127	R127H	0.66	379	P379C	0.97
127	R127I	0.64	379	P379E	0.83
127	R127K	0.90	379	P379F	1.22
127	R127L	0.55	379	P379G	1.03
127	R127M	0.79	379	P379H	0.95
127	R127Q	0.76	379	P379L	1.09
127	R127S	0.74	379	P379N	1.15
127	R127T	0.83	379	P379Q	1.07
127	R127V	0.51	379	P379R	1.20
127	R127W	0.72	379	P379S	1.12
127	R127Y	0.77	379	P379T	1.06
305	Y305H	0.61	379	P379V	1.05
305	Y305L	0.52	379	P379W	1.13
305	Y305N	0.55	379	P379Y	1.01
305	Y305Q	0.65	419	T419A	1.11
305	Y305R	0.84	419	T419C	0.85
305	Y305W	0.90	419	T419D	1.03
320	Q320A	0.55	419	T419E	1.09
320	Q320C	0.67	419	T419F	1.18
320	Q320D	0.61	419	T419G	1.03
320	Q320E	0.51	419	T419H	1.33
320	Q320F	0.55	419	T419K	1.11
320	Q320G	0.88	419	T419L	0.93
320	Q320H	0.65	419	T419M	1.08
320	Q320I	0.57	419	T419N	1.13
320	Q320M	0.79	419	T419P	1.22
320	Q320N	0.94	419	T419Q	1.11
320	Q320R	0.79	419	T419R	1.60
320	Q320S	0.64	419	T419S	1.11

Position	Variant	PI
419	T419V	1.16
419	T419W	1.17
419	T419Y	1.06
453	L453A	0.94
453	L453C	0.91
453	L453D	0.93
453	L453F	1.17
453	L453G	1.05
453	L453H	1.22
453	L453I	1.06
453	L453K	1.06
453	L453M	1.14
453	L453N	0.96
453	L453P	0.84
453	L453Q	1.04
453	L453R	1.00
453	L453S	0.99
453	L453T	1.03
453	L453V	1.05
453	L453W	1.00
453	L453Y	1.16

Table 10-4 ACE-Q Variants (73) with $PI \geq 0.5$ for CS-28, pH 10, 50°C Wash Performance

Position	Variant	PI		Position	Variant	PI
127	R127C	0.72		379	P379Y	0.86
127	R127G	0.71		419	T419A	0.83
305	Y305A	1.79		419	T419C	0.51
305	Y305E	1.56		419	T419D	0.59
305	Y305G	0.74		419	T419E	0.58
305	Y305L	2.96		419	T419F	0.69
305	Y305N	0.64		419	T419G	0.64
305	Y305R	2.44		419	T419H	0.92
305	Y305S	0.68		419	T419K	0.70
305	Y305W	2.61		419	T419L	0.77
320	Q320C	2.58		419	T419M	0.77
320	Q320D	4.81		419	T419N	0.68
320	Q320E	4.25		419	T419P	0.82
320	Q320H	4.88		419	T419Q	0.67
320	Q320I	1.47		419	T419R	0.56
320	Q320M	2.20		419	T419S	0.62
320	Q320N	2.12		419	T419V	0.91
320	Q320R	1.87		419	T419W	0.82
320	Q320Y	0.83		419	T419Y	0.90
379	P379A	0.90		453	L453A	1.41
379	P379C	0.93		453	L453C	1.43
379	P379E	0.74		453	L453D	1.39
379	P379F	0.89		453	L453F	1.20
379	P379G	1.02		453	L453G	1.50
379	P379H	0.81		453	L453H	2.01
379	P379L	0.83		453	L453I	1.17
379	P379N	1.03		453	L453K	1.60
379	P379Q	0.95		453	L453M	1.66
379	P379R	1.11		453	L453N	1.48
379	P379S	0.81		453	L453P	1.36
379	P379T	1.08		453	L453Q	1.53
379	P379V	1.01		453	L453R	2.03
379	P379W	0.97		453	L453S	1.61

Position	Variant	PI
453	L453T	1.90
453	L453V	1.71
453	L453W	2.12
453	L453Y	2.62
475	G475H	0.53
475	G475L	0.51
475	G475W	0.73

EXAMPLE 11**Thermostability of BASE Single-Mutation Variants**

[00370] Twenty-three single mutation variants of BASE were selected from the SEL screen for accurate $T_{50\%}$ determination in heat-inactivated 100% Persil (Henkel) HDL. Heat inactivation of commercial detergents serves to destroy the activity of any enzymatic components while retaining the properties of non-enzymatic components. The selection was based on improved performance indices in the thermostability and 10% Persil HDL stability assays, as performed in the SEL screen. The selected variants with their respective $T_{50\%}$ values in 100% Persil HDL are listed in Table 11-1.

Table 11-1 Thermostability of Amylase Variants Having Single Substitutions		
BASE Variant No.	Mutation	$T_{50\%}$ in 100 % Persil HDL (°C)
1	Wild Type BASE	35.4
2	N128C	40.3
3	T131I	38.1
4	T134P	36.8
5	Q138E	36.8
6	Y160I	38.7
7	T165I	39.3
8	T165V	37.8
9	K178L	44.7
10	T182A	38.2
11	T182C	41.0
12	T182D	37.2
13	T182M	38.3
14	T182F	36.5
15	T182N	36.5
16	T182G	42.0
17	T182P	38.9
18	T182Q	38.4
19	A185D	40.4
20	A185E	37.3
21	E189P	35.4
22	S243D	40.4
23	S243E	44.1
24	S243Q	42.7

EXAMPLE 12**Thermostability of BASE Combinatorial Variants**

[00371] Five positions (N128C, K178L, T182G, A185D, and S243Q) of the BASE reference amylase were chosen for the construction of a combinatorial library as described in Example 5. Of the possible 32 variants of this combinatorial library (designated BASE-S1 to BASE-S32), 30 variants were assayed for stability in heat-inactivated Persil HDL. The variants, their respective mutations and

the measured $T_{50\%}$ in Persil HDL are listed in Table 12-1 in comparison to wild type BASE (reference amylase), ACE, and ACE-S243Q amylase variants.

Table 12-1 Thermostability of Amylase Variants						
	Amino acid positions mutated					
Variant	N128	K178	T182	A185	S243	$T_{50\%}$ in 100% Persil HDL (°C)
BASE-WT						36.3
BASE-S1			G	D		45.9
BASE-S2			G			45.6
BASE-S3		L	G	D		49.8
BASE-S4		L	G			52.7
BASE-S5				D		40.4
BASE-S6						40.1
BASE-S7		L		D		43.9
BASE-S8		L				39.9
BASE-S9			G	D	Q	48.1
BASE-S10			G		Q	51.1
BASE-S11		L	G	D	Q	53.7
BASE-S12		L	G		Q	57.1
BASE-S13				D	Q	42.7
BASE-S14					Q	45.3
BASE-S15		L		D	Q	53.2
BASE-S16		L			Q	48.9
BASE-S17	C		G	D		45.8
BASE-S18	C		G			47.8
BASE-S19	C	L	G	D		51.4
BASE-S20	C	L	G			56.3
BASE-S21	C			D		42.5
BASE-S23	C	L		D		48.2
BASE-S24	C	L				44.0
BASE-S25	C		G	D	Q	51.5
BASE-S26	C		G		Q	55.9
BASE-S27	C	L	G	D	Q	57.3
BASE-S28	C	L	G		Q	63.2
BASE-S29	C			D	Q	48.1
BASE-S31	C	L		D	Q	56.4
ACE						60.1
ACE-S243Q						64.0

- 5 [00372] The results show that ACE and ACE-S243Q have significantly increased thermostability compared to WT BASE. Moreover, several combinatorial variants also demonstrated significantly increased thermostability, with six variants (BASE-S26, BASE-S20, BASE-S31, BASE-S12, BASE-S27, BASE-S28) showing more than a 50% increase in stability.

EXAMPLE 13**Cleaning Performance and Thermostability of BASE Combinatorial Variants**

[00373] Thirteen combinatorial BASE variants were constructed as described in Example 5, incorporating performance enhancing mutations and stability enhancing mutations. The alpha-amylase variants BASE-W1 to BASE-W13 were assayed for cleaning performance (PI or performance index) on CS28 microswatches under two different conditions (pH 8, 16°C, and pH 8, 32°C) and for thermostability ($T_{50\%}$) in MOPS buffer and in 100% Persil HDL as shown in Table 13-1.

Table 13-1 Cleaning Performance and Thermostability of Amylase Variants				
Variant	PI CS28, pH 8 16°C	PI CS28, pH 8 32°C	$T_{50\%}$ MOPS (°C)	$T_{50\%}$ Persil HDL (°C)
BASE-WT	1.0	1.0	62	36
BASE-W1	2.4	1.7	86	63
BASE-W2	2.8	1.4	86	64
BASE-W3	2.8	1.8	76	52
BASE-W4	2.8	1.5	87	64
BASE-W5	1.9	0.5	88	66
BASE-W6	2.5	1.7	87	64
BASE-W7	2.2	1.6	84	60
BASE-W8	2.7	1.5	87	64
BASE-W9	3.7	2.1	86	64
BASE-W10*	3.8	2.0	86	64
BASE-W11	3.4	2.3	86	64
BASE-W12	3.0	1.9	84	59
BASE-W13	2.7	1.9	86	63

*The BASE-W10 variant further comprises a F202Y substitution.

[00374] The results show that several combinatorial variants demonstrate significantly increased cleaning performance and detergent stability compared to WT BASE. Three of the variants, BASE-W9, BASE-W10, and BASE-W11, show more than three fold improvement in low temperature cleaning, while at the same time showing more than 1.75 fold improved detergent stability.

EXAMPLE 14**Cleaning Performance of BASE Variants W9, W10, W11, and ACE-QK**

[00375] The wash performance of BASE variants W9, W10 and W11, and ACE-QK was tested in a laundry detergent application. Stain removal was measured on CFT CS-28 rice starch on cotton (Center for Testmaterials BV, Vlaardingen, Netherlands) and EMPA161 starch on cotton (Test materials AG, St. Gallen, Switzerland) in launder-o-meter experiments using heat inactivated Ariel detergent (Proctor and Gamble), purchased from the local supermarket. Ariel detergent was inactivated at 90°C to 100°C in a Brother Hi-Speed microwave for 8 minutes. The detergent was allowed to cool down to less than 50°C and then heated again in the microwave for 7 min. This step was repeated once.

