



(86) Date de dépôt PCT/PCT Filing Date: 2010/06/03  
(87) Date publication PCT/PCT Publication Date: 2010/12/09  
(85) Entrée phase nationale/National Entry: 2011/11/30  
(86) N° demande PCT/PCT Application No.: US 2010/037328  
(87) N° publication PCT/PCT Publication No.: 2010/141779  
(30) Priorité/Priority: 2009/06/03 (US61/183,959)

(51) Cl.Int./Int.Cl. *C12N 9/42* (2006.01)

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(54) Titre : VARIANTS DE CELLULOSE A EXPRESSION, ACTIVITE ET/OU STABILITE AMELIOREE(S), ET  
UTILISATION ASSOCIEE

(54) Title: CELLULASE VARIANTS WITH IMPROVED EXPRESSION, ACTIVITY AND/OR STABILITY, AND USE  
THEREOF

(57) **Abrégé/Abstract:**

The present disclosure relates to cellulase variants. In particular the present disclosure relates to cellulase variants having improved expression, activity and/or stability. Also described are nucleic acids encoding the cellulase variants, compositions comprising the cellulase variants, and methods of use thereof.



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## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau

(43) International Publication Date  
9 December 2010 (09.12.2010)



(10) International Publication Number  
**WO 2010/141779 A1**

(51) International Patent Classification:  
*C12N 9/42* (2006.01)

(21) International Application Number:  
PCT/US2010/037328

(22) International Filing Date:  
3 June 2010 (03.06.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
61/183,959 3 June 2009 (03.06.2009) US

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(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

## Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: CELLULASE VARIANTS WITH IMPROVED EXPRESSION, ACTIVITY AND/OR STABILITY, AND USE THEREOF

(57) Abstract: The present disclosure relates to cellulase variants. In particular the present disclosure relates to cellulase variants having improved expression, activity and/or stability. Also described are nucleic acids encoding the cellulase variants, compositions comprising the cellulase variants, and methods of use thereof.



WO 2010/141779 A1

**CELLULASE VARIANTS WITH IMPROVED EXPRESSION, ACTIVITY AND/OR  
STABILITY, AND USE THEREOF**

**STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY  
SPONSORED RESEARCH AND DEVELOPMENT**

**CROSS REFERENCE TO RELATED APPLICATION**

[01] This application claims the benefit of U.S. Provisional Application No. 61/183,959, filed on June 3, 2009, which is hereby incorporated by reference in its entirety.

[02] This invention was made with Government support under conditional award no: DE-FC36-08GO18078 awarded by the Department of Energy. The Government has certain rights in this invention.

**I. FIELD**

[03] The present disclosure relates to enzymes and in particular cellulase variants. Also described are nucleic acids encoding the cellulase variants, compositions comprising the cellulase variants, methods of identifying additional useful cellulase variants and methods of use thereof.

**II. BACKGROUND**

[04] Cellulose and hemicellulose are the most abundant plant materials produced by photosynthesis. They can be degraded and used as an energy source by numerous microorganisms (*e.g.*, bacteria, yeast and fungi) that produce extracellular enzymes capable of hydrolysis of the polymeric substrates to monomeric sugars (Aro et al., J Biol Chem, 276: 24309-24314, 2001). As the limits of non-renewable resources approach, the potential of cellulose to become a major renewable energy resource is enormous (Krishna et al., Bioresource Tech, 77: 193-196, 2001). The effective utilization of cellulose through biological processes is one approach to overcoming the shortage of foods, feeds, and fuels (Ohmiya et al., Biotechnol Gen Engineer Rev, 14: 365-414, 1997).

[06] Cellulases are enzymes that hydrolyze cellulose (beta-1,4-glucan or beta D-glucosidic linkages) resulting in the formation of glucose, cellobiose, cellooligosaccharides, and the like. Cellulases have been traditionally divided into three major classes: endoglucanases (EC 3.2.1.4) ("EG"), exoglucanases or cellobiohydrolases (EC 3.2.1.91) ("CBH") and beta-glucosidases ([beta]-D-glucoside glucohydrolase; EC 3.2.1.21) ("BG") (Knowles et al., TIBTECH 5: 255-261, 1987; and Schulein, Methods Enzymol, 160: 234-243, 1988).

Endoglucanases act mainly on the amorphous parts of the cellulose fibre, whereas cellobiohydrolases are also able to degrade crystalline cellulose (Nevalainen and Penttila, Mycota, 303-319, 1995). Thus, the presence of a cellobiohydrolase in a cellulase system is required for efficient solubilization of crystalline cellulose (Suurnakki et al., Cellulose 7: 189-209, 2000). Beta-glucosidase acts to liberate D-glucose units from cellobiose, cellooligosaccharides, and other glucosides (Freer, J Biol Chem, 268: 9337-9342, 1993).

[07] Cellulases are known to be produced by a large number of bacteria, yeast and fungi. Certain fungi produce a complete cellulase system capable of degrading crystalline forms of cellulose, such that the cellulases are readily produced in large quantities via fermentation. Filamentous fungi play a special role since many yeast, such as *Saccharomyces cerevisiae*, lack the ability to hydrolyze cellulose (See, e.g., Wood et al., Methods in Enzymology, 160: 87-116, 1988).

[08] The fungal cellulase classifications of CBH, EG and BG can be further expanded to include multiple components within each classification. For example, multiple CBHs, EGs and BGs have been isolated from a variety of fungal sources including *Trichoderma reesei* (also referred to as *Hypocrea jecorina*), which contains known genes for two CBHs, i.e., CBH I ("CBH1") and CBH II ("CBH2"), at least 8 EGs, i.e., EG I, EG II, EG III, EGIV, EGV, EGVII and EGVIII, and at least 5 BGs, i.e., BG1, BG2, BG3, BG4 and BG5. EGIV, EGVII and EGVIII also have xyloglucanase activity.

[09] In order to efficiently convert crystalline cellulose to glucose the complete cellulase system comprising components from each of the CBH, EG and BG classifications is required, with isolated components less effective in hydrolyzing crystalline cellulose (Filho et al., Can J Microbiol, 42:1-5, 1996). A synergistic relationship has been observed between cellulase components from different classifications. In particular, the EG-type cellulases and CBH-type cellulases synergistically interact to more efficiently degrade cellulose.

[10] Cellulases are known in the art to be useful in the treatment of textiles for the purposes of enhancing the cleaning ability of detergent compositions, for use as a softening

agent, for improving the feel and appearance of cotton fabrics, and the like (Kumar et al., Textile Chemist and Colorist, 29:37-42, 1997). Cellulase-containing detergent compositions with improved cleaning performance (U.S. Pat. No. 4,435,307; GB App. Nos. 2,095,275 and 2,094,826) and for use in the treatment of fabric to improve the feel and appearance of the textile (U.S. Pat. Nos. 5,648,263, 5,691,178, and 5,776,757; and GB App. No. 1,358,599), have been described. Hence, cellulases produced in fungi and bacteria have received significant attention. In particular, fermentation of *Trichoderma* spp. (e.g., *Trichoderma longibrachiatum* or *Trichoderma reesei*) has been shown to produce a complete cellulase system capable of degrading crystalline forms of cellulose.

[11] Although cellulase compositions have been previously described, there remains a need for new and improved cellulase compositions. Improved cellulase compositions find used in household detergents, textile treatments, biomass conversion and paper manufacturing. Cellulases that exhibit improved expression, activity and stability are of particular interest.

### III. SUMMARY

[12] The present disclosure relates to enzymes and in particular cellulase variants. Also described are nucleic acids encoding the cellulase variants, compositions comprising the cellulase variants, methods of identifying additional useful cellulase variants and methods of use thereof.

[13] The present disclosure provides cellulase variants, wherein the variants are mature forms having cellulase activity and comprising a substitution at one or more positions selected from the group consisting of: 5, 18, 19, 28, 30, 32, 35, 38, 79, 80, 89, 100, 102, 103, 104, 105, 111, 117, 119, 121, 125, 126, 133, 137, 138, 139, 140, 141, 143, 150, 158, 162, 177, 180, 181, 182, 185, 186, 188, 190, 191, 192, 193, 196, 201, 207, 225, 226, 228, 229, 230, 233, 234, 236, 240, 243, 245, 251, 252, 258, 267, 268, 274, 292, 293, 303, 304, 306, 307, 313, 319, 322, 328, 331, 338, 340, 346, 361, 362, 363, 364, 365, 371, 384, 394, 396, 400, 406, 407, 414, 417, 422, 427, 431, 433, 436, 440, 441, 443, 444, 445 and 447, wherein the positions are numbered by correspondence with the amino acid sequence of a reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3. In some embodiments, the substitution at one or more positions results in a cellulase variant with improved expression, activity and/or stability in comparison to the reference CBH2. In some embodiments, the variant comprises a further substitution at one or more further positions selected from: (i) a

first group consisting of 63, 77, 129, 146, 147, 151, 153, 157, 161, 189, 194, 197, 203, 204, 208, 211, 237, 239, 244, 247, 254, 277, 281, 285, 288, 289, 294, 327, 339, 344, 356, 378, 382 and 405; or (ii) a second group consisting of 94, 98, 107, 120, 134, 144, 147, 154, 178, 179, 206, 210, 214, 231, 232, 266, 272, 275, 316, 323, 343, 360, 380, 381, 386, 399, 410, 413, 416, 426, and 429, wherein the further positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3. In some embodiments, the substitution at one or more positions is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 positions.

[14] In another aspect the disclosure provides cellulase variants, wherein the variants are mature forms having cellulase activity and comprising a substitution at one or more positions selected from the group consisting of: 63, 77, 129, 146, 147, 151, 153, 157, 161, 189, 194, 197, 203, 204, 208, 211, 237, 239, 244, 247, 254, 277, 281, 285, 288, 289, 294, 327, 339, 344, 356, 378, 382 and 405, wherein the positions are numbered by correspondence with the amino acid sequence of a reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3 and wherein when the cellulase variant consists of a single substitution, the single substitution is not a K/E, R/Q, N/D, Q/E, D/N or E/Q substitution. In some embodiments, the substitution at one or more positions results in a cellulase variant with improved expression, activity and/or stability in comparison to the reference CBH2. In some embodiments, the isolated cellulase variant comprises a further substitution at one or more further positions selected from: (i) a first group consisting of 5, 18, 19, 28, 30, 32, 35, 38, 79, 80, 89, 100, 102, 103, 104, 105, 111, 117, 119, 121, 125, 126, 133, 137, 138, 139, 140, 141, 143, 150, 158, 162, 177, 180, 181, 182, 185, 186, 188, 190, 191, 192, 193, 196, 201, 207, 225, 226, 228, 229, 230, 233, 234, 236, 240, 243, 245, 251, 252, 258, 267, 268, 274, 292, 293, 303, 304, 306, 307, 313, 319, 322, 328, 331, 338, 340, 346, 361, 362, 363, 364, 365, 371, 384, 394, 396, 400, 406, 407, 414, 417, 422, 427, 431, 433, 436, 440, 441, 443, 444, 445 and 447; or (ii) a second group consisting of 94, 98, 107, 120, 134, 144, 147, 154, 178, 179, 206, 210, 214, 231, 232, 266, 272, 275, 316, 323, 343, 360, 380, 381, 386, 399, 410, 413, 416, 426, and 429, wherein the further positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3. In some embodiments, the substitution at one or more positions is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 positions.

[15] In another aspect the disclosure provides cellulase variants, wherein the variants are mature forms having cellulase activity and comprising a substitution at one or more positions

selected from the group consisting of: 94, 98, 107, 120, 134, 144, 147, 154, 178, 179, 206, 210, 214, 231, 232, 266, 272, 275, 316, 323, 343, 360, 380, 381, 386, 399, 410, 413, 416, 426, and 429, wherein the positions are numbered by correspondence with the amino acid sequence of a reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3, and wherein

5 when the cellulase variant consists of a single substitution, the single substitution is not one of the group consisting of V94E, P98L, E107Q, M120L, M134G, M134L, M134V, L144G, L144R, L144S, T154A, A178V, L179C, V206L, S210L, S210R, T214M, T214Y, G231P, G231I, G231A, G231N, G231S, G231T, T232V, H266S, H266A, W272A, W272D, W272Y, N275L, S316P, V323L, V323N, V323Y, G360R, S380T, A381T, E399N, E399D, R413Y,

10 R413P, and A416G. In some embodiments, the substitution at one or more positions results in a cellulase variant with improved expression, activity and/or stability in comparison to the reference CBH2. In some embodiments, the variant comprises a further substitution at one or more further positions selected from: (i) a first group consisting of 5, 18, 19, 28, 30, 32, 35, 38, 79, 80, 89, 100, 102, 103, 104, 105, 111, 117, 119, 121, 125, 126, 133, 137, 138, 139,

15 140, 141, 143, 150, 158, 162, 177, 180, 181, 182, 185, 186, 188, 190, 191, 192, 193, 196, 201, 207, 225, 226, 228, 229, 230, 233, 234, 236, 240, 243, 245, 251, 252, 258, 267, 268, 274, 292, 293, 303, 304, 306, 307, 313, 319, 322, 328, 331, 338, 340, 346, 361, 362, 363, 364, 365, 371, 384, 394, 396, 400, 406, 407, 414, 417, 422, 427, 431, 433, 436, 440, 441, 443, 444, 445 and 447; or (ii) a second group consisting of 63, 77, 129, 146, 147, 151, 153,

20 157, 161, 189, 194, 197, 203, 204, 208, 211, 237, 239, 244, 247, 254, 277, 281, 285, 288, 289, 294, 327, 339, 344, 356, 378, 382 and 405, wherein the further positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3. In some embodiments, the substitution at one or more positions is selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 positions.

25 **[16]** The present disclosure further provides cellulase variants, wherein the variants are mature forms having cellulase activity and comprising a substitution at from one to six positions selected from the group consisting of 111, 144, 154, 162, 410 and 413, wherein the cellulase variant comprises: a leucine or a serine at position 111; a leucine, a glutamine or a tryptophan at position 144; a threonine, a cysteine or a valine at position 154; a tyrosine or an

30 asparagine at position 162; an arginine or a serine at position 410; and a serine, a tryptophan or a tyrosine at position 413; wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3,

and wherein when the cellulase variant consists of a single substitution, the single substitution is not a S413Y substitution.

[17] Also provided by the present disclosure are cellulase variants, wherein the variants are mature forms having cellulase activity and comprising a substitution at from one to six  
5 positions selected from the group consisting of 98, 194, 313, 316, 384 and 443, wherein the cellulase variant comprises: a proline or a leucine at position 98; a lysine, a cysteine or a glutamic acid at position 194; a serine or a threonine at position 313; a serine or a proline at position 316; a glycine, a cysteine or a glutamine at position 384; and an asparagine or an isoleucine at position 443; wherein the positions are numbered by correspondence with the  
10 amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3, and wherein when the cellulase variant consists of a single substitution, the single substitution is not one of the group selected from a P98L substitution, a K194E substitution, and a S316P substitution.

[18] In addition the present disclosure provides cellulase variants, wherein the variants are  
15 mature forms having cellulase activity and comprising a substitution at from one to six positions selected from the group consisting of 153, 161, 203, 233, 422 and 444, wherein the cellulase variant comprises: an arginine or a glutamine at position 153; an asparagine, an alanine or a tryptophan at position 161; an arginine or a histidine at position 203; a proline or an aspartic acid at position 233; a glutamine or a valine at position 422; and a proline or a  
20 glutamine at position 443; wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3, and wherein when the cellulase variant consists of a single substitution, the single substitution is not a R153Q substitution.

[19] Moreover the present disclosure provides cellulase variants, wherein the variants are  
25 mature forms having cellulase activity and comprising a substitution at from one to seven positions selected from the group consisting of 98, 111, 144, 313, 316, 413 and 422, wherein the cellulase variant comprises: a proline or a leucine at position 98; a leucine or a serine at position 111; a leucine or a tryptophan at position 144; a serine or a threonine at position 313; a serine or a proline at position 316; a serine or a tryptophan at position 413; and a glutamine  
30 or a valine at position 422; wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3, and wherein when the cellulase variant consists of a single substitution, the single substitution is not one of the group selected from a P98L substitution, and a S316P substitution.

[20] In further aspects of the disclosure cellulase variants are provided, wherein the variants comprise a glutamine at position 98, and a substitution selected from the group consisting of a T138C, a S316P, a S343Q, a Q362I, a S386C, a C400S and a S406P, wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3.

[21] Additionally, cellulase variants are provided which comprise a cysteine at position 138, and a substitution selected from the group consisting of a S316P, a S343Q, a Q362I, a S386C, a C400S and a S406P, wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3.

[22] In another aspect, cellulase variants are provided which comprise a proline at position 316, and a substitution selected from the group consisting of a S343Q, a Q362I, a S386C, a C400S and a S406P, wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3.

[23] Moreover, the present disclosure provides cellulase variants comprising a glutamine at position 343, and a substitution selected from the group consisting of a Q362I, a S386C, a C400S and a S406P, wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3.

[24] The present disclosure also provides cellulase variants comprising an isoleucine at position 362, and a substitution selected from the group consisting of a S386C, a C400S and a S406P, wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3.

[25] In one aspect, cellulase variants are provided comprising a cysteine at position 386, and a substitution selected from the group consisting of a C400S and a S406P, wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3. In some embodiments, the variants comprise a serine at position 400 and a proline at position 406.

[26] In addition, the present disclosure provides cellulase variants, wherein the variant is a mature form having cellulase activity and comprising a substitution at from one to seven positions selected from the group consisting of 98, 111, 182, 291, 316, 362 and 400, wherein the cellulase variant comprises: proline, leucine or glutamine at position 98; leucine or serine at position 111; asparagine or tryptophan at position 182; serine or a glutamic acid at position 291; serine or proline at position 316; glutamine, isoleucine or leucine at position 362; and

cysteine or serine at position 400; wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3, and wherein when the cellulase variant consists of a single substitution, the single substitution is not a P98L substitution or a S316P substitution. In some embodiments, the  
5 variant further comprises a L439P substitution or a T74S substitution.

[27] In some preferred embodiments of the disclosure, the cellulase variants of any of the preceding paragraphs of the summary are derived from a parent cellulase selected from the group consisting of *Hypocrea jecorina* CBH2, *Hypocrea koningii* CBH2, *Humicola insolens* CBH2, *Acremonium cellulolyticus* CBH2, *Agaricus bisporus* CBH2, *Fusarium osysporum*  
10 *EG*, *Phanerochaete chrysosporium* CBH2, *Talaromyces emersonii* CBH2, *Thermobifida fusca* 6B/E3 CBH2, *Thermobifida fusca* 6A/E2 *EG*, and *Cellulomonas fimi* *CenA EG*. In some embodiments, the cellulase variant is derived from a parent cellulase whose amino acid sequence is at least 75% 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a member of the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID  
15 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, and SEQ ID NO:13. In some embodiments, the variants are isolated. Also provided are compositions comprising a cellulase variant. In some preferred embodiments, the composition is enriched in the cellulase variant.

[28] In other aspects, the present disclosure provides an isolated nucleic acid encoding the  
20 cellulase variant of any of the preceding paragraphs of the summary. An expression vector comprising the isolated nucleic acid operably linked to a regulatory sequence is also provided, as is a host cell comprising the expression vector. In some embodiments, methods are provided for producing a cellulase variant, comprising culturing the host cells in a culture medium under suitable conditions to produce the cellulase variant.

[29] Also provided are compositions comprising the cellulase variant of any of the preceding paragraphs of the summary. In some preferred embodiments, the compositions further comprising at least one additional enzyme selected from the group consisting of a subtilisin, a neutral metalloprotease, a lipase, a cutinase, an amylase, a carbohydrase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, an oxidase, and a peroxidase.  
25 The disclosure further provides methods of cleaning or fabric care comprising contacting a surface or an article comprising a fabric with the composition. Additionally, methods are provided for depilling and surface finishing a fabric comprising contacting a surface or an article comprising a fabric with the composition.  
30

[30] In some preferred embodiments, methods are provided for converting biomass to sugars comprising contacting the biomass with the cellulase variant of any of the preceding paragraphs of the summary. In some preferred embodiments methods are provided for producing a fuel comprising: contacting a biomass composition with an enzymatic composition comprising the cellulase variant of any of the preceding paragraphs to yield a sugar solution; and culturing the sugar solution with a fermentative microorganism under conditions sufficient to produce a fuel.

[31] Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and specific examples, while indicating preferred embodiments of the disclosure, are given by way of illustration only, since various changes and modifications within the scope and spirit of the disclosure will become apparent to one skilled in the art from reading this detailed description.

#### IV. BRIEF DESCRIPTION OF THE DRAWINGS

[32] FIG. 1 provides an alignment of the amino acid sequences of the mature form of various cellulases: *Hypocrea jecorina* (also known as *T. reesei*) CBH2 (SEQ ID NO:3), *Hypocrea koningii* CBH2 (SEQ ID NO:4), *Humicola insolens* CBH2 (SEQ ID NO:5), *Acremonium cellulolyticus* CBH2 (SEQ ID NO:6), *Agaricus bisporus* CBH2 (SEQ ID NO:7), *Fusarium osysporum* EG (SEQ ID NO:8), *Phanerochaete chrysosporium* CBH2 (SEQ ID NO:9), *Talaromyces emersonii* CBH2 (SEQ ID NO:10), *Thermobifida. fusca* 6B/E3 CBH2 (SEQ ID NO:11), *Thermobifida fusca* 6A/E2 EG (SEQ ID NO:12), and *Cellulomonas fimi* CenA EG (SEQ ID NO:13).

[33] FIG. 2A provides a graph of the relative frequency of observed over expected pretreated corn stover (PCS) assay winners as a product of charge change of the CBH2 SELs. Decreasing CBH2 charge results in a significantly higher frequency of PCS assay winners. FIG. 2B provides a graph of the relative frequency of observed over expected phosphoric acid swollen cellulose (PASC) assay winners as a product of charge change of the CBH2 SELs. A significantly higher frequency of PASC winners is observed for variants without a charge change.

[34] FIG. 3 provides a plasmid map for pTTTpyrG-cbh2.

[35] FIG. 4 provides a schematic of the *T. reesei* ku80 deletion cassette.

[36] FIG. 5 illustrates the inactivation of the native ku80 gene as a consequence of its replacement with an als selectable marker.

[37] FIG. 6 provides a schematic of the *T. reesei* pyr2 deletion cassette.

5 [38] FIG. 7 illustrates the inactivation of the native pyr2 gene as a consequence of its replacement with a hygR selectable marker and an amdS fragment.

[39] FIG. 8 provides a schematic of the *T. reesei* hygR deletion cassette

[40] FIG. 9 illustrates the inactivation of the heterologous hygR gene as a consequence of its replacement with a pyr2 selectable marker.

[41] FIG. 10 provides a schematic of the *T. reesei* bgl1 deletion cassette

10 [42] FIG. 11 provides a schematic of the *T. reesei* egl3 deletion cassette

[43] FIG. 12 provides a schematic of the *T. reesei* telomeric plasmid vector used for expression of cre recombinase.

[44] FIG. 13 illustrates the inactivation of the pyr2 selectable marker and activation of the amdS selectable marker as a consequence of introduction of a polynucleotide of a gene of  
15 interest (GOI). In exemplary embodiments, the GOI encodes a CBH2 variant.

[45] FIG. 14 provides a schematic illustrating the derivation of the MAD6 host strain, from the quad-deleted strain.

## V. DETAILED DESCRIPTION OF VARIOUS EMBODIMENTS

20 [46] The present disclosure relates to enzymes and in particular cellulase variants. Also described are nucleic acids encoding the cellulase variants, compositions comprising the cellulase variants, methods of identifying additional useful cellulase variants and methods of using the compositions.

[47] It is to be understood that both the foregoing general description and the following  
25 detailed description are exemplary and explanatory only and are not restrictive of the compositions and methods described herein. Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In this application, the use of the singular includes the plural unless specifically stated otherwise. The use of “or” means  
30 “and/or” unless state otherwise. Likewise, the terms “comprise,” “comprising,” “comprises,” “include,” “including” and “includes” are not intended to be limiting. All patents and publications, including all amino acid and nucleotide sequences disclosed within such patents and publications, referred to herein are expressly incorporated by reference. The headings

provided herein are not limitations of the various aspects or embodiments of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms herein are more fully defined by reference to the specification as a whole.

[48] Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Singleton, et al., *DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY*, 2D ED., John Wiley and Sons, New York (1994), and Hale & Marham, *THE HARPER COLLINS DICTIONARY OF BIOLOGY*, Harper Perennial, NY (1991) provide one of skill with a general dictionary of many of the terms used in this disclosure. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, the preferred methods and materials are described. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxyl orientation, respectively. Practitioners are particularly directed to Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL* (Second Edition), Cold Spring Harbor Press, Plainview, N.Y., 1989, and Ausubel FM et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N.Y., 1993, for definitions and terms of the art. It is to be understood that this disclosure is not limited to the particular methodology, protocols, and reagents described, as these may vary.

## **I. Definitions**

[49] The terms below are more fully defined by reference to the specification as a whole.

[50] The term "polypeptide" as used herein refers to a compound made up of a single chain of amino acid residues linked by peptide bonds. The term "protein" as used herein may be synonymous with the term "polypeptide".

[51] "Variant" means a protein which is derived from a precursor protein (e.g., the native protein) by addition of one or more amino acids to either or both the C- and N-terminal end, substitution of one or more amino acids at one or a number of different sites in the amino acid sequence, or deletion of one or more amino acids at either or both ends of the protein or at one or more sites in the amino acid sequence. The preparation of a cellulase variant may be performed by any means known in the art. In preferred embodiments, a cellulase variant is prepared by modifying a DNA sequence which encodes for the native protein, transformation

of the modified DNA sequence into a suitable host, and expression of the modified DNA sequence to form the variant enzyme. The variant cellulase of the disclosure includes peptides comprising altered amino acid sequences in comparison with a precursor enzyme amino acid sequence wherein the variant cellulase retains the characteristic cellulolytic nature of the precursor enzyme but which may have altered properties in some specific aspect. For example, a variant cellulase may have an increased pH optimum or increased temperature or oxidative stability or decreased affinity or binding to non-cellulosic materials but will retain its characteristic cellulolytic activity. It is contemplated that the variants according to the present disclosure may be derived from a DNA fragment encoding a cellulase variant wherein the functional activity of the expressed cellulase variant is retained. For example, a DNA fragment encoding a cellulase may further include a DNA sequence or portion thereof encoding a hinge or linker attached to the cellulase DNA sequence at either the 5' or 3' end wherein the functional activity of the encoded cellulase domain is retained. The terms variant and derivative may be used interchangeably herein.

[52] "Equivalent residues" may also be defined by determining homology at the level of tertiary structure for a precursor cellulase whose tertiary structure has been determined by x-ray crystallography. Equivalent residues are defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of a cellulase and *Hypocrea jecorina* CBH2 (N on N, CA on CA, C on C and O on O) are within 0.13 nm and preferably 0.1 nm after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the cellulase in question to the *H. jecorina* CBH2. The best model is the crystallographic model giving the lowest R factor for experimental diffraction data at the highest resolution available see for examples US 2006/0205042.

[53] Equivalent residues which are functionally analogous to a specific residue of *H. jecorina* CBH2 are defined as those amino acids of a cellulase which may adopt a conformation such that they either alter, modify or contribute to protein structure, substrate binding or catalysis in a manner defined and attributed to a specific residue of the *H. jecorina* CBH2. Further, they are those residues of the cellulase (for which a tertiary structure has been obtained by x-ray crystallography) which occupy an analogous position to the extent that, although the main chain atoms of the given residue may not satisfy the criteria of equivalence on the basis of occupying a homologous position, the atomic coordinates of at least two of the side chain atoms of the residue lie within 0.13 nm of the corresponding side

chain atoms of *H. jecorina* CBH2. The crystal structure of *H. jecorina* CBH2 is shown in Zou et al. (1999) (Ref. 5, supra).

[54] The term "nucleic acid molecule" includes RNA, DNA and cDNA molecules. It will be understood that, as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding a given protein such as CBH2 and/or variants thereof may be produced. The present disclosure contemplates every possible variant nucleotide sequence, encoding variant cellulase such as CBH2, all of which are possible given the degeneracy of the genetic code.

[55] A "heterologous" nucleic acid construct or sequence has a portion of the sequence which is not native to the cell in which it is expressed. Heterologous, with respect to a control sequence refers to a control sequence (i.e. promoter or enhancer) that does not function in nature to regulate the same gene the expression of which it is currently regulating. Generally, heterologous nucleic acid sequences are not endogenous to the cell or part of the genome in which they are present, and have been added to the cell, by infection, transfection, transformation, microinjection, electroporation, or the like. A "heterologous" nucleic acid construct may contain a control sequence/DNA coding sequence combination that is the same as, or different from a control sequence/DNA coding sequence combination found in the native cell.

[56] As used herein, the term "vector" refers to a nucleic acid construct designed for transfer between different host cells. An "expression vector" refers to a vector that has the ability to incorporate and express heterologous DNA fragments in a foreign cell. Many prokaryotic and eukaryotic expression vectors are commercially available. Selection of appropriate expression vectors is within the knowledge of those having skill in the art.

[57] Accordingly, an "expression cassette" or "expression vector" is a nucleic acid construct generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid sequence to be transcribed and a promoter.

[58] As used herein, the term "plasmid" refers to a circular double-stranded (ds) DNA construct used as a cloning vector, and which forms an extrachromosomal self-replicating genetic element in many bacteria and some eukaryotes.

[59] As used herein, the term "selectable marker-encoding nucleotide sequence" refers to a nucleotide sequence which is capable of expression in cells and where expression of the selectable marker confers to cells containing the expressed gene the ability to grow in the presence of a corresponding selective agent, or under corresponding selective growth  
5 conditions.

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

[61] "Chimeric gene" or "heterologous nucleic acid construct", as defined herein refers to a  
15 non-native gene (i.e., one that has been introduced into a host) that may be composed of parts of different genes, including regulatory elements. A chimeric gene construct for transformation of a host cell is typically composed of a transcriptional regulatory region (promoter) operably linked to a heterologous protein coding sequence, or, in a selectable marker chimeric gene, to a selectable marker gene encoding a protein conferring, for  
20 example, antibiotic resistance to transformed cells. A typical chimeric gene of the present disclosure, for transformation into a host cell, includes a transcriptional regulatory region that is constitutive or inducible, a protein coding sequence, and a terminator sequence. A chimeric gene construct may also include a second DNA sequence encoding a signal peptide if secretion of the target protein is desired.

[62] A nucleic acid is "operably linked" when it is placed into a functional relationship  
25 with another nucleic acid sequence. For example, DNA encoding a secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably  
30 linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such

sites do not exist, the synthetic oligonucleotide adaptors, linkers or primers for PCR are used in accordance with conventional practice.

[63] As used herein, the term "gene" means the segment of DNA involved in producing a polypeptide chain, that may or may not include regions preceding and following the coding region, e.g. 5' untranslated (5' UTR) or "leader" sequences and 3' UTR or "trailer" sequences, as well as intervening sequences (introns) between individual coding segments (exons).

[64] In general, nucleic acid molecules which encode the variant cellulase such as CBH2 will hybridize, under moderate to high stringency conditions to the wild type sequence such as provided herein as SEQ ID NO:1. However, in some cases a CBH2-encoding nucleotide sequence is employed that possesses a substantially different codon usage, while the protein encoded by the CBH2-encoding nucleotide sequence has the same or substantially the same amino acid sequence as the native protein. For example, the coding sequence may be modified to facilitate faster expression of CBH2 in a particular prokaryotic or eukaryotic expression system, in accordance with the frequency with which a particular codon is utilized by the host (Te'o et al., FEMS Microbiology Letters, 190: 13-19, 2000, for example, describes the optimization of genes for expression in filamentous fungi).

[65] A nucleic acid sequence is considered to be "selectively hybridizable" to a reference nucleic acid sequence if the two sequences specifically hybridize to one another under moderate to high stringency hybridization and wash conditions. Hybridization conditions are based on the melting temperature ( $T_m$ ) of the nucleic acid binding complex or probe. For example, "maximum stringency" typically occurs at about  $T_m - 5^\circ\text{C}$ . ( $5^\circ\text{C}$  below the  $T_m$  of the probe); "high stringency" at about  $5 - 10^\circ\text{C}$  below the  $T_m$ ; "moderate" or "intermediate stringency" at about  $10 - 20^\circ\text{C}$ . below the  $T_m$  of the probe; and "low stringency" at about  $20 - 25^\circ\text{C}$ . below the  $T_m$ . Functionally, maximum stringency conditions may be used to identify sequences having strict identity or near-strict identity with the hybridization probe; while high stringency conditions are used to identify sequences having about 80% or more sequence identity with the probe.

[66] Moderate and high stringency hybridization conditions are well known in the art (see, for example, Sambrook, et al, 1989, Chapters 9 and 11, and in Ausubel, F. M., et al., 1993, expressly incorporated by reference herein). An example of high stringency conditions includes hybridization at about  $42^\circ\text{C}$ . in 50% formamide, 5xSSC, 5.times.Denhardt's solution, 0.5% SDS and 100 ug/ml denatured carrier DNA followed by washing two times in

2.times.SSC and 0.5% SDS at room temperature and two additional times in 0.1x SSC and 0.5% SDS at 42° degree. C.

[67] The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, 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.

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

[69] 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.

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

[71] It follows that the term "CBH2 expression" refers to transcription and translation of the *cbh2* gene or variants thereof, the products of which include precursor RNA, mRNA, polypeptide, post-translationally processed polypeptides, and derivatives thereof, including CBH2 from related species such as *Trichoderma koningii*, *Hypocrea jecorina* (also known as *Trichoderma longibrachiatum*, *Trichoderma reesei* or *Trichoderma viride*) and *Hypocrea schweinitzii*. By way of example, assays for CBH2 expression include Western blot for CBH2 protein, Northern blot analysis and reverse transcriptase polymerase chain reaction (RT-PCR) assays for *cbh2* mRNA, and Phosphoric Acid Swollen Cellulose (PASC) and p-hydroxybenzoic acid hydrazide (PAHBAH) assays as described in the following: (a) PASC: (Karlsson, J. et al. (2001), Eur. J. Biochem, 268, 6498-6507, Wood, T. (1988) in Methods in Enzymology, Vol.160. Biomass Part a Cellulose and Hemicellulose (Wood, W. & Kellog, S.

Eds.), pp.19-25, Academic Press, San Diego, Calif., USA) and (b) PAHBAH: (Lever, M. (1972) Analytical Biochemistry, 47, 273, Blakeney, A. B. & Mutton, L. L. (1980) Journal of Science of Food and Agriculture, 31, 889, Henry, R. J. (1984) Journal of the Institute of Brewing, 90, 37).

5 [72] The term "alternative splicing" refers to the process whereby multiple polypeptide isoforms are generated from a single gene, and involves the splicing together of nonconsecutive exons during the processing of some, but not all, transcripts of the gene. Thus a particular exon may be connected to any one of several alternative exons to form messenger RNAs. The alternatively-spliced mRNAs produce polypeptides ("splice variants") in which  
10 some parts are common while other parts are different.

[73] The term "signal sequence" refers to a sequence of amino acids at the N-terminal portion of a protein that facilitates the secretion of the mature form of the protein outside the cell. The mature form of the extracellular protein lacks the signal sequence that is cleaved off during the secretion process.

15 [74] By the term "host cell" is meant a cell that contains a vector and supports the replication, and/or transcription or transcription and translation (expression) of the expression construct. Host cells for use in the present disclosure can be prokaryotic cells, such as *E. coli*, or eukaryotic cells such as yeast, plant, insect, amphibian, or mammalian cells. In general, host cells are filamentous fungi.

20 [75] The term "filamentous fungi" means any and all filamentous fungi recognized by those of skill in the art. A preferred fungus is selected from the group consisting of *Aspergillus*, *Trichoderma*, *Fusarium*, *Chrysosporium*, *Penicillium*, *Humicola*, *Neurospora*, or alternative sexual forms thereof such as *Emericella*, *Hypocrea*. It has now been demonstrated that the asexual industrial fungus *Trichoderma reesei* is a clonal derivative of the ascomycete  
25 *Hypocrea jecorina* (See, Kuhls et al., PNAS, 93:7755-7760, 1996).

[76] The term "cellooligosaccharide" refers to oligosaccharide groups containing from 2-8 glucose units and having .beta.-1,4 linkages, e.g., cellobiose.

[77] The terms "cellulase" "cellulolytic enzymes" or "cellulase enzymes" refer to a category of enzymes capable of hydrolyzing cellulose polymers to shorter cello-  
30 oligosaccharide oligomers, cellobiose and/or glucose. Numerous examples of cellulases, such as exoglucanases, exocellobiohydrolases, endoglucanases, and glucosidases have been obtained from cellulolytic organisms, particularly including fungi, plants and bacteria. The enzymes made by these microbes are mixtures of proteins with three types of actions useful

in the conversion of cellulose to glucose: endoglucanases (EG), cellobiohydrolases (CBH), and beta-glucosidase. These three different types of cellulase enzymes act synergistically to convert cellulose and its derivatives to glucose.

[78] Many microbes make enzymes that hydrolyze cellulose, including the wood rotting fungus *Trichoderma*, the compost bacteria *Thermomonospora*, *Bacillus*, and *Cellulomonas*; *Streptomyces*; and the fungi *Humicola*, *Aspergillus* and *Fusarium*.

[79] CBH2 from *Hypocrea jecorina* is a member of the Glycosyl Hydrolase Family 6 (hence Cel6) and, specifically, was the first member of that family identified in *Hypocrea jecorina* (hence Cel6A). The Glycosyl Hydrolase Family 6 contains both Endoglucanases and Cellobiohydrolases/exoglucanases, and that CBH2 is the latter. Thus, the phrases CBH2, CBH2-type protein and Cel6 cellobiohydrolases may be used interchangeably herein.

[80] The term "cellulose binding domain" as used herein refers to portion of the amino acid sequence of a cellulase or a region of the enzyme that is involved in the cellulose binding activity of a cellulase or derivative thereof. Cellulose binding domains generally function by non-covalently binding the cellulase to cellulose, a cellulose derivative or other polysaccharide equivalent thereof. Cellulose binding domains permit or facilitate hydrolysis of cellulose fibers by the structurally distinct catalytic core region, and typically function independent of the catalytic core. Thus, a cellulose binding domain will not possess the significant hydrolytic activity attributable to a catalytic core. In other words, a cellulose binding domain is a structural element of the cellulase enzyme protein tertiary structure that is distinct from the structural element which possesses catalytic activity. Cellulose binding domain and cellulose binding module may be used interchangeably herein.

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

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

5 [83] As used herein, the term "cotton-containing fabric" refers to sewn or unsewn fabrics, yarns or fibers made of pure cotton or cotton blends including cotton woven fabrics, cotton knits, cotton denims, cotton yarns, raw cotton and the like.

[84] As used herein, the term "stonewashing composition" refers to a formulation for use in stonewashing cellulose containing fabrics. Stonewashing compositions are used to modify  
10 cellulose containing fabrics prior to sale, i.e., during the manufacturing process. In contrast, detergent compositions are intended for the cleaning of soiled garments and are not used during the manufacturing process.

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

[86] As used herein, the term "decrease or elimination in expression of the cbh2 gene"  
20 means that either that the cbh2 gene has been deleted from the genome and therefore cannot be expressed by the recombinant host microorganism; or that the cbh2 gene or transcript has been modified such that a functional CBH2 enzyme is not produced by the host microorganism or at levels that are significantly less than the unmodified cbh2 gene or transcript.

25 [87] The term "variant cbh2 gene" means that the nucleic acid sequence of the cbh2 gene from *H. jecorina* has been altered by removing, adding, and/or manipulating the coding sequence.

[88] As used herein, the term "purifying" generally refers to subjecting transgenic nucleic acid or protein containing cells to biochemical purification and/or column chromatography.

30 [89] As used herein, the terms "active" and "biologically active" refer to a biological activity associated with a particular protein and are used interchangeably herein. For example, the enzymatic activity associated with a protease is proteolysis and, thus, an active

protease has proteolytic activity. It follows that the biological activity of a given protein refers to any biological activity typically attributed to that protein by those of skill in the art.

[90] As used herein, the term "enriched" means that the cellulase such as CBH2 is found in a concentration that is greater relative to the CBH2 concentration found in a wild-type, or naturally occurring, fungal cellulase composition. The terms enriched, elevated and enhanced may be used interchangeably herein.

[91] A wild type fungal cellulase composition is one produced by a naturally occurring fungal source and which comprises one or more BGL, CBH and EG components wherein each of these components is found at the ratio produced by the fungal source. Thus, an enriched CBH composition would have CBH at an altered ratio wherein the ratio of CBH to other cellulase components (i.e., EGs, beta-glucosidases and other endoglucanases) is elevated. This ratio may be increased by either increasing CBH or decreasing (or eliminating) at least one other component by any means known in the art.

[92] The term "isolated" or "purified" as used herein refers to a nucleic acid or amino acid that is removed from at least one component with which it is naturally associated.

[93] Thus, to illustrate, a naturally occurring cellulase system may be purified into substantially pure components by recognized separation techniques well published in the literature, including ion exchange chromatography at a suitable pH, affinity chromatography, size exclusion and the like. For example, in ion exchange chromatography (usually anion exchange chromatography), it is possible to separate the cellulase components by eluting with a pH gradient, or a salt gradient, or both a pH and a salt gradient. The purified CBH may then be added to the enzymatic solution resulting in an enriched CBH solution. It is also possible to elevate the amount of CBH produced by a microbe using molecular genetics methods to overexpress the gene encoding CBH, possibly in conjunction with deletion of one or more genes encoding other cellulases.

[94] Fungal cellulases may contain more than one CBH component. The different components generally have different isoelectric points which allow for their separation via ion exchange chromatography and the like. Either a single CBH component or a combination of CBH components may be employed in an enzymatic solution.

[95] When employed in enzymatic solutions, the homolog or variant CBH2 component is generally added in an amount sufficient to allow the highest rate of release of soluble sugars from the biomass. The amount of homolog or variant CBH2 component added depends, upon the type of biomass to be saccharified, which can be readily determined by the skilled artisan

when employed, the weight percent of the homolog or variant CBH2 component present in the cellulase composition is from preferably between 1 and 100 with illustrative examples being about 1, preferably about 5, preferably about 10, preferably about 15, or preferably about 20 weight percent to preferably about 25, preferably about 30, preferably about 35, preferably about 40, preferably about 45 or preferably about 50 weight percent. Furthermore, preferred ranges may be about 0.5 to about 15 weight percent, about 0.5 to about 20 weight percent, from about 1 to about 10 weight percent, from about 1 to about 15 weight percent, from about 1 to about 20 weight percent, from about 1 to about 25 weight percent, from about 5 to about 20 weight percent, from about 5 to about 25 weight percent, from about 5 to about 30 weight percent, from about 5 to about 35 weight percent, from about 5 to about 40 weight percent, from about 5 to about 45 weight percent, from about 5 to about 50 weight percent, from about 10 to about 20 weight percent, from about 10 to about 25 weight percent, from about 10 to about 30 weight percent, from about 10 to about 35 weight percent, from about 10 to about 40 weight percent, from about 10 to about 45 weight percent, from about 10 to about 50 weight percent, from about 15 to about 60 weight percent, from about 15 to about 65 weight percent, from about 15 to about 70 weight percent, from about 15 to about 75 weight percent, from about 15 to about 80 weight percent, from about 15 to about 85 weight percent, from about 15 to about 95 weight percent. However, when employed, the weight percent of the homolog or variant CBH2 component relative to any EG type components present in the cellulase composition is from preferably about 1, preferably about 5, preferably about 10, preferably about 15, or preferably about 20 weight percent to preferably about 25, preferably about 30, preferably about 35, preferably about 40, preferably about 45 or preferably about 50 weight percent. Furthermore, preferred ranges may be about 0.5 to about 15 weight percent, about 0.5 to about 20 weight percent, from about 1 to about 10 weight percent, from about 1 to about 15 weight percent, from about 1 to about 20 weight percent, from about 1 to about 25 weight percent, from about 5 to about 20 weight percent, from about 5 to about 25 weight percent, from about 5 to about 30 weight percent, from about 5 to about 35 weight percent, from about 5 to about 40 weight percent, from about 5 to about 45 weight percent, from about 5 to about 50 weight percent, from about 10 to about 20 weight percent, from about 10 to about 25 weight percent, from about 10 to about 30 weight percent, from about 10 to about 35 weight percent, from about 10 to about 40 weight percent, from about 10 to about 45 weight percent, from about 10 to about 50 weight percent, from about 15 to about 20 weight percent, from about 15 to about 25 weight percent, from about 15 to about

30 weight percent, from about 15 to about 35 weight percent, from about 15 to about 30 weight percent, from about 15 to about 45 weight percent, from about 15 to about 50 weight percent.

## 5 II. Cellulases

[96] Cellulases are known in the art as enzymes that hydrolyze cellulose (beta-1,4-glucan or beta D-glucosidic linkages) resulting in the formation of glucose, cellobiose, cellooligosaccharides, and the like. As set forth above, cellulases have been traditionally divided into three major classes: endoglucanases (EC 3.2.1.4) ("EG"), exoglucanases or  
10 cellobiohydrolases (EC 3.2.1.91) ("CBH") and beta-glucosidases (EC 3.2.1.21) ("BG").

[97] Certain fungi produce complete cellulase systems which include exo-cellobiohydrolases or CBH-type cellulases, endoglucanases or EG-type cellulases and beta-glucosidases or BG-type cellulases. However, sometimes these systems lack CBH-type cellulases and bacterial cellulases also typically include little or no CBH-type cellulases. In  
15 addition, it has been shown that the EG components and CBH components synergistically interact to more efficiently degrade cellulose. The different components, i.e., the various endoglucanases and exocellobiohydrolases in a multi-component or complete cellulase system, generally have different properties, such as isoelectric point, molecular weight, degree of glycosylation, substrate specificity and enzymatic action patterns.

[98] It is believed that endoglucanase-type cellulases hydrolyze internal beta-1,4-glucosidic bonds in regions of low crystallinity of the cellulose and exo-cellobiohydrolase-type cellulases hydrolyze cellobiose from the reducing or non-reducing end of cellulose. It follows that the action of endoglucanase components can greatly facilitate the action of exo-cellobiohydrolases by creating new chain ends which are recognized by exo-  
25 cellobiohydrolase components. Further, beta-glucosidase-type cellulases have been shown to catalyze the hydrolysis of alkyl and/or aryl .beta.-D-glucosides such as methyl .beta.-D-glucoside and p-nitrophenyl glucoside as well as glycosides containing only carbohydrate residues, such as cellobiose. This yields glucose as the sole product for the microorganism and reduces or eliminates cellobiose which inhibits cellobiohydrolases and endoglucanases.

[99] Cellulases also find a number of uses in detergent compositions including to enhance cleaning ability, as a softening agent and to improve the feel of cotton fabrics (Hemmpel, ITB Dyeing/Printing/Finishing 3:5-14, 1991; Tyndall, Textile Chemist and Colorist 24:23-26, 1992; and Kumar et al., Textile Chemist and Colorist, 29:37-42, 1997). While the mechanism

is not part of the disclosure, softening and color restoration properties of cellulase have been attributed to the alkaline endoglucanase components in cellulase compositions, as exemplified by U.S. Pat. Nos. 5,648,263, 5,691,178, and 5,776,757, which disclose that detergent compositions containing a cellulase composition enriched in a specified alkaline endoglucanase component impart color restoration and improved softening to treated garments as compared to cellulase compositions not enriched in such a component. In addition, the use of such alkaline endoglucanase components in detergent compositions has been shown to complement the pH requirements of the detergent composition (e.g., by exhibiting maximal activity at an alkaline pH of 7.5 to 10, as described in U.S. Pat. Nos. 5,648,263, 5,691,178, and 5,776,757).

[100] Cellulase compositions have also been shown to degrade cotton-containing fabrics, resulting in reduced strength loss in the fabric (U.S. Pat. No. 4,822,516), contributing to reluctance to use cellulase compositions in commercial detergent applications. Cellulase compositions comprising endoglucanase components have been suggested to exhibit reduced strength loss for cotton-containing fabrics as compared to compositions comprising a complete cellulase system.

[101] Cellulases have also been shown to be useful in degradation of cellulase biomass to ethanol (wherein the cellulase degrades cellulose to glucose and yeast or other microbes further ferment the glucose into ethanol), in the treatment of mechanical pulp (Pere et al., In Proc. Tappi Pulping Conf., Nashville, Tenn., 27-31, pp. 693-696, 1996), for use as a feed additive (WO 91/04673) and in grain wet milling.

[102] Most CBHs and EGs have a multidomain structure consisting of a core domain separated from a cellulose binding domain (CBD) by a linker peptide (Suurnakki et al., 2000). The core domain contains the active site whereas the CBD interacts with cellulose by binding the enzyme to it (van Tilbeurgh et al., FEBS Lett. 204:223-227, 1986; Tomme et al., Eur. J. Biochem. 170:575-581, 1988). The CBDs are particularly important in the hydrolysis of crystalline cellulose. It has been shown that the ability of cellobiohydrolases to degrade crystalline cellulose clearly decreases when the CBD is absent (Linder and Teeri, J. Biotechnol. 57:15-28, 1997). However, the exact role and action mechanism of CBDs is still a matter of speculation. It has been suggested that the CBD enhances the enzymatic activity merely by increasing the effective enzyme concentration at the surface of cellulose (Stahlberg et al., Bio/Technol. 9:286-290, 1991), and/or by loosening single cellulose chains from the cellulose surface (Tormo et al., EMBO J. vol. 15, no. 21, pp. 5739-5751, 1996). Most studies

concerning the effects of cellulase domains on different substrates have been carried out with core proteins of cellobiohydrolases, as their core proteins can easily be produced by limited proteolysis with papain (Tomme et al., 1988). Numerous cellulases have been described in the scientific literature, examples of which include: from *Trichoderma reesei*: Shoemaker, S. et al., Bio/Technology, 1:691-696, 1983, which discloses CBH1; Teeri, T. et al., Gene, 51:43-52, 1987, which discloses CBH2. Cellulases from species other than *Trichoderma* have also been described e.g., Ooi et al., Nucleic Acids Research, vol. 18, no. 19, 1990, which discloses the cDNA sequence coding for endoglucanase F1-CMC produced by *Aspergillus aculeatus*; Kawaguchi T et al., Gene 173(2):287-8, 1996, which discloses the cloning and sequencing of the cDNA encoding beta-glucosidase 1 from *Aspergillus aculeatus*; Sakamoto et al., Curr. Genet. 27:435-439, 1995, which discloses the cDNA sequence encoding the endoglucanase CMCase-1 from *Aspergillus kawachii* IFO 4308; Saarilahti et al., Gene 90:9-14, 1990, which discloses an endoglucanase from *Erwinia carotovora*; Spilliaert R, et al., Eur J Biochem. 224(3):923-30, 1994, which discloses the cloning and sequencing of bglA, coding for a thermostable beta-glucanase from *Rhodothermus marinus*; and Halldorsdottir S et al., Appl Microbiol Biotechnol. 49(3):277-84, 1998, which discloses the cloning, sequencing and overexpression of a *Rhodothermus marinus* gene encoding a thermostable cellulase of glycosyl hydrolase family 12. However, there remains a need for identification and characterization of novel cellulases, with improved properties, such as improved performance under conditions of thermal stress or in the presence of surfactants, increased specific activity, altered substrate cleavage pattern, and/or high level expression in vitro.

[103] The development of new and improved cellulase compositions that comprise varying amounts CBH-type, EG-type and BG-type cellulases is of interest for use: (1) in detergent compositions that exhibit enhanced cleaning ability, function as a softening agent and/or improve the feel of cotton fabrics (e.g., "stone washing" or "biopolishing"); (2) in compositions for degrading wood pulp or other biomass into sugars (e.g., for bio-fuel production); and/or (3) in feed compositions.

[104] Also provided herein are whole cellulase preparation comprising cellulase variants. As used herein, the phrase "whole cellulase preparation" refers to both naturally occurring and non-naturally occurring cellulase containing compositions. A "naturally occurring" composition is one produced by a naturally occurring source and which comprises one or more cellobiohydrolase-type, one or more endoglucanase-type, and one or more beta-glucosidase components wherein each of these components is found at the ratio produced by

the source. A naturally occurring composition is one that is produced by an organism unmodified with respect to the cellulolytic enzymes such that the ratio of the component enzymes is unaltered from that produced by the native organism. A “non-naturally occurring” composition encompasses those compositions produced by: (1) combining  
 5 component cellulolytic enzymes either in a naturally occurring ratio or non-naturally occurring, i.e., altered, ratio; or (2) modifying an organism to overexpress or underexpress one or more cellulolytic enzyme; or (3) modifying an organism such that at least one cellulolytic enzyme is deleted. Accordingly, in some embodiments, the whole cellulase preparation can have one or more of the various EGs and/or CBHs, and/or beta-glucosidase  
 10 deleted. For example, EG1 may be deleted alone or in combination with other EGs and/or CBHs.

[105] In general, the whole cellulase preparation includes enzymes including, but are not limited to: (i) endoglucanases (EG) or 1,4- $\beta$ -d-glucan-4-glucanohydrolases (EC 3.2.1.4), (ii) exoglucanases, including 1,4- $\beta$ -d-glucan glucanohydrolases (also known as cellodextrinases)  
 15 (EC 3.2.1.74) and 1,4- $\beta$ -d-glucan cellobiohydrolases (exo-cellobiohydrolases, CBH) (EC 3.2.1.91), and (iii)  $\beta$ -glucosidase (BG) or  $\beta$ -glucoside glucohydrolases (EC 3.2.1.21).

[106] In the present disclosure, the whole cellulase preparation can be from any microorganism that is useful for the hydrolysis of a cellulosic material. In some embodiments, the whole cellulase preparation is a filamentous fungi whole cellulase.  
 20 “Filamentous fungi” include all filamentous forms of the subdivision Eumycota and Oomycota. In some embodiments, the whole cellulase preparation is a Acremonium, Aspergillus, Emericella, Fusarium, Humicola, Mucor, Myceliophthora, Neurospora, Scytalidium, Thielavia, Tolypocladium, or Trichoderma species, whole cellulase. In some embodiments, the whole cellulase preparation is an Aspergillus aculeatus, Aspergillus  
 25 awamori, Aspergillus foetidus, Aspergillus japonicus, Aspergillus nidulans, Aspergillus niger, or Aspergillus oryzae whole cellulase. In another aspect, whole cellulase preparation is a Fusarium bactridioides, Fusarium cerealis, Fusarium crookwellense, Fusarium culmorum, Fusarium graminearum, Fusarium graminum, Fusarium heterosporum, Fusarium negundi, Fusarium oxysporum, Fusarium reticulatum, Fusarium roseum, Fusarium sambucinum,  
 30 Fusarium sarcochroum, Fusarium sporotrichioides, Fusarium sulphureum, Fusarium torulosum, Fusarium trichothecioides, or Fusarium venenatum whole cellulase. In another aspect, the whole cellulase preparation is a Humicola insolens, Humicola lanuginosa, Mucor miehei, Myceliophthora thermophila, Neurospora crassa, Scytalidium thermophilum, or

Thielavia terrestris whole cellulase. In another aspect, the whole cellulase preparation a Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Trichoderma reesei e.g., RL-P37 (Sheir-Neiss et al., Appl. Microbiol. Biotechnology, 20 (1984) pp. 46-53; Montenecourt B.S., Can., 1-20, 1987), QM9414 (ATCC No. 26921), NRRL 15709, ATCC 5 13631, 56764, 56466, 56767, or Trichoderma viride e.g., ATCC 32098 and 32086, whole cellulase. In some embodiments, the whole cellulase preparation is a Trichoderma reesei RutC30 whole cellulase, which is available from the American Type Culture Collection as Trichoderma reesei ATCC 56765.

[107] Examples of commercial cellulase preparations suitable for use in the present 10 disclosure include, for example, CELLUCLAST<sup>TM</sup> (available from Novozymes A/S) and LAMINEX<sup>TM</sup>, IndiAge<sup>TM</sup> and Primafast<sup>TM</sup> LAMINEX BG enzyme (available Genencor Division, Danisco US. Inc.)

[108] In the present disclosure, the whole cellulase preparation can be from any 15 microorganism cultivation method known in the art resulting in the expression of enzymes capable of hydrolyzing a cellulosic material. Fermentation can include shake flask cultivation, small- or large-scale fermentation, such as continuous, batch, fed-batch, or solid state fermentations in laboratory or industrial fermenters performed in a suitable medium and under conditions allowing the cellulase to be expressed or isolated.

[109] Generally, the microorganism is cultivated in a cell culture medium suitable for 20 production of enzymes capable of hydrolyzing a cellulosic material. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures known in the art. Suitable culture media, temperature ranges and other conditions suitable for growth and cellulase production are known in the art. As a non-limiting example, the normal temperature range for the production of cellulases by 25 *Trichoderma reesei* is 24°C to 28°C.

[110] Generally, the whole cellulase preparation is used as is produced by fermentation with no or minimal recovery and/or purification. For example, once cellulases are secreted by a cell into the cell culture medium, the cell culture medium containing the cellulases can be used. In some embodiments the whole cellulase preparation comprises the unfractionated 30 contents of fermentation material, including cell culture medium, extracellular enzymes and cells. Alternatively, the whole cellulase preparation can be processed by any convenient method, e.g., by precipitation, centrifugation, affinity, filtration or any other method known in the art. In some embodiments, the whole cellulase preparation can be concentrated, for

example, and then used without further purification. In some embodiments the whole cellulase preparation comprises chemical agents that decrease cell viability or kills the cells. In some embodiments, the cells are lysed or permeabilized using methods known in the art.

### 5 III. Molecular Biology

[111] In one embodiment this disclosure provides for the expression of variant *cbh2* genes under control of a promoter functional in a filamentous fungus. Therefore, this disclosure relies on routine techniques in the field of recombinant genetics (See, e.g., Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd ed., 1989; Kriegler, Gene Transfer and  
10 Expression: A Laboratory Manual, 1990; and Ausubel et al., eds., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing and Wiley-Interscience, New York, 1994).

#### Methods of Mutating *cbh2* Nucleic Acid Sequences

[112] Any method known in the art that can introduce mutations is contemplated by the  
15 present disclosure.

[113] The present disclosure relates to the expression, purification and/or isolation and use of variant CBH2. These enzymes are preferably prepared by recombinant methods utilizing the *cbh2* gene from *H. jecorina*. The fermentation broth may be used with or without purification.

20 [114] After the isolation and cloning of the *cbh2* gene from *H. jecorina*, other methods known in the art, such as site directed mutagenesis, are used to make the substitutions, additions or deletions that correspond to substituted amino acids in the expressed CBH2 variant. Again, site directed mutagenesis and other methods of incorporating amino acid changes in expressed proteins at the DNA level are known in the art (Sambrook et al., supra;  
25 and Ausubel et al., supra).

[115] DNA encoding an amino acid sequence variant of the *H. jecorina* CBH2 is prepared by a variety of methods known in the art. These methods include, but are not limited to, preparation by site-directed (or oligonucleotide-mediated) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared DNA encoding the *H. jecorina* CBH2.

30 [116] Site-directed mutagenesis is a preferred method for preparing substitution variants. This technique is well known in the art (see, e.g., Carter et al. Nucleic Acids Res. 13:4431-4443 (1985) and Kunkel et al., Proc. Natl. Acad. Sci. USA 82:488 (1987)). Briefly, in carrying out site-directed mutagenesis of DNA, the starting DNA is altered by first

hybridizing an oligonucleotide encoding the desired mutation to a single strand of such starting DNA. After hybridization, a DNA polymerase is used to synthesize an entire second strand, using the hybridized oligonucleotide as a primer, and using the single strand of the starting DNA as a template. Thus, the oligonucleotide encoding the desired mutation is  
5 incorporated in the resulting double-stranded DNA.

[117] PCR mutagenesis is also suitable for making amino acid sequence variants of the starting polypeptide, i.e., H. jecorina CBH2. See Higuchi, in PCR Protocols, pp. 177-183 (Academic Press, 1990); and Vallette et al., Nuc. Acids Res. 17:723-733 (1989). See, also, for example Cadwell et al., PCR Methods and Applications, Vol 2, 28-33 (1992). Briefly,  
10 when small amounts of template DNA are used as starting material in a PCR, primers that differ slightly in sequence from the corresponding region in a template DNA can be used to generate relatively large quantities of a specific DNA fragment that differs from the template sequence only at the positions where the primers differ from the template.

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

[119] Alternatively, or additionally, the desired amino acid sequence encoding a variant  
30 CBH2 can be determined, and a nucleic acid sequence encoding such amino acid sequence variant can be generated synthetically.

[120] The variant CBH2(s) so prepared may be subjected to further modifications, oftentimes depending on the intended use of the cellulase. Such modifications may involve

further alteration of the amino acid sequence, fusion to heterologous polypeptide(s) and/or covalent modifications.

#### IV. **cbh2 Nucleic Acids And CBH2 Polypeptides**

5

##### A. **Variant cbh2-Type Nucleic acids**

[121] The nucleic acid sequence for the wild type cbh2 is shown in SEQ ID NO:1. The disclosure encompasses a nucleic acid molecule encoding the variant cellulases described herein. The nucleic acid may be a DNA molecule.

10 [122] After DNA sequences that encode the CBH2 variants have been cloned into DNA constructs, the DNA is used to transform microorganisms. The microorganism to be transformed for the purpose of expressing a variant CBH2 according to the present disclosure may advantageously comprise a strain derived from *Trichoderma sp.* Thus, a preferred mode for preparing variant CBH2 cellulases according to the present disclosure comprises  
15 transforming a *Trichoderma sp.* host cell with a DNA construct comprising at least a fragment of DNA encoding a portion or all of the variant CBH2. The DNA construct will generally be functionally attached to a promoter. The transformed host cell is then grown under conditions so as to express the desired protein. Subsequently, the desired protein product may be purified to substantial homogeneity.

20 [123] However, it may in fact be that the best expression vehicle for a given DNA encoding a variant CBH2 may differ from *H. jecorina*. Thus, it may be that it will be most advantageous to express a protein in a transformation host that bears phylogenetic similarity to the source organism for the variant CBH2. In an alternative embodiment, *Aspergillus niger* can be used as an expression vehicle. For a description of transformation techniques with *A.*  
25 *niger*, see WO 98/31821, the disclosure of which is incorporated by reference in its entirety.

[124] Accordingly, the present description of an *Aspergillus spp.* expression system is provided for illustrative purposes only and as one option for expressing the variant CBH2 of the disclosure. One of skill in the art, however, may be inclined to express the DNA encoding variant CBH2 in a different host cell if appropriate and it should be understood that the  
30 source of the variant CBH2 should be considered in determining the optimal expression host. Additionally, the skilled worker in the field will be capable of selecting the best expression system for a particular gene through routine techniques utilizing the tools available in the art.

## B. Variant CBH2 Polypeptides

[125] The variant CBH2's of this disclosure have amino acid sequences that are derived from the amino acid sequence of a precursor CBH2. The amino acid sequence of the CBH2 variant differs from the precursor CBH2 amino acid sequence by the substitution, deletion or insertion of one or more amino acids of the precursor amino acid sequence. In a preferred embodiment, the precursor CBH2 is *Hypocrea jecorina* CBH2. The mature amino acid sequence of *H. jecorina* CBH2 is shown in Example 2 (SEQ ID NO:3). Thus, this disclosure is directed to CBH2 variants which contain amino acid residues at positions which are equivalent to the particular identified residue in *H. jecorina* CBH2. A residue (amino acid) of an CBH2 homolog is equivalent to a residue of *Hypocrea jecorina* CBH2 if it is either homologous (i.e., corresponding in position in either primary or tertiary structure) or is functionally analogous to a specific residue or portion of that residue in *Hypocrea jecorina* CBH2 (i.e., having the same or similar functional capacity to combine, react, or interact chemically or structurally). As used herein, numbering is intended to correspond to that of the mature CBH2 amino acid sequence (SEQ ID NO:3).

[126] Alignment of amino acid sequences to determine homology is preferably determined by using a "sequence comparison algorithm." Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection. Visual inspection may utilize graphics packages such as, for example, MOE by Chemical Computing Group, Montreal Canada.

[127] An example of an algorithm that is suitable for determining sequence similarity is the BLAST algorithm, which is described in Altschul, et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. These initial neighborhood word hits act as starting points to find longer HSPs containing them. The word hits are

expanded in both directions along each of the two sequences being compared for as far as the cumulative alignment score can be increased. Extension of the word hits is stopped when: the cumulative alignment score falls off by the quantity X from a maximum achieved value; the cumulative score goes to zero or below; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M<sup>5</sup>, N<sup>-4</sup>, and a comparison of both strands.

[128] The BLAST algorithm then performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Nat'l. Acad. Sci. USA 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, an amino acid sequence is considered similar to a protease if the smallest sum probability in a comparison of the test amino acid sequence to a protease amino acid sequence is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[129] For purposes of the present disclosure, the degree of identity may be suitably determined by means of computer programs known in the art, such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wis., USA 53711) (Needleman, S. B. and Wunsch, C. D., (1970), Journal of Molecular Biology, 48, 443-45), using GAP with the following settings for polynucleotide sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3.

[130] A structural alignment between a *T. reesei* CBH2 and other cellulases may be used to identify equivalent/corresponding positions in other cellulases having a moderate to high degree of homology, e.g., about 50%, 55%, 60 %, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or even 99%, with *T. reesei* CBH2 (SEQ ID NO: 3). One method of obtaining the 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 hydrophobic cluster analysis (Gaboriaud *et al.*, FEBS Letters, 224:149-155, 1987) and reverse threading (Huber and Torda, Protein Science, 7:142-149, 1998).

[131] An exemplary alignment of the mature form of various reference cellulases is provided as Figure 1. The reference cellulases include: *Hypocrea jecorina* (also known as *T. reesei*) CBH2 (SEQ ID NO:3), *Hypocrea koningii* CBH2 (SEQ ID NO:4), *Humicola insolens* CBH2 (SEQ ID NO:5), *Acremonium cellulolyticus* CBH2 (SEQ ID NO:6), *Agaricus bisporus* CBH2 (SEQ ID NO:7), *Fusarium osysporum* EG (SEQ ID NO:8), *Phanerochaete chrysosporium* CBH2 (SEQ ID NO:9), *Talaromyces emersonii* CBH2 (SEQ ID NO:10), *Thermobifida. fusca* 6B/E3 CBH2 (SEQ ID NO:11), *Thermobifida fusca* 6A/E2 EG (SEQ ID NO:12), and *Cellulomonas fimi* CenA EG (SEQ ID NO:13). Sequences were aligned using the ClustalW and MUSCLE multiple sequence alignment algorithms. A matrix showing the percent identity of cellulases of the sequence alignment of Figure 1 is provided in Table 1.

**Table 1. Cellulase Percent Identity Matrix\***

| Percent_ID | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   |
|------------|------|------|------|------|------|------|------|------|------|------|------|
| 3          | 100  | 95.5 | 62.3 | 64.7 | 59.6 | 63.1 | 55.4 | 63.4 | 31.9 | 13.5 | 27   |
| 4          | 95.5 | 100  | 61.6 | 64   | 59.1 | 63.6 | 54.7 | 63   | 32.9 | 13.5 | 26.8 |
| 5          | 62.3 | 61.6 | 100  | 59.1 | 57.6 | 61.3 | 54   | 58.8 | 31.9 | 15.9 | 26.6 |
| 6          | 64.7 | 64   | 59.1 | 100  | 58.6 | 56.4 | 54   | 72.6 | 32.8 | 13.5 | 29.2 |
| 7          | 59.6 | 59.1 | 57.6 | 58.6 | 100  | 55.8 | 69.1 | 58.1 | 34.9 | 17.5 | 27.6 |
| 8          | 63.1 | 63.6 | 61.3 | 56.4 | 55.8 | 100  | 48.7 | 54.8 | 31.1 | 13.9 | 25.2 |
| 9          | 55.4 | 54.7 | 54   | 54   | 69.1 | 48.7 | 100  | 52.6 | 32.4 | 15.4 | 25.6 |
| 10         | 63.4 | 63   | 58.8 | 72.6 | 58.1 | 54.8 | 52.6 | 100  | 33.9 | 13.2 | 27.3 |
| 11         | 31.9 | 32.9 | 31.9 | 32.8 | 34.9 | 31.1 | 32.4 | 33.9 | 100  | 15.9 | 36.3 |
| 12         | 13.5 | 13.5 | 15.9 | 13.5 | 17.5 | 13.9 | 15.4 | 13.2 | 15.9 | 100  | 12.8 |
| 13         | 27   | 26.8 | 26.6 | 29.2 | 27.6 | 25.2 | 25.6 | 27.3 | 36.3 | 12.8 | 100  |

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

15

[132] Sequence searches are typically carried out using the BLASTN program when evaluating a given nucleic acid sequence relative to nucleic acid sequences in the GenBank DNA Sequences and other public databases. The BLASTX program is preferred for searching nucleic acid sequences that have been translated in all reading frames against amino acid sequences in the GenBank Protein Sequences and other public databases. Both BLASTN and BLASTX are run using default parameters of an open gap penalty of 11.0, and an extended gap penalty of 1.0, and utilize the BLOSUM-62 matrix. (See, e.g., Altschul, et al., 1997.)

## 25 V. Expression Of Recombinant CBH2 Variants

[133] The methods of the disclosure rely on the use cells to express variant CBH2, with no particular method of CBH2 expression required. The variant CBH2 is preferably secreted from the cells. The disclosure provides host cells which have been transduced, transformed or transfected with an expression vector comprising a variant CBH2-encoding nucleic acid sequence. The culture conditions, such as temperature, pH and the like, are those previously used for the parental host cell prior to transduction, transformation or transfection and will be apparent to those skilled in the art.

[134] In one approach, a filamentous fungal cell or yeast cell is transfected with an expression vector having a promoter or biologically active promoter fragment or one or more (e.g., a series) of enhancers which functions in the host cell line, operably linked to a DNA segment encoding variant CBH2, such that variant CBH2 is expressed in the cell line.

#### **A. Nucleic Acid Constructs/Expression Vectors.**

[135] Natural or synthetic polynucleotide fragments encoding variant CBH2 ("CBH2-encoding nucleic acid sequences") may be incorporated into heterologous nucleic acid constructs or vectors, capable of introduction into, and replication in, a filamentous fungal or yeast cell. The vectors and methods disclosed herein are suitable for use in host cells for the expression of variant CBH2. Any vector may be used as long as it is replicable and viable in the cells into which it is introduced. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. Cloning and expression vectors are also described in Sambrook et al., 1989, Ausubel F M et al., 1989, and Strathern et al., The Molecular Biology of the Yeast *Saccharomyces*, 1981, each of which is expressly incorporated by reference herein. Appropriate expression vectors for fungi are described in van den Hondel, C. A. M. J. J. et al. (1991) In: Bennett, J. W. and Lasure, L. L. (eds.) More Gene Manipulations in Fungi. Academic Press, pp. 396-428. The appropriate DNA sequence may be inserted into a plasmid or vector (collectively referred to herein as "vectors") by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by standard procedures. Such procedures and related sub-cloning procedures are deemed to be within the scope of knowledge of those skilled in the art.

[136] Recombinant filamentous fungi comprising the coding sequence for variant CBH2 may be produced by introducing a heterologous nucleic acid construct comprising the variant CBH2 coding sequence into the cells of a selected strain of the filamentous fungi.

[137] Once the desired form of a variant cbh2 nucleic acid sequence is obtained, it may be modified in a variety of ways. Where the sequence involves non-coding flanking regions, the flanking regions may be subjected to resection, mutagenesis, etc. Thus, transitions, transversions, deletions, and insertions may be performed on the naturally occurring  
5 sequence.

[138] A selected variant cbh2 coding sequence may be inserted into a suitable vector according to well-known recombinant techniques and used to transform filamentous fungi capable of CBH2 expression. Due to the inherent degeneracy of the genetic code, other nucleic acid sequences which encode substantially the same or a functionally equivalent  
10 amino acid sequence may be used to clone and express variant CBH2. Therefore it is appreciated that such substitutions in the coding region fall within the sequence variants covered by the present disclosure. Any and all of these sequence variants can be utilized in the same way as described herein for a parent CBH2-encoding nucleic acid sequence.

[139] The present disclosure also includes recombinant nucleic acid constructs comprising  
15 one or more of the variant CBH2-encoding nucleic acid sequences as described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the disclosure has been inserted, in a forward or reverse orientation.

[140] Heterologous nucleic acid constructs may include the coding sequence for variant cbh2. (i) in isolation; (ii) in combination with additional coding sequences; such as fusion  
20 protein or signal peptide coding sequences, where the cbh2 coding sequence is the dominant coding sequence; (iii) in combination with non-coding sequences, such as introns and control elements, such as promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host; and/or (iv) in a vector or host environment in which the cbh2 coding sequence is a heterologous gene.

[141] In one aspect of the present disclosure, a heterologous nucleic acid construct is employed to transfer a variant CBH2-encoding nucleic acid sequence into a cell in vitro, with established filamentous fungal and yeast lines preferred. For long-term, production of variant CBH2, stable expression is preferred. It follows that any method effective to generate stable transformants may be used in practicing the disclosure.

[142] Appropriate vectors are typically equipped with a selectable marker-encoding nucleic acid sequence, insertion sites, and suitable control elements, such as promoter and termination sequences. The vector may comprise regulatory sequences, including, for  
30 example, non-coding sequences, such as introns and control elements, i.e., promoter and

terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in host cells (and/or in a vector or host cell environment in which a modified soluble protein antigen coding sequence is not normally expressed), operably linked to the coding sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, many of which are commercially available and/or are described in Sambrook, et al., (supra).

[143] Exemplary promoters include both constitutive promoters and inducible promoters, examples of which include a CMV promoter, an SV40 early promoter, an RSV promoter, an EF-1.alpha. promoter, a promoter containing the tet responsive element (TRE) in the tet-on or tet-off system as described (ClonTech and BASF), the beta actin promoter and the metallothionine promoter that can upregulated by addition of certain metal salts. A promoter sequence is a DNA sequence which is recognized by the particular filamentous fungus for expression purposes. It is operably linked to DNA sequence encoding a variant CBH2 polypeptide. Such linkage comprises positioning of the promoter with respect to the initiation codon of the DNA sequence encoding the variant CBH2 polypeptide in the disclosed expression vectors. The promoter sequence contains transcription and translation control sequence which mediate the expression of the variant CBH2 polypeptide. Examples include the promoters from the *Aspergillus niger*, *A. awamori* or *A. oryzae* glucoamylase, alpha-amylase, or alpha-glucosidase encoding genes; the *A. nidulans* *gpdA* or *trpC* Genes; the *Neurospora crassa* *cbh1* or *trp1* genes; the *A. niger* or *Rhizomucor miehei* aspartic proteinase encoding genes; the *H. jecorina* (*T. reesei*) *cbh1*, *cbh2*, *egl1*, *egl2*, or other cellulase encoding genes.

[144] The choice of the proper selectable marker will depend on the host cell, and appropriate markers for different hosts are well known in the art. Typical selectable marker genes include *argB* from *A. nidulans* or *T. reesei*, *amdS* from *A. nidulans*, *pyr4* from *Neurospora crassa* or *T. reesei*, *pyrG* from *Aspergillus niger* or *A. nidulans*. Additional exemplary selectable markers include, but are not limited to *trpc*, *trp1*, *oliC31*, *niaD* or *leu2*, which are included in heterologous nucleic acid constructs used to transform a mutant strain such as *trp-*, *pyr-*, *leu-* and the like.

[145] Such selectable markers confer to transformants the ability to utilize a metabolite that is usually not metabolized by the filamentous fungi. For example, the *amdS* gene from *H. jecorina* which encodes the enzyme acetamidase that allows transformant cells to grow on acetamide as a nitrogen source. The selectable marker (e.g. *pyrG*) may restore the ability of

an auxotrophic mutant strain to grow on a selective minimal medium or the selectable marker (e.g. olic31) may confer to transformants the ability to grow in the presence of an inhibitory drug or antibiotic.

[146] The selectable marker coding sequence is cloned into any suitable plasmid using methods generally employed in the art. Exemplary plasmids include pUC18, pBR322, pRAX and pUC100. The pRAX plasmid contains AMAL sequences from *A. nidulans*, which make it possible to replicate in *A. niger*.

[147] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Sambrook et al., 1989; Freshney, *Animal Cell Culture*, 1987; Ausubel, et al., 1993; and Coligan et al., *Current Protocols in Immunology*, 1991.

## **B. Host Cells and Culture Conditions For CBH2 Production**

### **(i) Filamentous Fungi**

[148] Thus, the present disclosure provides filamentous fungi comprising cells which have been modified, selected and cultured in a manner effective to result in variant CBH2 production or expression relative to the corresponding non-transformed parental fungi.

[149] Examples of species of parental filamentous fungi that may be treated and/or modified for variant CBH2 expression include, but are not limited to *Trichoderma*, e.g., *Trichoderma reesei*, *Trichoderma longibrachiatum*, *Trichoderma viride*, *Trichoderma koningii*; *Penicillium* sp., *Humicola* sp., including *Humicola insolens*; *Aspergillus* sp., *Chrysosporium* sp., *Fusarium* sp., *Hypocrea* sp., and *Emericella* sp.

[150] CBH2 expressing cells are cultured under conditions typically employed to culture the parental fungal line. Generally, cells are cultured in a standard medium containing physiological salts and nutrients, such as described in Pourquie, J. et al., *Biochemistry and Genetics of Cellulose Degradation*, eds. Aubert, J. P. et al., Academic Press, pp. 71-86, 1988 and Ilmen, M. et al., *Appl. Environ. Microbiol.* 63:1298-1306, 1997. Culture conditions are also standard, e.g., cultures are incubated at 28.degree. C. in shaker cultures or fermenters until desired levels of CBH2 expression are achieved.

[151] Preferred culture conditions for a given filamentous fungus may be found in the scientific literature and/or from the source of the fungi such as the American Type Culture

Collection (ATCC; www.atcc.org/). After fungal growth has been established, the cells are exposed to conditions effective to cause or permit the expression of variant CBH2.

[152] In cases where a CBH2 coding sequence is under the control of an inducible promoter, the inducing agent, e.g., a sugar, metal salt or antibiotics, is added to the medium at a concentration effective to induce CBH2 expression.

[153] In one embodiment, the strain comprises *Aspergillus niger*, which is a useful strain for obtaining overexpressed protein. For example *A. niger* var *awamori* dgr246 is known to secrete elevated amounts of secreted cellulases (Goedegebuur et al., Curr. Genet (2002) 41: 89-98). Other strains of *Aspergillus niger* var *awamori* such as GCDAP3, GCDAP4 and GAP3-4 are known Ward et al (Ward, M, Wilson, L. J. and Kodama, K. H., 1993, Appl. Microbiol. Biotechnol. 39:738-743).

[154] In another embodiment, the strain comprises *Trichoderma reesei*, which is a useful strain for obtaining overexpressed protein. For example, RL-P37, described by Sheir-Neiss, et al., Appl. Microbiol. Biotechnol. 20:46-53 (1984) is known to secrete elevated amounts of cellulase enzymes. Functional equivalents of RL-P37 include *Trichoderma reesei* strain RUT-C30 (ATCC No. 56765) and strain QM9414 (ATCC No. 26921). It is contemplated that these strains would also be useful in overexpressing variant CBH2.

[155] Where it is desired to obtain the variant CBH2 in the absence of potentially detrimental native cellulolytic activity, it is useful to obtain a *Trichoderma* host cell strain which has had one or more cellulase genes deleted prior to introduction of a DNA construct or plasmid containing the DNA fragment encoding the variant CBH2. Such strains may be prepared by the method disclosed in U.S. Pat. No. 5,246,853 and WO 92/06209, which disclosures are hereby incorporated by reference. By expressing a variant CBH2 cellulase in a host microorganism that is missing one or more cellulase genes, the identification and subsequent purification procedures are simplified. Any gene from *Trichoderma* sp. which has been cloned can be deleted, for example, the *cbh1*, *cbh2*, *egl1*, and *egl2* genes as well as those encoding EG III and/or EGV protein (see e.g., U.S. Pat. No. 5,475,101 and WO 94/28117, respectively).

[156] Gene deletion may be accomplished by inserting a form of the desired gene to be deleted or disrupted into a plasmid by methods known in the art. The deletion plasmid is then cut at an appropriate restriction enzyme site(s), internal to the desired gene coding region, and the gene coding sequence or part thereof replaced with a selectable marker. Flanking DNA sequences from the locus of the gene to be deleted or disrupted, preferably between about 0.5

to 2.0 kb, remain on either side of the selectable marker gene. An appropriate deletion plasmid will generally have unique restriction enzyme sites present therein to enable the fragment containing the deleted gene, including flanking DNA sequences, and the selectable marker gene to be removed as a single linear piece.