[00376] EMPA swatches were measured before and after treatment by optical reflectance using a Tristimulus Minolta Meter CR- 400. The difference in the L, a, b values was converted to total color difference (dE), as defined by the CIE-LAB color space. Cleaning of the stains is expressed as percent stain removal index (%SRI) by taking a ratio between the color difference before and after washing and comparing it to the difference of unwashed soils (before wash) to unsoiled fabric.

[00377] Wash treatment was conducted in a launder-o-meter at 30°C. The wash time was 45 minutes (15 minutes to reach 30°C, then 30 minutes at 30°C) with a rinse time of 5 minutes with cold tap water. The water hardness was adjusted to 8.5° GH and heat inactivated Ariel was used at 4.5 ml/L. The soil load consisted of 2 ea. EMPA 161 and 2 CFT CS-28 swatches per beaker plus 6 steal balls. After the wash treatment, all swatches were spin-dried followed by air drying and read for optical reflectance as described above. The control consisted of a benchmark commercial enzyme.

The results are shown in Figure 5.

[00378] Generally, the presence of BASE variant in an amount of from about 0.05 to about 0.5 ppm improved performance compared to the control, with the relative performance of the BASE variants following the pattern W10>W11>W9>ACE-Q.

EXAMPLE 15

Cleaning Performance of BASE Variants X8C, W10EK, and ACE-QK

[00379] The wash performance of additional BASE variants BASE-X8C (*i.e.*, W11-T131I-T165I), BASE-W10EK (*i.e.*, BASE-N128C-K178L-T182G-S243E-Y305R-D319T-G475K), and ACE-S243Q-G475K (*i.e.*, ACE-QK) was tested in a laundry detergent application using a Terg-o-tometer.

The performance evaluation was conducted at 16°C. The soil load consisted of 2 ea. CS-28 Rice Starch (Center for Testmaterials BV, Vlaardingen, Netherlands), 2 ea. AS-10 Pigment Oil Milk (CFT of Holland), 2 ea. EMPA 161 Maize Starch, 2 ea. EMPA 160 Chocolate Cream, and 2 ea. EMPA 163 Porridge (EMPA Testmaterials AG, St. Gallen, Switzerland) swatches per beaker of the Terg-o-tometer, which was filled with 1 L of DI water. The water hardness was adjusted to 6 grains per gallon and heat-inactivated Great Value (Walmart) detergent was used at 1.0 ml/L. The wash time was 15 minutes. After the wash treatment, all swatches were spin-dried followed by air drying.

[00380] Each stain was measured before and after treatment by optical reflectance using a Minolta Reflectometer Chroma Meter Model CR-410 (Konica Minolta) set to a D65 (6500°K) standard illuminant. The difference in the L, a, b values was converted to total color difference (dE), as defined by the CIE-LAB color space. Cleaning of the stains was expressed as stain removal index (SRI) by taking a ratio between the color difference before and after washing. Results for the cleaning of EMPA 160 swatches are shown in Figure 6. All tested BASE variants showed effective cleaning compared to the control.

EXAMPLE 16

Laundry Application Synergy between BASE Variants and Subtilisin Protease

[00381] The synergy between BASE and subtilisin protease (*B. amyloliquefaciens* subtilisin BPN'-Y217L; BPN' Swissprot Accession Number P00782) for stain removal was measured on chocolate cream-stained EMPA 160 and porridge-stained EMPA 163 swatches in a full scale laundry application. Stain removal from EMPA 160 and EMPA 163 swatches was tested in buffered 5mM HEPES (Sigma, H4034) pH 8.0, and heat-inactivated TIDE® 2X coldwater detergent (Proctor & Gamble, Cincinnati, Ohio). Heat inactivation of commercial detergents serves to destroy the activity of enzymatic components while retaining the properties of nonenzymatic components. Heat inactivation was performed by placing pre-weighed liquid detergent (in a glass bottle) in a water bath at 95°C for 2 hours. The detergent was purchased from local supermarkets. Both unheated and heated detergents were assayed within 5 minutes of dissolving the detergent, to accurately determine the percentage deactivated. Enzyme activity was tested by AAPF and Ceralpha assays.

[00382] EMPA swatches were measured before and after treatment by optical reflectance using a Minolta Reflectometer CR-410 set to a D65 (6500°K) standard illuminant. The difference in the L, a, b values was converted to total color difference (dE), as defined by the CIE-LAB color space.

Cleaning of the stains was expressed as percent stain removal index (%SRI) by taking a ratio between the color difference before and after washing and comparing it to the difference of unwashed soils (before wash) to unsoiled fabric. Wash treatment was conducted in 44L in a Kenmore Washing Machine. The washing machine was filled using the 'Cold Auto Temperature' setting and the water hardness was adjusted to 6gpg using a 15,000gpg 3:1 Ca:Mg water hardness stock solution. TIDE® coldwater inactivated detergent (43.12 g) was added and the temperature adjusted to 32°C. Subtilisin protease was added to a final concentration of 0.6 ppm and ACE amylase was added to a final concentration of 0.1 ppm. Four swatches were used per wash condition and bleached cotton interlock knit was added as ballast to provide 40g/L total fabric load. The wash conditions were as follows: normal cycle (15.5 min) with second rinse (3 min), wash/rinse temperature of 89.6°F/32°C, fast agitation, and fast spin. After washing, the swatches were machine dried on low heat and read by optical reflectance as described above.

[00383] As shown in Figure 7A, the ACE alpha-amylase and the BPN'Y217L subtilisin protease produced a synergistic cleaning effect in laundry applications. Similar laundry application synergy tests were done with ACE-S243Q-G475K (ACE-QK) alpha-amylase and subtilisin protease (*B. amyloliquefaciens* subtilisin BPN'-Y217L variant). As shown in Figure 7B, the ACE-S243Q-G475K (ACE-QK) alpha-amylase and the BPN'Y217L subtilisin protease produced a synergistic cleaning effect in laundry applications, suggesting that BASE variants and proteases can be used in combination for superior cleaning in laundry applications.

EXAMPLE 17

Dose effects of BASE Variants and Subtilisin Protease

[00384] A dose efficiency curve of selected concentrations of ACE-S243Q and subtilisin protease (*B. amyloliquefaciens* subtilisin BPN'-Y217L; BPN' Swissprot Accession Number P00782) were generated using a Terg-o-tometer. The performance evaluation was conducted at both 20°C and 40°C. Typically, 2 ea. swatches of CS-28 Rice Starch (Center for Testmaterials BV, Vlaardingen, Netherlands), AS-10 Pigment Oil Milk (CFT of Holland), EMPA 161 Maize Starch, EMPA 160 Chocolate Cream, and EMPA 163 Porridge, (EMPA Testmaterials AG, St. Gallen, Switzerland) were placed in the steel container of the Terg-o-tometer, which was filled with 1 L of DI water and 1.0 g of commercial WISK® (Sun Products, purchased in USA) laundry detergent or 4.5 g OMO™ (Unilever, purchased in Denmark) laundry detergent. Two replicates were run at the same time. Unless otherwise stated, the tests were carried out for 12 minutes and the swatches were rinsed for 3 minutes. After washing, the swatches were air-dried.

[00385] Each stain was measured before and after treatment by optical reflectance using a Minolta Reflectometer Chroma Meter Model CR-410 (Konica Minolta) set to a D65 (6500°K) standard illuminant. The difference in the L, a, b values was converted to total color difference (dE), as defined by the CIE-LAB color space. Cleaning of the stains was expressed as stain removal index (SRI) by taking a ratio between the color difference before and after washing.

[00386] The results shown in Table 17-1 demonstrate that the combination of the BASE variant and protease produced significant cleaning benefits on several technical cleaning soils. These data demonstrate that a unique cleaning benefit is obtained using BASE variant in combination with a protease.

Table 17-1: Cleaning performance of ACE-S243Q with Protease

ACE-S243Q (ppm)	Protease (ppm)	EMPA161 SRI(dL)	CS-28 SRI(dE)	C-10 POM SRI(dE)	EMPA160 Choco SRI(dE)	EMPA163 Porridge SRI(dE)
0	0	-0.0317	0.2638	0.0846	0.125	0.0584
0	0	-0.0305	0.2634	0.1039	0.1105	0.0734