5 [157] A selectable marker must be chosen so as to enable detection of the transformed microorganism. Any selectable marker gene that is expressed in the selected microorganism will be suitable. For example, with *Aspergillus* sp., the selectable marker is chosen so that the presence of the selectable marker in the transformants will not significantly affect the properties thereof. Such a selectable marker may be a gene that encodes an assayable product.  
10 For example, a functional copy of an *Aspergillus* sp. gene may be used which if lacking in the host strain results in the host strain displaying an auxotrophic phenotype. Similarly, selectable markers exist for *Trichoderma* sp.

[158] In one embodiment, a *pyrG*- derivative strain of *Aspergillus* sp. is transformed with a functional *pyrG* gene, which thus provides a selectable marker for transformation. A *pyrG*-  
15 derivative strain may be obtained by selection of *Aspergillus* sp. strains that are resistant to fluoroarotic acid (FOA). The *pyrG* gene encodes orotidine-5'-monophosphate decarboxylase, an enzyme required for the biosynthesis of uridine. Strains with an intact *pyrG* gene grow in a medium lacking uridine but are sensitive to fluoroarotic acid. It is possible to select *pyrG*-  
derivative strains that lack a functional orotidine monophosphate decarboxylase enzyme and  
20 require uridine for growth by selecting for FOA resistance. Using the FOA selection technique it is also possible to obtain uridine-requiring strains which lack a functional orotate pyrophosphoribosyl transferase. It is possible to transform these cells with a functional copy of the gene encoding this enzyme (Berges & Barreau, *Curr. Genet.* 19:359-365 (1991), and van Hartingsveldt et al., (1986) Development of a homologous transformation system for  
25 *Aspergillus niger* based on the *pyrG* gene. *Mol. Gen. Genet.* 206:71-75). Selection of derivative strains is easily performed using the FOA resistance technique referred to above, and thus, the *pyrG* gene is preferably employed as a selectable marker.

[159] In a second embodiment, a *pyr4*. - derivative strain of *Hyphocrea* sp. (*Trichoderma* sp.) is transformed with a functional *pyr4* gene, which thus provides a  
30 selectable marker for transformation. A *pyr4*.sup.- derivative strain may be obtained by selection of *Hyphocrea* sp. (*Trichoderma* sp.) strains that are resistant to fluoroarotic acid (FOA). The *pyr4* gene encodes orotidine-5'-monophosphate decarboxylase, an enzyme required for the biosynthesis of uridine. Strains with an intact *pyr4* gene grow in a medium

lacking uridine but are sensitive to fluoroarotic acid. It is possible to select pyr4<sup>sup</sup>- derivative strains that lack a functional orotidine monophosphate decarboxylase enzyme and require uridine for growth by selecting for FOA resistance. Using the FOA selection technique it is also possible to obtain uridine-requiring strains which lack a functional orotate pyrophosphoribosyl transferase. It is possible to transform these cells with a functional copy of the gene encoding this enzyme (Berges & Barreau, 1991). Selection of derivative strains is easily performed using the FOA resistance technique referred to above, and thus, the pyr4 gene is preferably employed as a selectable marker.

[160] To transform pyrG<sup>-</sup> *Aspergillus* sp. or pyr4<sup>-</sup> *Hyphocrea* sp. (*Trichoderma* sp.) so as to be lacking in the ability to express one or more cellulase genes, a single DNA fragment comprising a disrupted or deleted cellulase gene is then isolated from the deletion plasmid and used to transform an appropriate pyr<sup>-</sup> *Aspergillus* or pyr<sup>-</sup> *Trichoderma* host.

Transformants are then identified and selected based on their ability to express the pyrG or pyr4, respectively, gene product and thus complement the uridine auxotrophy of the host strain. Southern blot analysis is then carried out on the resultant transformants to identify and confirm a double crossover integration event that replaces part or all of the coding region of the genomic copy of the gene to be deleted with the appropriate pyr selectable markers.

[161] Although the specific plasmid vectors described above relate to preparation of pyr<sup>-</sup> transformants, the present disclosure is not limited to these vectors. Various genes can be deleted and replaced in the *Aspergillus* sp. or *Hyphocrea* sp. (*Trichoderma* sp.) strain using the above techniques. In addition, any available selectable markers can be used, as discussed above. In fact, any host, e.g., *Aspergillus* sp. or *Hyphocrea* sp., gene that has been cloned, and thus identified, can be deleted from the genome using the above-described strategy.

[162] As stated above, the host strains used may be derivatives of *Hyphocrea* sp.

(*Trichoderma* sp.) that lack or have a nonfunctional gene or genes corresponding to the selectable marker chosen. For example, if the selectable marker of pyrG is chosen for *Aspergillus* sp., then a specific pyrG<sup>-</sup> derivative strain is used as a recipient in the transformation procedure. Also, for example, if the selectable marker of pyr4 is chosen for a *Hyphocrea* sp., then a specific pyr4<sup>-</sup> derivative strain is used as a recipient in the transformation procedure. Similarly, selectable markers comprising *Hyphocrea* sp. (*Trichoderma* sp.) genes equivalent to the *Aspergillus nidulans* genes amdS, argB, trpC, niaD may be used. The corresponding recipient strain must therefore be a derivative strain such as argB<sup>-</sup>, trpC<sup>-</sup>, niaD<sup>-</sup>, respectively.

[163] DNA encoding the CBH2 variant is then prepared for insertion into an appropriate microorganism. According to the present disclosure, DNA encoding a CBH2 variant comprises the DNA necessary to encode for a protein that has functional cellulolytic activity. The DNA fragment encoding the CBH2 variant may be functionally attached to a fungal promoter sequence, for example, the promoter of the *glaA* gene in *Aspergillus* or the promoter of the *cbh1* or *egl1* genes in *Trichoderma*.

[164] It is also contemplated that more than one copy of DNA encoding a CBH2 variant may be recombined into the strain to facilitate overexpression. The DNA encoding the CBH2 variant may be prepared by the construction of an expression vector carrying the DNA encoding the variant. The expression vector carrying the inserted DNA fragment encoding the CBH2 variant may be any vector which is capable of replicating autonomously in a given host organism or of integrating into the DNA of the host, typically a plasmid. In preferred embodiments two types of expression vectors for obtaining expression of genes are contemplated. The first contains DNA sequences in which the promoter, gene-coding region, and terminator sequence all originate from the gene to be expressed. Gene truncation may be obtained where desired by deleting undesired DNA sequences (e.g., coding for unwanted domains) to leave the domain to be expressed under control of its own transcriptional and translational regulatory sequences. A selectable marker may also be contained on the vector allowing the selection for integration into the host of multiple copies of the novel gene sequences.

[165] The second type of expression vector is preassembled and contains sequences required for high-level transcription and a selectable marker. It is contemplated that the coding region for a gene or part thereof can be inserted into this general-purpose expression vector such that it is under the transcriptional control of the expression cassettes promoter and terminator sequences.

[166] For example, in *Aspergillus*, pRAX is such a general-purpose expression vector. Genes or part thereof can be inserted downstream of the strong *glaa* promoter.

[167] For example, in *Hypocrea*, pTEX is such a general-purpose expression vector. Genes or part thereof can be inserted downstream of the strong *cbh1* promoter.

[168] In the vector, the DNA sequence encoding the CBH2 variant of the present disclosure should be operably linked to transcriptional and translational sequences, i.e., a suitable promoter sequence and signal sequence in reading frame to the structural gene. The promoter may be any DNA sequence that shows transcriptional activity in the host cell and may be

derived from genes encoding proteins either homologous or heterologous to the host cell. An optional signal peptide provides for extracellular production of the CBH2 variant. The DNA encoding the signal sequence is preferably that which is naturally associated with the gene to be expressed, however the signal sequence from any suitable source, for example an exo-  
5 cellobiohydrolase or endoglucanase from *Trichoderma*, is contemplated in the present disclosure.

[169] The procedures used to ligate the DNA sequences coding for the variant CBH2 of the present disclosure with the promoter, and insertion into suitable vectors are well known in the art.

10 [170] The DNA vector or construct described above may be introduced in the host cell in accordance with known techniques such as transformation, transfection, microinjection, microporation, biolistic bombardment and the like.

[171] In the preferred transformation technique, it must be taken into account that the permeability of the cell wall to DNA in *Hyprocrea* sp. (*Trichoderma* sp.) is very low.

15 Accordingly, uptake of the desired DNA sequence, gene or gene fragment is at best minimal. There are a number of methods to increase the permeability of the *Hyprocrea* sp. (*Trichoderma* sp.) cell wall in the derivative strain (i.e., lacking a functional gene corresponding to the used selectable marker) prior to the transformation process.

[172] The preferred method in the present disclosure to prepare *Aspergillus* sp. or  
20 *Hyprocrea* sp. (*Trichoderma* sp.) for transformation involves the preparation of protoplasts from fungal mycelium. See Campbell et al. Improved transformation efficiency of *A. niger* using homologous *niaD* gene for nitrate reductase. *Curr. Genet.* 16:53-56; 1989. The mycelium can be obtained from germinated vegetative spores. The mycelium is treated with an enzyme that digests the cell wall resulting in protoplasts. The protoplasts are then  
25 protected by the presence of an osmotic stabilizer in the suspending medium. These stabilizers include sorbitol, mannitol, potassium chloride, magnesium sulfate and the like. Usually the concentration of these stabilizers varies between 0.8 M and 1.2 M. It is preferable to use about a 1.2 M solution of sorbitol in the suspension medium.

[173] Uptake of the DNA into the host strain, (*Aspergillus* sp. or *Hyprocrea* sp.  
30 (*Trichoderma* sp.), is dependent upon the calcium ion concentration. Generally between about 10 mM  $\text{CaCl}_2$  and 50 mM  $\text{CaCl}_2$  is used in an uptake solution. Besides the need for the calcium ion in the uptake solution, other items generally included are a buffering system such as TE buffer (10 mM Tris, pH 7.4; 1 mM EDTA) or 10 mM MOPS, pH 6.0 buffer

(morpholinepropanesulfonic acid) and polyethylene glycol (PEG). It is believed that the polyethylene glycol acts to fuse the cell membranes thus permitting the contents of the medium to be delivered into the cytoplasm of the host cell, by way of example either *Aspergillus* sp. or *Hypocrea* sp. strain, and the plasmid DNA is transferred to the nucleus.

5 This fusion frequently leaves multiple copies of the plasmid DNA integrated into the host chromosome.

[174] Usually a suspension containing the *Aspergillus* sp. protoplasts or cells that have been subjected to a permeability treatment at a density of  $10^{5.5}$  to  $10^{6.6}$ /mL, preferably  $2 \times 10^{5.5}$ /mL are used in transformation. Similarly, a suspension containing the

10 *Hypocrea* sp. (*Trichoderma* sp.) protoplasts or cells that have been subjected to a permeability treatment at a density of  $10^{8.8}$  to  $10^{9.9}$ /mL, preferably  $2 \times 10^{8.8}$ /mL are used in transformation. A volume of 100  $\mu$ L of these protoplasts or cells in an appropriate solution (e.g., 1.2 M sorbitol; 50 mM  $\text{CaCl}_2$ ) are mixed with the desired DNA. Generally a high concentration of PEG is added to the uptake solution.

15 From 0.1 to 1 volume of 25% PEG 4000 can be added to the protoplast suspension. However, it is preferable to add about 0.25 volumes to the protoplast suspension. Additives such as dimethyl sulfoxide, heparin, spermidine, potassium chloride and the like may also be added to the uptake solution and aid in transformation.

[175] Generally, the mixture is then incubated at approximately 0°C. for a period of  
20 between 10 to 30 minutes. Additional PEG is then added to the mixture to further enhance the uptake of the desired gene or DNA sequence. The 25% PEG 4000 is generally added in volumes of 5 to 15 times the volume of the transformation mixture; however, greater and lesser volumes may be suitable. The 25% PEG 4000 is preferably about 10 times the volume of the transformation mixture. After the PEG is added, the transformation mixture is then  
25 incubated either at room temperature or on ice before the addition of a sorbitol and  $\text{CaCl}_2$  solution. The protoplast suspension is then further added to molten aliquots of a growth medium. This growth medium permits the growth of transformants only. Any growth medium can be used in the present disclosure that is suitable to grow the desired transformants. However, if  $\text{Pyr}^+$  transformants are being selected it is preferable to use a  
30 growth medium that contains no uridine. The subsequent colonies are transferred and purified on a growth medium depleted of uridine.

[176] At this stage, stable transformants may be distinguished from unstable transformants by their faster growth rate and, in *Trichoderma*, for example, the formation of circular

colonies with a smooth, rather than ragged outline on solid culture medium lacking uridine. Additionally, in some cases a further test of stability may be made by growing the transformants on solid non-selective medium (i.e. containing uridine), harvesting spores from this culture medium and determining the percentage of these spores which will subsequently germinate and grow on selective medium lacking uridine.

[177] In a particular embodiment of the above method, the CBH2 variant(s) are recovered in active form from the host cell after growth in liquid media as a result of the appropriate post translational processing of the CBH2 variant.

#### (ii) Yeast

[178] The present disclosure also contemplates the use of yeast as a host cell for CBH2 production. Several other genes encoding hydrolytic enzymes have been expressed in various strains of the yeast *S. cerevisiae*. These include sequences encoding for two endoglucanases (Penttila et al., *Yeast* vol. 3, pp 175-185, 1987), two cellobiohydrolases (Penttila et al., *Gene*, 63: 103-112, 1988) and one beta-glucosidase from *Trichoderma reesei* (Cummings and Fowler, *Curr. Genet.* 29:227-233, 1996), a xylanase from *Aureobasidium pullulans* (Li and Ljungdahl, *Appl. Environ. Microbiol.* 62, no.1, pp. 209-213, 1996), an alpha-amylase from wheat (Rothstein et al., *Gene* 55:353-356, 1987), etc. In addition, a cellulase gene cassette encoding the *Butyrivibrio fibrisolvens* endo-[beta]-1,4-glucanase (END1), *Phanerochaete chrysosporium* cellobiohydrolase (CBH1), the *Ruminococcus flavefaciens* cellodextrinase (CEL1) and the *Endomyces fibrilizer* cellobiase (Bgl1) was successfully expressed in a laboratory strain of *S. cerevisiae* (Van Rensburg et al., *Yeast*, vol. 14, pp. 67-76, 1998).

#### C. Introduction of a CBH2-Encoding Nucleic Acid Sequence into Host Cells.

[179] The disclosure further provides cells and cell compositions which have been genetically modified to comprise an exogenously provided variant CBH2-encoding nucleic acid sequence. A parental cell or cell line may be genetically modified (i.e., transduced, transformed or transfected) with a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc, as further described above.

[180] The methods of transformation of the present disclosure may result in the stable integration of all or part of the transformation vector into the genome of the filamentous

fungus. However, transformation resulting in the maintenance of a self-replicating extra-chromosomal transformation vector is also contemplated.

[181] Many standard transfection methods can be used to produce *Trichoderma reesei* cell lines that express large quantities of the heterologous protein. Some of the published methods for the introduction of DNA constructs into cellulase-producing strains of *Trichoderma* include Lorito, Hayes, DiPietro and Harman, 1993, *Curr. Genet.* 24: 349-356; Goldman, VanMontagu and Herrera-Estrella, 1990, *Curr. Genet.* 17:169-174; Penttila, Nevalainen, Ratto, Salminen and Knowles, 1987, *Gene* 6: 155-164, for *Aspergillus* Yelton, Hamer and Timberlake, 1984, *Proc. Natl. Acad. Sci. USA* 81: 1470-1474, for *Fusarium* Bajar, Podila and Kolattukudy, 1991, *Proc. Natl. Acad. Sci. USA* 88: 8202-8212, for *Streptomyces* Hopwood et al., 1985, The John Innes Foundation, Norwich, UK and for *Bacillus* Brigidi, DeRossi, Bertarini, Riccardi and Matteuzzi, 1990, *FEMS Microbiol. Lett.* 55: 135-138).

[182] Other methods for introducing a heterologous nucleic acid construct (expression vector) into filamentous fungi (e.g., *H. jecorina*) include, but are not limited to the use of a particle or gene gun, permeabilization of filamentous fungi cells walls prior to the transformation process (e.g., by use of high concentrations of alkali, e.g., 0.05 M to 0.4 M  $\text{CaCl}_2$  or lithium acetate), protoplast fusion or *Agrobacterium* mediated transformation. An exemplary method for transformation of filamentous fungi by treatment of protoplasts or spheroplasts with polyethylene glycol and  $\text{CaCl}_2$  is described in Campbell, E. I. et al., *Curr. Genet.* 16:53-56, 1989 and Penttila, M. et al., *Gene*, 63:11-22, 1988.

[183] Any of the well-known procedures for introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, polybrene, protoplast fusion, electroporation, biolistics, liposomes, microinjection, plasma vectors, viral vectors and any of the other well known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell (see, e.g., Sambrook et al., supra). Also of use is the *Agrobacterium*-mediated transfection method described in U.S. Pat. No. 6,255,115. It is only necessary that the particular genetic engineering procedure used be capable of successfully introducing at least one gene into the host cell capable of expressing the heterologous gene.

[184] In addition, heterologous nucleic acid constructs comprising a variant CBH2-encoding nucleic acid sequence can be transcribed in vitro, and the resulting RNA introduced into the host cell by well-known methods, e.g., by injection.

[185] The disclosure further includes novel and useful transformants of filamentous fungi such as *H. jecorina* and *A. niger* for use in producing fungal cellulase compositions. The disclosure includes transformants of filamentous fungi especially fungi comprising the variant CBH2 coding sequence, or deletion of the endogenous *cbh* coding sequence.

5 [186] Following introduction of a heterologous nucleic acid construct comprising the coding sequence for a variant *cbh2*, the genetically modified cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying expression of a variant CBH2-encoding nucleic acid sequence. The culture conditions, such as temperature, pH and the like, are those previously used for the host cell  
10 selected for expression, and will be apparent to those skilled in the art.

[187] The progeny of cells into which such heterologous nucleic acid constructs have been introduced are generally considered to comprise the variant CBH2-encoding nucleic acid sequence found in the heterologous nucleic acid construct.

[188] The disclosure further includes novel and useful transformants of filamentous fungi  
15 such as *H. jecorina* for use in producing fungal cellulase compositions. *Aspergillus niger* may also be used in producing the variant CBH2. The disclosure includes transformants of filamentous fungi especially fungi comprising the variant *cbh 2* coding sequence, or deletion of the endogenous *cbh2* coding sequence.

[189] Stable transformants of filamentous fungi can generally be distinguished from  
20 unstable transformants by their faster growth rate and, in *Trichoderma*, for example, the formation of circular colonies with a smooth rather than ragged outline on solid culture medium. Additionally, in some cases, a further test of stability can be made by growing the transformants on solid non-selective medium, harvesting the spores from this culture medium and determining the percentage of these spores which will subsequently germinate and grow  
25 on selective medium.

## **VI. Isolation And Purification Of Recombinant CBH2 Protein**

[190] In general, a variant CBH2 protein produced in cell culture is secreted into the medium and may be purified or isolated, e.g., by removing unwanted components from the  
30 cell culture medium. However, in some cases, a variant CBH2 protein may be produced in a cellular form necessitating recovery from a cell lysate. In such cases the variant CBH2 protein is purified from the cells in which it was produced using techniques routinely employed by those of skill in the art. Examples include, but are not limited to, affinity

chromatography (Tilbeurgh et al., FEBS Lett. 16:215, 1984), ion-exchange chromatographic methods (Goyal et al., Bioresource Technol. 36:37-50, 1991; Fliess et al., Eur. J. Appl. Microbiol. Biotechnol. 17:314-318, 1983; Bhikhabhai et al., J. Appl. Biochem. 6:336-345, 1984; Ellouz et al., J. Chromatography 396:307-317, 1987), including ion-exchange using  
5 materials with high resolution power (Medve et al., J. Chromatography A 808:153-165, 1998), hydrophobic interaction chromatography (Tomaz and Queiroz, J. Chromatography A 865:123-128, 1999), and two-phase partitioning (Brumbauer, et al., Bioseparation 7:287-295, 1999).

[191] Typically, the variant CBH2 protein is fractionated to segregate proteins having  
10 selected properties, such as binding affinity to particular binding agents, e.g., antibodies or receptors; or which have a selected molecular weight range, or range of isoelectric points.

[192] Once expression of a given variant CBH2 protein is achieved, the CBH2 protein thereby produced is purified from the cells or cell culture. Exemplary procedures suitable for such purification include the following: antibody-affinity column chromatography, ion  
15 exchange chromatography; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; and gel filtration using, e.g., Sephadex G-75. Various methods of protein purification may be employed and such methods are known in the art and described e.g. in Deutscher, Methods in Enzymology, vol. 182, no. 57, pp. 779, 1990;  
20 Scopes, Methods Enzymol. 90: 479-91, 1982. The purification step(s) selected will depend, e.g., on the nature of the production process used and the particular protein produced.

## VII. Utility of cbh2 and CBH2

[193] It can be appreciated that the variant cbh nucleic acids, the variant CBH2 protein and  
25 compositions comprising variant CBH2 protein activity find utility in a wide variety applications, some of which are described below.

[194] New and improved cellulase compositions that comprise varying amounts BG-type, EG-type and variant CBH-type cellulases find utility in detergent compositions that exhibit enhanced cleaning ability, function as a softening agent and/or improve the feel of cotton  
30 fabrics (e.g., "stone washing" or "biopolishing"), in compositions for degrading wood pulp into sugars (e.g., for bio-ethanol production), and/or in feed compositions. The isolation and characterization of cellulase of each type provides the ability to control the aspects of such compositions.

[195] In one approach, the cellulase of the disclosure finds utility in detergent compositions or in the treatment of fabrics to improve the feel and appearance.

[196] Since the rate of hydrolysis of cellulosic products may be increased by using a transformant having at least one additional copy of the cbh gene inserted into the genome, products that contain cellulose or heteroglycans can be degraded at a faster rate and to a greater extent. Products made from cellulose such as paper, cotton, cellulosic diapers and the like can be degraded more efficiently in a landfill. Thus, the fermentation product obtainable from the transformants or the transformants alone may be used in compositions to help degrade by liquefaction a variety of cellulose products that add to the overcrowded landfills.

[197] Separate saccharification and fermentation is a process whereby cellulose present in biomass, e.g., corn stover, is converted to glucose and subsequently yeast strains convert glucose into ethanol. Simultaneous saccharification and fermentation is a process whereby cellulose present in biomass, e.g., corn stover, is converted to glucose and, at the same time and in the same reactor, yeast strains convert glucose into ethanol. Thus, in another approach, the variant CBH type cellulase of the disclosure finds utility in the degradation of biomass to ethanol. Ethanol production from readily available sources of cellulose provides a stable, renewable fuel source.

[198] Cellulose-based feedstocks are comprised of agricultural wastes, grasses and woods and other low-value biomass such as municipal waste (e.g., recycled paper, yard clippings, etc.). Ethanol may be produced from the fermentation of any of these cellulosic feedstocks. However, the cellulose must first be converted to sugars before there can be conversion to ethanol.

[199] A large variety of feedstocks may be used with the inventive variant CBH and the one selected for use may depend on the region where the conversion is being done. For example, in the Midwestern United States agricultural wastes such as wheat straw, corn stover and bagasse may predominate while in California rice straw may predominate. However, it should be understood that any available cellulosic biomass may be used in any region.

[200] The methods of the present disclosure can be used in the production of monosaccharides, disaccharides, and polysaccharides as chemical or fermentation feedstocks for microorganism for the production of organic products, chemicals and fuels, plastics, and other products or intermediates. In particular, the value of processing residues (dried distillers grain, spent grains from brewing, sugarcane bagasse, etc.) can be increased by partial or complete solubilization of cellulose or hemicellulose. In addition to ethanol, some

chemicals that can be produced from cellulose and hemicellulose include, acetone, acetate, glycine, lysine, organic acids (e.g., lactic acid), 1,3-propanediol, butanediol, glycerol, ethylene glycol, furfural, polyhydroxyalkanoates, cis, cis-muconic acid, animal feed and xylose.

- 5 [201] A cellulase composition containing an enhanced amount of cellobiohydrolase finds utility in ethanol production. Ethanol from this process can be further used as an octane enhancer or directly as a fuel in lieu of gasoline which is advantageous because ethanol as a fuel source is more environmentally friendly than petroleum derived products. It is known that the use of ethanol will improve air quality and possibly reduce local ozone levels and
- 10 smog. Moreover, utilization of ethanol in lieu of gasoline can be of strategic importance in buffering the impact of sudden shifts in non-renewable energy and petrochemical supplies.
- [202] Ethanol can be produced via saccharification and fermentation processes from cellulosic biomass such as trees, herbaceous plants, municipal solid waste and agricultural and forestry residues. However, the ratio of individual cellulase enzymes within a naturally
- 15 occurring cellulase mixture produced by a microbe may not be the most efficient for rapid conversion of cellulose in biomass to glucose. It is known that endoglucanases act to produce new cellulose chain ends which themselves are substrates for the action of cellobiohydrolases and thereby improve the efficiency of hydrolysis of the entire cellulase system. Therefore, the use of increased or optimized cellobiohydrolase activity may greatly enhance the production
- 20 of ethanol.
- [203] Thus, the inventive cellobiohydrolase finds use in the hydrolysis of cellulose to its sugar components. In one embodiment, a variant cellobiohydrolase is added to the biomass prior to the addition of a fermentative organism. In a second embodiment, a variant cellobiohydrolase is added to the biomass at the same time as a fermentative organism.
- 25 Optionally, there may be other cellulase components present in either embodiment.
- [204] In another embodiment the cellulosic feedstock may be pretreated. Pretreatment may be by elevated temperature and the addition of either of dilute acid, concentrated acid or dilute alkali solution. The pretreatment solution is added for a time sufficient to at least partially hydrolyze the hemicellulose components and then neutralized.
- 30 [205] The major product of CBH2 action on cellulose is cellobiose which is available for conversion to glucose by BG activity (for instance in a fungal cellulase product). Either by the pretreatment of the cellulosic biomass or by the enzymatic action on the biomass, other sugars, in addition to glucose and cellobiose, can be made available from the biomass. The

hemi-cellulose content of the biomass can be converted (by hemi-cellulases) to sugars such as xylose, galactose, mannose and arabinose. Thus, in a biomass conversion process, enzymatic saccharification can produce sugars that are made available for biological or chemical conversions to other intermediates or end-products. Therefore, the sugars generated from biomass find use in a variety of processes in addition to the generation of ethanol. Examples of such conversions are fermentation of glucose to ethanol (as reviewed by M. E. Himmel et al. pp 2-45, in "Fuels and Chemicals from Biomass", ACS Symposium Series 666, ed B. C. Saha and J. Woodward, 1997) and other biological conversions of glucose to 2,5-diketo-D-gluconate (U.S. Pat. No. 6,599,722), lactic acid (R. Datta and S-P. Tsai pp 224-236, *ibid*), succinate (R. R. Gokarn, M. A. Eiteman and J. Sridhar pp 237-263, *ibid*), 1,3-propanediol (A-P. Zheng, H. Biebl and W-D. Deckwer pp 264-279, *ibid*), 2,3-butanediol (C. S. Gong, N. Cao and G. T. Tsao pp 280-293, *ibid*), and the chemical and biological conversions of xylose to xylitol (B. C. Saha and R. J. Bothast pp 307-319, *ibid*). See also, for example, WO 98/21339.

[206] The detergent compositions of this disclosure may employ besides the cellulase composition (irrespective of the cellobiohydrolase content, i.e., cellobiohydrolase-free, substantially cellobiohydrolase-free, or cellobiohydrolase enhanced), a surfactant, including anionic, non-ionic and ampholytic surfactants, a hydrolase, building agents, bleaching agents, bluing agents and fluorescent dyes, caking inhibitors, solubilizers, cationic surfactants and the like. All of these components are known in the detergent art. The cellulase composition as described above can be added to the detergent composition either in a liquid diluent, in granules, in emulsions, in gels, in pastes, and the like. Such forms are well known to the skilled artisan. When a solid detergent composition is employed, the cellulase composition is preferably formulated as granules. Preferably, the granules can be formulated so as to contain a cellulase protecting agent. For a more thorough discussion, see U.S. Pat. No. 6,162,782 entitled "Detergent compositions containing cellulase compositions deficient in CBH2 type components," which is incorporated herein by reference.

[207] Preferably the cellulase compositions are employed from about 0.00005 weight percent to about 5 weight percent relative to the total detergent composition. More preferably, the cellulase compositions are employed from about 0.0002 weight percent to about 2 weight percent relative to the total detergent composition.

[208] In addition the variant CBH2 nucleic acid sequence finds utility in the identification and characterization of related nucleic acid sequences. A number of techniques useful for determining (predicting or confirming) the function of related genes or gene products include,

but are not limited to, (A) DNA/RNA analysis, such as (1) overexpression, ectopic expression, and expression in other species; (2) gene knock-out (reverse genetics, targeted knock-out, viral induced gene silencing (VIGS, see Baulcombe, 100 Years of Virology, Calisher and Horzinek eds., Springer-Verlag, New York, N.Y. 15:189-201, 1999); (3) analysis of the methylation status of the gene, especially flanking regulatory regions; and (4) in situ hybridization; (B) gene product analysis such as (1) recombinant protein expression; (2) antisera production, (3) immunolocalization; (4) biochemical assays for catalytic or other activity; (5) phosphorylation status; and (6) interaction with other proteins via yeast two-hybrid analysis; (C) pathway analysis, such as placing a gene or gene product within a particular biochemical or signaling pathway based on its overexpression phenotype or by sequence homology with related genes; and (D) other analyses which may also be performed to determine or confirm the participation of the isolated gene and its product in a particular metabolic or signaling pathway, and help determine gene function.

**EXPERIMENTAL**

[209] 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. The attached figures are meant to be considered as integral parts of the specification and description of the disclosure. The following examples are offered to illustrate, but not to limit the claimed disclosure

[210] In the experimental disclosure which follows, the following abbreviations apply: M (molar); mM (millimolar);  $\mu$ M (micromolar); nM (nanomolar); mol (moles); mmol (millimoles);  $\mu$ mol (micromoles); nmol (nanomoles); gm (grams); mg (milligrams);  $\mu$ g (micrograms); pg (picograms); L (liters); ml and mL (milliliters);  $\mu$ l and  $\mu$ L (microliters); cm (centimeters); mm (millimeters);  $\mu$ m (micrometers); nm (nanometers); U (units); V (volts); MW (molecular weight); sec (seconds); min(s) (minute/minutes); h(s) and hr(s) (hour/hours); °C (degrees Centigrade); QS (quantity sufficient); ND (not done); NA (not applicable); rpm (revolutions per minute); H<sub>2</sub>O (water); dH<sub>2</sub>O (deionized water); HCl (hydrochloric acid); aa (amino acid); bp (base pair); kb (kilobase pair); kD (kilodaltons); cDNA (copy or complementary DNA); DNA (deoxyribonucleic acid); ssDNA (single stranded DNA); dsDNA (double stranded DNA); dNTP (deoxyribonucleotide triphosphate); RNA (ribonucleic acid); MgCl<sub>2</sub> (magnesium chloride); NaCl (sodium chloride); w/v (weight to volume); v/v (volume to volume); g (gravity); OD (optical density); CNPG (chloro-nitro-phenyl-beta-D-glucoside); CNP (2-chloro-4-nitrophenol); APB (acid-pretreated bagasse); PASC (phosphoric acid swollen cellulose) PCS (acid-pretreated corn stover); Pi or PI (performance index); HPLC (high pressure liquid chromatography); PAGE (polyacrylamide gel electrophoresis); PCR (polymerase chain reaction); RT-PCR (reverse transcription PCR); and SEL (site evaluation library).

**EXAMPLE 1****Assays**

[211] The following assays were used in the examples described below. Any deviations from the protocols provided below are indicated in the examples. In these experiments, a spectrophotometer was used to measure the absorbance of the products formed after the completion of the reactions.

**A. Hexokinase Assay For Measurement Of Residual Glucose**

[212] Residual glucose from *H. jecorina* culture supernatants expressing CBH2 variants was measured using a hexokinase assay. A volume of 5 µl of supernatant was added to 195 µl glucose hexokinase assay (Instrumentation Laboratory, Breda, Netherlands) in a 96-well microtiterplate (Costar Flat Bottom PS). The plates were incubated at room temperature for 15 min. Following incubation, the absorbance was measured at 340 nm OD. Supernatants of cultures expressing residual glucose were excluded from pooling for further studies.

**B. HPLC Assay for Protein Content Determination**

[213] The concentration of CBH2 variant proteins from pooled culture supernatants was determined using an Agilent 1100 (Hewlett Packard) HPLC equipped with a Proswift RP 2H column (Dionex). Ten microliters of sample, mixed with 50 µl of 10% acetonitrile in filtered demineralized water was injected following equilibration of the HPLC column with 10% acetonitrile containing 0.01% trifluoroacetic acid. Compounds were eluted using a gradient of 10% to 30% acetonitrile from 0.3 min to 1 min, followed by a gradient of 30% to 65% from 1 min to 4 mins. Protein concentrations of CBH2 variants were determined from a calibration curve generated using purified wild-type CBH2 (6.25, 12.5, 25, 50 µg/ml). To calculate performance index ( $P_i$  or PI), the ratio of the (average) total protein produced by a variant and (average) total protein produced by the wild-type at the same dose were averaged.

**C. Specific Activity Determination by Phosphoric Acid Swollen Cellulose (PASC)****Hydrolysis Assay**

[214] *Cellulose hydrolysis*: Phosphoric acid swollen cellulose (PASC) was prepared from Avicel according to a published method (Walseth, Tappi 35:228, 1971; and Wood, Biochem J, 121:353-362, 1971). This material was diluted with buffer and water to achieve a 1% w/v mixture such that the final concentration of sodium acetate was 50mM, pH 5.0. One hundred microliters of a 1% suspension of PASC in 50 mM sodium acetate buffer (pH5.0) was dispensed in a 96-well microtiterplate (Costar Flat Bottom PS). Ten microliters of a 5 mg/ml culture supernatant from a CBH2 deleted strain was added to the PASC, and 5, 10, 15, or 20 µl of pooled culture supernatants from *H. jecorina* cells expressing either wild-type CBH2 or CBH2 variants were added to it. Deletion of the CBH2 gene from *Hypocrea jecorina* (also referred to as *Trichoderma reesei*) is described in U. S. Pat. Nos. 5,861,271 and 5,650,322. Compensating volumes of acetate buffer were added to make up for differences in total

volume. The microtiterplate was sealed and incubated in a thermostatted incubator at 50°C under continuous shaking at 900 rpm. After two hours, the hydrolysis reaction was stopped by the addition of 100 µl glycine buffer, pH 10 to each well. To calculate performance index (P<sub>i</sub> or PI), the ratio of the (average) total sugar produced by a variant and (average) total sugar produced by the wild-type at the same dose were averaged. The hydrolysis reaction products were analyzed with the PAHBAH assay. *PAHBAH assay*: Aliquots of 150 µl of PAHBAH reducing sugar reagent (5% w/v p-hydroxybenzoic acid hydrazide (PAHBAH, Sigma # H9882, dissolved in 0.5 N HCl), (Lever, Anal Biochem, 47:273-279, 1972) were added to all wells of an empty microtiter plate. Ten microliters of the hydrolysis reaction supernatants were added to the PABA reaction plate. All plates were sealed and incubated at 69°C under continuous shaking of 900 rpm. After one hour the plates were placed on ice for five minutes and centrifuged at 720 x g at room temperature for five minutes. Samples of 80 µL of the developed PAHBAH reaction mixtures were transferred to a fresh (read) plate and absorbance was measured at 410 nm in a spectrophotometer. A cellobiose standard was included as control. A dose response curve was generated for wild-type CBH2 protein.

#### **D. Specific Activity Determination By Hydrolysis Of Dilute Acid Pretreated Corn Stover (PCS)**

[215] *Pretreated Corn Stover (PCS)*: Corn stover was pretreated with 2% w/w H<sub>2</sub>SO<sub>4</sub> as described (Schell et al., J Appl Biochem Biotechnol, 105:69-86, 2003) and followed by multiple washes with deionized water to obtain a paste having a pH of 4.5. Sodium acetate buffer (pH 5.0) was then added to a final concentration of 50mM sodium acetate and, if necessary, this mixture was then titrated to pH 5.0 using 1N NaOH. The cellulase concentration in the reaction mixture was approximately 7%. Sixty-five microliters of this cellulose suspension was added per well to a 96-well microtiterplate (Nunc Flat Bottom PS). Ten microliters of a 5 mg/ml culture supernatant from a CBH2 deleted strain was added to the PCS, and 5, 10, 15, or 20 µl of pooled culture supernatants from *H. jecorina* cells expressing either wild-type CBH2 or CBH2 variants were added to it. Compensating volumes of acetate buffer were added to make up for differences in total volume. After sealing of the plate, the plates were placed in a thermostatted incubator at 50°C under continuous shaking of 1300 rpm for 5 minutes. The plates were then incubated at 50°C while shaking at 220 rpm under 80% humidity to prevent drying. After 7 days the plates were put on ice for 5 min and the hydrolysis reaction was stopped by the addition of 100 µl glycine

buffer, pH 10 to each well. The hydrolysis reaction products were analyzed with the PAHBAH assay. To calculate performance index ( $P_i$  or PI), the ratio of the (average) total sugar produced by a variant and (average) total sugar produced by the wild-type at the same dose were averaged. *PAHBAH assay*: Aliquots of 150  $\mu$ l of PAHBAH reducing sugar reagent (5% w/v p-hydroxybenzoic acid hydrazide (PAHBAH, Sigma # H9882, dissolved in 0.5 N HCl), (Lever, Anal Biochem, 47:273-279, 1972) were added to all wells of an empty microtiter plate. Ten microliters of the hydrolysis reaction supernatants were added to the PABA reaction plate. All plates were sealed and incubated at 69°C under continuous shaking of 900 rpm. After one hour the plates were placed on ice for five minutes and centrifuged at 720 x g at room temperature for five minutes. Samples of 80  $\mu$ L of the developed PAHBAH reaction mixtures were transferred to a fresh (read) plate and absorbance was measured at 410 nm in a spectrophotometer. A cellobiose standard was included as control. A dose response curve was generated for wild-type CBH2 protein.

#### 15 **E. Specific Activity Determination By Hydrolysis Of Ammonia Pretreated Corncob (CC)**

[216] Corn cob was ground to pass through a 0.9mm screen then pretreated as described in Example 4 of patent application WO2006110901 (herein incorporated by reference for this method). Pretreated corn cob was used as a 7% cellulose suspension in 50 mM sodium acetate pH 5.0. Sixty-five microliters of the suspension was added per well to a 96-well microtiterplate (Nunc Flat Bottom PS). To each well, 20  $\mu$ l of 3.4 mg/ml supernatant from a strain ( $\Delta$ egl1,  $\Delta$ egl2,  $\Delta$ cbh1,  $\Delta$ cbh2) supplemented with 0.23 mg/ml purified *T. reesei* beta-glucosidase 1 (Cel3A) was added. Twenty or forty microliters of pooled culture supernatants from *H. jecorina* cells expressing either wild-type CBH2 or CBH2 variants were added.

Compensating volumes of acetate buffer were added to make up for differences in total volume. The plate was incubated at 50°C while shaking at 220 rpm under 80% humidity to prevent drying. After 3 days the plate was put on ice for 5 min and 100  $\mu$ l of 100 mM glycine pH 10.0 was added. After mixing, the plate was centrifuged at 3000 rpm for 5 min. A volume of 10  $\mu$ l supernatant was diluted in 190  $\mu$ l water. Twenty  $\mu$ l of the diluted solution was transferred to a new 96-well microtiterplate (Costar Flat Bottom PS) containing 100  $\mu$ l ABTS glucose assay mixture (2.74 mg/ml 2,2' azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid, 0.333 U/ml horseradish peroxidase type VI, 1 U/ml glucose oxidase) and increase in  $A_{420}$  was recorded in a microtiterplate spectrophotometer (Spectramax Plus 384, Molecular

Devices). A range of glucose concentrations was included as a standard on each plate (6.25, 12.5, 25, 50, 100, 200, 400 mM). Assays were done in duplicate. A dose response curve was generated for the wild-type CBH2 by fitting the data with the Langmuir equation ( $y = (x*a)/(x+b)$ ) and the activities of the CBH2 variants were divided by a calculated activity of wild-type CBH2 of the same plate to yield a performance index.

#### F. Stability Of CBH2 Variants In Presence Of Ethanol

[217] The stability of wild-type CBH2 and CBH2 variants was tested in the presence of 4.5% ethanol (EtOH) at 49°C. Pooled culture supernatants (80 µL) of *H. jecorina* cells expressing CBH2 variants were added to a 96-well plate (Greiner V-bottom PS) containing 10 µl of 40.5% EtOH per well. The plates were sealed and incubated in a thermostated incubator at 49°C for 16 hours with shaking at 900 rpm. Following incubation, the plates were placed on ice for 5 minutes. Residual CBH2 activity was determined using the phosphoric acid swollen cellulose (PASC) hydrolysis assay as described above.

[218] To calculate residual activity, the value of the product formed by the addition of 5, 10, 15 and 20 µl of EtOH-incubated CBH2 to the residual activity PASC assay was divided by the value of the product formed by the addition of 5, 10, 15 and 20 µl of EtOH-free CBH2 to the PASC assay. The individual values of these four ratios were then averaged to give the average residual activity. To determine PI value for the variant, the value of average residual activity for the variants was then divided by the average of the residual activity values of the wild-type CBH2 controls.

#### G. Thermostability Of CBH2 Variants

[219] The thermostability of wild-type CBH2 and CBH2 variants was tested at 53°C. Pooled culture supernatant (80uL) of *H. jecorina* cells expressing CBH2 variants were added to a 96-well plate (Greiner V-bottom PS). The plates were sealed and incubated in a thermostatted incubator at 53°C for 16 hours with shaking at 900 rpm. Following incubation, the plates were placed on ice for 5 minutes. Residual CBH2 activity was determined using the phosphoric acid swollen cellulose (PASC) hydrolysis assay as described above.

[220] To calculate residual activity, the value of the product formed by the addition of 5, 10, 15 and 20 µl of heat-treated CBH2 to the residual activity PASC assay was divided by the value of the product formed by the addition of 5, 10, 15 and 20 µl of unheated CBH2 to the PASC assay. The individual values of these four ratios were then averaged to give the

average residual activity. To determine PI value for the variant, the value of average residual activity for the variants was then divided by the average of the residual activity values of the wild-type CBH2 controls.

5

## EXAMPLE 2

### Generation of *Hypocrea jecorina* CBH2 Site Evaluation and Combinatorial Libraries

[221] The pTTTPyr-cbh2 plasmid containing the *Hypocrea jecorina* CBH2 protein encoding sequence (SEQ ID NO:1) was sent to BASEClear (Leiden, The Netherlands), GeneArt AG (Regensburg, Germany), and Sloning BioTechnology GmbH (Puchheim, Germany) for the generation of Site Evaluation Libraries (SELs). A map of pTTTPyr-cbh2 is provided in Figure 3. A request was made to the vendors for the generation of positional libraries at each of the sites in *Hypocrea jecorina* CBH2 mature protein (SEQ ID NO:3) shown in Table 2-1. The amino acid sequence of CBH2 full length protein is shown in SEQ ID NO:2.

15 [222] SEQ ID NO:1 sets forth the reference *Hypocrea jecorina* CBH2 coding DNA sequence:

atgattgtcggcattctcaccacgctggctacgctggccacactcgcagctagtgtgcctctagaggagcggcaagcttgctcaagcgt  
ctggggccaatgtggtggccagaattggctcgggtccgacttgctgtgcttccggaagcacatgcgtctactccaacgactattactccc  
agtgtcttcccggcgctgcaagctcaagctcgtccacgcgcgcgcgctgcacgacttctcgagtatccccacacatcccggtcga  
20 gctccgcgacgcctccacctggttctactactaccagagtacctccagtcggatcgggaaccgctacgtattcaggcaaccctttgttg  
gggtcactccttgggccaatgcatattacgcctctgaagttagcagcctcgtattcctagcttgactggagccatggccactgctgcag  
cagctgtcgcgaaagggtccctctttatgtggctagatactcttgacaagaccctctcatggagcaaaccctggccgacatccgcaccg  
ccaacaagaatggcggtaactatgccggacagtttggtgtatgacttgccggatcgcgattgcgctgcccttgccctgaatggcgaa  
tactctattgccgatggtggcgtcgccaaatataagaactatatcgacaccattcgtcaaattgtcgtggaattccgatatccggaccct  
25 cctggttattgagcctgactctcttgccaacctggtgaccaacctcggactccaaagtgtgccaatgctcagtcagcctaccttgagtgc  
atcaactacgccgtcacacagctgaaccttccaaatgttgcatgtatttgacgctggccatgcaggatggcttggtggccggcaaa  
ccaagaccggcgcgtcagctatttgcaaagtgttacaagaatgcatcgtctccgagagctcttcgcggattggcaaccaatgtcgcca  
actacaacgggtggaacattaccagccccccatcgtaacgcaaggcaacgctgtctacaacgagaagctgtacatccacgctattg  
gacctcttcttgccaatcacggctggtccaacgccttctcatcactgatcaagtcgatcgggaaagcagcctaccggacagcaaca  
30 gtggggagactggtgcaatgtgatcggcaccggatttggtattcgccatccgcaaactggggactcgttgctggattcgtttgtctg  
ggtcaagccaggcggcgagtgtgacggcaccagcgacagcagtgccacgatttgactcccactgtgcgctcccagatgccttgc  
aaccggcgcctcaagctggtgcttggtccaagcctactttgtgcagcttctcacaacgcaaaccatcgttcctgtaa.

[223] SEQ ID NO:2 sets forth the *Hypocrea jecorina* CBH2 full length protein sequence:

MIVGILTTLATLATLAASVPLEERQACSSVWGQCGGQNWSGPTCCASGSTCVYSNDYYSQCL  
 PGAASSSSSTRAASTTSRVSPSTTSRSSSATPPPGSTTTRVPPVGS GTATYSGNPFVGVTPWA  
 NAYYASEVSSLAIPSLTGAMATAAAVAKVP SFMWLDTLDKTPLMEQTLADIRTANKNGGNY  
 5 AGQFVVYDLPDRDCAALASNGEYSIADGGVAKYKNYIDTIRQIVVEYS DIRTLLVIEPDSL  
 NLVTNLGTPKCANAQSAYLECINYAVTQLNLPNVAMYLDAGHAGWLGWPANQDPAAQLFANV  
 YKNASSPRALRGLATNVANYNGWNITSPPSYTQGNVYNEKLYIHAIGPLL ANHGWSNAFFI  
 TDQGRSGKQPTGQQQWGDWCNVIGTGFGIRPSANTGDSL LDSFVWVKPGGEC DGTSDSSAPR  
 FDSHCALPDALQPAPQAGAWFQAYFVQLLTNANPSFL.

10

[224] SEQ ID NO:3 sets forth the *Hypocrea jecorina* CBH2 mature protein sequence:

QACSSVWGQCGGQNWSGPTCCASGSTCVYSNDYYSQCLPGAASSSSSTRAASTTSRVSPSTTS  
 RSSSATPPPGSTTTRVPPVGS GTATYSGNPFVGVTPWANAYYASEVSSLAIPSLTGAMATAA  
 AAVAKVP SFMWLDTLDKTPLMEQTLADIRTANKNGGNYAGQFVVYDLPDRDCAALASNGEYS  
 15 IADGGVAKYKNYIDTIRQIVVEYS DIRTLLVIEPDSL ANLVTNLGTPKCANAQSAYLECINY  
 AVTQLNLPNVAMYLDAGHAGWLGWPANQDPAAQLFANVYKNASSPRALRGLATNVANYNGWN  
 ITSPPSYTQGNVYNEKLYIHAIGPLL ANHGWSNAFFITDQGRSGKQPTGQQQWGDWCNVIG  
 TGFGIRPSANTGDSL LDSFVWVKPGGEC DGTSDSSAPR FDSHCALPDALQPAPQAGAWFQAY  
 FVQLLTNANPSFL.

20

[225] For each of the 162 sites listed in Table 2-1, an average of 18 substitution variants were generated. The libraries were received as purified plasmids encoding CBH2 variant proteins.

25 **Table 2-1: Selected Positions of the Mature CBH2 Protein**

| Region    | CBH2<br>Position |
|-----------|------------------|
| CBM       | 5                |
| CBM       | 18               |
| CBM       | 19               |
| CBM       | 28               |
| CBM       | 30               |
| CBM       | 32               |
| CBM       | 35               |
| CBM       | 38               |
| Linker    | 63               |
| Linker    | 77               |
| Linker    | 79               |
| Linker    | 80               |
| Catalytic | 89               |
| Catalytic | 94               |
| catalytic | 98               |
| catalytic | 100              |
| catalytic | 102              |
| catalytic | 103              |
| catalytic | 104              |
| catalytic | 105              |
| catalytic | 107              |
| catalytic | 111              |
| catalytic | 117              |
| catalytic | 119              |
| catalytic | 120              |
| catalytic | 121              |
| catalytic | 125              |
| catalytic | 126              |
| Catalytic | 129              |
| Catalytic | 133              |

| Region    | CBH2<br>Position |
|-----------|------------------|
| Catalytic | 134              |
| Catalytic | 137              |
| catalytic | 138              |
| catalytic | 139              |
| catalytic | 140              |
| catalytic | 141              |
| catalytic | 143              |
| catalytic | 144              |
| catalytic | 147              |
| catalytic | 150              |
| catalytic | 153              |
| catalytic | 154              |
| catalytic | 157              |
| catalytic | 158              |
| catalytic | 161              |
| catalytic | 162              |
| catalytic | 177              |
| catalytic | 178              |
| catalytic | 179              |
| catalytic | 180              |
| catalytic | 181              |
| catalytic | 182              |
| catalytic | 185              |
| catalytic | 186              |
| catalytic | 188              |
| catalytic | 189              |
| Catalytic | 190              |
| Catalytic | 191              |

| Region    | CBH2<br>Position |
|-----------|------------------|
| Catalytic | 192              |
| Catalytic | 193              |
| Catalytic | 194              |
| Catalytic | 196              |
| Catalytic | 197              |
| Catalytic | 201              |
| Catalytic | 203              |
| Catalytic | 204              |
| Catalytic | 206              |
| Catalytic | 207              |
| Catalytic | 210              |
| Catalytic | 214              |
| Catalytic | 225              |
| Catalytic | 226              |
| Catalytic | 228              |
| Catalytic | 229              |
| Catalytic | 230              |
| Catalytic | 231              |
| Catalytic | 232              |
| Catalytic | 233              |
| Catalytic | 234              |
| Catalytic | 236              |
| Catalytic | 237              |
| Catalytic | 239              |
| Catalytic | 240              |
| Catalytic | 243              |
| catalytic | 245              |
| catalytic | 247              |
| catalytic | 251              |
| catalytic | 252              |

| <b>Region</b> | <b>CBH2<br/>Position</b> |
|---------------|--------------------------|
| catalytic     | 254                      |
| catalytic     | 258                      |
| catalytic     | 266                      |
| catalytic     | 267                      |
| catalytic     | 268                      |
| catalytic     | 272                      |
| catalytic     | 274                      |
| catalytic     | 275                      |
| catalytic     | 281                      |
| catalytic     | 285                      |
| catalytic     | 288                      |
| catalytic     | 289                      |
| catalytic     | 291                      |
| catalytic     | 292                      |
| catalytic     | 293                      |
| catalytic     | 294                      |
| catalytic     | 303                      |
| catalytic     | 304                      |
| catalytic     | 306                      |
| catalytic     | 307                      |
| catalytic     | 312                      |
| catalytic     | 313                      |
| catalytic     | 316                      |
| catalytic     | 319                      |
| catalytic     | 322                      |
| catalytic     | 323                      |
| catalytic     | 327                      |
| catalytic     | 328                      |
| catalytic     | 331                      |
| catalytic     | 338                      |

| <b>Region</b> | <b>CBH2<br/>Position</b> |
|---------------|--------------------------|
| catalytic     | 339                      |
| catalytic     | 340                      |
| catalytic     | 343                      |
| catalytic     | 344                      |
| catalytic     | 346                      |
| catalytic     | 356                      |
| catalytic     | 360                      |
| catalytic     | 361                      |
| catalytic     | 362                      |
| catalytic     | 363                      |
| catalytic     | 364                      |
| catalytic     | 365                      |
| catalytic     | 371                      |
| catalytic     | 378                      |
| catalytic     | 380                      |
| catalytic     | 381                      |
| catalytic     | 382                      |
| catalytic     | 384                      |
| catalytic     | 386                      |
| catalytic     | 394                      |
| catalytic     | 396                      |
| catalytic     | 399                      |
| Catalytic     | 400                      |
| Catalytic     | 405                      |
| Catalytic     | 406                      |
| Catalytic     | 407                      |
| Catalytic     | 410                      |
| Catalytic     | 413                      |

| <b>Region</b> | <b>CBH2<br/>Position</b> |
|---------------|--------------------------|
| Catalytic     | 414                      |
| Catalytic     | 416                      |
| Catalytic     | 417                      |
| Catalytic     | 422                      |
| Catalytic     | 426                      |
| Catalytic     | 427                      |
| Catalytic     | 429                      |
| Catalytic     | 431                      |
| Catalytic     | 433                      |
| Catalytic     | 436                      |
| Catalytic     | 440                      |
| Catalytic     | 441                      |
| Catalytic     | 443                      |
| Catalytic     | 444                      |
| Catalytic     | 445                      |
| Catalytic     | 447                      |

[226] Four synthetic CBH2 combinatorial libraries were also produced by Sloning Biotechnology GmbH (Puchheim, Germany) and BaseClear (Leiden, The Netherlands). Tables 2-2 to 2-5 list the substitutions that could be present in members of the synthetic CBH2 combinatorial libraries (numbered according to the CBH2 mature amino acid sequence).

5

| <b>Table 2-2: CBH2 Combinatorial Library 1 Design (RCL 1)</b> |                          |                     |
|---------------------------------------------------------------|--------------------------|---------------------|
| <b>Targeted Position</b>                                      | <b>Wild-Type Residue</b> | <b>Substitution</b> |
| 111                                                           | L                        | L,S                 |
| 144                                                           | L                        | L,Q,W               |
| 154                                                           | T                        | T,C,V               |
| 162                                                           | Y                        | Y,N                 |
| 410                                                           | R                        | R,S                 |
| 413                                                           | S                        | S,W,Y               |

| <b>Table 2-3: CBH2 Combinatorial Library 2 Design (RCL 2)</b> |                          |                     |
|---------------------------------------------------------------|--------------------------|---------------------|
| <b>Targeted Position</b>                                      | <b>Wild-Type Residue</b> | <b>Substitution</b> |
| 98                                                            | P                        | P,L                 |
| 194                                                           | K                        | K,C,E               |
| 313                                                           | S                        | S,T                 |
| 316                                                           | S                        | S,P                 |
| 384                                                           | G                        | G,C,Q               |
| 443                                                           | N                        | N,I                 |

| Table 2-4: CBH2 Combinatorial Library 3 Design (RCL 3) |                   |              |
|--------------------------------------------------------|-------------------|--------------|
| Targeted Position                                      | Wild-Type Residue | Substitution |
| 153                                                    | R                 | R,Q          |
| 161                                                    | N                 | N,A,W        |
| 203                                                    | R                 | R,H          |
| 233                                                    | P                 | P,D          |
| 422                                                    | Q                 | Q,V          |
| 444                                                    | P                 | P,Q          |

| Table 2-5: CBH2 Combinatorial Library 4 Design (RCL 4) |                   |              |
|--------------------------------------------------------|-------------------|--------------|
| Targeted Position                                      | Wild-Type Residue | Substitution |
| 98                                                     | P                 | P,L          |
| 111                                                    | L                 | L,S          |
| 144                                                    | L                 | L,W          |
| 313                                                    | S                 | S,T          |
| 316                                                    | S                 | S,P          |
| 413                                                    | S                 | S,W          |
| 422                                                    | Q                 | Q,V          |

### Production of CBH2 Variant Proteins

- 5 [227] Purified pTTTpyr-cbh2 plasmids (p<sub>cbh1</sub>, Amp<sup>R</sup>, Acetamide<sup>R</sup>) containing open reading frames encoding CBH2 variant sequences were obtained from the vendors specified above. Protoplasts of a quad-deleted *H. jecorina* strain ( $\Delta$ egl1,  $\Delta$ egl2,  $\Delta$ cbh1  $\Delta$ cbh2) were transformed with the pTTTpyrG constructs and grown on selective agar containing acetamide at 28°C for 7 days as described (WO 2009/048488). Genes encoding cellobiohydrolase I
- 10 (CBHI, Cel7a), cellobiohydrolase II (CBHII, Cel6a), endoglucanase I (EGI, Cel7b), and endoglucanase II (EGII, Cel5a) have been inactivated in the quad-deleted strain. Spores were harvested, replated on acetamide agar, and incubated at 28°C for 7 days. In addition, spores were harvested in 15% glycerol and stored at -20°C for further use. For CBH2 variant protein production, a volume of 10  $\mu$ l spore suspension was added to 200  $\mu$ l glycine minimal
- 15 medium supplemented with 2% glucose/sophorose mixture in a PVDF filter plate: 6.0 g/L glycine, 4.7 g/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; 5.0 g/L KH<sub>2</sub>PO<sub>4</sub>; 1.0 g/L MgSO<sub>4</sub>·7H<sub>2</sub>O; 33.0 g/L PIPPS; pH 5.5;

with post sterile addition of ~2% glucose/sophorose mixture as the carbon source, 10 ml/L of 100g/L of CaCl<sub>2</sub>, 2.5 ml/L of *T. reesei* trace elements (400X): 175g/L Citric acid anhydrous; 200g/L FeSO<sub>4</sub>·7H<sub>2</sub>O; 16g/L ZnSO<sub>4</sub>·7H<sub>2</sub>O; 3.2 g/L CuSO<sub>4</sub>·5H<sub>2</sub>O; 1.4 g/L MnSO<sub>4</sub>·H<sub>2</sub>O; 0.8 g/L H<sub>3</sub>BO<sub>3</sub>. Each CBH2 variant was grown in quadruplicate. After sealing the plate with an oxygen permeable membrane, the plates were incubated at 28°C for 6 days, while shaking at 220 rpm. Supernatant was harvested by transferring the culture medium to a microtiter plate under low pressure and tested for residual glucose using the hexokinase assay as described in Example 1.

### EXAMPLE 3

#### Expression, Activity and Stability of CBH2 Variants

[228] *H. jecorina* CBH2 SEL and combinatorial variant proteins were tested various properties of interest. In particular, the cellulase variants were tested for protein expression using the HPLC assay (HPLC), specific activity using the PASC hydrolysis assay (Act. PASC) and the PCS hydrolysis assay (Act. PCS), stability in the presence of ethanol (EtOH ratio) and thermostability (heat ratio) as described in Example 1. Combinatorial variants were also tested for specific activity by hydrolysis of ammonia pretreated corncob (Sp. Act. CC) as described in Example 1. Performance data for CBH2 SEL variants are shown in Table 3-1, and performance data for the CBH2 combinatorial variants are shown in Table 3-2. Rows of Table 3-2 lacking performance data correspond to CBH2 combinatorial variants that were not expressed in initial tests.

[229] Performance index (Pi or PI) is the ratio of performance of the variant to the parent or reference cellulase. 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 = 0.05; combinable mutations are those mutations for which the variant has PI values = 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. Positions at which mutations occur are classed as follows: Non-restrictive positions have ≥ 20% neutral mutations for at least one property; and Restrictive positions have < 20% neutral mutations for activity and stability.

[230] This data may be used to engineer any CBH. Even if the CBH to be engineered has an amino acid different from that of CBH2 at a particular position, this data may be used to find a substitution that will alter the desired properties by identifying the best choice substitution, including substitution to the CBH2 wild type amino acid

[231] Table 3-1 shows performance index values (Pi or PI) for 2,828 variants of *Hypocrea jecorina* CBH2 at 162 positions. Performance indices less than or equal to 0.05 were fixed to 0.05 and indicated in bold italics in the table.