0	0	-0.023	0.2667	0.1297	0.1277	0.0991
0	0	-0.0326	0.2667	0.1112	0.1221	0.0953
0	0	-0.0426	0.256	0.08	0.1351	0.0569
0	0	-0.0338	0.2652	0.0951	0.1198	0.0572
0	0	-0.0262	0.2674	0.1036	0.1748	0.0982
0	0	-0.045	0.2744	0.0871	0.1201	0.0832
0.025	0.1	0.0941	0.4659	0.125	0.3105	0.1405
0.025	0.1	0.0924	0.472	0.125	0.2764	0.1496
0.025	0.1	0.0635	0.4515	0.1599	0.2836	0.1736
0.025	0.1	0.0696	0.4502	0.1668	0.2741	0.1915
0.025	0.1	0.0892	0.4752	0.1253	0.2989	0.1357
0.025	0.1	0.0945	0.468	0.1341	0.2571	0.1493
0.025	0.1	0.0481	0.4509	0.1216	0.1705	0.0854
0.025	0.1	0.0289	0.4503	0.1555	0.1645	0.1027
0.05	0.1	0.1269	0.5335	0.118	0.2627	0.1658
0.05	0.1	0.0679	0.5345	0.1202	0.2838	0.1457
0.05	0.1	0.0877	0.4801	0.1326	0.1666	0.1763
0.05	0.1	0.0847	0.4912	0.1288	0.1684	0.1426
0.05	0.1	0.1287	0.4941	0.1647	0.2747	0.1522
0.05	0.1	0.1485	0.4936	0.1752	0.2893	0.1709
0.05	0.1	0.1573	0.4516	0.1163	0.2708	0.1692
0.05	0.1	0.0691	0.4769	0.1446	0.2478	0.2253
0.1	0.1	0.1783	0.5517	0.1142	0.2292	0.1887
0.1	0.1	0.1162	0.5434	0.0983	0.2159	0.2012
0.1	0.1	0.1727	0.5376	0.1801	0.2397	0.2531
0.1	0.1	0.1691	0.531	0.1898	0.2349	0.2028
0.1	0.1	0.1521	0.5573	0.0993	0.2364	0.1533
0.1	0.1	0.1801	0.5637	0.1044	0.207	0.142
0.1	0.1	0.1308	0.5347	0.1027	0.2872	0.2478
0.1	0.1	0.1707	0.5418	0.1035	0.2893	0.2757
0.2	0.1	0.2517	0.5683	0.1457	0.171	0.1027
0.2	0.1	0.2308	0.5607	0.1336	0.1701	0.1243
0.2	0.1	0.1529	0.599	0.1891	0.2939	0.2218
0.2	0.1	0.1517	0.5998	0.1825	0.3002	0.2319
0.2	0.1	0.2836	0.545	0.1719	0.2939	0.199
0.2	0.1	0.2602	0.551	0.1602	0.2824	0.2124
0.2	0.1	0.2778	0.5795	0.1427	0.2494	0.1844
0.2	0.1	0.2722	0.5759	0.117	0.2897	0.1684
0.025	0.25	0.0897	0.4656	0.1653	0.2815	0.2177
0.025	0.25	0.0884	0.4726	0.1748	0.2942	0.1982
0.025	0.25	0.0281	0.4853	0.1637	0.3068	0.1646
0.025	0.25	0.0568	0.4647	0.1689	0.32	0.1591
0.025	0.25	0.0261	0.4335	0.1734	0.2168	0.1189
0.025	0.25	0.0225	0.4272	0.1437	0.255	0.1714
0.025	0.25	0.0537	0.4875	0.1489	0.2697	0.21
0.025	0.25	0.0559	0.4747	0.1466	0.2958	0.2055
0.05	0.25	0.0762	0.4241	0.1381	0.2479	0.236
0.05	0.25	0.0794	0.4164	0.1418	0.2423	0.162
0.05	0.25	0.1164	0.4943	0.2099	0.327	0.2089
0.05	0.25	0.1471	0.4842	0.1907	0.3064	0.2044
0.05	0.25	0.0312	0.4733	0.1543	0.2584	0.185
0.05	0.25	0.0557	0.4611	0.1556	0.2958	0.2273

0.05	0.25	0.0906	0.4782	0.1635	0.2914	0.1589
0.05	0.25	0.0697	0.4747	0.1693	0.2698	0.2226
0.1	0.25	0.255	0.4986	0.1794	0.2805	0.2547
0.1	0.25	0.2447	0.5058	0.1858	0.31	0.2679
0.1	0.25	0.153	0.5276	0.163	0.2489	0.2432
0.1	0.25	0.1541	0.5416	0.1647	0.2585	0.2319
0.1	0.25	0.1735	0.5578	0.1359	0.2877	0.144
0.1	0.25	0.1567	0.5608	0.1353	0.3235	0.1351
0.1	0.25	0.0987	0.561	0.1419	0.2798	0.2194
0.1	0.25	0.1201	0.5542	0.1334	0.2491	0.1942
0.2	0.25	0.2803	0.5574	0.1839	0.2461	0.2486
0.2	0.25	0.2197	0.577	0.1748	0.2125	0.2301
0.2	0.25	0.214	0.5644	0.1732	0.3019	0.1514
0.2	0.25	0.1824	0.5827	0.1926	0.2925	0.1492
0.2	0.25	0.2513	0.5869	0.1629	0.3038	0.2395
0.2	0.25	0.2354	0.5877	0.1467	0.2961	0.2396
0.2	0.25	0.2589	0.5823	0.151	0.3097	0.2109
0.2	0.25	0.2176	0.5652	0.1526	0.3123	0.1749
0.025	0.5	0.0754	0.4867	0.1756	0.261	0.1599
0.025	0.5	0.0645	0.4902	0.2003	0.2744	0.1638
0.025	0.5	0.0578	0.4306	0.2164	0.2246	0.1775
0.025	0.5	0.0629	0.4232	0.2266	0.2339	0.1971
0.025	0.5	0.0261	0.3563	0.1708	0.2715	0.1783
0.025	0.5	0.0433	0.3837	0.1746	0.3044	0.171
0.025	0.5	0.084	0.4094	0.1793	0.3157	0.1723
0.025	0.5	0.1339	0.4221	0.1921	0.3159	0.1576
0.05	0.5	0.1018	0.4777	0.178	0.313	0.2437
0.05	0.5	0.1068	0.4955	0.171	0.2915	0.2507
0.05	0.5	0.1313	0.4776	0.2014	0.2261	0.1646
0.05	0.5	0.1468	0.4737	0.2122	0.2926	0.186
0.05	0.5	0.0959	0.4573	0.2034	0.2519	0.2163
0.05	0.5	0.1652	0.4721	0.1935	0.2562	0.1837
0.05	0.5	0.0732	0.4849	0.1834	0.3303	0.1187
0.05	0.5	0.0845	0.4961	0.2198	0.3601	0.1213
0.1	0.5	0.1838	0.5488	0.2121	0.2669	0.2804
0.1	0.5	0.1763	0.5582	0.1892	0.2824	0.2767
0.1	0.5	0.2292	0.5212	0.2358	0.2773	0.2334
0.1	0.5	0.2217	0.5137	0.2441	0.2902	0.1984
0.1	0.5	0.2083	0.5468	0.1476	0.2162	0.0908
0.1	0.5	0.2082	0.5448	0.1403	0.2113	0.0992
0.1	0.5	0.2037	0.5514	0.2085	0.1865	0.1317
0.1	0.5	0.14	0.527	0.2297	0.2348	0.1383
0.2	0.5	0.2851	0.5694	0.1949	0.2853	0.2283
0.2	0.5	0.316	0.5664	0.1852	0.2861	0.2113
0.2	0.5	0.2059	0.597	0.2343	0.3013	0.2598
0.2	0.5	0.2227	0.5838	0.237	0.3184	0.2889
0.2	0.5	0.3379	0.5854	0.1716	0.2117	0.2698
0.2	0.5	0.3356	0.588	0.1762	0.2159	0.2599
0.2	0.5	0.2862	0.5867	0.2147	0.2314	0.1106
0.2	0.5	0.2693	0.5893	0.1964	0.2658	0.1203
0.025	1	0.0873	0.4258	0.1974	0.3064	0.1162
0.025	1	0.0751	0.4501	0.1789	0.2839	0.1214

0.025	1	0.0882	0.4631	0.2165	0.3237	0.2614
0.025	1	0.0819	0.4879	0.2068	0.34	0.2637
0.025	1	0.123	0.4284	0.2148	0.2782	0.1592
0.025	1	0.0524	0.4391	0.2232	0.2916	0.2253
0.025	1	0.021	0.4215	0.2235	0.2801	0.1232
0.025	1	0.0376	0.4478	0.2031	0.2564	0.1307
0.05	1	0.1742	0.5086	0.1707	0.2801	0.1726
0.05	1	0.1647	0.5138	0.1614	0.2746	0.1651
0.05	1	0.1588	0.529	0.3072	0.2718	0.2403
0.05	1	0.1597	0.5241	0.261	0.2598	0.2451
0.05	1	0.1846	0.4865	0.2127	0.2889	0.1236
0.05	1	0.1018	0.4942	0.2157	0.3268	0.1537
0.05	1	0.1215	0.4998	0.1923	0.2883	0.2071
0.05	1	0.101	0.4956	0.1961	0.3312	0.1918
0.1	1	0.1312	0.5636	0.2155	0.3115	0.2694
0.1	1	0.1378	0.542	0.2202	0.3166	0.2764
0.1	1	0.1452	0.533	0.2445	0.3908	0.1793
0.1	1	0.1184	0.5202	0.2501	0.3644	0.1741
0.1	1	0.1635	0.5755	0.2163	0.3158	0.1417
0.1	1	0.2388	0.5909	0.2233	0.351	0.1628
0.1	1	0.1531	0.5478	0.2591	0.2813	0.2639
0.1	1	0.1577	0.5626	0.2561	0.2801	0.2355
0.2	1	0.2264	0.5704	0.2121	0.2499	0.1882
0.2	1	0.2565	0.5849	0.2178	0.2291	0.172
0.2	1	0.3134	0.5953	0.2341	0.3278	0.2834
0.2	1	0.2699	0.6076	0.2487	0.3373	0.3069
0.2	1	0.2516	0.5654	0.2298	0.2916	0.3055
0.2	1	0.2486	0.5815	0.2341	0.2906	0.2825
0.2	1	0.2545	0.5617	0.2738	0.2637	0.1517
0.2	1	0.3077	0.5693	0.2667	0.2681	0.1791

[00387] The laundry application synergies between additional BASE variants [*i.e.*, BASE-X8C (*i.e.*, W11-T131I-T165I), BASE W10EK (*i.e.*, BASE-N128C-K178L-T182G-S243E-Y305R-D319T-G475K), and ACE-S243Q-G475K (*i.e.*, ACE-QK) in combination with the BPN^Y217L subtilisin protease were further tested on EMPA 160 and EMPA 163 swatches as described above. The results are shown in Figure 8 (BASE-X8C), Figure 9 (BASE W10EK), and Figure 10 (ACE-QK). These data demonstrate that a unique cleaning benefit is obtained using BASE variants in combination with a protease.

[00388] All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the disclosure pertains. Those of skill in the art readily appreciate that the present disclosure is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The compositions and methods described herein are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the disclosure. It is readily apparent to one skilled in the art that varying substitutions and modifications may be made to the disclosure disclosed herein without departing from the scope and spirit of the disclosure.

[00389] The disclosure illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the disclosure claimed. Thus, it should be understood that although the present disclosure has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this disclosure as defined by herein.

[00390] The disclosure has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of the disclosure with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not excised material is specifically recited herein.