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 5                                                     | S005A   | 1.73 | 1.06            | 1.01           | 0.99         | 0.95         |
| 5                                                     | S005C   | 0.34 | 1.03            | 0.92           | 0.95         | 0.76         |
| 5                                                     | S005D   | 0.08 | 0.69            | 0.44           | 1.87         | 2.70         |
| 5                                                     | S005E   | 0.82 | 1.10            | 1.00           | 0.85         | 0.63         |
| 5                                                     | S005F   | 0.35 | 2.69            | 0.87           | 0.99         | 0.98         |
| 5                                                     | S005G   | 0.73 | 1.06            | 0.84           | 0.97         | 0.89         |
| 5                                                     | S005H   | 0.14 | 0.77            | 0.50           | 1.06         | 0.68         |
| 5                                                     | S005I   | 1.35 | 0.57            | 0.54           | 1.77         | 1.07         |
| 5                                                     | S005K   | 0.52 | 0.98            | 0.68           | 0.97         | 0.94         |
| 5                                                     | S005L   | 1.25 | 1.10            | 0.99           | 0.99         | 0.96         |
| 5                                                     | S005M   | 0.44 | 1.11            | 0.82           | 0.96         | 0.89         |
| 5                                                     | S005N   | 0.28 | 0.74            | 0.41           | 1.78         | 3.67         |
| 5                                                     | S005P   | 0.46 | 0.66            | 0.63           | 1.46         | 1.25         |
| 5                                                     | S005Q   | 0.72 | 1.17            | 0.98           | 0.94         | 0.92         |
| 5                                                     | S005R   | 0.36 | 1.01            | 0.73           | 0.89         | 0.82         |
| 5                                                     | S005T   | 0.28 | 1.05            | 0.73           | 0.98         | 0.84         |
| 5                                                     | S005V   | 0.40 | 1.15            | 0.79           | 0.91         | 0.85         |
| 5                                                     | S005W   | 0.61 | 1.11            | 1.00           | 0.95         | 0.87         |
| 5                                                     | S005Y   | 0.98 | 1.08            | 0.84           | 0.95         | 0.96         |
| 18                                                    | P18A    | 0.57 | 1.11            | 1.05           | 0.94         | 0.90         |
| 18                                                    | P18C    | 0.16 | 0.63            | 0.48           | 1.14         | 1.09         |
| 18                                                    | P18D    | 0.20 | 0.66            | 0.93           | 0.52         | 1.20         |
| 18                                                    | P18E    | 0.51 | 0.87            | 0.42           | 1.01         | 1.09         |
| 18                                                    | P18F    | 0.22 | 0.69            | 0.37           | 1.02         | 0.92         |
| 18                                                    | P18G    | 0.88 | 1.03            | 0.57           | 0.31         | 0.18         |
| 18                                                    | P18H    | 0.88 | 1.08            | 0.89           | 0.92         | 0.98         |
| 18                                                    | P18I    | 0.77 | 1.00            | 0.89           | 0.88         | 0.90         |
| 18                                                    | P18K    | 0.24 | 0.50            | 0.39           | 2.14         | 2.11         |
| 18                                                    | P18L    | 0.56 | 1.14            | 0.77           | 0.91         | 0.91         |
| 18                                                    | P18M    | 1.38 | 0.98            | 0.84           | 0.97         | 1.01         |
| 18                                                    | P18N    | 0.24 | 0.46            | 0.35           | 4.62         | 7.95         |
| 18                                                    | P18Q    | 0.12 | 0.28            | <b>0.05</b>    | 10.15        | 19.36        |
| 18                                                    | P18R    | 0.39 | 0.98            | 0.75           | 0.93         | 0.81         |
| 18                                                    | P18S    | 0.88 | 1.12            | 0.86           | 0.94         | 1.02         |
| 18                                                    | P18T    | 0.46 | 0.69            | 0.45           | 0.85         | 1.14         |
| 18                                                    | P18V    | 0.62 | 0.95            | 0.93           | 0.93         | 0.89         |
| 18                                                    | P18W    | 0.70 | 1.09            | 0.98           | 0.92         | 0.93         |
| 18                                                    | P18Y    | 0.91 | 1.08            | 0.91           | 0.91         | 0.95         |
| 19                                                    | T19A    | 0.96 | 1.06            | 0.96           | 0.89         | 0.87         |
| 19                                                    | T19C    | 0.08 | 0.12            | <b>0.05</b>    | 3.22         | 3.56         |
| 19                                                    | T19E    | 0.68 | 1.17            | 0.87           | 0.88         | 0.89         |
| 19                                                    | T19F    | 0.75 | 1.15            | 0.91           | 0.92         | 0.95         |
| 19                                                    | T19G    | 1.92 | 1.03            | 0.99           | 0.94         | 1.00         |
| 19                                                    | T19I    | 1.08 | 1.19            | 1.01           | 0.85         | 0.95         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 19                                                    | T19K    | 1.01 | 1.25            | 1.00           | 0.91         | 0.94         |
| 19                                                    | T19L    | 0.45 | 1.18            | 0.92           | 0.83         | 0.85         |
| 19                                                    | T19M    | 1.71 | 1.04            | 0.80           | 0.89         | 1.04         |
| 19                                                    | T19N    | 0.11 | 0.20            | 0.07           | 4.18         | 10.48        |
| 19                                                    | T19P    | 0.77 | 1.08            | 1.08           | 0.87         | 0.98         |
| 19                                                    | T19Q    | 0.67 | 1.07            | 0.84           | 0.92         | 0.95         |
| 19                                                    | T19R    | 1.11 | 1.08            | 0.89           | 0.91         | 1.04         |
| 19                                                    | T19S    | 0.81 | 1.13            | 0.84           | 0.87         | 0.95         |
| 19                                                    | T19V    | 0.09 | 0.18            | 0.09           | 13.65        | 56.16        |
| 19                                                    | T19W    | 0.25 | 0.51            | 0.47           | 0.77         | 0.59         |
| 19                                                    | T19Y    | 0.69 | 1.08            | 0.97           | 0.85         | 0.92         |
| 28                                                    | V28A    | 0.55 | 0.97            | 1.10           | 0.99         | 0.94         |
| 28                                                    | V28C    | 0.82 | 1.00            | 0.96           | 0.92         | 0.71         |
| 28                                                    | V28D    | 0.11 | 0.05            | 0.29           | 1.43         | 6.68         |
| 28                                                    | V28E    | 0.43 | 0.90            | 0.75           | 0.96         | 0.94         |
| 28                                                    | V28F    | 0.47 | 0.93            | 0.71           | 0.95         | 0.95         |
| 28                                                    | V28G    | 0.88 | 0.92            | 0.84           | 0.95         | 0.99         |
| 28                                                    | V28H    | 0.08 | 0.05            | 0.15           | 5.35         | 18.58        |
| 28                                                    | V28I    | 1.01 | 1.02            | 0.75           | 1.00         | 1.00         |
| 28                                                    | V28K    | 0.67 | 1.03            | 0.88           | 0.96         | 0.96         |
| 28                                                    | V28L    | 0.86 | 1.02            | 0.83           | 0.92         | 1.05         |
| 28                                                    | V28M    | 0.71 | 1.02            | 0.71           | 0.95         | 0.98         |
| 28                                                    | V28N    | 0.72 | 0.53            | 0.65           | 0.81         | 0.97         |
| 28                                                    | V28P    | 0.80 | 0.52            | 0.72           | 0.83         | 0.93         |
| 28                                                    | V28Q    | 0.56 | 0.97            | 0.73           | 0.95         | 0.98         |
| 28                                                    | V28R    | 0.76 | 1.01            | 0.74           | 0.95         | 0.98         |
| 28                                                    | V28S    | 0.52 | 0.96            | 0.88           | 0.97         | 0.98         |
| 28                                                    | V28T    | 0.52 | 1.03            | 0.84           | 1.02         | 1.00         |
| 28                                                    | V28W    | 0.42 | 0.84            | 0.75           | 0.95         | 0.90         |
| 28                                                    | V28Y    | 0.51 | 0.95            | 0.83           | 0.95         | 0.90         |
| 30                                                    | S30A    | 1.09 | 1.06            | 1.01           | 1.08         | 1.06         |
| 30                                                    | S30C    | 1.26 | 0.99            | 0.96           | 1.08         | 1.09         |
| 30                                                    | S30D    | 0.30 | 0.57            | 0.50           | 0.57         | 0.94         |
| 30                                                    | S30E    | 0.14 | 0.44            | 0.25           | 0.69         | 1.00         |
| 30                                                    | S30F    | 0.58 | 0.91            | 0.67           | 0.96         | 0.97         |
| 30                                                    | S30G    | 0.70 | 0.98            | 0.72           | 1.10         | 1.02         |
| 30                                                    | S30H    | 0.30 | 0.61            | 0.56           | 0.84         | 2.68         |
| 30                                                    | S30I    | 0.66 | 1.02            | 0.72           | 1.03         | 1.02         |
| 30                                                    | S30K    | 1.31 | 0.94            | 0.70           | 1.12         | 1.16         |
| 30                                                    | S30L    | 0.46 | 0.93            | 0.44           | 0.93         | 0.85         |
| 30                                                    | S30M    | 0.81 | 1.03            | 0.89           | 1.14         | 1.00         |
| 30                                                    | S30N    | 0.19 | 0.57            | 0.42           | 0.95         | 1.12         |
| 30                                                    | S30P    | 0.31 | 0.42            | 0.50           | 0.88         | 1.02         |
| 30                                                    | S30Q    | 0.63 | 0.65            | 0.43           | 0.85         | 1.65         |
| 30                                                    | S30R    | 0.50 | 0.67            | 0.45           | 1.11         | 1.37         |
| 30                                                    | S30T    | 0.81 | 0.99            | 0.76           | 1.09         | 1.05         |
| 30                                                    | S30V    | 0.76 | 1.04            | 0.73           | 1.02         | 1.02         |
| 30                                                    | S30W    | 0.41 | 0.92            | 0.59           | 0.93         | 0.78         |
| 30                                                    | S30Y    | 0.21 | 0.52            | 0.40           | 1.11         | 1.14         |
| 32                                                    | D32A    | 0.51 | 1.10            | 1.32           | 1.00         | 1.10         |
| 32                                                    | D32C    | 0.24 | 0.93            | 1.02           | 0.92         | 0.89         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 32                                                    | D32E    | 0.44 | 1.31            | 0.42           | 0.44         | 0.47         |
| 32                                                    | D32F    | 0.18 | 0.65            | 0.45           | 0.77         | 0.93         |
| 32                                                    | D32G    | 0.32 | 1.17            | 1.08           | 0.97         | 0.92         |
| 32                                                    | D32H    | 0.41 | 0.65            | 0.63           | 0.78         | 1.12         |
| 32                                                    | D32I    | 0.64 | 1.10            | 1.06           | 0.99         | 1.00         |
| 32                                                    | D32K    | 0.39 | 1.00            | 0.77           | 0.95         | 0.93         |
| 32                                                    | D32L    | 0.43 | 1.10            | 1.17           | 0.94         | 1.02         |
| 32                                                    | D32M    | 0.37 | 1.14            | 0.96           | 0.90         | 0.91         |
| 32                                                    | D32N    | 0.61 | 0.73            | 0.55           | 0.94         | 1.12         |
| 32                                                    | D32P    | 0.49 | 0.99            | 0.80           | 0.59         | 0.56         |
| 32                                                    | D32Q    | 0.65 | 1.11            | 1.00           | 0.94         | 0.97         |
| 32                                                    | D32R    | 0.28 | 1.05            | 0.78           | 0.87         | 0.87         |
| 32                                                    | D32S    | 0.31 | 0.91            | 0.78           | 0.91         | 0.87         |
| 32                                                    | D32T    | 0.38 | 0.74            | 0.67           | 1.00         | 1.10         |
| 32                                                    | D32V    | 0.49 | 1.01            | 0.96           | 1.02         | 1.04         |
| 32                                                    | D32W    | 0.69 | 0.91            | 0.93           | 0.97         | 0.99         |
| 32                                                    | D32Y    | 0.82 | 1.02            | 1.04           | 1.00         | 1.03         |
| 35                                                    | S35A    | 0.86 | 0.92            | 0.87           | 1.05         | 0.92         |
| 35                                                    | S35C    | 0.59 | 0.84            | 0.99           | 0.92         | 0.68         |
| 35                                                    | S35E    | 0.29 | 0.91            | 0.98           | 1.06         | 0.94         |
| 35                                                    | S35F    | 0.08 | 1.17            | 0.90           | 1.02         | 0.89         |
| 35                                                    | S35G    | 0.67 | 0.88            | 0.76           | 1.03         | 0.93         |
| 35                                                    | S35I    | 0.58 | 0.61            | 0.65           | 0.78         | 0.70         |
| 35                                                    | S35L    | 0.37 | 0.93            | 1.02           | 0.92         | 0.85         |
| 35                                                    | S35M    | 0.57 | 0.95            | 1.01           | 0.96         | 0.87         |
| 35                                                    | S35N    | 0.42 | 0.88            | 1.04           | 1.00         | 1.01         |
| 35                                                    | S35P    | 0.55 | 0.48            | 0.74           | 0.82         | 0.86         |
| 35                                                    | S35Q    | 0.53 | 0.90            | 0.88           | 0.98         | 0.94         |
| 35                                                    | S35R    | 0.38 | 0.91            | 0.99           | 0.87         | 0.83         |
| 35                                                    | S35V    | 0.89 | 0.65            | 0.57           | 0.86         | 0.93         |
| 35                                                    | S35W    | 1.60 | 1.01            | 1.03           | 1.10         | 1.05         |
| 35                                                    | S35Y    | 0.64 | 0.97            | 0.85           | 0.87         | 0.79         |
| 38                                                    | L38A    | 0.64 | 0.86            | 0.96           | 1.06         | 1.00         |
| 38                                                    | L38E    | 0.65 | 0.94            | 0.98           | 0.95         | 1.02         |
| 38                                                    | L38P    | 0.22 | 0.23            | 0.42           | 0.91         | 0.72         |
| 38                                                    | L38S    | 0.53 | 0.84            | 0.71           | 0.99         | 0.91         |
| 38                                                    | L38W    | 0.30 | 0.51            | 0.77           | 0.84         | 0.53         |
| 63                                                    | R63A    | 1.21 | 1.03            | 0.94           | 0.97         | 1.00         |
| 63                                                    | R63C    | 1.47 | 1.02            | 1.05           | 0.96         | 1.06         |
| 63                                                    | R63D    | 0.12 | 0.11            | 0.19           | 0.05         | 1.02         |
| 63                                                    | R63E    | 0.88 | 0.94            | 0.98           | 0.96         | 1.05         |
| 63                                                    | R63F    | 0.91 | 0.95            | 0.85           | 0.94         | 1.09         |
| 63                                                    | R63G    | 0.71 | 0.96            | 0.95           | 1.08         | 1.20         |
| 63                                                    | R63H    | 0.68 | 0.94            | 0.92           | 1.04         | 1.00         |
| 63                                                    | R63I    | 0.81 | 0.93            | 1.11           | 1.03         | 1.09         |
| 63                                                    | R63K    | 0.23 | 0.53            | 0.77           | 0.81         | 0.91         |
| 63                                                    | R63L    | 0.94 | 0.96            | 0.91           | 1.06         | 1.29         |
| 63                                                    | R63M    | 1.22 | 0.99            | 1.10           | 1.05         | 1.09         |
| 63                                                    | R63N    | 1.23 | 1.02            | 1.11           | 1.03         | 1.07         |
| 63                                                    | R63P    | 0.96 | 0.99            | 1.17           | 1.04         | 1.09         |
| 63                                                    | R63Q    | 0.87 | 0.99            | 1.01           | 0.99         | 1.13         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 63                                                    | R63S    | 0.59 | 0.92            | 1.09           | 1.00         | 0.94         |
| 63                                                    | R63T    | 0.43 | 0.87            | 0.94           | 0.91         | 0.84         |
| 63                                                    | R63V    | 1.00 | 0.99            | 0.89           | 1.03         | 1.06         |
| 63                                                    | R63W    | 0.98 | 0.97            | 0.91           | 1.08         | 1.10         |
| 63                                                    | R63Y    | 1.29 | 1.03            | 0.99           | 1.06         | 1.07         |
| 77                                                    | R77F    | 0.25 | 0.81            | 0.58           | 0.92         | 0.81         |
| 77                                                    | R77G    | 0.41 | 1.02            | 0.75           | 1.00         | 0.93         |
| 77                                                    | R77L    | 0.14 | 0.53            | 0.36           | 0.94         | 0.79         |
| 77                                                    | R77N    | 0.83 | 0.99            | 0.87           | 1.00         | 1.02         |
| 79                                                    | P79C    | 0.96 | 1.09            | 0.93           | 1.15         | 0.92         |
| 79                                                    | P79D    | 0.33 | 1.08            | 1.17           | 1.02         | 0.94         |
| 79                                                    | P79E    | 0.64 | 1.11            | 0.96           | 1.05         | 0.98         |
| 79                                                    | P79F    | 0.27 | 0.79            | 0.74           | 1.00         | 0.91         |
| 79                                                    | P79H    | 0.21 | 0.76            | 0.68           | 0.92         | 0.77         |
| 79                                                    | P79I    | 0.37 | 0.91            | 0.98           | 0.93         | 0.93         |
| 79                                                    | P79K    | 0.16 | 1.09            | 0.62           | 0.79         | 0.70         |
| 79                                                    | P79L    | 0.66 | 1.12            | 0.97           | 1.10         | 1.09         |
| 79                                                    | P79M    | 0.74 | 0.94            | 1.04           | 1.06         | 1.06         |
| 79                                                    | P79N    | 0.74 | 1.10            | 0.94           | 1.04         | 0.96         |
| 79                                                    | P79R    | 0.41 | 1.11            | 0.86           | 1.04         | 0.97         |
| 79                                                    | P79S    | 0.89 | 1.04            | 0.86           | 1.20         | 1.02         |
| 79                                                    | P79V    | 0.32 | 0.87            | 0.84           | 0.97         | 0.99         |
| 79                                                    | P79W    | 0.53 | 1.02            | 1.09           | 1.05         | 0.98         |
| 80                                                    | P80C    | 0.85 | 1.11            | 1.04           | 1.14         | 0.92         |
| 80                                                    | P80D    | 1.19 | 1.07            | 1.14           | 1.26         | 1.08         |
| 80                                                    | P80E    | 0.41 | 0.88            | 0.86           | 1.14         | 0.88         |
| 80                                                    | P80F    | 0.25 | 1.07            | 0.85           | 0.97         | 0.92         |
| 80                                                    | P80G    | 0.87 | 1.10            | 1.05           | 1.14         | 0.97         |
| 80                                                    | P80H    | 0.33 | 0.83            | 0.83           | 1.11         | 0.90         |
| 80                                                    | P80I    | 0.30 | 0.84            | 0.74           | 0.90         | 0.88         |
| 80                                                    | P80K    | 0.52 | 0.71            | 0.53           | 1.06         | 1.01         |
| 80                                                    | P80L    | 1.23 | 1.09            | 0.96           | 1.09         | 1.05         |
| 80                                                    | P80Q    | 0.77 | 1.10            | 0.87           | 1.06         | 1.05         |
| 80                                                    | P80R    | 0.25 | 1.04            | 1.23           | 1.06         | 0.96         |
| 80                                                    | P80T    | 0.90 | 0.88            | 0.99           | 1.94         | 1.80         |
| 80                                                    | P80V    | 0.36 | 0.99            | 1.06           | 0.93         | 0.92         |
| 80                                                    | P80W    | 1.13 | 1.01            | 0.95           | 1.21         | 1.08         |
| 89                                                    | S89A    | 0.19 | 0.70            | 1.04           | 0.73         | 0.50         |
| 89                                                    | S89C    | 0.27 | 0.82            | 1.09           | 0.83         | 0.90         |
| 89                                                    | S89D    | 1.45 | 1.06            | 1.13           | 1.05         | 1.02         |
| 89                                                    | S89E    | 0.28 | 0.87            | 0.87           | 0.94         | 0.74         |
| 89                                                    | S89F    | 0.26 | 0.79            | 0.79           | 0.97         | 0.84         |
| 89                                                    | S89G    | 0.27 | 0.82            | 0.82           | 0.88         | 0.78         |
| 89                                                    | S89H    | 1.57 | 1.03            | 0.86           | 1.02         | 1.04         |
| 89                                                    | S89I    | 0.51 | 1.01            | 0.92           | 1.03         | 1.07         |
| 89                                                    | S89K    | 0.51 | 0.93            | 0.76           | 0.99         | 0.96         |
| 89                                                    | S89L    | 0.69 | 1.02            | 0.97           | 0.90         | 0.87         |
| 89                                                    | S89M    | 0.22 | 0.77            | 1.02           | 0.85         | 0.92         |
| 89                                                    | S89N    | 0.28 | 0.85            | 0.68           | 0.83         | 0.88         |
| 89                                                    | S89P    | 0.64 | 0.98            | 1.18           | 0.99         | 0.92         |
| 89                                                    | S89Q    | 0.51 | 0.80            | 0.53           | 0.98         | 1.15         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 89                                                    | S89R    | 0.57 | 1.00            | 0.83           | 1.03         | 0.94         |
| 89                                                    | S89T    | 0.88 | 1.03            | 0.95           | 1.02         | 1.03         |
| 89                                                    | S89V    | 0.16 | 0.65            | 1.02           | 0.73         | 0.61         |
| 89                                                    | S89W    | 0.82 | 1.03            | 1.02           | 0.92         | 0.79         |
| 89                                                    | S89Y    | 0.70 | 1.02            | 0.97           | 1.00         | 1.00         |
| 94                                                    | V94A    | 1.18 | 0.98            | 1.45           | 1.11         | 1.06         |
| 94                                                    | V94C    | 0.38 | 0.79            | 0.66           | 0.97         | 0.76         |
| 94                                                    | V94D    | 1.13 | 0.99            | 0.92           | 1.10         | 0.97         |
| 94                                                    | V94F    | 0.28 | 0.73            | 0.55           | 0.89         | 0.89         |
| 94                                                    | V94G    | 0.25 | 0.68            | 0.43           | 0.93         | 0.79         |
| 94                                                    | V94H    | 0.23 | 0.64            | 0.52           | 1.03         | 0.97         |
| 94                                                    | V94K    | 0.50 | 0.98            | 0.73           | 1.07         | 1.08         |
| 94                                                    | V94P    | 0.13 | 0.14            | 0.17           | 0.16         | 1.34         |
| 94                                                    | V94R    | 0.22 | 0.90            | 0.58           | 0.76         | 1.03         |
| 94                                                    | V94S    | 0.24 | 0.76            | 0.44           | 0.85         | 0.77         |
| 94                                                    | V94T    | 0.22 | 0.80            | 0.37           | 0.71         | 0.86         |
| 98                                                    | P98A    | 0.90 | 1.09            | 0.94           | 1.01         | 0.99         |
| 98                                                    | P98C    | 0.53 | 1.01            | 1.23           | 1.06         | 1.03         |
| 98                                                    | P98D    | 0.28 | 0.26            | 0.35           | 1.32         | 2.58         |
| 98                                                    | P98E    | 0.30 | 0.54            | 0.35           | 0.99         | 1.28         |
| 98                                                    | P98F    | 0.30 | 0.77            | 0.79           | 0.67         | 0.75         |
| 98                                                    | P98G    | 0.61 | 0.97            | 0.71           | 0.92         | 1.00         |
| 98                                                    | P98H    | 0.29 | 0.67            | 0.35           | 0.62         | 0.87         |
| 98                                                    | P98I    | 0.51 | 1.05            | 0.84           | 1.15         | 0.88         |
| 98                                                    | P98K    | 0.29 | 0.30            | 0.23           | 1.06         | 2.07         |
| 98                                                    | P98L    | 0.80 | 1.12            | 0.89           | 1.16         | 1.45         |
| 98                                                    | P98M    | 0.58 | 1.03            | 0.91           | 1.05         | 0.92         |
| 98                                                    | P98N    | 0.33 | 0.42            | 0.38           | 1.00         | 1.12         |
| 98                                                    | P98Q    | 0.50 | 1.03            | 0.90           | 1.14         | 1.46         |
| 98                                                    | P98R    | 0.28 | 0.30            | 0.05           | 0.93         | 1.55         |
| 98                                                    | P98S    | 0.32 | 0.27            | 0.16           | 1.23         | 1.36         |
| 98                                                    | P98T    | 0.19 | 0.32            | 0.05           | 1.31         | 1.98         |
| 98                                                    | P98V    | 0.60 | 1.06            | 1.08           | 1.05         | 1.08         |
| 98                                                    | P98W    | 0.37 | 0.38            | 0.34           | 0.82         | 1.01         |
| 98                                                    | P98Y    | 0.25 | 0.65            | 0.19           | 0.87         | 0.93         |
| 100                                                   | A100C   | 0.37 | 0.91            | 0.83           | 0.71         | 0.61         |
| 100                                                   | A100D   | 0.14 | 0.38            | 0.27           | 0.09         | 0.39         |
| 100                                                   | A100E   | 0.18 | 0.67            | 0.48           | 0.51         | 0.22         |
| 100                                                   | A100F   | 0.11 | 0.34            | 0.27           | 0.42         | 0.74         |
| 100                                                   | A100G   | 0.26 | 0.79            | 0.54           | 0.36         | 0.18         |
| 100                                                   | A100H   | 0.11 | 0.09            | 0.14           | 1.53         | 0.51         |
| 100                                                   | A100K   | 0.16 | 0.97            | 0.59           | 0.71         | 0.65         |
| 100                                                   | A100L   | 0.25 | 0.78            | 0.49           | 0.62         | 0.42         |
| 100                                                   | A100N   | 0.31 | 0.92            | 0.70           | 0.53         | 0.26         |
| 100                                                   | A100P   | 0.14 | 0.65            | 0.53           | 0.63         | 0.44         |
| 100                                                   | A100R   | 0.14 | 0.37            | 0.26           | 0.53         | 0.81         |
| 100                                                   | A100S   | 0.33 | 0.90            | 0.64           | 0.68         | 0.48         |
| 100                                                   | A100T   | 0.39 | 0.87            | 0.70           | 0.86         | 0.89         |
| 100                                                   | A100V   | 0.13 | 0.77            | 0.48           | 0.93         | 0.61         |
| 100                                                   | A100W   | 0.59 | 0.92            | 0.60           | 0.62         | 0.29         |
| 100                                                   | A100Y   | 0.25 | 0.65            | 0.62           | 0.81         | 0.42         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 102                                                   | A102C   | 0.11 | 0.05            | 0.20           | 1.02         | 5.64         |
| 102                                                   | A102D   | 0.60 | 0.98            | 1.01           | 0.82         | 0.64         |
| 102                                                   | A102E   | 0.77 | 0.98            | 0.69           | 1.01         | 1.01         |
| 102                                                   | A102F   | 0.57 | 0.97            | 0.86           | 0.91         | 0.81         |
| 102                                                   | A102G   | 0.55 | 1.00            | 0.90           | 0.93         | 0.89         |
| 102                                                   | A102H   | 0.96 | 1.05            | 0.87           | 0.91         | 0.96         |
| 102                                                   | A102I   | 0.43 | 0.88            | 0.73           | 0.77         | 0.75         |
| 102                                                   | A102K   | 0.50 | 0.97            | 0.91           | 0.95         | 1.02         |
| 102                                                   | A102L   | 0.58 | 0.99            | 0.82           | 0.91         | 0.86         |
| 102                                                   | A102M   | 0.56 | 0.94            | 0.75           | 0.93         | 0.87         |
| 102                                                   | A102N   | 0.62 | 1.01            | 0.75           | 0.93         | 0.92         |
| 102                                                   | A102P   | 0.45 | 0.87            | 0.63           | 0.80         | 0.50         |
| 102                                                   | A102Q   | 0.10 | 0.09            | 0.14           | 1.47         | 2.14         |
| 102                                                   | A102R   | 0.55 | 1.02            | 0.83           | 0.99         | 1.02         |
| 102                                                   | A102S   | 0.51 | 0.99            | 0.84           | 0.95         | 0.96         |
| 102                                                   | A102T   | 0.49 | 0.96            | 0.81           | 0.69         | 0.54         |
| 102                                                   | A102V   | 0.49 | 0.95            | 0.74           | 0.73         | 0.55         |
| 102                                                   | A102W   | 0.11 | 0.06            | 0.05           | 44.39        | 31.37        |
| 102                                                   | A102Y   | 0.55 | 0.97            | 0.87           | 0.84         | 0.72         |
| 103                                                   | Y103A   | 0.89 | 1.01            | 1.02           | 0.64         | 0.45         |
| 103                                                   | Y103C   | 0.38 | 0.76            | 0.55           | 0.63         | 0.64         |
| 103                                                   | Y103D   | 0.62 | 0.97            | 0.40           | 0.21         | 0.10         |
| 103                                                   | Y103E   | 0.90 | 0.95            | 0.92           | 0.92         | 0.61         |
| 103                                                   | Y103F   | 1.32 | 0.93            | 0.88           | 1.01         | 0.98         |
| 103                                                   | Y103G   | 0.54 | 0.85            | 0.50           | 0.47         | 0.12         |
| 103                                                   | Y103H   | 1.18 | 0.96            | 0.98           | 1.12         | 1.15         |
| 103                                                   | Y103I   | 0.09 | 0.12            | 0.05           | 0.31         | 1.44         |
| 103                                                   | Y103K   | 0.83 | 0.90            | 0.54           | 0.96         | 0.99         |
| 103                                                   | Y103L   | 1.03 | 1.02            | 0.94           | 0.88         | 0.66         |
| 103                                                   | Y103M   | 0.08 | 0.09            | 0.09           | 0.53         | 0.91         |
| 103                                                   | Y103P   | 0.51 | 0.85            | 0.47           | 0.44         | 0.21         |
| 103                                                   | Y103Q   | 0.81 | 0.96            | 0.48           | 0.90         | 0.66         |
| 103                                                   | Y103R   | 0.82 | 0.88            | 0.67           | 1.00         | 0.99         |
| 103                                                   | Y103S   | 0.42 | 0.85            | 0.56           | 0.50         | 0.17         |
| 103                                                   | Y103T   | 0.41 | 0.74            | 0.33           | 0.44         | 0.42         |
| 103                                                   | Y103V   | 1.02 | 0.93            | 0.63           | 0.95         | 0.87         |
| 103                                                   | Y103W   | 0.93 | 0.96            | 0.72           | 0.75         | 0.56         |
| 104                                                   | Y104A   | 0.36 | 0.82            | 0.27           | 0.32         | 0.17         |
| 104                                                   | Y104C   | 0.19 | 0.51            | 0.05           | 0.31         | 0.18         |
| 104                                                   | Y104D   | 0.23 | 0.44            | 0.14           | 0.40         | 0.22         |
| 104                                                   | Y104E   | 0.12 | 0.05            | 0.05           | 4.53         | 29.82        |
| 104                                                   | Y104F   | 0.92 | 1.02            | 0.66           | 0.49         | 0.12         |
| 104                                                   | Y104G   | 0.28 | 0.50            | 0.05           | 0.35         | 0.28         |
| 104                                                   | Y104H   | 0.12 | 0.10            | 0.05           | 2.55         | 1.45         |
| 104                                                   | Y104I   | 0.27 | 0.61            | 0.14           | 0.35         | 0.16         |
| 104                                                   | Y104K   | 0.38 | 0.45            | 0.15           | 0.24         | 0.21         |
| 104                                                   | Y104L   | 0.50 | 0.87            | 0.23           | 0.23         | 0.13         |
| 104                                                   | Y104M   | 0.41 | 0.73            | 0.26           | 0.27         | 0.15         |
| 104                                                   | Y104N   | 0.22 | 0.15            | 0.14           | 1.06         | 1.75         |
| 104                                                   | Y104P   | 0.22 | 0.52            | 0.11           | 0.60         | 0.25         |
| 104                                                   | Y104Q   | 0.20 | 0.08            | 0.05           | 1.63         | 1.16         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 104                                                   | Y104R   | 0.37 | 0.80            | 0.45           | 0.15         | 0.14         |
| 104                                                   | Y104S   | 0.38 | 0.48            | 0.26           | 0.46         | 0.17         |
| 104                                                   | Y104T   | 0.21 | 0.51            | 0.18           | 0.31         | 0.21         |
| 104                                                   | Y104V   | 0.23 | 0.28            | 0.09           | 0.15         | 0.47         |
| 104                                                   | Y104W   | 0.77 | 0.91            | 0.60           | 0.37         | 0.16         |
| 105                                                   | A105D   | 0.17 | 0.36            | 0.58           | 0.20         | 0.05         |
| 105                                                   | A105G   | 0.21 | 0.55            | 0.64           | 0.89         | 0.52         |
| 105                                                   | A105P   | 0.11 | 0.16            | 0.30           | 0.14         | 0.71         |
| 105                                                   | A105R   | 0.42 | 0.97            | 0.89           | 1.02         | 1.01         |
| 105                                                   | A105T   | 0.34 | 0.92            | 0.72           | 0.51         | 0.18         |
| 105                                                   | A105V   | 0.35 | 0.78            | 0.74           | 0.60         | 0.24         |
| 105                                                   | A105Y   | 0.44 | 0.56            | 0.41           | 0.09         | 0.13         |
| 107                                                   | E107D   | 0.19 | 0.27            | 0.40           | 0.07         | 0.23         |
| 107                                                   | E107I   | 0.78 | 0.82            | 0.40           | 0.15         | 0.10         |
| 107                                                   | E107K   | 0.23 | 0.46            | 0.53           | 0.57         | 0.31         |
| 107                                                   | E107N   | 0.38 | 0.62            | 0.60           | 0.09         | 0.16         |
| 107                                                   | E107P   | 0.26 | 0.42            | 0.25           | 0.05         | 0.26         |
| 107                                                   | E107Q   | 1.19 | 0.94            | 0.54           | 0.58         | 0.14         |
| 107                                                   | E107R   | 0.25 | 0.53            | 0.21           | 0.05         | 0.08         |
| 107                                                   | E107S   | 0.89 | 0.90            | 0.39           | 0.06         | 0.12         |
| 107                                                   | E107T   | 0.53 | 0.72            | 0.55           | 0.05         | 0.09         |
| 107                                                   | E107V   | 1.46 | 1.00            | 0.47           | 0.06         | 0.08         |
| 107                                                   | E107W   | 0.69 | 0.73            | 0.37           | 0.06         | 0.18         |
| 107                                                   | E107Y   | 0.88 | 0.82            | 0.37           | 0.05         | 0.08         |
| 111                                                   | L111A   | 1.36 | 1.06            | 1.25           | 0.84         | 0.30         |
| 111                                                   | L111C   | 0.57 | 0.81            | 0.99           | 1.00         | 0.93         |
| 111                                                   | L111D   | 0.28 | 0.65            | 0.54           | 0.67         | 0.48         |
| 111                                                   | L111E   | 0.69 | 1.35            | 1.34           | 0.91         | 0.75         |
| 111                                                   | L111F   | 0.52 | 0.67            | 0.49           | 1.02         | 0.76         |
| 111                                                   | L111G   | 0.52 | 1.18            | 0.88           | 0.58         | 0.19         |
| 111                                                   | L111H   | 0.55 | 0.90            | 0.80           | 0.86         | 0.82         |
| 111                                                   | L111I   | 0.93 | 0.77            | 0.90           | 0.86         | 0.80         |
| 111                                                   | L111K   | 0.43 | 0.90            | 0.68           | 0.94         | 0.68         |
| 111                                                   | L111M   | 1.99 | 1.19            | 0.97           | 1.00         | 0.82         |
| 111                                                   | L111N   | 0.26 | 0.76            | 0.80           | 0.73         | 0.53         |
| 111                                                   | L111P   | 0.19 | 0.49            | 0.29           | 0.05         | 0.05         |
| 111                                                   | L111Q   | 0.96 | 1.34            | 1.11           | 0.95         | 0.82         |
| 111                                                   | L111R   | 0.52 | 1.11            | 0.84           | 0.84         | 0.56         |
| 111                                                   | L111S   | 0.61 | 0.92            | 0.92           | 1.00         | 1.04         |
| 111                                                   | L111T   | 0.66 | 1.17            | 0.84           | 0.81         | 0.55         |
| 111                                                   | L111V   | 0.73 | 0.76            | 0.62           | 0.53         | 0.23         |
| 111                                                   | L111W   | 0.52 | 0.89            | 0.77           | 0.68         | 0.41         |
| 111                                                   | L111Y   | 0.45 | 0.75            | 0.57           | 1.11         | 0.84         |
| 117                                                   | T117A   | 0.96 | 1.07            | 0.80           | 0.85         | 0.85         |
| 117                                                   | T117C   | 0.61 | 0.94            | 0.94           | 0.87         | 0.96         |
| 117                                                   | T117D   | 0.95 | 0.99            | 1.08           | 0.94         | 0.85         |
| 117                                                   | T117E   | 0.62 | 1.14            | 0.97           | 1.01         | 1.03         |
| 117                                                   | T117F   | 0.51 | 1.14            | 0.82           | 0.88         | 0.90         |
| 117                                                   | T117G   | 0.63 | 1.01            | 0.81           | 0.73         | 0.64         |
| 117                                                   | T117H   | 0.12 | 0.23            | 0.51           | 3.12         | 8.65         |
| 117                                                   | T117K   | 0.69 | 1.03            | 0.82           | 0.95         | 1.05         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 117                                                   | T117L   | 0.51 | 0.99            | 1.00           | 0.80         | 0.77         |
| 117                                                   | T117M   | 0.62 | 0.99            | 1.02           | 0.92         | 0.88         |
| 117                                                   | T117N   | 1.35 | 1.03            | 0.84           | 0.99         | 0.98         |
| 117                                                   | T117P   | 0.32 | 0.88            | 0.62           | 0.69         | 0.46         |
| 117                                                   | T117Q   | 0.28 | 0.93            | 0.71           | 0.95         | 0.99         |
| 117                                                   | T117R   | 0.60 | 1.04            | 0.78           | 0.94         | 0.93         |
| 117                                                   | T117S   | 0.95 | 1.03            | 0.93           | 1.06         | 1.16         |
| 117                                                   | T117V   | 1.00 | 1.03            | 0.70           | 0.92         | 0.94         |
| 117                                                   | T117W   | 0.72 | 1.01            | 0.66           | 0.80         | 0.74         |
| 117                                                   | T117Y   | 1.18 | 1.05            | 0.84           | 0.95         | 0.91         |
| 119                                                   | A119C   | 0.28 | 1.02            | 0.98           | 0.86         | 0.61         |
| 119                                                   | A119D   | 0.31 | 0.84            | 0.79           | 0.67         | 0.57         |
| 119                                                   | A119E   | 0.25 | 1.16            | 0.99           | 0.86         | 0.84         |
| 119                                                   | A119F   | 0.28 | 0.89            | 0.60           | 0.68         | 0.58         |
| 119                                                   | A119G   | 0.69 | 0.97            | 0.86           | 0.89         | 0.73         |
| 119                                                   | A119H   | 0.49 | 1.03            | 0.85           | 0.91         | 0.71         |
| 119                                                   | A119I   | 0.31 | 0.74            | 0.62           | 0.77         | 0.62         |
| 119                                                   | A119K   | 0.33 | 0.89            | 0.65           | 1.00         | 0.72         |
| 119                                                   | A119L   | 0.55 | 1.11            | 0.94           | 0.84         | 0.59         |
| 119                                                   | A119M   | 0.30 | 0.71            | 0.36           | 0.69         | 0.77         |
| 119                                                   | A119N   | 0.85 | 1.04            | 0.66           | 1.00         | 0.84         |
| 119                                                   | A119P   | 0.71 | 1.02            | 1.10           | 1.07         | 0.97         |
| 119                                                   | A119Q   | 0.58 | 1.17            | 0.82           | 1.09         | 0.88         |
| 119                                                   | A119R   | 0.56 | 0.80            | 0.42           | 0.92         | 0.88         |
| 119                                                   | A119S   | 1.44 | 0.99            | 0.97           | 1.05         | 0.53         |
| 119                                                   | A119T   | 0.80 | 0.86            | 0.76           | 0.94         | 0.68         |
| 119                                                   | A119V   | 0.36 | 0.89            | 0.45           | 0.90         | 0.91         |
| 119                                                   | A119W   | 0.38 | 0.95            | 0.78           | 0.61         | 0.52         |
| 119                                                   | A119Y   | 0.38 | 0.54            | 0.47           | 0.79         | 0.99         |
| 120                                                   | M120A   | 0.51 | 1.00            | 0.73           | 0.51         | 0.28         |
| 120                                                   | M120C   | 0.52 | 0.88            | 0.74           | 0.66         | 0.58         |
| 120                                                   | M120D   | 0.31 | 0.95            | 0.76           | 0.32         | 0.31         |
| 120                                                   | M120E   | 0.18 | 0.43            | 0.09           | 1.51         | 1.97         |
| 120                                                   | M120F   | 0.53 | 1.04            | 0.70           | 0.87         | 0.80         |
| 120                                                   | M120G   | 0.35 | 0.49            | 0.30           | 0.92         | 1.21         |
| 120                                                   | M120H   | 0.34 | 0.70            | 0.54           | 0.77         | 1.16         |
| 120                                                   | M120I   | 0.27 | 0.85            | 0.44           | 0.74         | 0.70         |
| 120                                                   | M120K   | 0.34 | 1.10            | 0.49           | 0.72         | 0.76         |
| 120                                                   | M120L   | 1.64 | 1.08            | 0.68           | 1.07         | 1.17         |
| 120                                                   | M120N   | 0.17 | 0.35            | 0.05           | 0.40         | 0.62         |
| 120                                                   | M120P   | 0.28 | 0.53            | 0.33           | 0.65         | 0.71         |
| 120                                                   | M120Q   | 0.30 | 0.61            | 0.34           | 1.36         | 1.41         |
| 120                                                   | M120R   | 0.31 | 0.80            | 0.14           | 0.31         | 0.28         |
| 120                                                   | M120S   | 0.40 | 1.04            | 0.58           | 0.64         | 0.31         |
| 120                                                   | M120T   | 0.35 | 0.95            | 0.52           | 0.67         | 0.45         |
| 120                                                   | M120V   | 0.30 | 0.60            | 0.47           | 0.91         | 1.12         |
| 120                                                   | M120W   | 0.53 | 1.06            | 0.77           | 1.01         | 0.98         |
| 120                                                   | M120Y   | 0.70 | 0.84            | 0.47           | 0.91         | 0.81         |
| 121                                                   | A121C   | 0.17 | 0.79            | 0.59           | 0.64         | 0.38         |
| 121                                                   | A121D   | 0.08 | 1.27            | 0.35           | 0.31         | 0.22         |
| 121                                                   | A121E   | 0.11 | 0.30            | 0.05           | 0.99         | 1.43         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 121                                                   | A121F   | 0.23 | 0.68            | 0.56           | 0.30         | 0.24         |
| 121                                                   | A121G   | 0.13 | 0.49            | 0.37           | 14.85        | 14.78        |
| 121                                                   | A121H   | 0.08 | 0.39            | 0.49           | 0.36         | 0.28         |
| 121                                                   | A121I   | 0.12 | 0.39            | 0.32           | 1.82         | 1.58         |
| 121                                                   | A121K   | 0.23 | 0.78            | 0.62           | 0.95         | 0.91         |
| 121                                                   | A121L   | 0.40 | 0.66            | 0.46           | 0.76         | 0.76         |
| 121                                                   | A121M   | 0.11 | 0.85            | 0.48           | 0.85         | 0.53         |
| 121                                                   | A121N   | 0.09 | 0.54            | 0.15           | 0.56         | 0.59         |
| 121                                                   | A121P   | 0.10 | 0.30            | 0.18           | 1.03         | 1.05         |
| 121                                                   | A121Q   | 0.05 | 0.05            | 0.05           | 0.05         | 0.05         |
| 121                                                   | A121R   | 0.52 | 1.10            | 0.85           | 0.98         | 1.01         |
| 121                                                   | A121S   | 0.14 | 0.74            | 0.85           | 0.60         | 0.24         |
| 121                                                   | A121T   | 0.25 | 0.62            | 0.46           | 0.49         | 0.27         |
| 121                                                   | A121V   | 0.15 | 0.73            | 0.57           | 0.81         | 0.75         |
| 121                                                   | A121W   | 0.33 | 0.46            | 0.45           | 0.26         | 0.31         |
| 121                                                   | A121Y   | 0.35 | 0.77            | 0.57           | 0.36         | 0.16         |
| 125                                                   | A125C   | 0.50 | 0.37            | 0.23           | 0.39         | 0.71         |
| 125                                                   | A125D   | 0.13 | 0.46            | 0.21           | 1.24         | 2.42         |
| 125                                                   | A125E   | 0.70 | 0.66            | 0.40           | 0.96         | 1.07         |
| 125                                                   | A125F   | 0.42 | 0.86            | 0.50           | 0.29         | 0.39         |
| 125                                                   | A125G   | 0.17 | 0.50            | 0.10           | 1.45         | 2.19         |
| 125                                                   | A125H   | 0.46 | 1.06            | 0.44           | 0.39         | 0.30         |
| 125                                                   | A125I   | 0.31 | 0.60            | 0.37           | 0.91         | 1.27         |
| 125                                                   | A125K   | 0.60 | 1.12            | 0.76           | 0.71         | 0.62         |
| 125                                                   | A125L   | 0.36 | 1.09            | 0.38           | 0.69         | 0.60         |
| 125                                                   | A125M   | 0.60 | 1.01            | 0.64           | 0.79         | 0.71         |