CLAIMS

What is claimed is:

1. A cleaning composition comprising:

5 (a) an alpha-amylase having an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 2; and

(b) a protease;

wherein the composition provides a level of cleaning greater than that observed for corresponding compositions lacking the alpha amylase or lacking the protease.

10 2. The cleaning composition of claim 1, wherein the at least one additional enzyme is a subtilisin.

15 3. The cleaning composition of claim 2, wherein the at least one additional enzyme is subtilisin BPN' or a variant, thereof.

4. The cleaning composition of claim 3, wherein the at least one additional enzyme is the Y217L variant of subtilisin BPN'.

20 5. The cleaning composition of any of the preceding claims, further comprising at least one surfactant.

25 6. The cleaning composition of any of the preceding claims, further comprising at least one additional enzyme selected from the group consisting of a lipase, a cutinase, a carbohydrase, a cellulase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, an oxidase, a perhydrolase, a pectate lyase, and a peroxidase.

30 7. The cleaning composition of any of the preceding claims, wherein the alpha-amylase is derived from a parent alpha-amylase selected from the group consisting of BASE, ACE, ACE-Q, and ACE-QK.

8. The cleaning composition of any of the preceding claims, wherein the alpha-amylase is a mature form of a variant alpha-amylase having amylase activity, and comprising a substitution at one or more positions selected from the group consisting of 1, 2, 3, 4, 5, 7, 15, 16, 17, 18, 19, 22, 25, 26,

28, 29, 30, 32, 35, 36, 37, 50, 51, 52, 53, 54, 55, 56, 59, 60, 70, 71, 72, 73, 75, 78, 83, 87, 90, 91, 93, 94, 95, 104, 105, 107, 108, 110, 112, 113, 116, 118, 125, 126, 128, 129, 130, 131, 134, 136, 138, 142, 144, 147, 149, 150, 152, 154, 156, 158, 160, 161, 162, 165, 166, 168, 169, 170, 172, 174, 177, 178, 182, 183, 185, 189, 192, 195, 197, 201, 202, 203, 207, 210, 214, 217, 221, 228, 234, 236, 237, 246, 250, 254, 255, 257, 264, 267, 269, 270, 272, 275, 279, 283, 284, 298, 301, 303, 305, 306, 310, 311, 314, 318, 319, 320, 322, 323, 336, 337, 338, 339, 340, 344, 359, 374, 375, 376, 377, 379, 381, 382, 393, 394, 399, 401, 407, 408, 419, 433, 436, 438, 444, 447, 448, 451, 453, 459, 465, 470, 475, 476, 483, and 484;

wherein the positions correspond to amino acid residues in the amino acid sequence set forth in SEQ ID NO: 2; and

wherein the substitution of the naturally-occurring amino acid residue at the one or more positions for a different amino acid residue produces an alpha-amylase variant having a performance index > 1.0 for a measure of stability, and a performance index > 1.0 for a measure of activity.

9. The cleaning composition of claim 8, wherein the variant alpha-amylase comprises a substitution at one or more positions selected from the group consisting of 7, 29, 35, 53, 60, 72, 87, 108, 116, 126, 128, 129, 130, 131, 134, 136, 138, 142, 156, 161, 165, 178, 182, 185, 189, 192, 195, 197, 202, 210, 214, 217, 221, 234, 246, 269, 303, 310, 337, 340, 374, 401, and 438, and wherein the substitution of the naturally-occurring amino acid residue for a different amino acid residue, produces an alpha-amylase variant having a performance index > 1.5 for a measure of activity and a performance index > 1.0 for a measure of stability.

10. The cleaning composition of claim 8, wherein the variant alpha-amylase comprises a substitution at one or more positions selected from the group consisting of 2, 7, 22, 25, 28, 30, 37, 70, 75, 83, 87, 91, 93, 108, 128, 160, 165, 178, 182, 183, 217, 269, 270, 279, 283, 298, 305, 306, 310, 320, 374, 375, 376, 407, 419, 475, and 476, wherein the substitution of the naturally-occurring amino acid residue for a different amino acid residue, produces an alpha-amylase variant having a performance index > 1.5 for a measure of stability and a performance index > 1.0 for a measure of activity.

11. The cleaning composition of claim 8, wherein the variant alpha-amylase comprises a substitution at one or more positions selected from the group consisting of 83, 125, 128, 131, 160, 178, 182, 183, 185, 189, 279, 305, 319, 320, 379, 407, 433, 453, 475, 476, and 483.

12. The cleaning composition of any of claims 1-7, wherein the alpha-amylase is a mature form of a variant alpha-amylase comprising a substitution at one or more positions selected from the group consisting of 83, 125, 128, 131, 160, 178, 182, 183, 185, 189, 279, 305, 319, 320, 379, 407,

433, 453, 475, 476, and 483, wherein the positions correspond to amino acid residues in the amino acid sequence set forth in SEQ ID NO: 2, and wherein the substitution provides at least one beneficial effect selected from the group consisting of improved cleaning performance, improved detergent stability, improved thermostability, and improved protein expression.

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13. The cleaning composition of any of claims 1-7, wherein the alpha-amylase is a mature form of a variant alpha-amylase comprising a substitution at one or more positions selected from the group consisting of 5, 32, 83, 95, 154, 214, 221, 228, 322, 401, 407, 419, 444, 447, 459, 470, 483, and 484;

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wherein the positions correspond to amino acid residues in the amino acid sequence set forth in SEQ ID NO: 2; and

wherein the substitution of the naturally-occurring amino acid residue for a different amino acid residue, produces an alpha-amylase variant having a performance index value of 0.5 or better for activity at pH 8, activity at pH 10, activity at 16°C, and activity at 32°C, and a performance index

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value of 0.5 or better for stability in detergent and for thermostability.

14. The cleaning composition of any of claims 8-13, wherein alpha-amylase variant further comprises a substitution at position 243 corresponding to the amino acid sequence set forth in SEQ ID NO: 2.

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15. The cleaning composition of any of claims 8-13, wherein alpha-amylase variant further comprises a deletion at position 180 and/or position 181, corresponding to the amino acid sequence set forth in SEQ ID NO: 2.

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16. The cleaning composition of any of the preceding claims, wherein the alpha-amylase variant is derived from a parent alpha-amylase having an amino acid sequence that is at least 75% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14.

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17. The cleaning composition of any of the preceding claims, wherein the alpha-amylase variant has at least 75% sequence identity with the amino acid sequence set forth in SEQ ID NO: 2.

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18. The cleaning composition of any of the preceding claims, wherein the alpha-amylase variant has at least 80% sequence identity with the amino acid sequence set forth in SEQ ID NO: 2.

19. The cleaning composition of any of the preceding claims, wherein the alpha-amylase variant has at least 90% sequence identity with the amino acid sequence set forth in SEQ ID NO: 2.

20. The cleaning composition of any of the preceding claims, wherein said variant comprises
5 a substitution at one or more positions selected from the group consisting of 128, 178, 182, 185, and 189 corresponding to the amino acid sequence set forth in SEQ ID: 2, wherein the substitution provides improved cleaning performance or improved detergent stability.

21. The cleaning composition of any of the preceding claims, wherein said alpha-amylase
10 variant comprises:

- (a) an alanine at position 125,
a cysteine at position 128,
an isoleucine at position 131,
an isoleucine at position 165,
15 a leucine at position 178,
a glycine at position 182,
a tyrosine at position 202,
an arginine at position 305,
a threonine at position 319, or
20 an arginine at position 475;
- (b) the substitutions N128C+K178L+T182G+Y305R+G475K, and
at least one additional substitution selected from the group consisting of S125A, T131I,
T165I, F202Y, and D319T; or
- (c) the substitutions
25 N128C+K178L+T182G+F202Y+Y305R+D319T+G475K,
S125A+N128C+K178L+T182G+Y305R+G475K, or
S125A+N128C+T131I+T165I+K178L+T182G+Y305R+G475K;

wherein the variant optionally further comprises a substitution at position 243 and/or a deletion
at position 180 and/or position 181; and

30 wherein the positions correspond to the amino acid sequence set forth in SEQ ID NO: 2.

22. The cleaning composition of any of the preceding claims, wherein said alpha-amylase variant comprises a substitution at position 475.

35 23. The cleaning composition of any of the preceding claims, wherein said alpha-amylase variant comprises a substitution at position 243 and/or a deletion at position 180 and/or position 181.

24. The cleaning composition of any of the preceding claims, wherein said alpha-amylase variant comprises a substitution at position 243 and/or a deletion at position 180 and/or position 181.

5 25. The cleaning composition of any of the preceding claims, wherein the composition provides a level of cleaning greater than that observed for corresponding compositions lacking the alpha amylase or lacking the protease, as measured at a temperature below about 25°C.

10 26. The cleaning composition of any of the preceding claims, wherein the composition provides a level of cleaning greater than that observed for corresponding compositions lacking the alpha amylase or lacking the protease, as measured at a temperature below about 20°C.

27. The cleaning composition of any of the preceding claims, wherein the alpha-amylase and protease are co-formulated.

15 28. A method of cleaning a fabric or hard surface, comprising contacting the fabric or hard surface with the cleaning composition of any of the preceding claims.

20 29. The method of claim 28, wherein the method is performed at a temperature below about 25°C.