| 125                                                   | A125N   | 0.40 | 0.97            | 0.49           | 0.46         | 0.34         |
| 125                                                   | A125P   | 0.43 | 0.55            | 0.33           | 0.31         | 0.45         |
| 125                                                   | A125Q   | 0.29 | 0.74            | 0.34           | 0.78         | 0.73         |
| 125                                                   | A125R   | 0.56 | 1.09            | 0.82           | 0.74         | 0.69         |
| 125                                                   | A125S   | 0.65 | 0.93            | 0.47           | 0.87         | 0.71         |
| 125                                                   | A125T   | 0.50 | 1.00            | 0.43           | 0.50         | 0.28         |
| 125                                                   | A125V   | 0.46 | 0.94            | 0.60           | 0.54         | 0.50         |
| 125                                                   | A125W   | 0.50 | 0.94            | 0.49           | 0.28         | 0.13         |
| 125                                                   | A125Y   | 0.34 | 0.63            | 0.43           | 0.61         | 0.91         |
| 126                                                   | A126D   | 0.22 | 0.80            | 0.45           | 0.23         | 0.31         |
| 126                                                   | A126E   | 1.46 | 1.02            | 0.84           | 1.06         | 1.04         |
| 126                                                   | A126F   | 0.61 | 0.88            | 0.89           | 0.89         | 0.66         |
| 126                                                   | A126G   | 0.19 | 0.46            | 0.67           | 0.80         | 0.67         |
| 126                                                   | A126H   | 0.27 | 0.86            | 1.16           | 0.84         | 0.75         |
| 126                                                   | A126I   | 0.23 | 0.65            | 0.41           | 0.37         | 0.26         |
| 126                                                   | A126K   | 0.80 | 1.00            | 0.86           | 1.10         | 1.15         |
| 126                                                   | A126L   | 0.44 | 1.10            | 0.89           | 0.88         | 0.63         |
| 126                                                   | A126M   | 0.30 | 0.67            | 0.71           | 0.95         | 0.83         |
| 126                                                   | A126N   | 0.79 | 0.92            | 0.78           | 1.04         | 0.75         |
| 126                                                   | A126P   | 0.17 | 0.26            | 0.62           | 1.39         | 0.83         |
| 126                                                   | A126Q   | 1.31 | 0.96            | 0.97           | 1.11         | 1.05         |
| 126                                                   | A126R   | 0.82 | 0.88            | 0.82           | 0.97         | 0.95         |
| 126                                                   | A126S   | 0.58 | 0.95            | 0.80           | 1.11         | 1.00         |
| 126                                                   | A126T   | 0.59 | 0.49            | 0.60           | 0.65         | 0.56         |
| 126                                                   | A126V   | 0.62 | 0.96            | 0.68           | 0.97         | 0.70         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 126                                                   | A126W   | 0.50 | 0.85            | 0.95           | 0.65         | 0.52         |
| 126                                                   | A126Y   | 0.75 | 0.96            | 0.80           | 0.82         | 0.68         |
| 129                                                   | K129A   | 0.23 | 1.03            | 0.81           | 0.74         | 0.57         |
| 129                                                   | K129L   | 0.80 | 0.94            | 0.80           | 0.82         | 0.71         |
| 129                                                   | K129N   | 0.15 | 0.93            | 0.65           | 0.73         | 0.61         |
| 129                                                   | K129Q   | 0.41 | 1.30            | 0.89           | 1.01         | 0.99         |
| 129                                                   | K129S   | 0.37 | 1.05            | 0.92           | 0.84         | 0.84         |
| 129                                                   | K129T   | 0.16 | 0.87            | 0.37           | 0.81         | 0.69         |
| 129                                                   | K129V   | 0.17 | 0.93            | 0.91           | 0.63         | 0.45         |
| 129                                                   | K129Y   | 0.35 | 0.95            | 0.82           | 0.68         | 0.46         |
| 133                                                   | F133A   | 0.19 | 0.25            | 0.13           | 0.84         | 0.80         |
| 133                                                   | F133C   | 0.15 | 0.20            | 0.09           | 0.71         | 1.32         |
| 133                                                   | F133D   | 0.16 | 0.16            | 0.20           | 0.26         | 2.20         |
| 133                                                   | F133G   | 0.17 | 0.12            | 0.19           | 0.87         | 5.31         |
| 133                                                   | F133H   | 0.14 | 0.22            | 0.06           | 0.90         | 0.76         |
| 133                                                   | F133K   | 0.11 | 0.16            | 0.05           | 0.64         | 8.77         |
| 133                                                   | F133P   | 0.12 | 0.24            | 0.05           | 0.33         | 0.41         |
| 133                                                   | F133Q   | 0.12 | 0.26            | 0.16           | 0.58         | 1.12         |
| 133                                                   | F133S   | 0.12 | 0.27            | 0.05           | 1.08         | 2.34         |
| 133                                                   | F133T   | 0.11 | 0.24            | 0.05           | 0.21         | 0.52         |
| 133                                                   | F133W   | 0.13 | 0.52            | 0.27           | 0.42         | 0.21         |
| 133                                                   | F133Y   | 0.13 | 0.60            | 0.25           | 0.53         | 0.74         |
| 134                                                   | M134A   | 1.09 | 0.96            | 1.06           | 0.79         | 0.76         |
| 134                                                   | M134C   | 0.91 | 1.01            | 0.84           | 0.80         | 1.20         |
| 134                                                   | M134D   | 0.42 | 0.69            | 0.50           | 0.89         | 1.52         |
| 134                                                   | M134E   | 0.82 | 1.10            | 0.94           | 0.88         | 0.97         |
| 134                                                   | M134F   | 1.53 | 1.12            | 0.91           | 1.02         | 1.19         |
| 134                                                   | M134G   | 0.33 | 1.11            | 0.80           | 1.16         | 1.47         |
| 134                                                   | M134H   | 0.93 | 1.17            | 0.66           | 0.79         | 0.53         |
| 134                                                   | M134I   | 1.16 | 1.13            | 0.72           | 1.20         | 1.10         |
| 134                                                   | M134K   | 0.53 | 0.56            | 0.38           | 2.39         | 3.02         |
| 134                                                   | M134L   | 1.45 | 1.15            | 0.75           | 1.67         | 1.64         |
| 134                                                   | M134N   | 0.90 | 1.25            | 0.93           | 1.09         | 0.76         |
| 134                                                   | M134P   | 0.37 | 0.40            | 0.28           | 2.37         | 3.64         |
| 134                                                   | M134Q   | 0.57 | 1.27            | 0.83           | 0.99         | 0.93         |
| 134                                                   | M134R   | 0.84 | 1.54            | 0.86           | 1.74         | 1.18         |
| 134                                                   | M134S   | 0.55 | 1.15            | 0.70           | 1.31         | 1.40         |
| 134                                                   | M134T   | 0.65 | 1.54            | 0.64           | 1.33         | 1.29         |
| 134                                                   | M134V   | 1.50 | 1.24            | 0.69           | 1.74         | 1.84         |
| 134                                                   | M134W   | 0.39 | 0.93            | 0.57           | 0.98         | 1.47         |
| 134                                                   | M134Y   | 0.93 | 1.15            | 0.68           | 1.48         | 0.96         |
| 137                                                   | D137A   | 0.56 | 0.95            | 0.80           | 1.06         | 1.06         |
| 137                                                   | D137C   | 0.35 | 0.31            | 0.30           | 0.92         | 1.04         |
| 137                                                   | D137E   | 0.48 | 0.85            | 0.50           | 1.00         | 0.93         |
| 137                                                   | D137F   | 0.28 | 0.46            | 0.44           | 0.80         | 0.88         |
| 137                                                   | D137G   | 0.23 | 0.49            | 0.39           | 1.24         | 1.25         |
| 137                                                   | D137H   | 0.14 | 0.24            | 0.05           | 1.77         | 1.76         |
| 137                                                   | D137I   | 0.20 | 0.28            | 0.19           | 0.96         | 0.85         |
| 137                                                   | D137K   | 0.29 | 0.30            | 0.17           | 0.72         | 1.06         |
| 137                                                   | D137L   | 0.23 | 0.21            | 0.31           | 0.90         | 1.53         |
| 137                                                   | D137M   | 0.23 | 0.34            | 0.21           | 0.72         | 1.06         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 137                                                   | D137N   | 2.26 | 0.75            | 0.52           | 1.03         | 1.13         |
| 137                                                   | D137P   | 0.41 | 1.22            | 0.48           | 1.03         | 1.23         |
| 137                                                   | D137Q   | 0.31 | 0.49            | 0.25           | 0.82         | 0.82         |
| 137                                                   | D137R   | 0.25 | 0.24            | 0.34           | 4.67         | 1.96         |
| 137                                                   | D137S   | 0.37 | 0.94            | 0.77           | 0.91         | 1.05         |
| 137                                                   | D137T   | 0.55 | 0.85            | 0.47           | 1.01         | 1.10         |
| 137                                                   | D137V   | 0.24 | 0.27            | 0.15           | 1.14         | 1.33         |
| 137                                                   | D137W   | 0.34 | 0.42            | 0.15           | 0.99         | 0.72         |
| 137                                                   | D137Y   | 0.36 | 0.50            | 0.28           | 0.83         | 0.91         |
| 138                                                   | T138A   | 3.42 | 1.04            | 1.06           | 1.05         | 1.26         |
| 138                                                   | T138C   | 2.40 | 0.97            | 0.95           | 1.03         | 1.30         |
| 138                                                   | T138D   | 0.23 | 0.11            | 0.05           | 0.76         | 1.68         |
| 138                                                   | T138E   | 2.03 | 0.49            | 0.56           | 0.78         | 1.58         |
| 138                                                   | T138F   | 0.64 | 0.53            | 0.37           | 1.42         | 4.28         |
| 138                                                   | T138G   | 0.51 | 0.75            | 0.63           | 0.99         | 0.94         |
| 138                                                   | T138H   | 0.58 | 0.51            | 0.47           | 1.14         | 1.06         |
| 138                                                   | T138I   | 0.54 | 0.86            | 0.75           | 0.95         | 0.99         |
| 138                                                   | T138K   | 0.40 | 0.39            | 0.18           | 0.86         | 1.68         |
| 138                                                   | T138L   | 1.26 | 1.01            | 0.90           | 1.01         | 1.19         |
| 138                                                   | T138M   | 0.75 | 0.97            | 0.97           | 1.09         | 1.14         |
| 138                                                   | T138N   | 0.51 | 0.52            | 0.43           | 2.14         | 2.93         |
| 138                                                   | T138P   | 0.40 | 0.66            | 0.71           | 1.23         | 1.63         |
| 138                                                   | T138Q   | 0.91 | 0.98            | 0.85           | 0.96         | 1.01         |
| 138                                                   | T138R   | 0.40 | 0.67            | 0.57           | 0.71         | 1.00         |
| 138                                                   | T138S   | 1.29 | 0.95            | 1.05           | 1.13         | 1.25         |
| 138                                                   | T138V   | 0.99 | 0.98            | 0.74           | 1.04         | 1.20         |
| 138                                                   | T138W   | 0.67 | 0.81            | 0.83           | 0.95         | 0.84         |
| 138                                                   | T138Y   | 0.34 | 0.83            | 0.54           | 1.37         | 1.26         |
| 139                                                   | L139A   | 0.40 | 1.16            | 0.39           | 0.97         | 0.82         |
| 139                                                   | L139C   | 1.15 | 1.04            | 0.89           | 0.95         | 1.04         |
| 139                                                   | L139D   | 1.03 | 0.94            | 0.95           | 0.98         | 1.07         |
| 139                                                   | L139E   | 0.17 | 0.74            | 0.78           | 1.07         | 0.99         |
| 139                                                   | L139F   | 0.46 | 0.77            | 0.48           | 1.04         | 1.25         |
| 139                                                   | L139G   | 0.60 | 1.04            | 0.95           | 1.07         | 1.06         |
| 139                                                   | L139H   | 0.45 | 0.40            | 0.50           | 0.73         | 0.94         |
| 139                                                   | L139I   | 0.19 | 0.87            | 0.93           | 0.89         | 0.98         |
| 139                                                   | L139K   | 0.65 | 1.03            | 0.97           | 0.97         | 1.10         |
| 139                                                   | L139M   | 0.29 | 0.77            | 0.76           | 0.88         | 0.43         |
| 139                                                   | L139N   | 0.30 | 0.94            | 0.85           | 0.97         | 1.02         |
| 139                                                   | L139P   | 0.18 | 0.19            | 0.29           | 40.66        | 1.02         |
| 139                                                   | L139Q   | 0.76 | 0.96            | 0.98           | 1.05         | 1.25         |
| 139                                                   | L139R   | 0.51 | 0.98            | 0.68           | 1.00         | 0.96         |
| 139                                                   | L139S   | 0.46 | 1.19            | 1.02           | 1.09         | 1.07         |
| 139                                                   | L139T   | 0.37 | 1.32            | 1.05           | 1.00         | 1.13         |
| 139                                                   | L139V   | 0.73 | 1.04            | 0.64           | 0.95         | 1.05         |
| 139                                                   | L139W   | 0.50 | 0.85            | 0.69           | 1.05         | 0.84         |
| 139                                                   | L139Y   | 1.51 | 1.00            | 0.50           | 2.68         | 1.87         |
| 140                                                   | D140A   | 0.98 | 1.14            | 1.00           | 1.01         | 1.09         |
| 140                                                   | D140C   | 0.24 | 0.71            | 0.75           | 0.73         | 0.90         |
| 140                                                   | D140E   | 0.47 | 0.87            | 0.63           | 0.85         | 0.87         |
| 140                                                   | D140F   | 0.38 | 0.75            | 0.45           | 0.85         | 0.73         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 140                                                   | D140G   | 0.25 | 0.87            | 0.63           | 0.72         | 0.72         |
| 140                                                   | D140H   | 0.26 | 0.77            | 0.72           | 0.86         | 0.94         |
| 140                                                   | D140I   | 0.45 | 0.96            | 0.84           | 1.09         | 1.05         |
| 140                                                   | D140K   | 0.48 | 0.75            | 0.62           | 0.77         | 1.01         |
| 140                                                   | D140L   | 0.28 | 0.80            | 0.81           | 0.95         | 0.91         |
| 140                                                   | D140M   | 0.34 | 1.08            | 0.89           | 1.08         | 1.00         |
| 140                                                   | D140N   | 0.29 | 0.58            | 0.37           | 0.81         | 1.02         |
| 140                                                   | D140P   | 0.10 | 0.14            | 0.17           | 10.60        | 12.72        |
| 140                                                   | D140Q   | 1.14 | 1.06            | 0.94           | 1.06         | 1.10         |
| 140                                                   | D140R   | 0.36 | 0.75            | 0.47           | 1.09         | 1.02         |
| 140                                                   | D140S   | 0.65 | 1.06            | 0.92           | 1.04         | 1.05         |
| 140                                                   | D140T   | 0.16 | 0.34            | 0.20           | 1.64         | 1.96         |
| 140                                                   | D140V   | 0.24 | 0.85            | 0.55           | 0.88         | 1.11         |
| 140                                                   | D140W   | 0.94 | 0.98            | 0.92           | 0.98         | 1.09         |
| 140                                                   | D140Y   | 0.34 | 0.78            | 0.64           | 0.91         | 1.01         |
| 141                                                   | K141A   | 0.83 | 0.94            |                | 0.54         | 0.54         |
| 141                                                   | K141C   | 1.16 | 1.10            |                | 0.56         | 0.51         |
| 141                                                   | K141D   | 0.72 | 0.61            |                | 1.39         | 0.68         |
| 141                                                   | K141E   | 0.65 | 0.29            |                | 2.54         | 2.73         |
| 141                                                   | K141F   | 0.52 | 0.51            |                | 2.02         | 1.67         |
| 141                                                   | K141G   | 0.49 | 0.58            |                | 1.62         | 1.45         |
| 141                                                   | K141H   | 1.32 | 1.17            |                | 0.67         | 0.83         |
| 141                                                   | K141I   | 1.18 | 0.57            |                | 1.58         | 1.16         |
| 141                                                   | K141L   | 0.87 | 0.35            |                | 2.74         | 1.77         |
| 141                                                   | K141M   | 0.45 | 0.51            |                | 1.93         | 1.54         |
| 141                                                   | K141N   | 0.54 | 0.60            |                | 2.08         | 1.22         |
| 141                                                   | K141R   | 0.63 | 1.11            |                | 1.14         | 0.32         |
| 141                                                   | K141S   | 0.81 | 0.72            |                | 1.14         | 1.63         |
| 141                                                   | K141T   | 0.45 | 0.60            |                | 2.03         | 1.72         |
| 141                                                   | K141V   | 0.40 | 0.46            |                | 2.92         | 2.99         |
| 141                                                   | K141Y   | 0.65 | 0.07            |                | 1.05         | 3.34         |
| 143                                                   | P143A   | 0.46 | 1.10            | 0.93           | 1.04         | 0.83         |
| 143                                                   | P143C   | 2.28 | 0.75            | 0.82           | 0.68         | 0.66         |
| 143                                                   | P143D   | 0.29 | 0.30            | 0.14           | 5.14         | 4.04         |
| 143                                                   | P143E   | 1.87 | 0.96            | 0.98           | 1.05         | 1.14         |
| 143                                                   | P143F   | 0.57 | 1.29            | 0.70           | 0.98         | 0.90         |
| 143                                                   | P143G   | 0.67 | 0.71            | 1.06           | 0.92         | 1.04         |
| 143                                                   | P143H   | 0.49 | 0.92            | 0.76           | 0.97         | 0.97         |
| 143                                                   | P143I   | 0.86 | 1.06            | 1.03           | 1.02         | 1.10         |
| 143                                                   | P143K   | 0.30 | 0.78            | 0.93           | 0.93         | 0.79         |
| 143                                                   | P143L   | 1.12 | 0.74            | 0.84           | 1.08         | 1.09         |
| 143                                                   | P143M   | 0.18 | 0.06            | 0.14           | 3.72         | 1.81         |
| 143                                                   | P143N   | 0.56 | 1.16            | 1.03           | 1.06         | 0.91         |
| 143                                                   | P143Q   | 1.57 | 1.09            | 0.90           | 1.09         | 1.16         |
| 143                                                   | P143R   | 0.36 | 0.77            | 0.97           | 0.99         | 0.77         |
| 143                                                   | P143S   | 1.92 | 1.11            | 1.05           | 1.06         | 1.25         |
| 143                                                   | P143T   | 0.49 | 1.33            | 0.96           | 0.86         | 0.85         |
| 143                                                   | P143V   | 0.51 | 0.73            | 0.68           | 0.81         | 0.90         |
| 143                                                   | P143W   | 1.09 | 0.91            | 1.02           | 0.95         | 0.99         |
| 143                                                   | P143Y   | 1.06 | 0.82            | 0.70           | 0.95         | 0.98         |
| 144                                                   | L144A   | 1.88 | 1.05            | 1.15           | 0.95         | 0.67         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 144                                                   | L144C   | 0.36 | 1.10            | 1.11           | 0.75         | 0.75         |
| 144                                                   | L144D   | 0.37 | 0.79            | 0.76           | 0.76         | 0.72         |
| 144                                                   | L144E   | 1.08 | 1.06            | 0.99           | 0.90         | 0.77         |
| 144                                                   | L144F   | 0.77 | 1.03            | 0.93           | 0.96         | 0.85         |
| 144                                                   | L144G   | 0.33 | 0.88            | 0.76           | 0.76         | 0.67         |
| 144                                                   | L144H   | 0.81 | 1.04            | 0.75           | 0.89         | 0.87         |
| 144                                                   | L144I   | 0.68 | 1.03            | 0.95           | 0.93         | 0.72         |
| 144                                                   | L144K   | 0.34 | 1.02            | 0.72           | 0.82         | 0.76         |
| 144                                                   | L144M   | 0.32 | 1.09            | 0.96           | 0.94         | 0.85         |
| 144                                                   | L144N   | 0.44 | 0.81            | 0.59           | 0.86         | 0.81         |
| 144                                                   | L144P   | 0.26 | 0.50            | 0.16           | 0.71         | 1.07         |
| 144                                                   | L144Q   | 0.36 | 1.00            | 0.77           | 1.00         | 1.31         |
| 144                                                   | L144S   | 0.35 | 0.19            | 0.24           | 1.57         | 2.43         |
| 144                                                   | L144T   | 0.27 | 0.93            | 0.63           | 0.74         | 0.78         |
| 144                                                   | L144V   | 0.32 | 0.83            | 0.70           | 0.76         | 0.77         |
| 144                                                   | L144W   | 0.33 | 1.15            | 1.00           | 0.84         | 0.98         |
| 144                                                   | L144Y   | 1.02 | 1.05            | 0.85           | 0.99         | 0.96         |
| 147                                                   | Q147A   | 0.09 | 0.94            | 0.82           | 0.91         | 0.87         |
| 147                                                   | Q147C   | 0.14 | 0.68            | 0.48           | 0.92         | 0.81         |
| 147                                                   | Q147E   | 0.86 | 1.00            | 0.88           | 1.07         | 1.05         |
| 147                                                   | Q147F   | 0.10 | 0.65            | 0.66           | 1.03         | 1.11         |
| 147                                                   | Q147G   | 0.39 | 1.01            | 0.93           | 1.02         | 1.00         |
| 147                                                   | Q147I   | 0.14 | 0.88            | 0.54           | 0.96         | 0.75         |
| 147                                                   | Q147K   | 0.13 | 0.85            | 0.61           | 0.87         | 0.89         |
| 147                                                   | Q147L   | 0.11 | 0.77            | 0.47           | 1.08         | 0.91         |
| 147                                                   | Q147M   | 0.23 | 0.85            | 0.56           | 0.75         | 0.69         |
| 147                                                   | Q147N   | 0.67 | 1.04            | 0.83           | 0.99         | 0.97         |
| 147                                                   | Q147P   | 0.20 | 0.25            | 0.26           | 0.66         | 1.19         |
| 147                                                   | Q147R   | 0.46 | 1.01            | 0.80           | 0.97         | 0.85         |
| 147                                                   | Q147S   | 0.63 | 0.97            | 0.79           | 1.12         | 1.02         |
| 147                                                   | Q147T   | 0.83 | 0.97            | 0.85           | 1.10         | 1.09         |
| 147                                                   | Q147V   | 0.19 | 0.71            | 0.59           | 0.89         | 0.94         |
| 147                                                   | Q147W   | 0.20 | 0.61            | 0.63           | 0.74         | 0.82         |
| 150                                                   | A150C   | 0.15 | 0.40            | 0.42           | 0.61         | 0.90         |
| 150                                                   | A150D   | 0.71 | 0.82            | 0.92           | 0.98         | 0.98         |
| 150                                                   | A150E   | 0.37 | 1.09            | 0.90           | 1.05         | 1.03         |
| 150                                                   | A150F   | 0.33 | 1.10            | 0.87           | 1.01         | 0.93         |
| 150                                                   | A150G   | 0.72 | 0.95            | 0.87           | 1.11         | 1.07         |
| 150                                                   | A150H   | 1.02 | 1.02            | 0.92           | 1.14         | 1.21         |
| 150                                                   | A150I   | 0.28 | 0.83            | 0.92           | 1.03         | 1.05         |
| 150                                                   | A150K   | 0.66 | 1.00            | 0.87           | 1.07         | 1.07         |
| 150                                                   | A150L   | 0.46 | 0.59            | 0.45           | 1.12         | 1.92         |
| 150                                                   | A150M   | 0.51 | 0.80            | 0.70           | 1.05         | 1.05         |
| 150                                                   | A150N   | 0.82 | 0.98            | 0.81           | 1.09         | 1.14         |
| 150                                                   | A150P   | 0.14 | 0.44            | 0.42           | 1.18         | 1.65         |
| 150                                                   | A150Q   | 0.64 | 1.02            | 0.86           | 1.17         | 1.15         |
| 150                                                   | A150R   | 0.36 | 0.91            | 0.87           | 0.94         | 1.05         |
| 150                                                   | A150S   | 0.53 | 1.05            | 0.95           | 0.99         | 1.05         |
| 150                                                   | A150T   | 0.13 | 0.96            | 0.67           | 0.99         | 0.83         |
| 150                                                   | A150V   | 0.35 | 0.81            | 0.87           | 0.99         | 0.98         |
| 150                                                   | A150W   | 0.61 | 0.82            | 0.77           | 1.01         | 0.99         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 150                                                   | A150Y   | 0.45 | 0.54            | 0.49           | 0.74         | 0.76         |
| 153                                                   | R153A   | 0.23 | 0.50            | 0.72           | 0.63         | 0.64         |
| 153                                                   | R153C   | 0.26 | 0.51            | 0.86           | 0.94         | 0.88         |
| 153                                                   | R153D   | 0.35 | 0.35            | 0.54           | 0.75         | 0.72         |
| 153                                                   | R153E   | 0.27 | 0.69            | 0.75           | 0.79         | 0.80         |
| 153                                                   | R153F   | 0.21 | 0.38            | 0.69           | 0.68         | 0.58         |
| 153                                                   | R153G   | 0.21 | 0.31            | 0.59           | 0.62         | 0.92         |
| 153                                                   | R153H   | 0.39 | 0.71            | 0.79           | 0.97         | 0.97         |
| 153                                                   | R153I   | 0.22 | 0.28            | 0.37           | 0.63         | 1.09         |
| 153                                                   | R153K   | 0.26 | 0.39            | 0.54           | 0.78         | 0.92         |
| 153                                                   | R153L   | 0.19 | 0.30            | 0.40           | 0.94         | 0.44         |
| 153                                                   | R153M   | 0.31 | 0.70            | 0.86           | 0.92         | 1.05         |
| 153                                                   | R153N   | 0.22 | 0.20            | 0.42           | 1.12         | 1.23         |
| 153                                                   | R153P   | 0.23 | 0.14            | 0.10           | 0.39         | 2.17         |
| 153                                                   | R153Q   | 0.24 | 0.52            | 0.99           | 0.77         | 1.05         |
| 153                                                   | R153S   | 0.41 | 0.78            | 0.99           | 0.93         | 0.93         |
| 153                                                   | R153T   | 0.31 | 0.63            | 0.74           | 0.84         | 0.94         |
| 153                                                   | R153V   | 0.23 | 0.28            | 0.59           | 0.63         | 0.81         |
| 153                                                   | R153W   | 0.42 | 0.77            | 1.07           | 0.90         | 0.87         |
| 153                                                   | R153Y   | 0.32 | 0.60            | 0.73           | 0.84         | 0.90         |
| 154                                                   | T154A   | 0.73 | 1.06            | 0.86           | 0.98         | 1.00         |
| 154                                                   | T154C   | 0.05 | 0.96            | 0.94           | 1.36         | 1.39         |
| 154                                                   | T154D   | 0.64 | 0.68            | 0.82           | 1.26         | 1.90         |
| 154                                                   | T154E   | 0.35 | 0.83            | 0.65           | 0.96         | 0.91         |
| 154                                                   | T154F   | 0.21 | 0.88            | 0.86           | 0.94         | 0.84         |
| 154                                                   | T154G   | 0.61 | 0.92            | 0.94           | 0.99         | 0.94         |
| 154                                                   | T154H   | 0.46 | 0.97            | 0.86           | 1.03         | 1.02         |
| 154                                                   | T154I   | 0.43 | 0.84            | 0.69           | 0.94         | 0.94         |
| 154                                                   | T154K   | 0.43 | 0.93            | 0.85           | 0.98         | 0.89         |
| 154                                                   | T154L   | 0.34 | 0.70            | 0.67           | 0.98         | 0.89         |
| 154                                                   | T154M   | 0.32 | 0.90            | 0.77           | 1.08         | 1.05         |
| 154                                                   | T154N   | 0.05 | 0.23            | 0.25           | 2.37         | 3.90         |
| 154                                                   | T154P   | 0.30 | 0.46            | 0.34           | 0.78         | 0.37         |
| 154                                                   | T154Q   | 0.17 | 0.71            | 1.01           | 0.87         | 0.93         |
| 154                                                   | T154R   | 0.40 | 0.96            | 1.10           | 1.04         | 0.95         |
| 154                                                   | T154S   | 0.79 | 1.03            | 0.91           | 1.05         | 1.06         |
| 154                                                   | T154V   | 0.33 | 0.86            | 0.99           | 1.01         | 0.96         |
| 154                                                   | T154W   | 0.43 | 0.97            | 0.98           | 0.93         | 0.94         |
| 154                                                   | T154Y   | 0.59 | 0.80            | 0.80           | 0.78         | 1.13         |
| 157                                                   | K157A   | 0.68 | 1.04            | 0.93           | 1.01         | 0.91         |
| 157                                                   | K157D   | 0.69 | 0.96            | 1.01           | 1.12         | 0.90         |
| 157                                                   | K157E   | 0.10 | 0.92            | 0.75           | 1.03         | 0.73         |
| 157                                                   | K157F   | 0.40 | 1.01            | 0.94           | 1.07         | 0.95         |
| 157                                                   | K157G   | 0.50 | 0.98            | 0.76           | 1.04         | 0.93         |
| 157                                                   | K157H   | 0.46 | 1.01            | 0.85           | 1.08         | 0.94         |
| 157                                                   | K157I   | 0.71 | 1.00            | 0.81           | 1.05         | 0.94         |
| 157                                                   | K157L   | 0.18 | 0.88            | 0.60           | 1.04         | 0.75         |
| 157                                                   | K157M   | 0.33 | 0.99            | 0.77           | 0.99         | 0.88         |
| 157                                                   | K157P   | 0.11 | 0.57            | 0.63           | 3.04         | 1.86         |
| 157                                                   | K157Q   | 0.50 | 1.00            | 0.70           | 1.00         | 0.88         |
| 157                                                   | K157R   | 0.82 | 1.02            | 1.19           | 0.98         | 0.92         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 157                                                   | K157T   | 0.14 | 0.79            | 0.47           | 1.02         | 0.83         |
| 157                                                   | K157V   | 0.46 | 0.99            | 0.86           | 0.99         | 0.91         |
| 157                                                   | K157W   | 1.06 | 0.95            | 0.84           | 1.04         | 0.93         |
| 157                                                   | K157Y   | 0.77 | 0.98            | 0.92           | 1.00         | 0.84         |
| 158                                                   | N158A   | 0.43 | 0.95            | 0.80           | 0.94         | 1.03         |
| 158                                                   | N158C   | 0.25 | 0.77            | 0.68           | 1.03         | 0.92         |
| 158                                                   | N158D   | 0.55 | 0.99            | 0.86           | 1.01         | 0.90         |
| 158                                                   | N158E   | 0.54 | 0.93            | 0.88           | 0.94         | 1.18         |
| 158                                                   | N158F   | 0.36 | 0.90            | 0.70           | 1.02         | 0.89         |
| 158                                                   | N158G   | 0.24 | 1.06            | 0.66           | 0.89         | 0.75         |
| 158                                                   | N158H   | 0.43 | 0.93            | 0.72           | 0.98         | 0.89         |
| 158                                                   | N158I   | 0.92 | 1.06            | 0.91           | 1.13         | 1.09         |
| 158                                                   | N158L   | 0.62 | 1.12            | 0.81           | 0.97         | 0.99         |
| 158                                                   | N158M   | 0.19 | 0.38            | 0.27           | 0.40         | 0.88         |
| 158                                                   | N158P   | 0.19 | 0.81            | 0.43           | 0.85         | 1.12         |
| 158                                                   | N158Q   | 0.41 | 1.05            | 0.95           | 0.93         | 1.06         |
| 158                                                   | N158R   | 0.23 | 0.74            | 0.43           | 0.76         | 0.74         |
| 158                                                   | N158S   | 0.61 | 0.92            | 0.61           | 0.88         | 0.84         |
| 158                                                   | N158T   | 0.18 | 0.63            | 0.85           | 0.80         | 2.47         |
| 158                                                   | N158V   | 0.65 | 0.92            | 0.75           | 1.00         | 0.87         |
| 158                                                   | N158W   | 0.28 | 0.90            | 0.65           | 0.80         | 0.73         |
| 158                                                   | N158Y   | 0.19 | 0.90            | 0.92           | 0.05         | 0.32         |
| 161                                                   | N161A   | 0.39 | 1.43            | 1.91           | 0.99         | 0.97         |
| 161                                                   | N161C   | 0.62 | 0.95            | 1.12           | 1.05         | 1.06         |
| 161                                                   | N161E   | 0.76 | 1.03            | 1.07           | 1.12         | 1.12         |
| 161                                                   | N161F   | 0.62 | 1.07            | 1.07           | 1.01         | 1.05         |
| 161                                                   | N161G   | 0.55 | 0.99            | 1.02           | 0.99         | 0.95         |
| 161                                                   | N161H   | 0.55 | 0.92            | 0.83           | 1.04         | 1.02         |
| 161                                                   | N161I   | 0.51 | 0.89            | 0.68           | 1.03         | 1.01         |
| 161                                                   | N161K   | 0.43 | 0.84            | 0.67           | 1.03         | 1.20         |
| 161                                                   | N161L   | 0.32 | 0.59            | 0.92           | 2.18         | 1.90         |
| 161                                                   | N161M   | 0.53 | 1.01            | 1.11           | 0.98         | 1.09         |
| 161                                                   | N161P   | 1.06 | 1.03            | 0.98           | 1.12         | 1.24         |
| 161                                                   | N161Q   | 0.68 | 1.02            | 0.97           | 1.04         | 1.07         |
| 161                                                   | N161R   | 0.40 | 0.75            | 0.34           | 0.41         | 0.44         |
| 161                                                   | N161S   | 0.58 | 1.01            | 1.06           | 0.90         | 0.90         |
| 161                                                   | N161T   | 1.06 | 1.05            | 0.96           | 1.01         | 1.18         |
| 161                                                   | N161V   | 0.59 | 1.00            | 0.97           | 1.00         | 1.12         |
| 161                                                   | N161W   | 0.46 | 0.91            | 1.31           | 1.02         | 0.95         |
| 161                                                   | N161Y   | 0.83 | 1.03            | 1.09           | 1.04         | 1.05         |
| 162                                                   | Y162A   | 0.15 | 0.78            | 0.73           | 0.97         | 1.11         |
| 162                                                   | Y162C   | 0.15 | 0.64            | 0.50           | 0.73         | 1.57         |
| 162                                                   | Y162D   | 0.11 | 0.39            | 0.61           | 1.14         | 1.90         |
| 162                                                   | Y162E   | 0.14 | 0.28            | 0.37           | 1.44         | 2.01         |
| 162                                                   | Y162F   | 0.76 | 1.04            | 0.87           | 1.11         | 1.03         |
| 162                                                   | Y162G   | 0.29 | 1.05            | 0.87           | 0.97         | 0.84         |
| 162                                                   | Y162H   | 0.61 | 1.04            | 0.91           | 1.12         | 1.16         |
| 162                                                   | Y162I   | 0.21 | 0.77            | 0.85           | 0.91         | 0.66         |
| 162                                                   | Y162K   | 0.16 | 0.83            | 0.93           | 1.06         | 0.89         |
| 162                                                   | Y162L   | 0.17 | 0.59            | 0.52           | 0.78         | 0.83         |
| 162                                                   | Y162M   | 0.17 | 0.66            | 0.61           | 0.79         | 0.81         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 162                                                   | Y162N   | 0.28 | 0.92            | 1.02           | 1.04         | 0.99         |
| 162                                                   | Y162P   | 0.14 | 0.33            | 0.21           | 0.95         | 1.01         |
| 162                                                   | Y162Q   | 0.18 | 0.93            | 0.81           | 0.86         | 0.75         |
| 162                                                   | Y162R   | 0.20 | 0.82            | 0.86           | 1.04         | 0.91         |
| 162                                                   | Y162S   | 0.20 | 1.05            | 0.67           | 1.01         | 0.91         |
| 162                                                   | Y162T   | 0.14 | 0.52            | 0.33           | 0.63         | 0.96         |
| 162                                                   | Y162V   | 0.20 | 0.67            | 0.89           | 1.02         | 0.87         |
| 162                                                   | Y162W   | 0.54 | 1.03            | 0.77           | 0.92         | 0.81         |
| 177                                                   | A177C   | 0.27 | 0.42            | 0.53           | 1.74         | 1.01         |
| 177                                                   | A177D   | 2.02 | 0.95            | 0.98           | 0.93         | 1.04         |
| 177                                                   | A177E   | 0.36 | 0.59            | 0.67           | 1.03         | 1.02         |
| 177                                                   | A177F   | 0.21 | 0.48            | 0.31           | 0.87         | 0.69         |
| 177                                                   | A177G   | 0.80 | 0.73            | 0.66           | 0.95         | 1.11         |
| 177                                                   | A177H   | 0.12 | 0.32            | 0.30           | 0.99         | 4.79         |
| 177                                                   | A177I   | 0.25 | 0.35            | 0.23           | 0.68         | 0.79         |
| 177                                                   | A177K   | 0.52 | 0.76            | 0.43           | 1.02         | 1.07         |
| 177                                                   | A177L   | 0.33 | 0.26            | 0.27           | 0.89         | 1.00         |
| 177                                                   | A177M   | 0.36 | 0.66            | 0.64           | 0.82         | 0.91         |
| 177                                                   | A177N   | 0.66 | 0.92            | 0.92           | 1.09         | 1.32         |
| 177                                                   | A177P   | 0.51 | 0.52            | 0.36           | 0.70         | 0.61         |
| 177                                                   | A177Q   | 0.21 | 0.46            | 0.42           | 1.40         | 1.12         |
| 177                                                   | A177R   | 0.25 | 0.54            | 0.42           | 1.04         | 1.04         |
| 177                                                   | A177S   | 1.45 | 1.01            | 0.84           | 1.06         | 1.31         |
| 177                                                   | A177T   | 0.45 | 0.62            | 0.46           | 0.86         | 0.74         |
| 177                                                   | A177V   | 0.30 | 0.58            | 0.42           | 0.78         | 0.63         |
| 177                                                   | A177W   | 0.37 | 0.56            | 0.28           | 1.22         | 0.85         |
| 177                                                   | A177Y   | 0.84 | 0.76            | 0.53           | 0.95         | 1.04         |
| 178                                                   | A178C   | 0.26 | 0.31            | 0.17           | 0.89         | 1.15         |
| 178                                                   | A178D   | 1.60 | 0.63            | 0.68           | 0.96         | 1.03         |
| 178                                                   | A178E   | 0.92 | 0.61            | 0.44           | 1.03         | 1.23         |
| 178                                                   | A178F   | 0.56 | 0.40            | 0.23           | 0.93         | 0.91         |
| 178                                                   | A178G   | 1.18 | 0.73            | 0.61           | 0.84         | 0.81         |
| 178                                                   | A178H   | 0.11 | 0.71            | 0.33           | 1.21         | 1.02         |
| 178                                                   | A178I   | 0.40 | 0.27            | 0.15           | 1.50         | 1.40         |
| 178                                                   | A178K   | 0.76 | 0.10            | 0.12           | 1.15         | 2.62         |
| 178                                                   | A178L   | 0.29 | 0.25            | 0.23           | 1.61         | 1.99         |
| 178                                                   | A178M   | 0.69 | 0.40            | 0.37           | 1.23         | 1.43         |
| 178                                                   | A178N   | 1.80 | 0.36            | 0.48           | 0.95         | 1.39         |
| 178                                                   | A178P   | 0.11 | 0.18            | 0.14           | 2.14         | 1.65         |
| 178                                                   | A178Q   | 1.22 | 0.51            | 0.49           | 1.17         | 1.36         |
| 178                                                   | A178R   | 0.43 | 0.19            | 0.23           | 1.45         | 1.85         |
| 178                                                   | A178S   | 0.35 | 0.80            | 0.62           | 1.05         | 0.93         |
| 178                                                   | A178T   | 0.21 | 0.39            | 0.30           | 2.08         | 1.42         |
| 178                                                   | A178V   | 1.07 | 0.46            | 0.36           | 1.10         | 1.21         |
| 178                                                   | A178W   | 0.88 | 0.16            | 0.05           | 1.65         | 2.37         |
| 178                                                   | A178Y   | 0.73 | 0.44            | 0.05           | 1.02         | 1.30         |
| 179                                                   | L179A   | 1.02 | 1.03            | 1.26           | 1.04         | 0.97         |
| 179                                                   | L179C   | 1.06 | 0.87            | 1.01           | 1.09         | 1.04         |
| 179                                                   | L179D   | 0.29 | 0.75            | 0.43           | 0.93         | 0.84         |
| 179                                                   | L179E   | 0.14 | 0.16            | 0.32           | 0.59         | 0.79         |
| 179                                                   | L179F   | 0.27 | 0.77            | 0.42           | 1.01         | 0.93         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 179                                                   | L179G   | 1.02 | 0.98            | 0.93           | 1.05         | 0.98         |
| 179                                                   | L179H   | 0.95 | 0.95            | 0.61           | 1.10         | 0.93         |
| 179                                                   | L179K   | 0.76 | 0.89            | 0.70           | 0.99         | 0.87         |
| 179                                                   | L179M   | 0.30 | 0.82            | 0.61           | 1.12         | 1.11         |
| 179                                                   | L179N   | 0.94 | 0.76            | 0.75           | 1.11         | 0.99         |
| 179                                                   | L179P   | 0.24 | 0.32            | 0.26           | 0.91         | 0.85         |
| 179                                                   | L179Q   | 0.34 | 0.85            | 1.03           | 1.18         | 1.19         |
| 179                                                   | L179R   | 0.13 | 0.17            | 0.05           | 0.27         | 0.92         |
| 179                                                   | L179S   | 0.78 | 0.95            | 1.02           | 1.08         | 1.10         |
| 179                                                   | L179T   | 0.79 | 0.90            | 0.86           | 1.11         | 1.00         |
| 179                                                   | L179V   | 0.82 | 0.96            | 0.79           | 1.06         | 1.03         |
| 179                                                   | L179W   | 0.40 | 0.94            | 0.54           | 0.85         | 0.71         |
| 179                                                   | L179Y   | 0.24 | 0.67            | 0.56           | 0.79         | 0.73         |
| 180                                                   | A180C   | 0.50 | 0.80            | 0.11           | 0.78         | 0.57         |
| 180                                                   | A180D   | 0.66 | 0.21            | 0.09           | 0.61         | 0.10         |
| 180                                                   | A180E   | 1.04 | 0.53            | 0.24           | 0.76         | 0.74         |
| 180                                                   | A180F   | 0.72 | 0.15            | 0.05           | 0.38         | 1.72         |
| 180                                                   | A180G   | 0.35 | 0.68            | 0.22           | 0.75         | 0.58         |
| 180                                                   | A180H   | 0.58 | 0.11            | 0.07           | 0.56         | 0.25         |
| 180                                                   | A180I   | 0.62 | 0.26            | 0.09           | 0.64         | 0.86         |
| 180                                                   | A180K   | 0.88 | 0.12            | 0.05           | 0.57         | 0.55         |
| 180                                                   | A180L   | 0.82 | 0.20            | 0.08           | 0.59         | 0.83         |
| 180                                                   | A180M   | 0.94 | 0.15            | 0.05           | 13.63        | 0.81         |
| 180                                                   | A180N   | 0.08 | 0.10            | 0.26           | 0.05         | 4.24         |
| 180                                                   | A180P   | 0.50 | 0.53            | 0.10           | 0.72         | 0.59         |
| 180                                                   | A180Q   | 0.85 | 0.35            | 0.13           | 0.58         | 0.58         |
| 180                                                   | A180R   | 0.69 | 0.05            | 0.11           | 4.95         | 3.56         |
| 180                                                   | A180S   | 1.32 | 0.89            | 0.77           | 1.04         | 1.01         |
| 180                                                   | A180T   | 0.76 | 0.44            | 0.18           | 0.60         | 0.55         |
| 180                                                   | A180V   | 0.32 | 0.19            | 0.23           | 0.85         | 0.84         |
| 180                                                   | A180W   | 1.14 | 0.09            | 0.05           | 0.65         | 1.85         |
| 180                                                   | A180Y   | 0.63 | 0.09            | 0.05           | 2.52         | 1.23         |
| 181                                                   | S181A   | 0.56 | 0.39            | 0.35           | 0.81         | 0.73         |
| 181                                                   | S181C   | 0.84 | 0.12            | 0.08           | 1.86         | 1.46         |
| 181                                                   | S181D   | 0.84 | 0.12            | 0.16           | 1.32         | 0.90         |
| 181                                                   | S181E   | 0.54 | 0.11            | 0.05           | 0.96         | 1.23         |
| 181                                                   | S181F   | 0.54 | 0.10            | 0.05           | 1.34         | 1.42         |
| 181                                                   | S181G   | 0.90 | 0.42            | 0.18           | 0.89         | 0.84         |
| 181                                                   | S181K   | 0.53 | 0.06            | 0.05           | 1.81         | 4.72         |
| 181                                                   | S181L   | 0.34 | 0.15            | 0.05           | 0.99         | 1.53         |
| 181                                                   | S181M   | 0.45 | 0.11            | 0.13           | 0.55         | 2.09         |
| 181                                                   | S181N   | 0.71 | 0.17            | 0.08           | 1.30         | 0.58         |
| 181                                                   | S181P   | 0.57 | 0.11            | 0.05           | 1.37         | 0.79         |
| 181                                                   | S181Q   | 0.42 | 0.11            | 0.08           | 2.41         | 1.67         |
| 181                                                   | S181R   | 0.58 | 0.07            | 0.05           | 0.18         | 1.33         |
| 181                                                   | S181T   | 1.01 | 0.17            | 0.09           | 1.53         | 1.19         |
| 181                                                   | S181V   | 0.58 | 0.13            | 0.09           | 1.76         | 1.08         |
| 181                                                   | S181W   | 0.76 | 0.05            | 0.05           | 0.96         | 3.23         |
| 181                                                   | S181Y   | 0.64 | 0.61            | 0.36           | 0.71         | 0.88         |
| 182                                                   | N182A   | 0.56 | 1.21            | 0.96           | 0.92         | 1.05         |
| 182                                                   | N182C   | 1.24 | 0.96            | 0.80           | 0.91         | 0.89         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 182                                                   | N182D   | 1.58 | 1.02            | 0.89           | 0.93         | 0.87         |
| 182                                                   | N182E   | 0.21 | 0.17            | 0.05           | 0.63         | 1.74         |
| 182                                                   | N182G   | 0.93 | 0.86            | 0.83           | 1.21         | 1.26         |
| 182                                                   | N182H   | 1.45 | 1.06            | 0.65           | 0.87         | 0.85         |
| 182                                                   | N182I   | 0.94 | 0.59            | 0.54           | 1.06         | 1.13         |
| 182                                                   | N182K   | 0.99 | 0.59            | 0.43           | 0.87         | 0.80         |
| 182                                                   | N182L   | 0.78 | 0.98            | 0.78           | 1.01         | 1.13         |
| 182                                                   | N182M   | 1.74 | 0.90            | 0.84           | 1.12         | 1.28         |
| 182                                                   | N182P   | 0.87 | 0.63            | 0.50           | 0.85         | 0.85         |
| 182                                                   | N182Q   | 0.18 | 0.13            | 0.05           | 1.70         | 1.48         |
| 182                                                   | N182R   | 0.69 | 0.73            | 0.46           | 1.03         | 1.03         |
| 182                                                   | N182S   | 1.09 | 1.07            | 0.98           | 0.91         | 0.92         |
| 182                                                   | N182T   | 1.06 | 0.95            | 0.89           | 0.94         | 0.99         |
| 182                                                   | N182V   | 1.10 | 0.74            | 0.59           | 0.76         | 0.77         |
| 182                                                   | N182W   | 2.82 | 1.06            | 1.36           | 1.10         | 1.26         |
| 182                                                   | N182Y   | 1.20 | 0.87            | 0.90           | 1.03         | 1.15         |
| 185                                                   | Y185A   | 0.07 | 0.05            | 0.29           | 2.19         | 1.88         |
| 185                                                   | Y185C   | 0.14 | 0.18            | 0.21           | 0.05         | 0.13         |
| 185                                                   | Y185D   | 0.17 | 0.08            | 0.16           | 0.55         | 1.10         |
| 185                                                   | Y185E   | 0.59 | 0.08            | 0.12           | 0.09         | 1.45         |
| 185                                                   | Y185F   | 0.86 | 1.08            | 0.91           | 1.06         | 1.05         |
| 185                                                   | Y185G   | 0.15 | 0.12            | 0.06           | 0.05         | 0.22         |
| 185                                                   | Y185H   | 0.59 | 0.88            | 0.34           | 0.64         | 0.46         |
| 185                                                   | Y185I   | 0.21 | 0.68            | 0.54           | 0.66         | 0.64         |
| 185                                                   | Y185L   | 1.88 | 1.19            | 1.11           | 1.02         | 1.09         |
| 185                                                   | Y185M   | 0.35 | 0.74            | 0.41           | 0.97         | 0.78         |
| 185                                                   | Y185P   | 0.22 | 0.10            | 0.20           | 0.33         | 0.89         |
| 185                                                   | Y185Q   | 0.12 | 0.22            | 0.14           | 0.05         | 0.62         |
| 185                                                   | Y185R   | 0.12 | 0.08            | 0.05           | 3.65         | 4.42         |
| 185                                                   | Y185S   | 0.16 | 0.41            | 0.16           | 0.32         | 0.26         |
| 185                                                   | Y185T   | 0.15 | 0.35            | 0.25           | 0.19         | 0.38         |
| 185                                                   | Y185V   | 0.24 | 0.16            | 0.15           | 0.60         | 0.37         |
| 185                                                   | Y185W   | 0.28 | 0.80            | 0.77           | 0.83         | 0.97         |
| 186                                                   | S186A   | 0.87 | 0.88            | 0.89           | 0.96         | 1.06         |
| 186                                                   | S186C   | 0.27 | 0.64            | 0.67           | 1.22         | 2.83         |
| 186                                                   | S186D   | 1.73 | 0.97            | 0.96           | 1.04         | 1.19         |
| 186                                                   | S186E   | 2.57 | 0.82            | 0.72           | 1.06         | 1.19         |
| 186                                                   | S186F   | 0.33 | 0.64            | 0.48           | 1.42         | 1.61         |
| 186                                                   | S186G   | 0.49 | 1.02            | 0.59           | 1.01         | 1.09         |
| 186                                                   | S186H   | 2.18 | 0.96            | 0.70           | 1.09         | 1.40         |
| 186                                                   | S186I   | 3.18 | 0.88            | 0.86           | 1.09         | 1.27         |
| 186                                                   | S186K   | 1.22 | 0.95            | 0.61           | 1.03         | 1.00         |
| 186                                                   | S186L   | 1.03 | 1.06            | 0.92           | 1.13         | 1.31         |
| 186                                                   | S186M   | 1.21 | 0.88            | 0.73           | 1.06         | 1.27         |
| 186                                                   | S186N   | 0.16 | 0.65            | 0.43           | 0.91         | 1.04         |
| 186                                                   | S186P   | 0.19 | 0.78            | 0.22           | 0.77         | 0.82         |
| 186                                                   | S186Q   | 0.69 | 1.10            | 0.95           | 1.07         | 1.21         |
| 186                                                   | S186R   | 2.78 | 0.90            | 0.73           | 0.89         | 1.07         |
| 186                                                   | S186T   | 0.48 | 1.08            | 0.87           | 1.14         | 1.21         |
| 186                                                   | S186V   | 0.92 | 1.03            | 0.91           | 1.03         | 1.26         |
| 186                                                   | S186W   | 0.64 | 0.94            | 0.61           | 1.01         | 1.14         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                 |              |              |
|-------------------------------------------------------|---------|------|-----------------|-----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac.  | Res.<br>EtOH | Res.<br>Heat |
| 186                                                   | S186Y   | 0.21 | 0.24            | <del>0.05</del> | 2.68         | 4.96         |
| 188                                                   | A188C   | 0.67 | 0.57            | 0.62            | 0.99         | 1.05         |
| 188                                                   | A188D   | 0.74 | 0.55            | 0.69            | 1.10         | 1.38         |
| 188                                                   | A188E   | 0.42 | 0.79            | 0.62            | 1.41         | 1.06         |
| 188                                                   | A188F   | 0.48 | 0.88            | 0.50            | 0.93         | 0.80         |
| 188                                                   | A188H   | 0.79 | 0.64            | 0.60            | 1.32         | 1.39         |
| 188                                                   | A188I   | 0.41 | 0.52            | 0.37            | 1.60         | 1.05         |
| 188                                                   | A188K   | 0.72 | 0.43            | 0.31            | 0.79         | 0.58         |
| 188                                                   | A188L   | 0.82 | 0.63            | 0.53            | 1.41         | 0.92         |
| 188                                                   | A188M   | 0.39 | 0.60            | 0.52            | 3.51         | 3.70         |
| 188                                                   | A188N   | 0.33 | 0.39            | 0.42            | 2.28         | 1.14         |
| 188                                                   | A188P   | 0.36 | 1.06            | 0.82            | 1.01         | 0.89         |
| 188                                                   | A188Q   | 0.12 | 0.85            | 0.72            | 0.88         | 0.79         |
| 188                                                   | A188R   | 0.36 | 0.87            | 0.49            | 0.93         | 0.83         |
| 188                                                   | A188S   | 0.63 | 1.21            | 1.17            | 1.05         | 1.00         |
| 188                                                   | A188T   | 1.02 | 0.67            | 0.60            | 1.24         | 1.29         |
| 188                                                   | A188V   | 0.55 | 0.92            | 0.63            | 0.96         | 0.94         |
| 188                                                   | A188W   | 0.53 | 0.61            | 0.57            | 0.90         | 1.02         |
| 188                                                   | A188Y   | 0.54 | 0.45            | 0.30            | 1.00         | 0.98         |
| 189                                                   | D189A   | 0.29 | 0.77            | 0.73            | 0.79         | 0.69         |
| 189                                                   | D189C   | 0.38 | 0.89            | 0.82            | 0.90         | 0.76         |
| 189                                                   | D189E   | 0.44 | 0.98            | 0.84            | 0.94         | 0.89         |
| 189                                                   | D189F   | 0.39 | 0.82            | 0.64            | 0.94         | 0.99         |
| 189                                                   | D189G   | 0.27 | 0.87            | 0.86            | 0.88         | 0.71         |
| 189                                                   | D189H   | 0.39 | 0.91            | 0.52            | 0.91         | 0.83         |
| 189                                                   | D189I   | 0.16 | 0.52            | 0.39            | 0.63         | 0.61         |
| 189                                                   | D189K   | 0.37 | 0.96            | 0.78            | 0.89         | 0.73         |
| 189                                                   | D189L   | 0.33 | 1.04            | 0.82            | 0.89         | 0.85         |
| 189                                                   | D189N   | 0.55 | 0.95            | 1.05            | 1.00         | 0.96         |
| 189                                                   | D189P   | 0.12 | 0.30            | 0.22            | 0.72         | 0.76         |
| 189                                                   | D189Q   | 0.73 | 0.98            | 0.72            | 0.93         | 0.86         |
| 189                                                   | D189R   | 0.38 | 0.96            | 0.82            | 0.94         | 0.88         |
| 189                                                   | D189S   | 0.14 | 0.60            | 0.32            | 0.86         | 1.22         |
| 189                                                   | D189T   | 0.21 | 0.84            | 0.84            | 0.85         | 0.76         |
| 189                                                   | D189V   | 0.36 | 1.03            | 0.99            | 1.03         | 0.91         |
| 189                                                   | D189W   | 0.13 | 0.18            | 0.34            | 0.45         | 1.06         |
| 189                                                   | D189Y   | 0.32 | 0.52            | 0.46            | 1.30         | 1.34         |
| 190                                                   | G190A   | 0.79 | 0.81            | 0.62            | 0.86         | 0.77         |
| 190                                                   | G190C   | 0.35 | 0.80            | 0.87            | 0.78         | 0.83         |
| 190                                                   | G190D   | 0.54 | 0.95            | 0.82            | 0.93         | 0.92         |
| 190                                                   | G190E   | 0.18 | 0.71            | 0.49            | 0.89         | 1.05         |
| 190                                                   | G190H   | 0.11 | 0.40            | 0.24            | 0.35         | 0.80         |
| 190                                                   | G190I   | 0.13 | 0.45            | 0.39            | 0.49         | 0.55         |
| 190                                                   | G190K   | 0.27 | 0.87            | 0.58            | 0.91         | 0.94         |
| 190                                                   | G190L   | 0.74 | 0.89            | 0.60            | 0.82         | 1.04         |
| 190                                                   | G190M   | 0.45 | 0.58            | 0.45            | 0.93         | 1.39         |
| 190                                                   | G190N   | 0.56 | 1.00            | 0.73            | 0.93         | 0.98         |
| 190                                                   | G190P   | 0.14 | 0.09            | 0.26            | 26.00        | 3.19         |
| 190                                                   | G190Q   | 0.59 | 1.04            | 0.80            | 0.95         | 1.04         |
| 190                                                   | G190R   | 0.49 | 0.74            | 0.44            | 0.74         | 1.10         |
| 190                                                   | G190S   | 0.86 | 1.15            | 0.96            | 0.99         | 1.13         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 190                                                   | G190T   | 0.50 | 0.84            | 0.79           | 1.06         | 1.15         |
| 190                                                   | G190V   | 0.25 | 0.90            | 0.57           | 0.88         | 0.83         |
| 190                                                   | G190W   | 0.53 | 0.79            | 0.42           | 0.90         | 0.89         |
| 190                                                   | G190Y   | 0.38 | 0.95            | 0.83           | 0.84         | 0.91         |
| 191                                                   | G191C   | 0.14 | 0.17            | 0.20           | 0.82         | 1.34         |
| 191                                                   | G191D   | 0.20 | 0.39            | 0.27           | 0.28         | 0.13         |
| 191                                                   | G191E   | 0.15 | 0.22            | 0.10           | 0.75         | 0.79         |
| 191                                                   | G191F   | 0.17 | 0.19            | 0.25           | 0.51         | 0.94         |
| 191                                                   | G191I   | 0.14 | 0.18            | 0.22           | 0.55         | 0.95         |
| 191                                                   | G191L   | 0.15 | 0.24            | 0.18           | 0.95         | 0.91         |
| 191                                                   | G191M   | 0.13 | 0.23            | 0.40           | 0.46         | 0.88         |
| 191                                                   | G191P   | 0.12 | 0.19            | 0.29           | 0.56         | 1.20         |
| 191                                                   | G191Q   | 0.19 | 0.65            | 0.51           | 0.89         | 0.74         |
| 191                                                   | G191R   | 0.13 | 0.21            | 0.24           | 0.62         | 1.52         |
| 191                                                   | G191S   | 0.09 | 0.38            | 0.05           | 0.75         | 1.30         |
| 191                                                   | G191T   | 0.12 | 0.23            | 0.10           | 0.47         | 1.18         |
| 191                                                   | G191V   | 0.13 | 0.21            | 0.17           | 0.51         | 1.34         |
| 191                                                   | G191W   | 0.12 | 0.18            | 0.12           | 0.07         | 1.08         |
| 192                                                   | V192A   | 0.42 | 0.95            | 0.73           | 1.00         | 0.80         |
| 192                                                   | V192C   | 0.87 | 0.68            | 0.52           | 0.88         | 0.73         |
| 192                                                   | V192D   | 0.38 | 0.39            | 0.34           | 0.48         | 0.50         |
| 192                                                   | V192E   | 0.69 | 1.02            | 0.82           | 0.98         | 0.98         |
| 192                                                   | V192F   | 0.20 | 0.19            | 0.06           | 0.05         | 0.08         |
| 192                                                   | V192G   | 0.16 | 0.46            | 0.21           | 0.47         | 0.53         |
| 192                                                   | V192H   | 0.30 | 0.75            | 0.40           | 0.83         | 0.86         |
| 192                                                   | V192K   | 0.34 | 0.58            | 0.32           | 0.77         | 0.72         |
| 192                                                   | V192L   | 0.76 | 1.04            | 0.55           | 1.03         | 1.00         |
| 192                                                   | V192M   | 0.46 | 0.96            | 0.44           | 1.01         | 0.95         |
| 192                                                   | V192P   | 0.35 | 0.80            | 0.29           | 0.93         | 0.83         |
| 192                                                   | V192R   | 0.60 | 1.01            | 0.76           | 1.02         | 0.99         |
| 192                                                   | V192S   | 0.29 | 0.76            | 0.46           | 0.93         | 0.85         |
| 192                                                   | V192T   | 0.34 | 0.47            | 0.22           | 0.75         | 0.87         |
| 192                                                   | V192W   | 0.25 | 0.53            | 0.25           | 0.72         | 0.65         |
| 192                                                   | V192Y   | 0.25 | 0.64            | 0.25           | 0.81         | 0.75         |
| 193                                                   | A193C   | 0.31 | 0.99            | 0.93           | 0.94         | 0.71         |
| 193                                                   | A193D   | 0.49 | 1.07            | 1.02           | 1.11         | 0.90         |
| 193                                                   | A193E   | 0.31 | 1.05            | 0.89           | 1.01         | 0.72         |
| 193                                                   | A193F   | 0.35 | 1.06            | 0.75           | 0.99         | 0.80         |
| 193                                                   | A193G   | 0.17 | 0.68            | 0.41           | 1.32         | 1.04         |
| 193                                                   | A193I   | 0.37 | 1.12            | 0.88           | 0.97         | 0.82         |
| 193                                                   | A193K   | 0.32 | 0.85            | 0.71           | 0.99         | 0.85         |
| 193                                                   | A193L   | 0.31 | 1.10            | 0.88           | 0.90         | 0.81         |
| 193                                                   | A193M   | 0.44 | 1.12            | 0.82           | 0.94         | 0.90         |
| 193                                                   | A193N   | 0.74 | 0.96            | 0.84           | 1.05         | 0.97         |
| 193                                                   | A193P   | 0.23 | 0.99            | 0.79           | 0.95         | 0.79         |
| 193                                                   | A193Q   | 0.48 | 1.11            | 0.82           | 1.02         | 0.96         |
| 193                                                   | A193R   | 0.37 | 1.06            | 0.73           | 0.98         | 0.89         |
| 193                                                   | A193S   | 0.48 | 0.98            | 0.81           | 0.95         | 0.85         |
| 193                                                   | A193T   | 0.10 | 0.40            | 0.31           | 1.16         | 0.77         |
| 193                                                   | A193V   | 0.45 | 1.06            | 0.85           | 0.99         | 0.87         |
| 193                                                   | A193W   | 0.49 | 1.11            | 1.05           | 0.98         | 0.87         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 193                                                   | A193Y   | 0.40 | 0.78            | 0.63           | 0.88         | 0.79         |
| 194                                                   | K194A   | 0.30 | 0.67            | 0.86           | 0.95         | 1.00         |
| 194                                                   | K194C   | 0.22 | 1.08            | 1.72           | 0.88         | 0.88         |
| 194                                                   | K194D   | 0.61 | 0.98            | 1.07           | 1.00         | 1.03         |
| 194                                                   | K194E   | 0.44 | 1.00            | 1.14           | 1.02         | 1.14         |
| 194                                                   | K194F   | 0.67 | 1.03            | 0.94           | 1.04         | 1.13         |
| 194                                                   | K194G   | 0.43 | 0.92            | 0.65           | 0.92         | 0.96         |
| 194                                                   | K194H   | 0.60 | 0.99            | 0.92           | 1.05         | 1.15         |
| 194                                                   | K194I   | 0.24 | 0.38            | 0.49           | 0.50         | 0.59         |
| 194                                                   | K194L   | 0.61 | 0.98            | 0.90           | 1.01         | 1.22         |
| 194                                                   | K194M   | 0.61 | 1.02            | 0.86           | 1.03         | 1.17         |
| 194                                                   | K194N   | 0.54 | 0.99            | 1.02           | 1.04         | 1.17         |
| 194                                                   | K194P   | 0.32 | 0.39            | 0.55           | 0.46         | 0.38         |
| 194                                                   | K194Q   | 0.64 | 1.00            | 1.00           | 1.03         | 1.17         |
| 194                                                   | K194R   | 0.66 | 1.04            | 0.88           | 1.01         | 1.23         |
| 194                                                   | K194S   | 0.66 | 1.00            | 0.83           | 1.12         | 1.03         |
| 194                                                   | K194T   | 0.08 | 0.11            | 1.09           | 1.17         | 9.80         |
| 194                                                   | K194V   | 0.23 | 0.30            | 0.71           | 0.55         | 0.62         |
| 194                                                   | K194W   | 0.43 | 0.93            | 0.98           | 0.98         | 1.01         |
| 194                                                   | K194Y   | 0.08 | 0.11            | 0.36           | 0.60         | 2.65         |
| 196                                                   | K196A   | 0.43 | 0.98            | 0.81           | 0.91         | 0.85         |
| 196                                                   | K196F   | 0.52 | 1.02            | 0.93           | 1.02         | 1.04         |
| 196                                                   | K196G   | 0.13 | 0.71            | 0.30           | 0.96         | 0.86         |
| 196                                                   | K196H   | 0.26 | 1.02            | 0.77           | 1.00         | 1.03         |
| 196                                                   | K196L   | 0.63 | 0.95            | 0.71           | 1.00         | 1.00         |
| 196                                                   | K196M   | 0.84 | 0.98            | 0.94           | 0.97         | 1.02         |
| 196                                                   | K196Q   | 0.67 | 0.98            | 0.74           | 0.97         | 1.05         |
| 196                                                   | K196W   | 0.31 | 0.84            | 0.51           | 0.92         | 0.86         |
| 196                                                   | K196Y   | 0.07 | 1.23            | 0.50           | 0.94         | 0.96         |
| 197                                                   | N197A   | 0.56 | 1.22            | 1.12           | 0.98         | 0.96         |
| 197                                                   | N197C   | 0.30 | 0.98            | 0.72           | 1.09         | 1.02         |
| 197                                                   | N197D   | 1.50 | 1.12            | 1.08           | 1.06         | 1.07         |
| 197                                                   | N197F   | 0.29 | 0.99            | 0.69           | 0.91         | 0.85         |
| 197                                                   | N197G   | 1.53 | 1.10            | 0.92           | 1.07         | 1.10         |
| 197                                                   | N197H   | 1.02 | 1.07            | 0.80           | 1.08         | 1.10         |
| 197                                                   | N197I   | 0.60 | 1.10            | 0.74           | 1.03         | 1.02         |
| 197                                                   | N197K   | 0.45 | 1.00            | 0.48           | 0.89         | 0.94         |
| 197                                                   | N197L   | 0.29 | 0.97            | 0.60           | 0.97         | 0.95         |
| 197                                                   | N197M   | 0.53 | 1.14            | 0.82           | 1.05         | 1.05         |
| 197                                                   | N197P   | 0.41 | 1.01            | 0.79           | 0.93         | 0.93         |
| 197                                                   | N197Q   | 0.39 | 0.95            | 0.72           | 0.98         | 0.98         |
| 197                                                   | N197R   | 0.26 | 0.98            | 0.74           | 0.93         | 1.05         |
| 197                                                   | N197T   | 0.68 | 1.07            | 0.78           | 1.04         | 1.00         |
| 197                                                   | N197V   | 0.67 | 1.12            | 0.87           | 0.97         | 1.04         |
| 197                                                   | N197W   | 0.46 | 0.99            | 0.77           | 0.98         | 1.01         |
| 197                                                   | N197Y   | 0.18 | 0.56            | 0.06           | 0.63         | 1.17         |
| 201                                                   | T201A   | 0.54 | 0.61            | 0.61           | 0.87         | 0.83         |
| 201                                                   | T201C   | 0.34 | 0.79            | 0.62           | 0.92         | 0.99         |
| 201                                                   | T201D   | 0.30 | 1.00            | 1.08           | 0.89         | 0.73         |
| 201                                                   | T201E   | 0.18 | 0.84            | 0.43           | 0.87         | 0.71         |
| 201                                                   | T201F   | 0.32 | 0.81            | 0.61           | 0.85         | 0.89         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 201                                                   | T201G   | 0.33 | 1.06            | 0.93           | 0.90         | 0.86         |
| 201                                                   | T201H   | 0.33 | 0.96            | 0.75           | 0.86         | 0.86         |
| 201                                                   | T201I   | 0.20 | 0.86            | 0.58           | 0.89         | 0.70         |
| 201                                                   | T201K   | 0.25 | 1.12            | 0.87           | 0.87         | 0.74         |
| 201                                                   | T201L   | 0.40 | 0.98            | 0.63           | 0.93         | 0.89         |
| 201                                                   | T201M   | 0.43 | 0.97            | 0.66           | 0.90         | 0.95         |
| 201                                                   | T201N   | 0.29 | 0.85            | 0.66           | 0.85         | 0.85         |
| 201                                                   | T201P   | 0.22 | 0.63            | 0.42           | 0.94         | 1.41         |
| 201                                                   | T201Q   | 0.42 | 0.95            | 0.70           | 0.86         | 0.95         |
| 201                                                   | T201R   | 0.33 | 0.56            | 0.39           | 1.28         | 1.26         |
| 201                                                   | T201S   | 0.38 | 1.01            | 0.70           | 0.90         | 0.83         |
| 201                                                   | T201V   | 0.28 | 0.56            | 0.53           | 1.07         | 1.11         |
| 201                                                   | T201W   | 0.23 | 0.66            | 0.50           | 0.92         | 0.93         |
| 201                                                   | T201Y   | 0.24 | 0.55            | 0.40           | 1.05         | 2.18         |
| 203                                                   | R203A   | 0.71 | 0.93            | 0.75           | 0.69         | 0.88         |
| 203                                                   | R203F   | 0.85 | 0.92            | 0.99           | 0.67         | 0.87         |
| 203                                                   | R203G   | 0.60 | 1.07            | 1.13           | 0.66         | 1.39         |
| 203                                                   | R203H   | 0.81 | 1.04            | 1.24           | 2.57         | 3.09         |
| 203                                                   | R203I   | 0.39 | 0.70            | 0.64           | 1.46         | 3.02         |
| 203                                                   | R203K   | 0.96 | 0.84            | 0.99           | 0.55         | 0.75         |
| 203                                                   | R203L   | 0.46 | 1.14            | 0.54           | 1.08         | 1.24         |
| 203                                                   | R203M   | 0.58 | 0.88            | 0.91           | 0.99         | 1.25         |
| 203                                                   | R203N   | 0.36 | 1.08            | 0.82           | 1.06         | 1.20         |
| 203                                                   | R203P   | 0.29 | 0.49            | 0.28           | 2.85         | 4.58         |
| 203                                                   | R203Q   | 0.80 | 0.99            | 0.78           | 0.84         | 0.86         |
| 203                                                   | R203S   | 0.54 | 1.19            | 0.53           | 1.99         | 1.96         |
| 203                                                   | R203T   | 0.66 | 1.04            | 0.82           | 0.99         | 0.91         |
| 203                                                   | R203V   | 0.85 | 0.94            | 0.89           | 0.73         | 0.78         |
| 203                                                   | R203W   | 0.39 | 1.10            | 0.63           | 2.28         | 2.42         |
| 203                                                   | R203Y   | 0.57 | 1.09            | 1.03           | 1.03         | 1.18         |
| 204                                                   | Q204A   | 0.91 | 0.98            | 0.98           | 0.99         | 0.99         |
| 204                                                   | Q204C   | 1.03 | 1.13            | 0.91           | 1.00         | 0.89         |
| 204                                                   | Q204D   | 1.23 | 1.04            | 0.73           | 1.00         | 1.00         |
| 204                                                   | Q204E   | 1.08 | 1.12            | 1.05           | 0.93         | 1.04         |
| 204                                                   | Q204F   | 0.48 | 0.74            | 0.94           | 1.13         | 0.94         |
| 204                                                   | Q204G   | 1.14 | 1.08            | 0.87           | 0.77         | 0.75         |
| 204                                                   | Q204H   | 1.30 | 1.12            | 0.84           | 1.16         | 0.91         |
| 204                                                   | Q204I   | 0.86 | 0.68            | 0.88           | 1.33         | 1.57         |
| 204                                                   | Q204K   | 1.13 | 0.75            | 0.91           | 1.61         | 1.28         |
| 204                                                   | Q204L   | 0.45 | 0.82            | 0.81           | 0.98         | 0.96         |
| 204                                                   | Q204M   | 0.44 | 0.85            | 0.87           | 0.78         | 0.76         |
| 204                                                   | Q204N   | 0.59 | 0.96            | 0.96           | 1.04         | 0.83         |
| 204                                                   | Q204P   | 0.36 | 0.50            | 0.83           | 1.09         | 1.19         |
| 204                                                   | Q204R   | 0.95 | 0.87            | 0.89           | 1.36         | 1.06         |
| 204                                                   | Q204S   | 0.68 | 0.53            | 1.00           | 0.97         | 1.95         |
| 204                                                   | Q204T   | 1.94 | 1.08            | 0.93           | 1.07         | 1.18         |
| 204                                                   | Q204V   | 0.85 | 0.78            | 0.74           | 1.29         | 1.32         |
| 204                                                   | Q204W   | 0.13 | 0.07            | 0.22           | 0.11         | 0.15         |
| 206                                                   | V206C   | 0.46 | 0.83            | 0.43           | 1.52         | 1.48         |
| 206                                                   | V206D   | 0.38 | 0.24            | 0.10           | 3.69         | 7.75         |
| 206                                                   | V206E   | 0.35 | 0.45            | 0.39           | 1.54         | 3.44         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 206                                                   | V206F   | 0.76 | 1.00            | 0.74           | 2.06         | 2.29         |
| 206                                                   | V206G   | 0.33 | 0.94            | 0.79           | 1.56         | 1.54         |
| 206                                                   | V206I   | 0.35 | 1.56            | 0.83           | 1.97         | 1.61         |
| 206                                                   | V206L   | 0.82 | 1.44            | 0.87           | 0.70         | 0.84         |
| 206                                                   | V206M   | 0.42 | 1.41            | 0.49           | 1.75         | 1.64         |
| 206                                                   | V206P   | 0.48 | 0.24            | 0.30           | 3.72         | 7.12         |
| 206                                                   | V206Q   | 0.27 | 1.35            | 0.40           | 2.88         | 2.34         |
| 206                                                   | V206R   | 0.24 | 0.72            | 0.45           | 7.05         | 6.46         |
| 206                                                   | V206S   | 0.44 | 0.48            | 0.44           | 1.64         | 2.37         |
| 206                                                   | V206T   | 0.71 | 1.30            | 0.69           | 1.07         | 1.06         |
| 206                                                   | V206W   | 0.77 | 0.50            | 0.29           | 0.71         | 2.48         |
| 206                                                   | V206Y   | 0.66 | 1.19            | 0.57           | 1.17         | 1.58         |
| 207                                                   | V207A   | 0.72 | 1.09            | 0.93           | 0.94         | 1.01         |
| 207                                                   | V207C   | 0.69 | 1.07            | 0.83           | 0.94         | 0.99         |
| 207                                                   | V207D   | 0.11 | 0.05            | 0.05           | 1.44         | 1.05         |
| 207                                                   | V207E   | 0.56 | 1.04            | 0.70           | 0.79         | 1.01         |
| 207                                                   | V207F   | 0.78 | 1.11            | 0.67           | 0.87         | 1.09         |
| 207                                                   | V207G   | 0.83 | 0.98            | 0.90           | 1.12         | 1.01         |
| 207                                                   | V207I   | 0.69 | 1.06            | 0.84           | 0.94         | 1.03         |
| 207                                                   | V207K   | 0.75 | 0.97            | 0.67           | 1.03         | 1.04         |
| 207                                                   | V207L   | 0.64 | 1.01            | 0.73           | 0.70         | 1.09         |
| 207                                                   | V207M   | 0.58 | 0.92            | 0.62           | 1.00         | 1.03         |
| 207                                                   | V207N   | 0.68 | 0.84            | 0.53           | 1.01         | 1.03         |
| 207                                                   | V207P   | 0.20 | 0.10            | 0.05           | 1.83         | 2.90         |
| 207                                                   | V207Q   | 0.62 | 1.04            | 0.72           | 0.89         | 1.01         |
| 207                                                   | V207R   | 0.99 | 0.93            | 0.69           | 0.86         | 1.10         |
| 207                                                   | V207S   | 0.18 | 0.05            | 0.05           | 11.51        | 0.58         |
| 207                                                   | V207W   | 0.96 | 0.95            | 0.58           | 1.13         | 1.06         |
| 207                                                   | V207Y   | 0.78 | 0.95            | 0.62           | 1.06         | 1.01         |
| 210                                                   | S210A   | 0.69 | 0.94            | 0.87           | 0.94         | 0.91         |
| 210                                                   | S210E   | 0.28 | 0.80            | 0.64           | 0.85         | 0.76         |
| 210                                                   | S210F   | 0.43 | 0.80            | 0.53           | 0.92         | 0.81         |
| 210                                                   | S210G   | 0.39 | 0.70            | 0.49           | 0.94         | 0.83         |
| 210                                                   | S210H   | 0.32 | 0.90            | 0.80           | 0.93         | 0.72         |
| 210                                                   | S210I   | 0.78 | 0.72            | 0.56           | 0.91         | 0.84         |
| 210                                                   | S210L   | 0.06 | 1.45            | 1.58           | 0.93         | 0.86         |
| 210                                                   | S210N   | 0.45 | 0.93            | 1.02           | 0.93         | 0.92         |
| 210                                                   | S210P   | 0.31 | 0.78            | 0.80           | 0.86         | 0.77         |
| 210                                                   | S210R   | 0.38 | 0.91            | 0.62           | 0.94         | 0.88         |
| 210                                                   | S210T   | 0.55 | 0.95            | 0.76           | 0.96         | 0.89         |
| 210                                                   | S210V   | 0.33 | 0.82            | 0.65           | 0.99         | 0.93         |
| 210                                                   | S210W   | 0.38 | 1.02            | 0.97           | 1.00         | 0.89         |
| 210                                                   | S210Y   | 0.28 | 0.77            | 0.58           | 0.96         | 0.87         |
| 214                                                   | T214A   | 0.23 | 1.00            | 0.60           | 0.84         | 1.11         |
| 214                                                   | T214C   | 0.95 | 1.08            | 0.98           | 0.92         | 0.94         |
| 214                                                   | T214D   | 0.13 | 0.57            | 0.55           | 0.65         | 1.29         |
| 214                                                   | T214E   | 0.16 | 0.54            | 0.32           | 0.90         | 1.02         |
| 214                                                   | T214F   | 0.19 | 0.89            | 0.57           | 0.62         | 0.84         |
| 214                                                   | T214G   | 0.11 | 0.62            | 0.47           | 0.61         | 0.75         |
| 214                                                   | T214H   | 0.88 | 1.05            | 0.97           | 1.04         | 1.13         |
| 214                                                   | T214I   | 1.00 | 1.08            | 1.00           | 1.06         | 1.03         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 214                                                   | T214K   | 0.25 | 0.41            | 0.25           | 0.87         | 0.74         |
| 214                                                   | T214L   | 0.33 | 1.07            | 0.31           | 0.61         | 0.40         |
| 214                                                   | T214N   | 1.80 | 1.04            | 1.04           | 1.10         | 1.20         |
| 214                                                   | T214P   | 0.62 | 0.99            | 0.90           | 1.03         | 0.95         |
| 214                                                   | T214Q   | 0.86 | 1.05            | 0.78           | 1.03         | 1.08         |
| 214                                                   | T214R   | 0.37 | 0.17            | 0.05           | 0.48         | 1.29         |
| 214                                                   | T214S   | 0.54 | 1.03            | 0.86           | 0.94         | 1.08         |
| 214                                                   | T214V   | 1.01 | 1.13            | 1.03           | 1.01         | 0.97         |
| 214                                                   | T214W   | 0.32 | 0.29            | 0.32           | 0.87         | 1.07         |
| 214                                                   | T214Y   | 0.36 | 0.50            | 0.47           | 0.68         | 0.62         |
| 225                                                   | N225A   | 0.26 | 0.36            | 0.51           | 0.54         | 0.38         |
| 225                                                   | N225C   | 0.15 | 0.13            | 0.41           | 1.24         | 0.81         |
| 225                                                   | N225D   | 0.34 | 0.36            | 0.47           | 0.54         | 0.25         |
| 225                                                   | N225E   | 0.15 | 0.11            | 0.21           | 0.87         | 0.31         |
| 225                                                   | N225F   | 0.13 | 0.17            | 0.29           | 0.77         | 0.42         |
| 225                                                   | N225G   | 0.10 | 0.31            | 0.20           | 0.70         | 0.69         |
| 225                                                   | N225H   | 0.12 | 0.13            | 0.17           | 0.80         | 0.30         |
| 225                                                   | N225I   | 0.08 | 0.17            | 0.22           | 0.80         | 0.37         |
| 225                                                   | N225K   | 0.26 | 0.12            | 0.17           | 0.98         | 2.90         |
| 225                                                   | N225L   | 0.11 | 0.24            | 0.36           | 0.87         | 1.11         |
| 225                                                   | N225M   | 0.12 | 0.74            | 0.47           | 0.99         | 0.78         |
| 225                                                   | N225P   | 0.14 | 0.29            | 0.44           | 0.58         | 0.74         |
| 225                                                   | N225Q   | 0.14 | 0.44            | 0.25           | 0.60         | 0.41         |
| 225                                                   | N225R   | 0.11 | 0.13            | 0.21           | 1.69         | 1.02         |
| 225                                                   | N225S   | 0.14 | 0.40            | 0.45           | 0.68         | 0.82         |
| 225                                                   | N225T   | 0.21 | 0.50            | 0.34           | 0.56         | 0.76         |
| 225                                                   | N225V   | 0.20 | 0.38            | 0.30           | 0.84         | 0.33         |
| 225                                                   | N225W   | 0.20 | 0.13            | 0.20           | 1.87         | 1.45         |
| 225                                                   | N225Y   | 0.19 | 0.20            | 0.17           | 0.75         | 0.52         |
| 226                                                   | L226C   | 0.15 | 0.65            | 0.39           | 0.73         | 0.56         |
| 226                                                   | L226D   | 0.16 | 0.18            | 0.31           | 0.68         | 1.08         |
| 226                                                   | L226E   | 0.20 | 0.29            | 0.31           | 0.93         | 1.23         |
| 226                                                   | L226F   | 0.21 | 0.57            | 0.34           | 0.80         | 0.74         |
| 226                                                   | L226G   | 0.16 | 0.29            | 0.15           | 0.41         | 0.51         |
| 226                                                   | L226H   | 0.19 | 0.32            | 0.29           | 0.81         | 0.95         |
| 226                                                   | L226M   | 0.67 | 1.02            | 0.80           | 1.12         | 1.05         |
| 226                                                   | L226N   | 0.12 | 0.35            | 0.21           | 0.64         | 0.81         |
| 226                                                   | L226P   | 0.21 | 0.21            | 0.26           | 1.02         | 1.62         |
| 226                                                   | L226R   | 0.14 | 0.24            | 0.16           | 0.73         | 1.18         |
| 226                                                   | L226S   | 0.23 | 0.59            | 0.27           | 0.70         | 0.80         |
| 226                                                   | L226T   | 0.14 | 0.46            | 0.15           | 0.71         | 0.68         |
| 226                                                   | L226V   | 0.26 | 0.86            | 0.43           | 0.85         | 0.83         |
| 226                                                   | L226W   | 0.18 | 0.26            | 0.24           | 0.71         | 1.94         |
| 226                                                   | L226Y   | 0.15 | 0.28            | 0.35           | 0.53         | 1.17         |
| 228                                                   | T228A   | 0.22 | 0.48            | 0.87           | 0.29         | 0.49         |
| 228                                                   | T228C   | 0.12 | 0.37            | 0.79           | 0.49         | 0.81         |
| 228                                                   | T228F   | 0.22 | 0.14            | 0.23           | 0.92         | 1.09         |
| 228                                                   | T228G   | 0.25 | 0.46            | 0.67           | 0.32         | 0.44         |
| 228                                                   | T228H   | 0.14 | 0.24            | 0.73           | 0.46         | 1.00         |
| 228                                                   | T228I   | 0.23 | 0.23            | 0.36           | 0.76         | 0.88         |
| 228                                                   | T228K   | 0.19 | 0.11            | 0.31           | 0.54         | 1.35         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 228                                                   | T228M   | 0.28 | 0.34            | 0.41           | 0.55         | 0.62         |
| 228                                                   | T228N   | 0.23 | 0.49            | 0.28           | 0.54         | 0.49         |
| 228                                                   | T228P   | 0.14 | 0.25            | 0.42           | 0.26         | 0.78         |
| 228                                                   | T228R   | 0.38 | 0.11            | 0.74           | 0.90         | 1.70         |
| 228                                                   | T228S   | 0.22 | 0.63            | 0.41           | 0.55         | 0.52         |
| 228                                                   | T228V   | 0.56 | 0.69            | 0.13           | 0.90         | 0.67         |
| 228                                                   | T228W   | 0.18 | 0.08            | 0.42           | 9.87         | 33.17        |
| 228                                                   | T228Y   | 0.28 | 0.12            | 0.43           | 0.05         | 1.28         |
| 229                                                   | N229A   | 0.56 | 0.88            | 0.80           | 0.75         | 0.68         |
| 229                                                   | N229C   | 0.19 | 1.06            | 0.65           | 0.90         | 1.09         |
| 229                                                   | N229D   | 0.16 | 0.36            | 0.05           | 0.87         | 0.78         |
| 229                                                   | N229E   | 0.42 | 0.73            | 0.54           | 0.89         | 0.69         |
| 229                                                   | N229F   | 0.24 | 0.44            | 0.50           | 0.97         | 0.90         |
| 229                                                   | N229G   | 0.52 | 0.77            | 0.78           | 0.77         | 0.70         |
| 229                                                   | N229H   | 0.66 | 0.76            | 0.60           | 0.95         | 1.00         |
| 229                                                   | N229I   | 0.49 | 0.78            | 0.81           | 0.89         | 0.93         |
| 229                                                   | N229K   | 0.84 | 0.81            | 0.64           | 0.90         | 0.86         |
| 229                                                   | N229L   | 0.39 | 0.48            | 0.20           | 0.61         | 0.61         |
| 229                                                   | N229M   | 0.54 | 0.77            | 0.43           | 0.90         | 0.86         |
| 229                                                   | N229P   | 0.35 | 0.67            | 0.34           | 0.80         | 0.85         |
| 229                                                   | N229Q   | 0.17 | 0.48            | 0.33           | 0.94         | 1.72         |
| 229                                                   | N229R   | 0.42 | 0.81            | 0.53           | 0.93         | 0.81         |
| 229                                                   | N229S   | 0.49 | 0.92            | 0.69           | 0.84         | 0.72         |
| 229                                                   | N229T   | 0.36 | 0.67            | 0.53           | 0.64         | 0.60         |
| 229                                                   | N229V   | 0.48 | 0.56            | 0.44           | 0.74         | 0.90         |
| 229                                                   | N229W   | 0.33 | 0.64            | 0.60           | 0.75         | 0.73         |
| 229                                                   | N229Y   | 0.30 | 0.90            | 0.66           | 0.96         | 0.93         |
| 230                                                   | L230A   | 0.18 | 0.85            | 0.43           | 0.54         | 0.28         |
| 230                                                   | L230C   | 0.27 | 0.77            | 0.51           | 0.62         | 0.39         |
| 230                                                   | L230D   | 0.23 | 0.45            | 0.26           | 0.55         | 0.43         |
| 230                                                   | L230E   | 0.30 | 0.85            | 0.45           | 0.85         | 0.51         |
| 230                                                   | L230F   | 0.46 | 0.88            | 0.42           | 0.80         | 0.62         |
| 230                                                   | L230G   | 0.33 | 0.70            | 0.35           | 0.84         | 0.39         |
| 230                                                   | L230H   | 0.37 | 0.57            | 0.17           | 3.13         | 1.86         |
| 230                                                   | L230I   | 0.22 | 0.63            | 0.48           | 0.71         | 0.62         |
| 230                                                   | L230K   | 0.11 | 1.05            | 0.15           | 0.86         | 0.52         |
| 230                                                   | L230M   | 0.14 | 0.65            | 0.59           | 0.80         | 0.76         |
| 230                                                   | L230N   | 0.55 | 0.97            | 0.50           | 0.71         | 0.51         |
| 230                                                   | L230P   | 0.26 | 0.71            | 0.32           | 0.49         | 0.40         |
| 230                                                   | L230Q   | 0.30 | 0.78            | 0.37           | 0.79         | 0.51         |
| 230                                                   | L230R   | 0.87 | 1.03            | 0.42           | 0.92         | 0.79         |
| 230                                                   | L230S   | 0.36 | 0.63            | 0.43           | 1.04         | 0.66         |
| 230                                                   | L230T   | 0.42 | 1.04            | 0.52           | 0.78         | 0.67         |
| 230                                                   | L230V   | 0.29 | 0.82            | 0.36           | 0.72         | 0.62         |
| 230                                                   | L230W   | 0.14 | 0.28            | 0.16           | 0.80         | 0.81         |
| 230                                                   | L230Y   | 0.65 | 0.92            | 0.72           | 0.87         | 0.79         |
| 231                                                   | G231A   | 1.45 | 0.98            | 1.13           | 1.00         | 0.98         |
| 231                                                   | G231C   | 0.43 | 0.72            | 0.24           | 0.89         | 0.66         |
| 231                                                   | G231E   | 0.13 | 0.15            | 0.10           | 0.67         | 1.05         |
| 231                                                   | G231F   | 0.54 | 0.82            | 0.22           | 0.86         | 0.70         |
| 231                                                   | G231H   | 0.39 | 0.78            | 0.32           | 0.88         | 0.80         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 231                                                   | G231I   | 0.17 | 0.14            | 0.56           | 1.07         | 1.44         |
| 231                                                   | G231K   | 0.33 | 0.75            | 0.62           | 0.84         | 0.74         |
| 231                                                   | G231L   | 0.45 | 0.92            | 0.65           | 0.74         | 0.58         |
| 231                                                   | G231M   | 0.16 | 0.24            | 0.60           | 0.81         | 1.41         |
| 231                                                   | G231N   | 0.58 | 0.87            | 0.93           | 0.97         | 0.98         |
| 231                                                   | G231P   | 0.16 | 0.21            | 0.45           | 0.72         | 0.81         |
| 231                                                   | G231Q   | 0.29 | 0.13            | 0.22           | 0.77         | 1.25         |
| 231                                                   | G231R   | 0.33 | 0.43            | 0.36           | 0.79         | 0.79         |
| 231                                                   | G231S   | 0.81 | 1.01            | 0.62           | 1.01         | 0.96         |
| 231                                                   | G231T   | 0.96 | 0.66            | 0.44           | 0.89         | 0.68         |
| 231                                                   | G231V   | 0.19 | 0.55            | 0.55           | 0.54         | 0.42         |