30. The method of claim 29, wherein the method is performed at a temperature below about 20°C.

	1	11	21	31	41	51
AAA63900	NTAPINETMMQYFEWDL	PNDGTLWTKVKNEA	ANLSSLGIT	LWLP	PAYKGT	SQSDVGYGV
AAA22240	--ANLNGTLMQYFEWY	MPNDGQHWKRLQND	SAYLA	EHGITAV	WIPPAYKGT	SQADVGYGA
AAA22241	-AAPFNGTMMQYFEWY	LPDDGTLWTKVANE	ANLSSLGIT	LWLP	PAYKGT	SRSDVGYGV
AAA22191	----VNGTLMQYFEWY	TPNDGQHWKRLQND	AEHLSDIGIT	AVWI	PPAYKGL	SQSDNGYGP
AAA22231	HHNGTNGTMMQYFEWY	LPNDGNHWNRLNSD	ASNLKSKGIT	AVWI	PPAWKGASQ	NDVGYGA
AAK00598	-DTVNNGTLMQYFEWY	APNDGNHWNRLR	TD	AE	LAQKGITSV	WIPPAYKGT
CAL48155	HHNGTNGTMMQYFEWY	LPNDGNHWNRLR	SDASNLKDKGIT	AVWI	PPAWKGASQ	NDVGYGA
AAE00432	HHNGTNGTMMQYFEWY	LPNDGNHWNRLR	DDASNLNRNGIT	AIWI	PPAWKGT	SQNDVGYGA
CAC16486	HHNGTNGTMMQYFEWY	LPNDGNHWNRLR	SDASNLKDKGIT	SAVWI	PPAWKGASQ	NDVGYGA
CAD35985	HHNGTNGTMMQYFEWY	LPNDGNHWNRLR	DDAANLKSKGIT	AVWI	PPAWKGT	SQNDVGYGA
CAJ00040	--DGLNGTMMQYFEWY	LENDGQHWNR	LHDDAAALSDAGIT	AIWI	PPAYKGT	NSQADVGYGA
	61	71	81	91	101	111
AAA63900	YDLYDLGEFNQKGTIR	TKYGTQYIQAIQA	AKAGMQVY	ADV	VFNH	KAGADGTEFVDAV
AAA22240	YDLYDLGEFNQKGTIR	TKYGTQKELQSAIK	SLHSRDIN	VYGD	VVNH	KGGADATEDVTAV
AAA22241	YDLYDLGEFNQKGTIR	TKYGTQKELQSAIK	AAHAGMQVY	ADV	FDH	KGGADGTEWVDAV
AAA22191	YDLYDLGEFNQKGTIR	TKYGTQKELQSAIK	SLHSRNVQVY	GDV	VVNH	KAGADATEDVTAV
AAA22231	YDLYDLGEFNQKGTIR	TKYGTQKELQSAIK	AAVTSKNGI	QVYGD	VVMNH	KGGADATEMVR
AAK00598	YDLYDLGEFNQKGTIR	TKYGTQKELQSAIK	ALHKNIDVY	GDV	VVMNH	KGGADYETETVAV
CAL48155	YDLYDLGEFNQKGTIR	TKYGTQKELQSAIK	ALHKNIDVY	GDV	VVMNH	KGGADATEWVR
AAE00432	YDLYDLGEFNQKGTIR	TKYGTQKELQSAIK	ALHKNIDVY	GDV	VVMNH	KGGADATEWVR
CAC16486	YDLYDLGEFNQKGTIR	TKYGTQKELQSAIK	ALHKNIDVY	GDV	VVMNH	KGGADATEWVR
CAD35985	YDLYDLGEFNQKGTIR	TKYGTQKELQSAIK	ALHKNIDVY	GDV	VVMNH	KGGADATEWVR
CAJ00040	YDLYDLGEFNQKGTIR	TKYGTQKELQSAIK	ALHKNIDVY	GDV	VVMNH	KGGADATEWVR
	121	131	141	151	161	171
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AAA22240	EVDPSNRNQETSGTYQ	IQAWTKFDFPGR	GNTYSSFKWRWY	HFDG	TWD	DESRL-NRIYKF
AAA22241	EVDPSNRNQETSGTYQ	IQAWTKFDFPGR	GNTYSSFKWRWY	HFDG	TWD	DESRL-NRIYKF
AAA22191	EVDPSNRNQETSGTYQ	IQAWTKFDFPGR	GNTYSSFKWRWY	HFDG	TWD	DESRL-NRIYKF
AAA22231	EVDPSNRNQETSGTYQ	IQAWTKFDFPGR	GNTYSSFKWRWY	HFDG	TWD	DESRL-NRIYKF
AAK00598	EVDPSNRNQETSGTYQ	IQAWTKFDFPGR	GNTYSSFKWRWY	HFDG	TWD	DESRL-NRIYKF
CAL48155	EVDPSNRNQETSGTYQ	IQAWTKFDFPGR	GNTYSSFKWRWY	HFDG	TWD	DESRL-NRIYKF
AAE00432	EVDPSNRNQETSGTYQ	IQAWTKFDFPGR	GNTYSSFKWRWY	HFDG	TWD	DESRL-NRIYKF
CAC16486	EVDPSNRNQETSGTYQ	IQAWTKFDFPGR	GNTYSSFKWRWY	HFDG	TWD	DESRL-NRIYKF
CAD35985	EVDPSNRNQETSGTYQ	IQAWTKFDFPGR	GNTYSSFKWRWY	HFDG	TWD	DESRL-NRIYKF
CAJ00040	EVDPSNRNQETSGTYQ	IQAWTKFDFPGR	GNTYSSFKWRWY	HFDG	TWD	DESRL-NRIYKF
	181	191	201	211	221	231
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AAA22240	QG--KAWDWEVSN	ENGNYDYL	MYADIDY	DPDVA	AEIKRWGTWY	ANELQLDGFR
AAA22241	RGIGKAWDWEVDTE	NGNYDYL	MYADLDM	HP	EVVTE	LKNWGTWYVNTTNIDGFR
AAA22191	RGEKAWDWEVSS	ENGNYDYL	MYADVDY	DPDVA	ETKKWGIWY	ANELSLDGFR
AAA22231	RGHGKAWDWEVDTE	NGNYDYL	MYADIDM	HP	EVVTE	LKNWGTWYVNTTNIDGFR
AAK00598	RGIGKAWDWEVSS	ENGNYDYL	MYADIDM	HP	EVVTE	LKNWGTWYVNTTNIDGFR
CAL48155	RGDGKAWDWEVDTE	NGNYDYL	MYADIDM	HP	EVVTE	LKNWGTWYVNTTNIDGFR
AAE00432	RGDGKAWDWEVSS	ENGNYDYL	MYADIDM	HP	EVVTE	LKNWGTWYVNTTNIDGFR
CAC16486	RGDGKAWDWEVDTE	NGNYDYL	MYADIDM	HP	EVVTE	LKNWGTWYVNTTNIDGFR
CAD35985	RGTGKAWDWEVDTE	NGNYDYL	MYADIDM	HP	EVVTE	LKNWGTWYVNTTNIDGFR
CAJ00040	AN--TNWNWRVDE	ENGNYDYL	LGSNIDF	SHPEVQ	DELKDWG	SWFTDEL

Figure 1

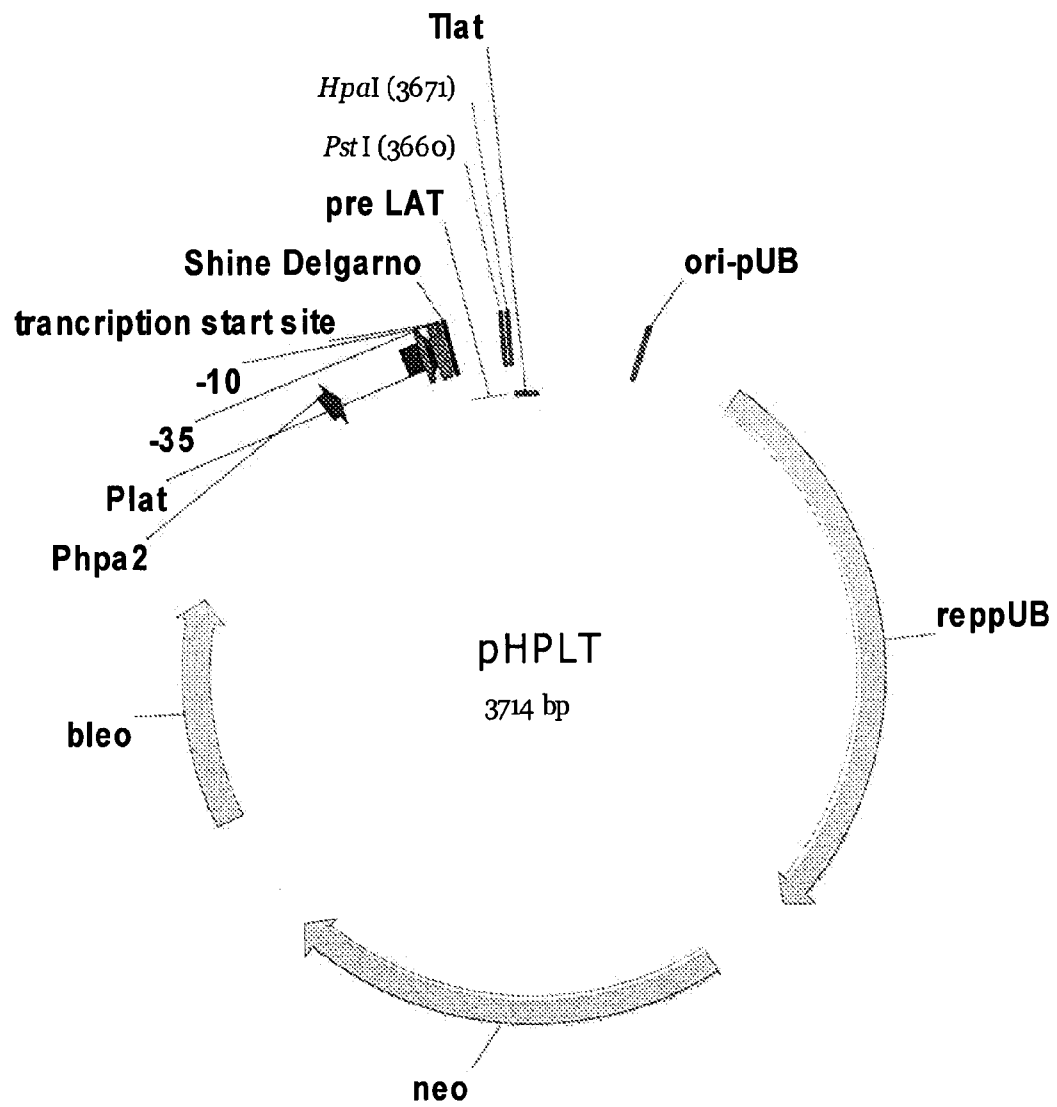
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AAA22241	IKFSFLRDWVNVHREKTGKEMFTVAEYWQNDLGALENYLNKTNFNHVSFVDVPLHYQFHAA					
AAA22191	IKFSFFPDWLSYVRSQTGKPLFTVGEYWSYDINKLHNYITKTNGTMSLFDAPLHNNFYTA					
AAA22231	IKYSFTRDWINHVRSATGKNMFAVAEFWKNDLGAIENYLQKTNNHNSVFDVPLHYNLYNA					
AAK00598	IDHEYLRDWNHVRQQTGKEMFAVAEYWQNDIQTLNNYLAKVNYNQSVFDAPLHYNFYHA					
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AAE00432	IKYSFTRDWLTHVRNATGKEMFAVAEFWKNDLGALENYLNKTNWNHNSVFDVPLHYNLYNA					
CAC16486	IKYSFTRDWINHVRSATGKNMFAVAEFWKNDLGAIENYLKTNWNHNSVFDVPLHYNLYNA					
CAD35985	IKYSYTRDWLTHVRNTTGKPMFAVAEFWKNDLAAIENYLKTNWNHNSVFDVPLHYNLYNA					
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AAA63900	301	311	321	331	341	351
AAA22240	SKSSGYFDMRYLLNNTLMKDQPSLAVTLVDNHDTPQGQSLQSWVEPWFKPLAYAFILTRQ					
AAA22241	STQGGGYDMRKLNGTVVSKHPLKSVTFVDNHDTPQGQSLQSWVEPWFKPLAYAFILTRQ					
AAA22191	SSQGGGYDMRRLLDGTVVSRHPEKAVTFVENHDTQPGQSLQSWVEPWFKPLAYAFILTRQ					
AAA22231	SKSGGNYDMRNIFNGTVVQRHPSHAVTFVDNHDSPQEEALESFVEEWFKPLAYALTLTRE					
AAK00598	SKGNGNYDMRNILKGTVVANHPTLAVTLVENHDSQPGQSLQSWVEPWFKPLAYAFILTRA					
CAL48155	SRSGGNYDMRQIFNGTVVQRHPTHAVTFVDNHDSPQEEALESFVEEWFKPLAYALTLTRD					
AAE00432	SNSGGNYDMAKLLNGTVVQKHPMHAVTFVDNHDSPQGESLESFVQEWFKPLAYALILTRE					
CAC16486	SKSGGNYDMRQIFNGTVVQRHPMHAVTFVDNHDSPQEEALESFVEEWFKPLAYALTLTRE					
CAD35985	SNSGGYFDMRNILNGSVVQKHPHIAVTFVDNHDSPQGEALESFVQSWFKPLAYALILTRE					
CAJ00040	SQQGGSYDMRNILRGLSLEAHPMHAVTFVDNHDTPQGESLESWVADWFKPLAYATILTRE					
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AAA22240	EGYPCVFYGDYYGI---PKYNIPLGLKSKIDPLLIARRDYAYGTQRDYIDHQDIIGWTREG					
AAA22241	SGYPQVFYGDYGTGKGSQREIPALKHKIEPILKARKQYAYGAQHDFDHHDIIGWTREG					
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AAK00598	QGYPSVFYGDYYGI---PTHGVPAMRSKIDPILEARQKYAYGKQNDYLDHNNIIGWTREG					
CAL48155	EGYPSVFYGDYGTGKGSNYEIPALKDKIDPILTARKNYAYGTQRDYFDHDPVIGWTREG					
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CAD35985	QGYPSVFYGDYYGI---PTHGVPAMRSKIDPILEARQKYAYGRQNDYLDHNNIIGWTREG					
CAJ00040	QGYPSVFYGDYYGI---PTHGVPMSKSKIDPLLQARQTYAYGTQHDYFDHDDIIGWTREG					
	GGYPNVFYGDYYGI---PNDNISAKKDMIDELLDARQNYAYGTQHDYFDHWDVVGWTREG					
AAA63900	421	431	441	451	461	471
AAA22240	IDTKPNSGLAALITDGPGGSKWMYVGKKHAGKVFYDLTGNRSDTVTINADGWGEFKVNGG					
AAA22241	DSSVANSGLAALITDGPGGAKRMYVGRQNAGETWHDITGNRSEPVVINSEGWGEFHVNGG					
AAA22191	VTEKPGSGLAALITDGPGGSKWMYVGKQHAGKVFYDLTGNRSDTVTINSDGWGEFKVNGG					
AAA22231	DSSAAKSGLAALITDGPGGSKRMYAGLKNAGETWYDITGNRSDTVKIGSDGWGEFHVNDG					
AAK00598	NTAHPNSGLATIMSDGAGGSKWMFVGRNKAGQVWSDITGNRTGTVTINADGWCNFSVNGG					
CAL48155	DSVHANSGLATLISDGPGGAKWMDVGGKNNAGEIWDITGNQNTNTVTINKDGWQQFQVSGG					
AAE00432	NTAHPNSGLATIMSDGPGGNKWMYVGRNKAGQVWRDITGNRSGTVTINADGWCNFSVNGG					
CAC16486	NTTHPNSGLATIMSDGPGGERKWMYVQNKAGQVWHDITGNKPGTVTINADGWANFSVNGG					
CAD35985	NTAHPNSGLATIMSDGAGGNKWMFVGRNKAGQVWTDITGNRAGTVTINADGWCNFSVNGG					
CAJ00040	DSSHPNSGLATIMSDGPGGNKWMYVGKHKAGQVWRDITGNRSGTVTINADGWCNFTVNGG					
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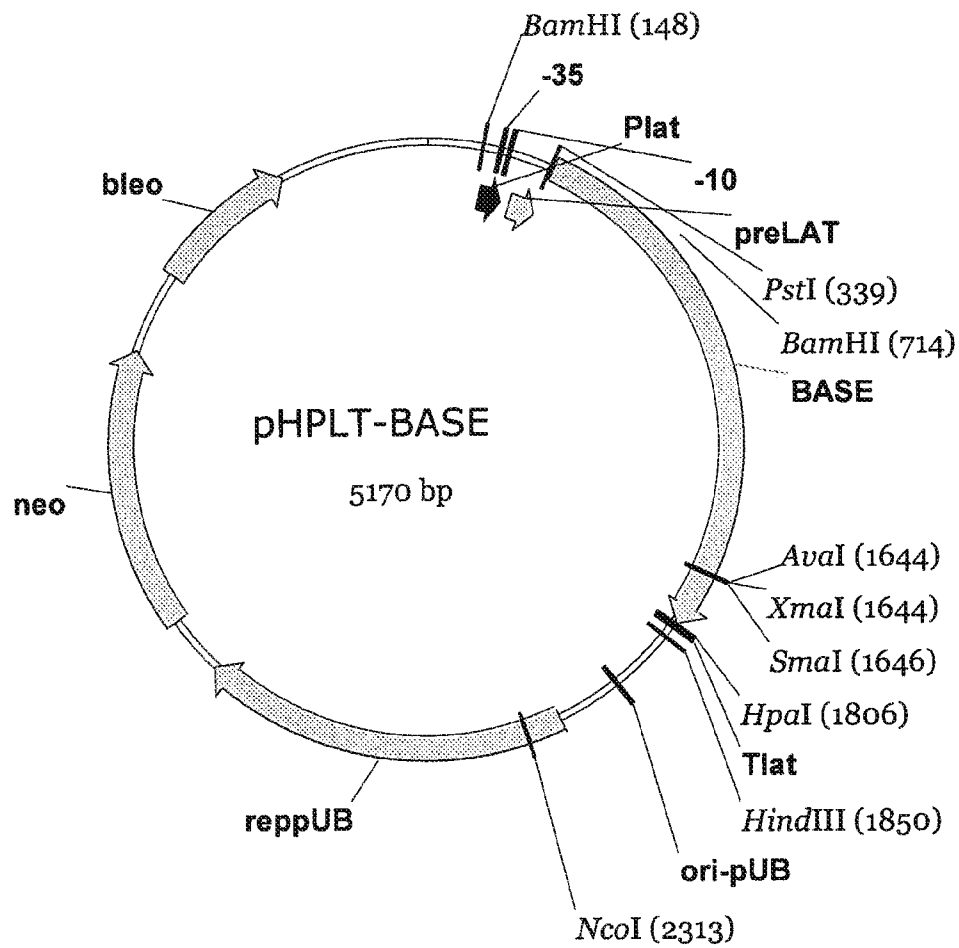
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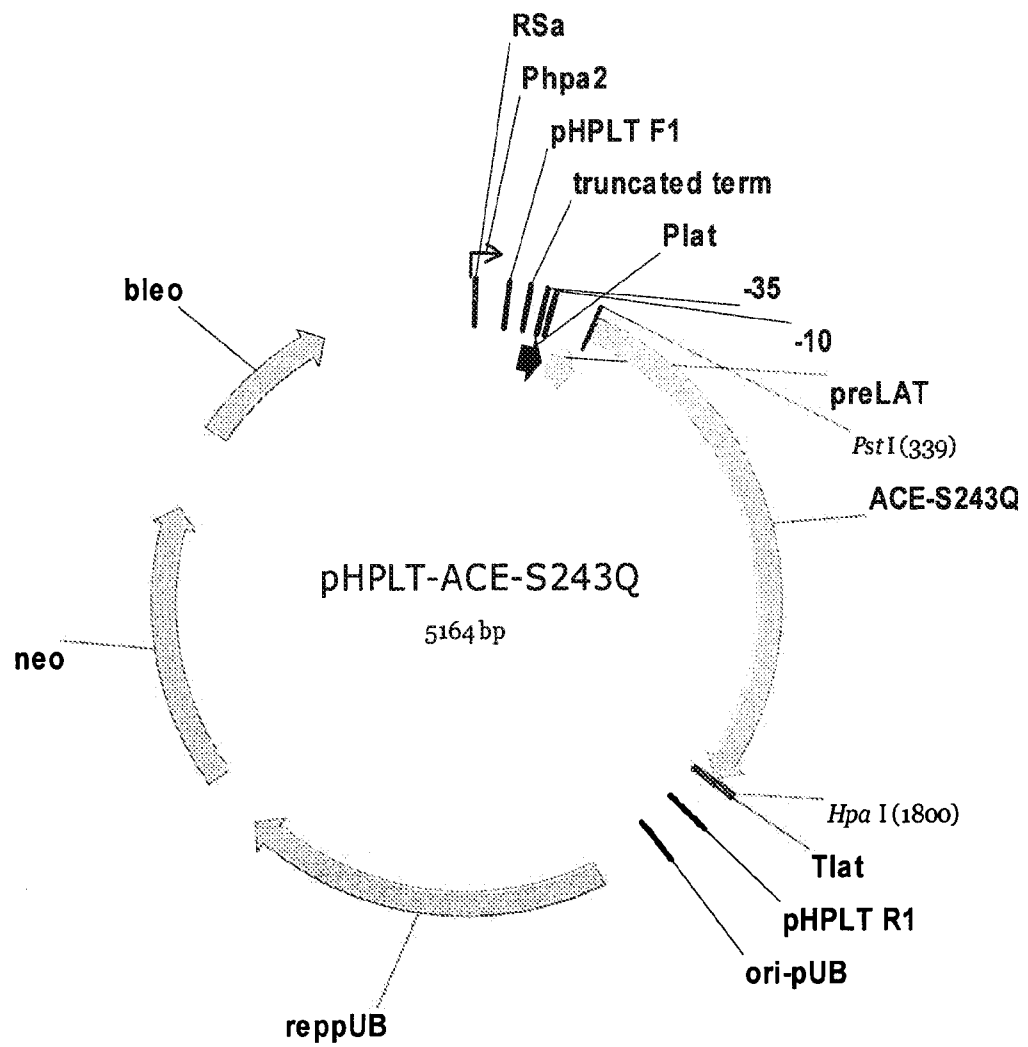
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AAA22240	SVSIYVQR-----	(SEQ ID NO: 5)
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AAA22191	SVSIYVQK-----	(SEQ ID NO: 7)
AAA22231	SVSIWVVK-----	(SEQ ID NO: 8)
AAK00598	SVSIYVQR-----	(SEQ ID NO: 9)
CAL48155	SVSIWVNN-----	(SEQ ID NO: 10)
AAE00432	SVSIWVKR-----	(SEQ ID NO: 11)
CAC16486	SVSIWVVK-----	(SEQ ID NO: 12)
CAD35985	AVSVWVKQ-----	(SEQ ID NO: 13)
CAJ00040	SVSVYVNO-----	(SEQ ID NO: 14)

Figure 1 (cont.)

**Figure 2**

**Figure 3**

**Figure 4**

Wash performance test-BASE variants W9, W 10, W11 and ACE Q (G475K)

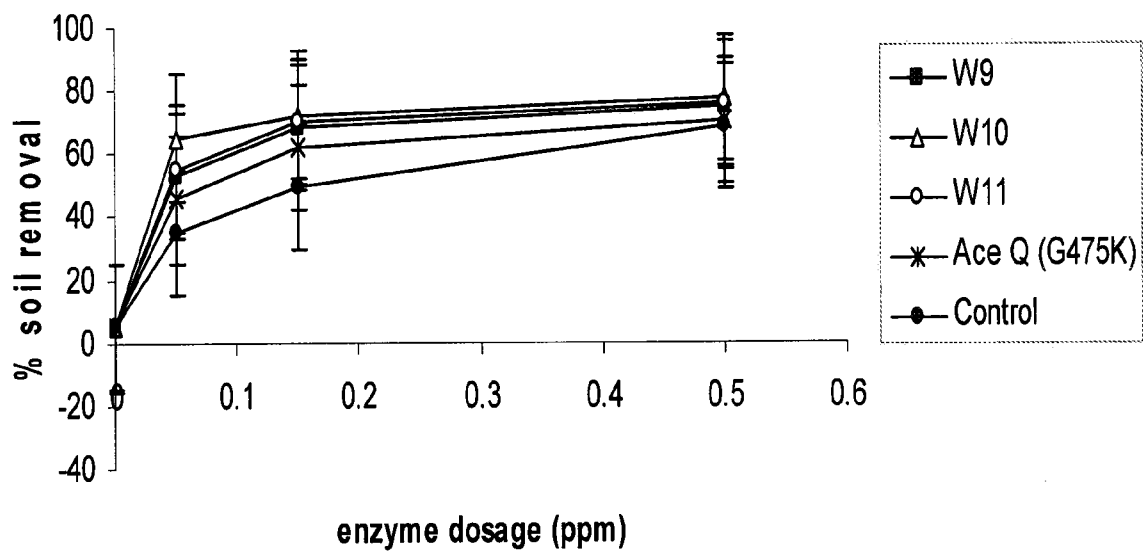
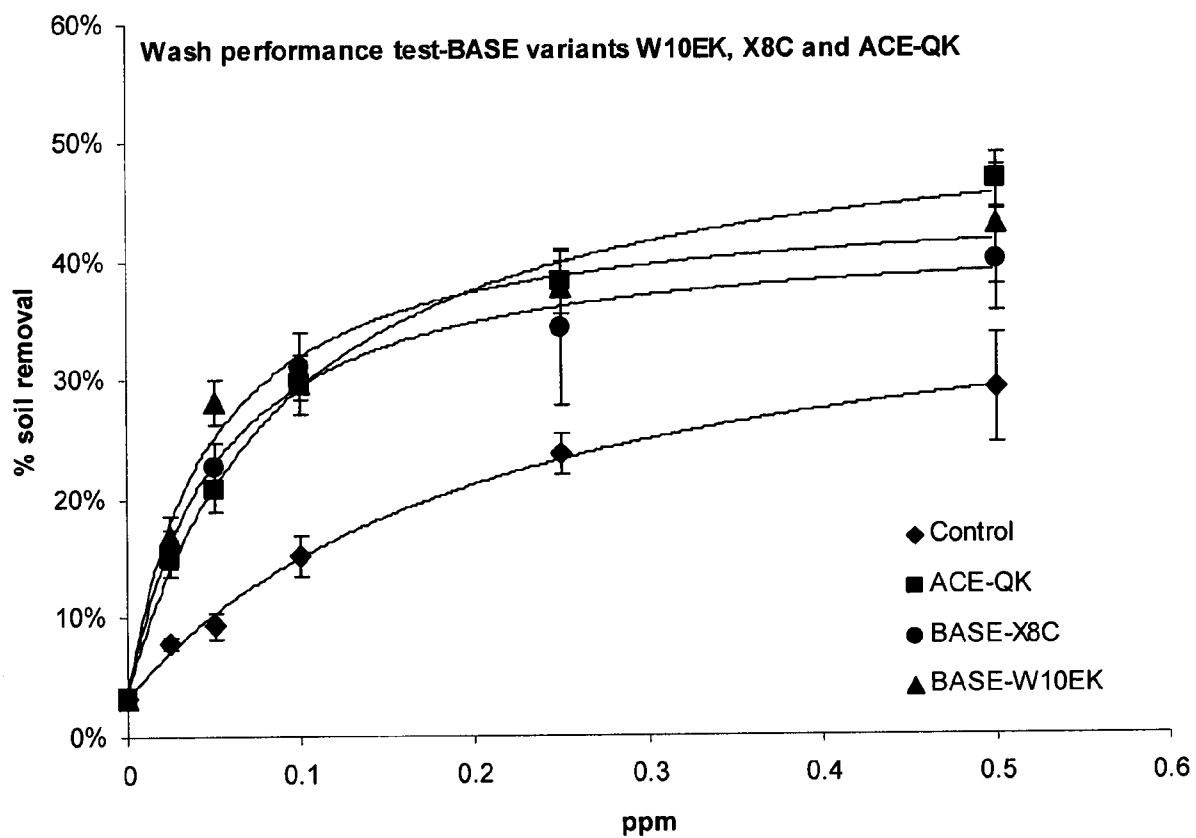


Figure 5

**Figure 6**

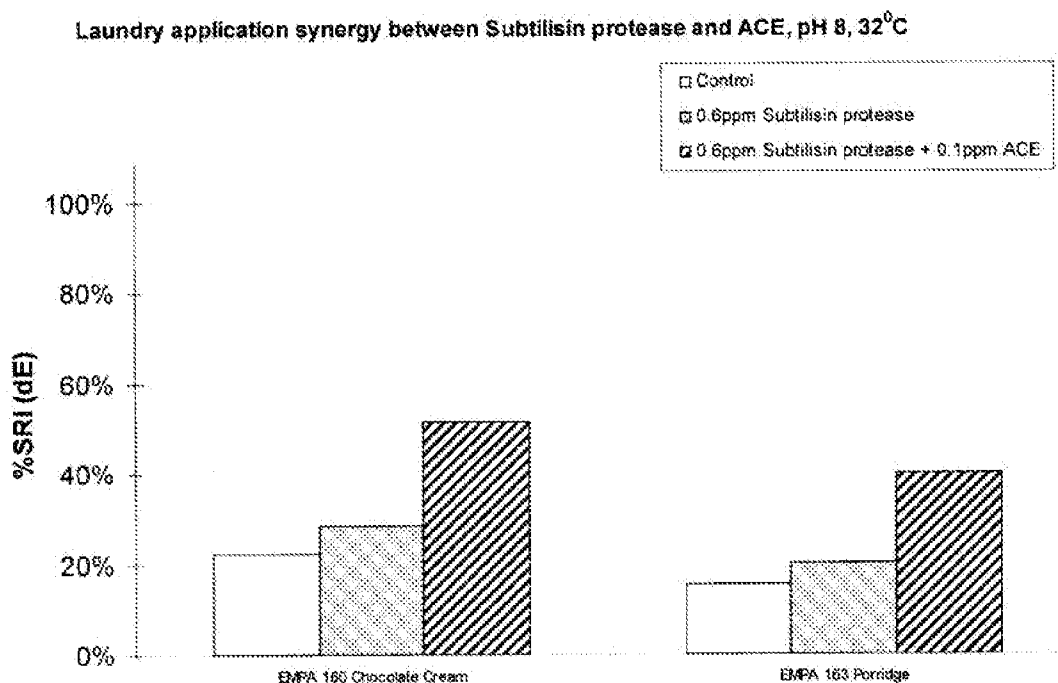


Figure 7A

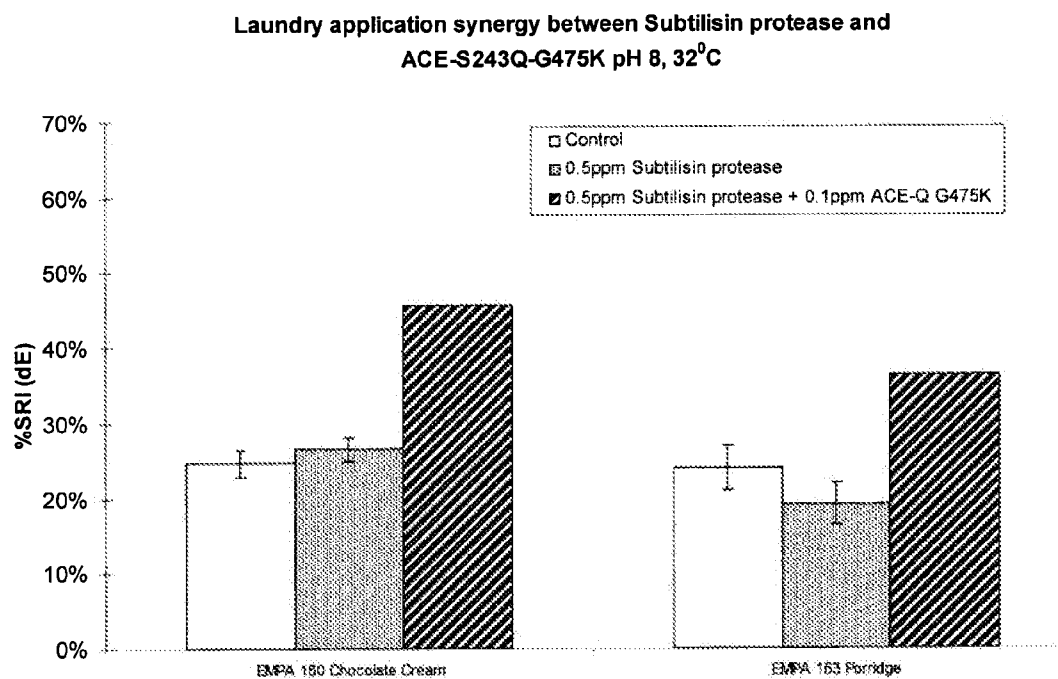
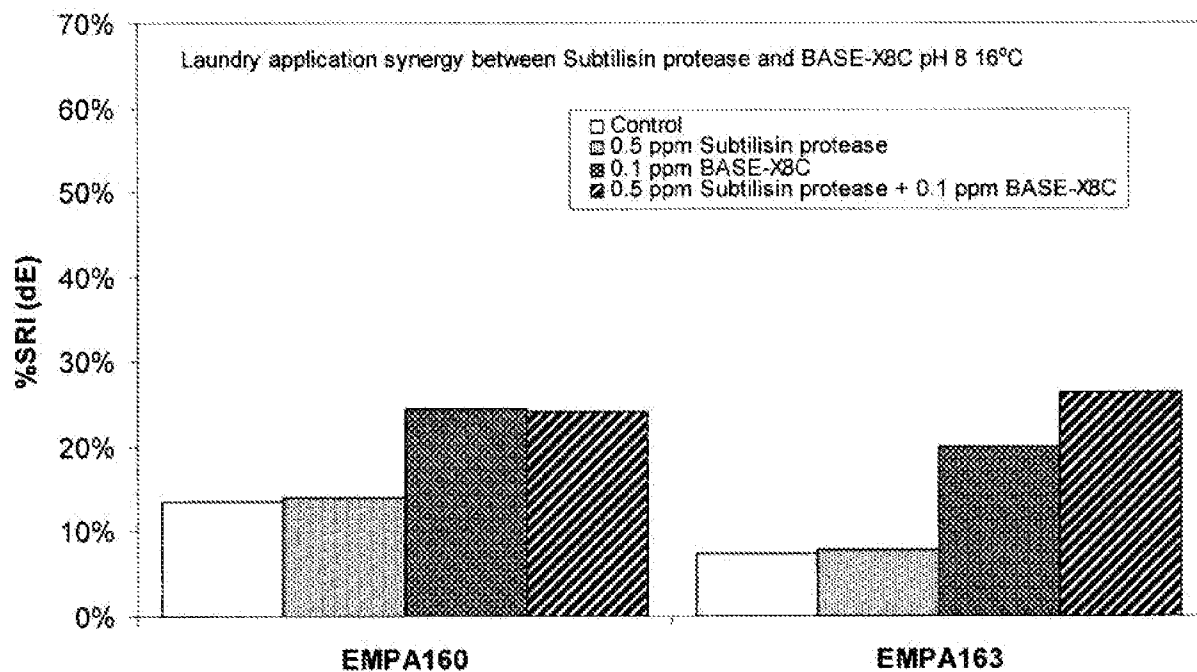
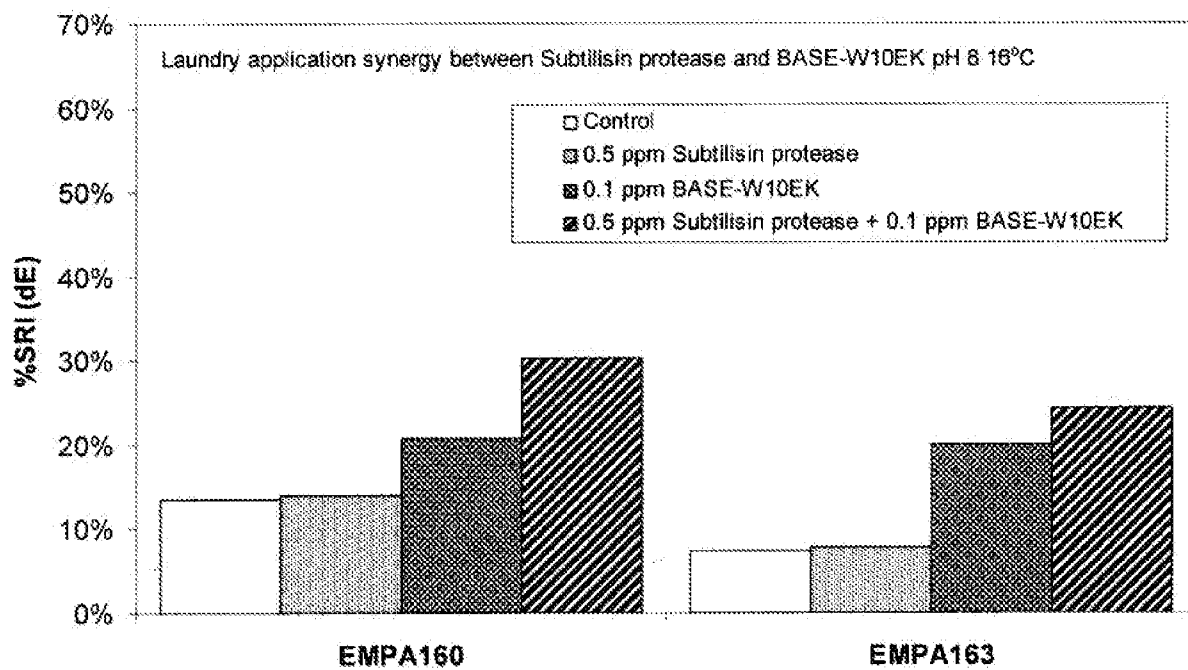
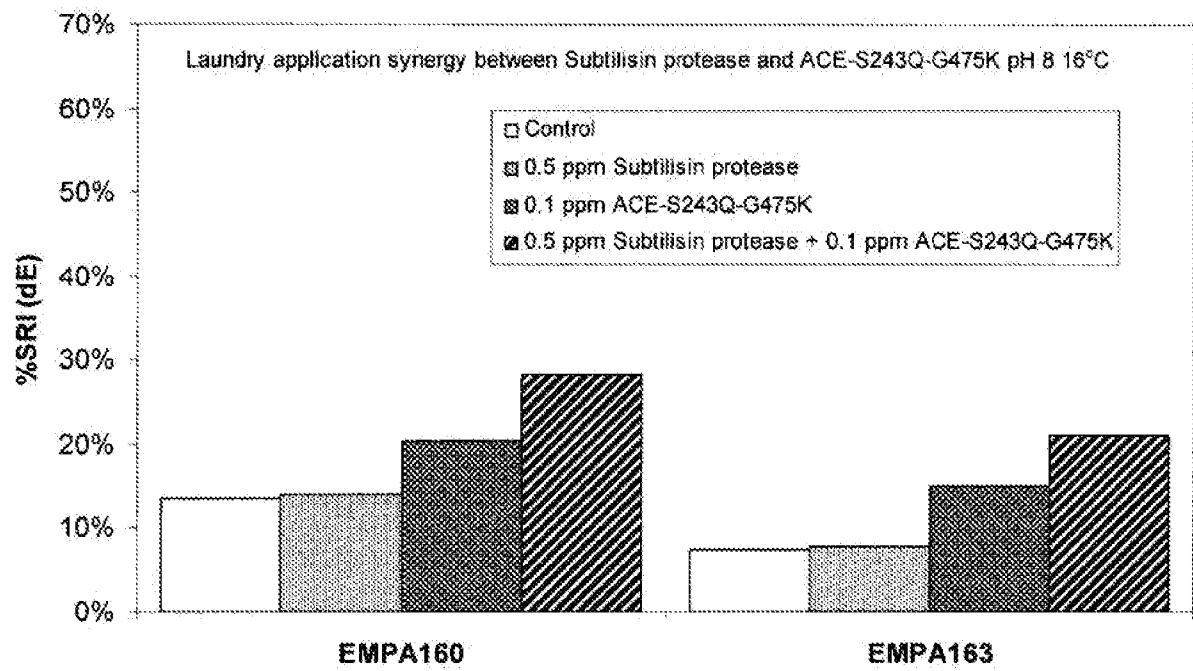


Figure 7B

**Figure 8****Figure 9**

**Figure 10**