| 231                                                   | G231W   | 0.73 | 0.80            | 0.16           | 0.91         | 0.74         |
| 231                                                   | G231Y   | 0.75 | 0.85            | 0.58           | 0.83         | 0.65         |
| 232                                                   | T232A   | 0.31 | 0.67            | 0.99           | 1.00         | 0.83         |
| 232                                                   | T232C   | 0.36 | 0.63            | 1.16           | 0.71         | 0.97         |
| 232                                                   | T232E   | 0.34 | 0.64            | 0.88           | 0.91         | 0.85         |
| 232                                                   | T232F   | 0.31 | 0.62            | 0.87           | 0.90         | 0.97         |
| 232                                                   | T232I   | 0.23 | 0.49            | 0.81           | 0.94         | 0.96         |
| 232                                                   | T232K   | 0.63 | 0.76            | 0.66           | 0.97         | 1.02         |
| 232                                                   | T232L   | 0.20 | 0.55            | 0.96           | 0.91         | 0.85         |
| 232                                                   | T232N   | 0.22 | 0.21            | 0.52           | 0.47         | 0.65         |
| 232                                                   | T232P   | 0.52 | 0.79            | 0.95           | 0.89         | 1.02         |
| 232                                                   | T232Q   | 0.33 | 0.57            | 0.89           | 0.83         | 0.98         |
| 232                                                   | T232S   | 0.34 | 0.72            | 0.76           | 0.94         | 1.06         |
| 232                                                   | T232V   | 0.50 | 0.67            | 0.89           | 1.42         | 1.42         |
| 232                                                   | T232W   | 0.25 | 0.43            | 0.47           | 0.73         | 0.59         |
| 232                                                   | T232Y   | 0.43 | 0.72            | 0.71           | 1.05         | 1.00         |
| 233                                                   | P233A   | 0.56 | 1.06            | 1.15           | 0.85         | 0.77         |
| 233                                                   | P233C   | 0.28 | 0.92            | 1.01           | 0.85         | 0.72         |
| 233                                                   | P233D   | 0.41 | 0.89            | 1.49           | 0.87         | 0.84         |
| 233                                                   | P233E   | 0.52 | 1.02            | 1.34           | 0.91         | 0.88         |
| 233                                                   | P233F   | 0.30 | 1.01            | 0.89           | 0.85         | 0.81         |
| 233                                                   | P233G   | 0.40 | 1.07            | 1.22           | 0.79         | 0.69         |
| 233                                                   | P233H   | 0.38 | 1.01            | 0.64           | 0.90         | 0.84         |
| 233                                                   | P233I   | 0.43 | 1.04            | 0.87           | 0.90         | 0.81         |
| 233                                                   | P233K   | 0.33 | 0.85            | 0.76           | 0.91         | 0.86         |
| 233                                                   | P233L   | 0.45 | 1.03            | 0.89           | 0.90         | 0.87         |
| 233                                                   | P233M   | 0.26 | 0.99            | 0.92           | 0.92         | 0.88         |
| 233                                                   | P233N   | 0.17 | 0.70            | 1.15           | 0.94         | 0.82         |
| 233                                                   | P233Q   | 0.35 | 1.03            | 1.19           | 0.94         | 0.87         |
| 233                                                   | P233R   | 0.27 | 0.77            | 0.47           | 0.77         | 0.96         |
| 233                                                   | P233S   | 0.32 | 0.60            | 0.62           | 0.98         | 1.01         |
| 233                                                   | P233T   | 0.27 | 0.77            | 0.84           | 0.87         | 0.90         |
| 233                                                   | P233V   | 0.33 | 0.76            | 0.80           | 0.78         | 0.92         |
| 233                                                   | P233W   | 0.21 | 0.75            | 0.70           | 0.98         | 0.82         |
| 233                                                   | P233Y   | 0.27 | 0.73            | 0.91           | 0.87         | 0.91         |
| 234                                                   | K234A   | 1.16 | 0.90            | 0.81           | 0.91         | 1.46         |
| 234                                                   | K234C   | 0.60 | 0.94            | 0.48           | 0.94         | 1.36         |
| 234                                                   | K234D   | 0.61 | 0.74            | 0.44           | 0.93         | 1.46         |
| 234                                                   | K234F   | 0.36 | 0.82            | 0.05           | 0.98         | 2.17         |
| 234                                                   | K234G   | 0.84 | 0.91            | 0.56           | 0.50         | 1.41         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 234                                                   | K234H   | 0.59 | 0.98            | 0.45           | 1.00         | 1.83         |
| 234                                                   | K234L   | 0.27 | 1.08            | 0.42           | 2.09         | 1.93         |
| 234                                                   | K234M   | 0.39 | 0.84            | 0.44           | 1.18         | 1.85         |
| 234                                                   | K234N   | 0.51 | 0.97            | 0.59           | 1.23         | 1.19         |
| 234                                                   | K234S   | 0.17 | 0.94            | 0.43           | 3.50         | 3.17         |
| 234                                                   | K234T   | 0.77 | 0.92            | 0.57           | 0.75         | 1.27         |
| 234                                                   | K234V   | 0.32 | 1.01            | 0.22           | 1.43         | 2.47         |
| 234                                                   | K234Y   | 0.28 | 1.01            | 0.39           | 1.29         | 1.86         |
| 236                                                   | A236C   | 0.12 | 0.18            | 0.33           | 3.03         | 3.16         |
| 236                                                   | A236D   | 0.39 | 0.97            | 0.91           | 1.03         | 0.61         |
| 236                                                   | A236E   | 0.45 | 1.02            | 0.79           | 0.94         | 0.82         |
| 236                                                   | A236F   | 0.42 | 0.96            | 0.62           | 0.95         | 0.93         |
| 236                                                   | A236G   | 0.58 | 0.84            | 0.59           | 0.88         | 0.68         |
| 236                                                   | A236H   | 0.62 | 0.97            | 0.54           | 0.93         | 0.90         |
| 236                                                   | A236I   | 0.22 | 0.86            | 0.50           | 0.89         | 0.49         |
| 236                                                   | A236K   | 0.34 | 1.08            | 0.56           | 0.95         | 0.69         |
| 236                                                   | A236L   | 0.32 | 0.94            | 0.74           | 0.99         | 0.79         |
| 236                                                   | A236M   | 0.41 | 1.01            | 0.71           | 0.88         | 0.90         |
| 236                                                   | A236N   | 0.30 | 0.97            | 0.72           | 0.94         | 0.88         |
| 236                                                   | A236P   | 0.14 | 0.29            | 0.22           | 0.38         | 0.87         |
| 236                                                   | A236Q   | 0.43 | 1.07            | 0.74           | 0.93         | 0.78         |
| 236                                                   | A236R   | 0.48 | 1.03            | 0.56           | 0.97         | 0.96         |
| 236                                                   | A236S   | 0.38 | 0.83            | 0.71           | 1.01         | 0.80         |
| 236                                                   | A236T   | 0.35 | 0.97            | 0.60           | 0.85         | 0.71         |
| 236                                                   | A236V   | 0.32 | 0.88            | 0.55           | 0.87         | 0.63         |
| 236                                                   | A236W   | 0.30 | 0.58            | 0.45           | 13.41        | 5.08         |
| 236                                                   | A236Y   | 0.42 | 0.54            | 0.53           | 3.39         | 1.31         |
| 237                                                   | N237A   | 0.75 | 1.02            | 0.91           | 0.80         | 0.88         |
| 237                                                   | N237C   | 0.43 | 1.26            | 0.77           | 1.02         | 1.28         |
| 237                                                   | N237F   | 0.52 | 0.91            | 0.17           | 0.76         | 1.34         |
| 237                                                   | N237G   | 0.71 | 1.06            | 0.38           | 1.02         | 0.95         |
| 237                                                   | N237H   | 0.57 | 0.98            | 0.56           | 0.99         | 1.03         |
| 237                                                   | N237I   | 0.66 | 0.96            | 0.23           | 0.68         | 1.05         |
| 237                                                   | N237K   | 0.63 | 1.04            | 0.38           | 1.17         | 1.31         |
| 237                                                   | N237L   | 0.76 | 1.04            | 0.58           | 0.64         | 1.40         |
| 237                                                   | N237P   | 0.24 | 0.59            | 0.07           | 1.96         | 3.82         |
| 237                                                   | N237Q   | 0.93 | 1.06            | 1.05           | 0.92         | 1.39         |
| 237                                                   | N237R   | 0.65 | 1.02            | 0.34           | 1.19         | 1.69         |
| 237                                                   | N237S   | 0.91 | 0.99            | 0.75           | 0.98         | 1.25         |
| 237                                                   | N237T   | 1.39 | 0.95            | 0.92           | 0.68         | 1.15         |
| 237                                                   | N237V   | 0.80 | 1.07            | 0.45           | 0.83         | 1.69         |
| 237                                                   | N237W   | 0.46 | 1.12            | 0.24           | 0.97         | 1.62         |
| 237                                                   | N237Y   | 0.30 | 0.63            | 0.16           | 2.26         | 3.41         |
| 239                                                   | Q239A   | 1.09 | 0.96            | 0.82           | 0.94         | 1.05         |
| 239                                                   | Q239C   | 0.63 | 0.99            | 0.94           | 0.98         | 0.97         |
| 239                                                   | Q239D   | 0.59 | 0.98            | 0.91           | 0.92         | 0.92         |
| 239                                                   | Q239E   | 0.70 | 1.10            | 1.16           | 1.00         | 1.04         |
| 239                                                   | Q239F   | 0.83 | 0.99            | 0.63           | 0.99         | 0.98         |
| 239                                                   | Q239G   | 0.39 | 1.21            | 0.58           | 0.91         | 0.97         |
| 239                                                   | Q239H   | 0.96 | 1.08            | 0.84           | 0.92         | 0.99         |
| 239                                                   | Q239I   | 0.59 | 1.07            | 0.43           | 0.88         | 0.90         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 239                                                   | Q239L   | 0.71 | 0.98            | 0.70           | 0.82         | 0.88         |
| 239                                                   | Q239M   | 0.85 | 1.08            | 0.77           | 0.97         | 0.99         |
| 239                                                   | Q239N   | 0.70 | 1.05            | 0.65           | 0.99         | 0.96         |
| 239                                                   | Q239P   | 0.31 | 0.51            | 0.40           | 0.47         | 0.90         |
| 239                                                   | Q239R   | 1.06 | 1.02            | 0.70           | 1.01         | 0.96         |
| 239                                                   | Q239S   | 0.88 | 1.08            | 1.04           | 0.90         | 1.02         |
| 239                                                   | Q239T   | 0.54 | 1.01            | 0.73           | 0.84         | 0.74         |
| 239                                                   | Q239V   | 0.84 | 0.97            | 0.71           | 1.03         | 0.92         |
| 239                                                   | Q239W   | 0.94 | 0.96            | 0.64           | 0.87         | 0.80         |
| 239                                                   | Q239Y   | 1.18 | 0.96            | 0.75           | 0.92         | 0.95         |
| 240                                                   | S240A   | 0.90 | 0.96            | 0.80           | 0.89         | 0.61         |
| 240                                                   | S240C   | 0.46 | 0.57            | 0.56           | 0.87         | 0.89         |
| 240                                                   | S240D   | 0.18 | 0.05            | 0.12           | 23.73        | 17.94        |
| 240                                                   | S240E   | 2.24 | 1.09            | 0.99           | 1.03         | 1.07         |
| 240                                                   | S240F   | 0.67 | 1.04            | 0.61           | 0.98         | 0.80         |
| 240                                                   | S240G   | 1.27 | 1.04            | 0.91           | 1.06         | 1.09         |
| 240                                                   | S240H   | 0.17 | 0.62            | 0.22           | 1.08         | 1.14         |
| 240                                                   | S240I   | 0.56 | 0.95            | 0.58           | 0.92         | 0.91         |
| 240                                                   | S240K   | 0.66 | 1.04            | 0.51           | 0.94         | 0.74         |
| 240                                                   | S240L   | 1.77 | 1.05            | 0.69           | 1.08         | 1.10         |
| 240                                                   | S240M   | 0.57 | 1.07            | 0.57           | 0.94         | 0.83         |
| 240                                                   | S240N   | 0.28 | 0.68            | 0.45           | 0.78         | 1.01         |
| 240                                                   | S240P   | 1.00 | 0.99            | 0.62           | 1.00         | 0.97         |
| 240                                                   | S240Q   | 0.73 | 0.96            | 0.68           | 0.94         | 0.74         |
| 240                                                   | S240R   | 0.66 | 0.68            | 0.48           | 0.98         | 1.67         |
| 240                                                   | S240T   | 0.17 | 0.31            | 0.24           | 0.95         | 1.11         |
| 240                                                   | S240V   | 1.16 | 1.08            | 0.73           | 0.95         | 0.97         |
| 240                                                   | S240W   | 0.56 | 0.69            | 0.44           | 1.15         | 1.33         |
| 240                                                   | S240Y   | 0.50 | 0.82            | 0.50           | 0.97         | 0.84         |
| 243                                                   | L243A   | 0.86 | 0.07            | 0.19           | 5.39         | 5.71         |
| 243                                                   | L243C   | 0.84 | 0.47            | 0.52           | 1.60         | 1.93         |
| 243                                                   | L243D   | 0.61 | 0.56            | 0.65           | 0.78         | 0.75         |
| 243                                                   | L243E   | 0.49 | 0.49            | 0.41           | 2.61         | 3.15         |
| 243                                                   | L243F   | 0.59 | 0.80            | 0.67           | 0.87         | 0.75         |
| 243                                                   | L243G   | 0.75 | 0.44            | 0.36           | 0.85         | 0.96         |
| 243                                                   | L243H   | 0.05 | 0.05            | 0.05           | 0.05         | 0.05         |
| 243                                                   | L243I   | 0.40 | 0.76            | 0.81           | 0.90         | 0.89         |
| 243                                                   | L243K   | 0.84 | 0.64            | 0.47           | 0.91         | 0.97         |
| 243                                                   | L243M   | 0.49 | 0.72            | 0.54           | 0.91         | 0.82         |
| 243                                                   | L243N   | 0.17 | 0.63            | 0.52           | 0.78         | 0.73         |
| 243                                                   | L243P   | 0.11 | 0.23            | 0.37           | 0.93         | 1.19         |
| 243                                                   | L243Q   | 0.58 | 0.75            | 0.67           | 1.04         | 0.95         |
| 243                                                   | L243R   | 0.74 | 0.58            | 0.46           | 1.01         | 0.96         |
| 243                                                   | L243S   | 0.95 | 0.58            | 0.44           | 0.86         | 0.74         |
| 243                                                   | L243T   | 0.44 | 0.67            | 0.50           | 0.91         | 0.82         |
| 243                                                   | L243V   | 0.34 | 0.36            | 0.42           | 0.75         | 0.80         |
| 243                                                   | L243W   | 0.48 | 0.47            | 0.31           | 0.89         | 0.99         |
| 243                                                   | L243Y   | 0.56 | 0.87            | 0.91           | 0.92         | 0.92         |
| 245                                                   | C245A   | 0.27 | 0.97            | 0.62           | 0.74         | 0.64         |
| 245                                                   | C245D   | 0.15 | 0.42            | 0.54           | 0.56         | 0.58         |
| 245                                                   | C245E   | 0.15 | 0.24            | 0.13           | 0.39         | 0.91         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 245                                                   | C245F   | 0.18 | 0.14            | 0.13           | 0.56         | 1.88         |
| 245                                                   | C245G   | 0.26 | 0.83            | 0.78           | 0.85         | 0.79         |
| 245                                                   | C245H   | 0.08 | 0.62            | 0.05           | 0.85         | 1.44         |
| 245                                                   | C245K   | 0.13 | 0.28            | 0.39           | 0.80         | 1.34         |
| 245                                                   | C245L   | 0.13 | 0.26            | 0.08           | 0.91         | 1.68         |
| 245                                                   | C245M   | 0.12 | 0.49            | 0.13           | 0.66         | 0.89         |
| 245                                                   | C245N   | 0.12 | 0.47            | 0.38           | 0.45         | 0.77         |
| 245                                                   | C245P   | 0.09 | 0.13            | 0.12           | 0.73         | 1.96         |
| 245                                                   | C245Q   | 0.11 | 0.22            | 0.22           | 0.75         | 1.30         |
| 245                                                   | C245R   | 0.14 | 0.20            | 0.35           | 0.40         | 1.61         |
| 245                                                   | C245S   | 0.26 | 0.63            | 0.37           | 0.57         | 0.95         |
| 245                                                   | C245T   | 0.13 | 0.46            | 0.28           | 0.74         | 0.88         |
| 245                                                   | C245V   | 0.14 | 0.50            | 0.45           | 0.43         | 0.72         |
| 245                                                   | C245W   | 0.15 | 0.14            | 0.20           | 1.16         | 1.54         |
| 245                                                   | C245Y   | 0.09 | 0.17            | 0.38           | 1.45         | 1.94         |
| 247                                                   | N247A   | 1.18 | 0.36            | 0.05           | 1.64         | 1.68         |
| 247                                                   | N247C   | 1.46 | 0.82            | 0.79           | 0.71         | 0.83         |
| 247                                                   | N247D   | 1.18 | 0.76            | 0.67           | 0.58         | 0.96         |
| 247                                                   | N247E   | 1.32 | 0.77            | 0.18           | 0.81         | 0.76         |
| 247                                                   | N247F   | 0.76 | 0.81            | 0.29           | 1.10         | 1.27         |
| 247                                                   | N247G   | 0.49 | 0.89            | 0.54           | 1.83         | 2.30         |
| 247                                                   | N247H   | 1.70 | 0.87            | 0.55           | 0.86         | 0.61         |
| 247                                                   | N247I   | 1.53 | 0.88            | 0.66           | 0.77         | 0.85         |
| 247                                                   | N247K   | 0.79 | 0.64            | 0.22           | 1.38         | 1.54         |
| 247                                                   | N247L   | 1.05 | 0.60            | 0.52           | 1.10         | 1.25         |
| 247                                                   | N247M   | 0.73 | 0.76            | 0.34           | 1.39         | 1.37         |
| 247                                                   | N247P   | 0.77 | 0.43            | 0.05           | 2.01         | 2.69         |
| 247                                                   | N247Q   | 0.49 | 0.60            | 0.41           | 1.77         | 1.71         |
| 247                                                   | N247R   | 0.71 | 0.46            | 0.16           | 1.64         | 1.85         |
| 247                                                   | N247S   | 0.73 | 0.52            | 0.05           | 2.08         | 1.91         |
| 247                                                   | N247T   | 0.62 | 0.88            | 0.68           | 1.16         | 1.39         |
| 247                                                   | N247V   | 0.49 | 0.67            | 0.34           | 2.16         | 1.64         |
| 247                                                   | N247Y   | 0.71 | 0.85            | 0.28           | 1.55         | 1.56         |
| 251                                                   | T251A   | 0.41 | 0.86            | 0.74           | 0.92         | 0.70         |
| 251                                                   | T251C   | 0.37 | 1.08            | 1.28           | 0.92         | 0.77         |
| 251                                                   | T251D   | 0.27 | 0.72            | 0.75           | 0.89         | 0.79         |
| 251                                                   | T251E   | 0.72 | 1.07            | 0.85           | 0.98         | 0.86         |
| 251                                                   | T251F   | 0.36 | 0.98            | 0.55           | 0.87         | 0.68         |
| 251                                                   | T251G   | 0.40 | 1.02            | 0.79           | 0.95         | 0.82         |
| 251                                                   | T251H   | 0.30 | 1.09            | 0.72           | 0.83         | 0.80         |
| 251                                                   | T251I   | 0.38 | 0.67            | 0.54           | 1.11         | 0.82         |
| 251                                                   | T251K   | 0.27 | 0.81            | 0.55           | 0.87         | 0.80         |
| 251                                                   | T251L   | 0.57 | 1.14            | 0.81           | 0.92         | 0.86         |
| 251                                                   | T251M   | 0.44 | 1.05            | 0.63           | 0.94         | 0.87         |
| 251                                                   | T251N   | 0.50 | 0.83            | 0.59           | 0.90         | 0.97         |
| 251                                                   | T251P   | 0.12 | 0.19            | 0.05           | 1.25         | 1.26         |
| 251                                                   | T251Q   | 0.48 | 1.01            | 0.77           | 0.95         | 0.92         |
| 251                                                   | T251R   | 0.58 | 1.02            | 0.62           | 0.96         | 0.89         |
| 251                                                   | T251S   | 0.67 | 1.05            | 0.78           | 0.89         | 0.87         |
| 251                                                   | T251V   | 0.91 | 1.04            | 0.77           | 0.99         | 0.98         |
| 251                                                   | T251W   | 0.30 | 0.83            | 0.54           | 0.97         | 0.90         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 251                                                   | T251Y   | 0.10 | 0.18            | 0.14           | 1.18         | 4.59         |
| 252                                                   | Q252A   | 0.43 | 0.95            | 0.85           | 0.95         | 0.73         |
| 252                                                   | Q252C   | 0.89 | 0.98            | 0.84           | 0.94         | 0.79         |
| 252                                                   | Q252D   | 0.21 | 0.65            | 0.65           | 1.00         | 0.61         |
| 252                                                   | Q252E   | 0.31 | 1.01            | 0.80           | 0.90         | 0.74         |
| 252                                                   | Q252F   | 0.39 | 0.82            | 0.52           | 1.15         | 0.72         |
| 252                                                   | Q252G   | 0.41 | 0.96            | 0.64           | 0.92         | 0.76         |
| 252                                                   | Q252H   | 0.78 | 0.97            | 0.63           | 0.98         | 0.84         |
| 252                                                   | Q252I   | 0.93 | 1.04            | 0.89           | 1.00         | 0.94         |
| 252                                                   | Q252K   | 0.60 | 0.63            | 0.53           | 1.53         | 1.18         |
| 252                                                   | Q252L   | 0.47 | 1.02            | 0.77           | 0.91         | 0.88         |
| 252                                                   | Q252M   | 0.54 | 1.04            | 0.69           | 0.94         | 0.86         |
| 252                                                   | Q252N   | 0.24 | 0.83            | 0.64           | 0.97         | 0.80         |
| 252                                                   | Q252P   | 0.16 | 0.12            | 0.09           | 1.25         | 1.12         |
| 252                                                   | Q252R   | 0.59 | 0.68            | 0.51           | 1.15         | 0.91         |
| 252                                                   | Q252S   | 0.53 | 1.01            | 0.82           | 0.92         | 0.88         |
| 252                                                   | Q252T   | 0.32 | 0.69            | 0.33           | 0.55         | 0.62         |
| 252                                                   | Q252V   | 0.52 | 0.83            | 0.40           | 1.12         | 1.12         |
| 252                                                   | Q252W   | 0.34 | 0.66            | 0.60           | 1.18         | 0.85         |
| 252                                                   | Q252Y   | 0.51 | 0.98            | 0.72           | 0.95         | 0.85         |
| 254                                                   | N254A   | 0.28 | 0.88            | 0.69           | 0.91         | 0.88         |
| 254                                                   | N254C   | 0.26 | 0.88            | 0.82           | 0.83         | 0.91         |
| 254                                                   | N254E   | 0.20 | 0.84            | 0.56           | 0.76         | 0.91         |
| 254                                                   | N254F   | 0.15 | 0.33            | 0.10           | 0.72         | 1.71         |
| 254                                                   | N254G   | 0.28 | 0.37            | 0.29           | 0.73         | 1.31         |
| 254                                                   | N254I   | 0.36 | 0.18            | 0.15           | 0.57         | 1.14         |
| 254                                                   | N254K   | 0.24 | 0.24            | 0.44           | 0.45         | 1.18         |
| 254                                                   | N254L   | 0.23 | 0.30            | 0.11           | 0.49         | 0.64         |
| 254                                                   | N254M   | 0.18 | 0.33            | 0.33           | 0.89         | 1.34         |
| 254                                                   | N254P   | 0.36 | 0.22            | 0.05           | 0.98         | 1.35         |
| 254                                                   | N254Q   | 0.39 | 0.26            | 0.14           | 0.85         | 1.07         |
| 254                                                   | N254R   | 0.36 | 0.14            | 0.08           | 1.49         | 1.94         |
| 254                                                   | N254S   | 0.29 | 0.49            | 0.19           | 0.87         | 1.24         |
| 254                                                   | N254T   | 0.22 | 0.53            | 0.50           | 0.97         | 1.34         |
| 254                                                   | N254V   | 1.22 | 0.98            | 0.90           | 1.05         | 1.08         |
| 254                                                   | N254W   | 0.26 | 0.17            | 0.12           | 1.05         | 1.02         |
| 254                                                   | N254Y   | 0.21 | 0.40            | 0.05           | 0.88         | 1.30         |
| 258                                                   | V258A   | 0.56 | 0.63            | 0.72           | 1.10         | 1.08         |
| 258                                                   | V258C   | 0.52 | 0.74            | 0.68           | 1.09         | 1.41         |
| 258                                                   | V258D   | 0.20 | 0.28            | 0.55           | 1.07         | 1.24         |
| 258                                                   | V258E   | 0.18 | 0.17            | 0.29           | 1.03         | 1.45         |
| 258                                                   | V258F   | 0.12 | 0.19            | 0.24           | 0.87         | 2.94         |
| 258                                                   | V258G   | 0.18 | 0.50            | 0.48           | 0.83         | 0.88         |
| 258                                                   | V258H   | 0.16 | 0.20            | 0.43           | 1.16         | 1.55         |
| 258                                                   | V258I   | 0.26 | 0.87            | 0.79           | 0.85         | 0.78         |
| 258                                                   | V258K   | 0.17 | 0.32            | 0.42           | 0.49         | 0.89         |
| 258                                                   | V258L   | 0.34 | 0.92            | 1.10           | 0.88         | 0.82         |
| 258                                                   | V258M   | 0.33 | 0.91            | 1.11           | 0.87         | 0.86         |
| 258                                                   | V258N   | 0.22 | 0.47            | 0.46           | 0.95         | 0.88         |
| 258                                                   | V258P   | 0.21 | 0.13            | 0.19           | 1.53         | 1.46         |
| 258                                                   | V258Q   | 0.18 | 0.23            | 0.38           | 0.53         | 1.08         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 258                                                   | V258R   | 0.23 | 0.15            | <i>0.05</i>    | 0.33         | 1.69         |
| 258                                                   | V258S   | 0.34 | 0.73            | 0.83           | 0.90         | 0.89         |
| 258                                                   | V258T   | 0.64 | 0.98            | 1.01           | 0.94         | 0.91         |
| 258                                                   | V258W   | 0.21 | 0.16            | 0.37           | 0.88         | 1.85         |
| 258                                                   | V258Y   | 0.18 | 0.14            | 0.14           | 1.13         | 1.36         |
| 266                                                   | H266A   | 0.26 | 0.41            | 0.39           | 0.42         | 0.57         |
| 266                                                   | H266C   | 0.08 | 0.15            | 0.28           | 1.20         | 2.86         |
| 266                                                   | H266D   | 0.17 | 0.24            | 0.23           | 1.06         | 1.47         |
| 266                                                   | H266E   | 0.20 | 0.20            | 0.30           | 2.06         | 3.59         |
| 266                                                   | H266I   | 0.21 | 0.14            | 0.16           | 3.49         | 1.64         |
| 266                                                   | H266K   | 0.18 | 0.25            | 0.13           | 1.55         | 0.94         |
| 266                                                   | H266L   | 0.24 | 0.14            | 0.20           | 0.85         | 1.74         |
| 266                                                   | H266M   | 0.18 | 0.19            | 0.29           | 6.85         | 0.91         |
| 266                                                   | H266N   | 0.49 | 0.73            | 0.48           | 0.79         | 0.63         |
| 266                                                   | H266P   | 0.14 | 0.14            | 0.26           | 0.98         | 1.25         |
| 266                                                   | H266R   | 0.30 | 0.17            | 0.23           | 1.16         | 1.30         |
| 266                                                   | H266S   | 0.19 | 0.29            | 0.26           | 0.68         | 0.87         |
| 266                                                   | H266T   | 0.20 | 0.27            | 0.18           | 2.35         | 1.95         |
| 266                                                   | H266V   | 0.21 | 0.20            | 0.30           | 0.67         | 1.54         |
| 266                                                   | H266W   | 0.22 | 0.27            | 0.11           | 0.86         | 0.32         |
| 266                                                   | H266Y   | 0.24 | 0.30            | 0.22           | 0.50         | 0.54         |
| 267                                                   | A267C   | 0.85 | 0.97            | 0.75           | 0.99         | 0.83         |
| 267                                                   | A267D   | 0.23 | 0.23            | 0.07           | 0.76         | 0.91         |
| 267                                                   | A267E   | 0.34 | 0.52            | 0.44           | 0.56         | 0.41         |
| 267                                                   | A267F   | 0.21 | 0.12            | <i>0.05</i>    | 1.58         | 0.93         |
| 267                                                   | A267G   | 0.86 | 1.00            | 0.36           | 0.44         | 0.13         |
| 267                                                   | A267H   | 0.38 | 0.35            | <i>0.05</i>    | 0.52         | 0.34         |
| 267                                                   | A267I   | 0.21 | 0.11            | <i>0.05</i>    | 0.82         | 0.86         |
| 267                                                   | A267K   | 0.20 | 0.21            | <i>0.05</i>    | 1.20         | 1.07         |
| 267                                                   | A267L   | 0.21 | 0.19            | 0.08           | 0.84         | 1.56         |
| 267                                                   | A267M   | 0.23 | 0.22            | 0.06           | 0.71         | 0.73         |
| 267                                                   | A267N   | 0.29 | 0.49            | <i>0.05</i>    | 0.47         | 0.45         |
| 267                                                   | A267P   | 0.21 | 0.28            | 0.14           | 0.72         | 0.95         |
| 267                                                   | A267R   | 0.18 | 0.16            | 0.20           | 1.08         | 0.95         |
| 267                                                   | A267S   | 0.80 | 0.93            | 0.67           | 0.83         | 0.55         |
| 267                                                   | A267T   | 0.34 | 0.68            | 0.17           | 0.46         | 0.24         |
| 267                                                   | A267V   | 0.23 | 0.52            | 0.21           | 0.28         | 0.31         |
| 267                                                   | A267W   | 0.25 | 0.22            | 0.16           | 0.91         | 0.83         |
| 267                                                   | A267Y   | 0.21 | 0.19            | 0.05           | 0.80         | 0.85         |
| 268                                                   | G268A   | 4.40 | 0.33            | 0.14           | 1.65         | 1.34         |
| 268                                                   | G268C   | 0.53 | 0.49            | 0.19           | 1.23         | 1.99         |
| 268                                                   | G268D   | 0.61 | 0.74            | 0.80           | 0.73         | 0.55         |
| 268                                                   | G268E   | 0.55 | 0.17            | 0.07           | 1.42         | 1.95         |
| 268                                                   | G268F   | 1.01 | 0.47            | 0.41           | 0.94         | 1.00         |
| 268                                                   | G268H   | 1.25 | <i>0.05</i>     | 0.35           | <i>0.05</i>  | <i>0.05</i>  |
| 268                                                   | G268I   | 0.76 | 0.65            | 0.80           | 1.21         | 0.84         |
| 268                                                   | G268K   | 0.86 | 0.75            | 0.77           | 0.83         | 0.75         |
| 268                                                   | G268L   | 0.76 | 0.44            | 0.27           | 1.10         | 1.31         |
| 268                                                   | G268M   | 0.55 | 0.36            | 0.14           | 0.49         | 0.31         |
| 268                                                   | G268N   | 0.62 | 0.37            | 0.33           | 0.86         | 1.32         |
| 268                                                   | G268P   | 0.49 | 0.53            | 0.53           | 1.16         | 1.29         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 268                                                   | G268Q   | 1.37 | 0.53            | 0.42           | 0.85         | 0.94         |
| 268                                                   | G268R   | 1.09 | 0.40            | 0.37           | 0.95         | 0.82         |
| 268                                                   | G268S   | 0.81 | 0.70            | 0.36           | 0.53         | 0.52         |
| 268                                                   | G268T   | 0.69 | 0.42            | 0.29           | 0.76         | 0.74         |
| 268                                                   | G268V   | 1.23 | 0.47            | 0.33           | 0.97         | 0.98         |
| 268                                                   | G268W   | 0.69 | 0.47            | 0.47           | 1.71         | 2.59         |
| 268                                                   | G268Y   | 0.61 | 0.25            | 0.06           | 0.76         | 1.28         |
| 272                                                   | W272A   | 0.93 | 0.52            | 0.20           | 0.59         | 0.33         |
| 272                                                   | W272C   | 0.57 | 0.43            | 0.13           | 0.40         | 0.34         |
| 272                                                   | W272D   | 0.84 | 0.51            | 0.24           | 0.41         | 0.10         |
| 272                                                   | W272E   | 0.67 | 0.47            | 0.12           | 0.55         | 0.41         |
| 272                                                   | W272F   | 0.59 | 0.73            | 0.23           | 0.72         | 0.46         |
| 272                                                   | W272G   | 0.89 | 0.54            | 0.13           | 0.74         | 0.43         |
| 272                                                   | W272H   | 0.07 | 0.27            | 0.05           | 1.91         | 0.37         |
| 272                                                   | W272I   | 0.67 | 0.55            | 0.16           | 0.44         | 0.20         |
| 272                                                   | W272K   | 0.74 | 0.31            | 0.05           | 0.35         | 0.53         |
| 272                                                   | W272L   | 0.67 | 0.59            | 0.20           | 0.53         | 0.42         |
| 272                                                   | W272M   | 1.42 | 0.62            | 0.14           | 0.61         | 0.32         |
| 272                                                   | W272N   | 0.92 | 0.51            | 0.10           | 0.48         | 0.33         |
| 272                                                   | W272P   | 0.61 | 0.49            | 0.14           | 0.62         | 0.44         |
| 272                                                   | W272Q   | 0.14 | 0.07            | 0.11           | 0.63         | 0.92         |
| 272                                                   | W272R   | 0.89 | 0.36            | 0.05           | 0.58         | 0.44         |
| 272                                                   | W272S   | 1.03 | 0.51            | 0.32           | 0.75         | 0.43         |
| 272                                                   | W272T   | 0.85 | 0.49            | 0.18           | 0.52         | 0.31         |
| 272                                                   | W272V   | 0.74 | 0.53            | 0.07           | 0.27         | 0.15         |
| 272                                                   | W272Y   | 0.85 | 0.67            | 0.28           | 0.50         | 0.45         |
| 274                                                   | A274C   | 0.23 | 0.47            | 0.52           | 2.95         | 0.43         |
| 274                                                   | A274D   | 0.16 | 0.58            | 0.55           | 0.79         | 0.43         |
| 274                                                   | A274E   | 0.32 | 0.99            | 0.87           | 0.98         | 0.80         |
| 274                                                   | A274F   | 0.33 | 0.85            | 0.42           | 0.91         | 0.89         |
| 274                                                   | A274G   | 0.34 | 1.02            | 0.53           | 0.82         | 0.65         |
| 274                                                   | A274H   | 0.40 | 0.83            | 0.37           | 0.91         | 0.67         |
| 274                                                   | A274I   | 0.26 | 0.88            | 0.34           | 0.84         | 0.71         |
| 274                                                   | A274K   | 0.26 | 0.78            | 0.33           | 0.91         | 0.85         |
| 274                                                   | A274L   | 0.42 | 0.88            | 0.49           | 0.96         | 0.75         |
| 274                                                   | A274M   | 0.31 | 0.63            | 0.48           | 1.23         | 0.84         |
| 274                                                   | A274N   | 0.41 | 0.97            | 0.67           | 0.95         | 0.89         |
| 274                                                   | A274P   | 0.26 | 0.74            | 0.43           | 0.95         | 0.51         |
| 274                                                   | A274Q   | 0.44 | 0.86            | 0.73           | 0.97         | 0.98         |
| 274                                                   | A274R   | 0.51 | 0.99            | 0.38           | 0.97         | 0.91         |
| 274                                                   | A274S   | 0.46 | 0.96            | 0.71           | 0.97         | 0.84         |
| 274                                                   | A274T   | 0.41 | 0.55            | 0.47           | 1.14         | 1.03         |
| 274                                                   | A274V   | 0.38 | 0.87            | 0.51           | 0.96         | 0.85         |
| 274                                                   | A274W   | 0.56 | 0.70            | 0.37           | 1.03         | 0.88         |
| 274                                                   | A274Y   | 0.11 | 0.07            | 0.10           | 1.25         | 0.33         |
| 275                                                   | N275A   | 1.11 | 0.93            | 0.87           | 1.02         | 0.97         |
| 275                                                   | N275C   | 0.74 | 0.76            | 0.71           | 1.01         | 0.95         |
| 275                                                   | N275D   | 0.40 | 0.78            | 0.68           | 0.97         | 1.00         |
| 275                                                   | N275E   | 0.65 | 0.90            | 0.57           | 1.02         | 0.96         |
| 275                                                   | N275F   | 1.34 | 0.86            | 0.71           | 0.98         | 0.98         |
| 275                                                   | N275G   | 0.20 | 0.67            | 0.17           | 0.31         | 0.18         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 275                                                   | N275K   | 0.27 | 0.84            | 0.34           | 0.91         | 0.94         |
| 275                                                   | N275L   | 0.98 | 0.90            | 0.70           | 0.92         | 1.00         |
| 275                                                   | N275M   | 0.65 | 0.87            | 0.63           | 1.04         | 0.98         |
| 275                                                   | N275Q   | 0.58 | 0.88            | 0.56           | 1.03         | 0.95         |
| 275                                                   | N275R   | 0.64 | 0.68            | 0.27           | 0.96         | 1.04         |
| 275                                                   | N275S   | 0.61 | 0.99            | 0.57           | 0.91         | 0.87         |
| 275                                                   | N275T   | 0.70 | 0.85            | 0.53           | 0.96         | 1.01         |
| 275                                                   | N275V   | 0.81 | 0.89            | 0.53           | 0.95         | 0.97         |
| 275                                                   | N275W   | 1.07 | 0.92            | 0.69           | 1.00         | 1.02         |
| 275                                                   | N275Y   | 0.37 | 0.64            | 0.43           | 0.94         | 0.96         |
| 281                                                   | Q281A   | 1.53 | 0.81            | 0.66           | 0.85         | 0.32         |
| 281                                                   | Q281C   | 1.70 | 0.88            | 0.70           | 0.73         | 0.43         |
| 281                                                   | Q281D   | 1.10 | 0.60            | 0.43           | 1.33         | 0.95         |
| 281                                                   | Q281E   | 2.50 | 0.89            | 0.85           | 1.10         | 0.63         |
| 281                                                   | Q281F   | 0.76 | 0.72            | 0.15           | 1.31         | 0.97         |
| 281                                                   | Q281G   | 1.04 | 0.75            | 0.46           | 1.02         | 1.35         |
| 281                                                   | Q281I   | 0.62 | 0.63            | 0.08           | 1.74         | 1.66         |
| 281                                                   | Q281L   | 0.78 | 0.68            | 0.31           | 1.79         | 1.51         |
| 281                                                   | Q281N   | 1.38 | 0.85            | 0.46           | 1.09         | 0.85         |
| 281                                                   | Q281T   | 0.59 | 0.72            | 0.22           | 1.64         | 1.10         |
| 281                                                   | Q281V   | 1.46 | 0.94            | 0.69           | 1.22         | 0.86         |
| 285                                                   | N285A   | 0.88 | 1.01            | 0.71           | 0.50         | 0.62         |
| 285                                                   | N285C   | 0.46 | 1.02            | 0.70           | 1.22         | 1.23         |
| 285                                                   | N285D   | 0.35 | 0.70            | 0.59           | 1.99         | 2.24         |
| 285                                                   | N285F   | 0.27 | 0.67            | 0.15           | 2.16         | 1.85         |
| 285                                                   | N285G   | 0.47 | 1.24            | 0.62           | 1.02         | 0.92         |
| 285                                                   | N285H   | 0.60 | 1.04            | 0.30           | 0.93         | 1.11         |
| 285                                                   | N285K   | 0.36 | 0.76            | 0.25           | 1.49         | 2.79         |
| 285                                                   | N285L   | 0.46 | 1.15            | 0.68           | 1.16         | 1.05         |
| 285                                                   | N285P   | 0.18 | 0.27            | 0.18           | 6.33         | 7.75         |
| 285                                                   | N285S   | 0.26 | 0.77            | 0.21           | 2.14         | 2.16         |
| 285                                                   | N285T   | 0.40 | 0.78            | 0.33           | 1.66         | 2.14         |
| 285                                                   | N285V   | 0.19 | 0.84            | 0.18           | 2.84         | 3.86         |
| 285                                                   | N285W   | 0.39 | 1.21            | 0.38           | 1.60         | 2.07         |
| 285                                                   | N285Y   | 0.72 | 1.15            | 1.19           | 1.15         | 0.91         |
| 288                                                   | K288A   | 0.41 | 0.61            | 0.68           | 1.52         | 2.84         |
| 288                                                   | K288C   | 0.41 | 0.95            | 0.63           | 0.72         | 1.49         |
| 288                                                   | K288D   | 0.23 | 0.23            | 0.05           | 5.77         | 5.10         |
| 288                                                   | K288E   | 0.38 | 0.89            | 0.81           | 0.65         | 1.60         |
| 288                                                   | K288F   | 0.36 | 0.75            | 0.29           | 1.53         | 2.74         |
| 288                                                   | K288G   | 0.23 | 0.40            | 0.12           | 2.22         | 4.09         |
| 288                                                   | K288H   | 0.32 | 0.90            | 0.48           | 1.09         | 1.54         |
| 288                                                   | K288I   | 0.40 | 0.75            | 0.37           | 1.51         | 2.48         |
| 288                                                   | K288L   | 0.80 | 0.96            | 0.81           | 0.60         | 0.82         |
| 288                                                   | K288N   | 0.71 | 0.98            | 0.84           | 1.08         | 1.68         |
| 288                                                   | K288P   | 0.26 | 0.34            | 0.05           | 5.00         | 5.39         |
| 288                                                   | K288Q   | 0.23 | 0.80            | 0.18           | 1.23         | 2.51         |
| 288                                                   | K288R   | 0.27 | 0.42            | 0.05           | 2.13         | 3.33         |
| 288                                                   | K288S   | 0.19 | 0.71            | 0.21           | 2.02         | 2.72         |
| 288                                                   | K288T   | 0.25 | 0.71            | 0.35           | 1.27         | 2.96         |
| 288                                                   | K288V   | 0.31 | 0.84            | 0.38           | 1.67         | 1.87         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 289                                                   | N289A   | 0.39 | 0.70            | 0.90           | 0.98         | 0.87         |
| 289                                                   | N289C   | 0.21 | 0.10            | 0.50           | 6.35         | 7.25         |
| 289                                                   | N289D   | 0.84 | 0.96            | 0.87           | 0.93         | 0.92         |
| 289                                                   | N289E   | 0.13 | 0.26            | 0.63           | 1.03         | 1.48         |
| 289                                                   | N289F   | 0.14 | 0.27            | 0.39           | 0.85         | 0.78         |
| 289                                                   | N289G   | 0.17 | 0.42            | 0.79           | 0.72         | 0.58         |
| 289                                                   | N289H   | 0.15 | 0.54            | 0.66           | 0.94         | 0.91         |
| 289                                                   | N289I   | 0.16 | 0.20            | 0.05           | 0.64         | 0.68         |
| 289                                                   | N289K   | 0.31 | 0.79            | 0.66           | 0.88         | 0.85         |
| 289                                                   | N289L   | 0.16 | 0.39            | 0.48           | 0.92         | 1.00         |
| 289                                                   | N289M   | 0.18 | 0.52            | 0.14           | 0.80         | 0.74         |
| 289                                                   | N289Q   | 0.18 | 0.49            | 0.45           | 0.63         | 0.66         |
| 289                                                   | N289R   | 0.41 | 0.87            | 0.23           | 0.91         | 0.84         |
| 289                                                   | N289S   | 0.44 | 1.05            | 0.85           | 0.84         | 0.88         |
| 289                                                   | N289T   | 0.20 | 0.58            | 0.79           | 0.90         | 0.86         |
| 289                                                   | N289V   | 0.22 | 0.64            | 0.33           | 0.92         | 0.83         |
| 289                                                   | N289W   | 0.19 | 0.43            | 0.59           | 0.95         | 0.98         |
| 289                                                   | N289Y   | 0.24 | 0.62            | 0.47           | 0.78         | 0.91         |
| 291                                                   | S291A   | 1.22 | 2.27            | 2.57           | 0.90         | 1.26         |
| 291                                                   | S291C   | 2.02 | 2.57            | 3.26           | 0.99         | 1.96         |
| 291                                                   | S291D   | 0.40 | 0.28            | 0.24           | 1.20         | 5.19         |
| 291                                                   | S291E   | 2.53 | 2.54            | 3.48           | 1.02         | 1.91         |
| 291                                                   | S291F   | 1.57 | 2.22            | 1.63           | 1.02         | 1.89         |
| 291                                                   | S291G   | 0.60 | 0.24            | 0.25           | 1.08         | 0.92         |
| 291                                                   | S291H   | 2.52 | 2.45            | 2.33           | 1.06         | 1.94         |
| 291                                                   | S291I   | 0.81 | 0.95            | 0.53           | 1.09         | 1.32         |
| 291                                                   | S291K   | 1.26 | 2.23            | 1.10           | 1.05         | 1.58         |
| 291                                                   | S291L   | 1.28 | 1.99            | 1.50           | 0.91         | 1.23         |
| 291                                                   | S291M   | 1.88 | 2.50            | 2.13           | 1.01         | 1.91         |
| 291                                                   | S291N   | 0.46 | 0.21            | 0.05           | 2.48         | 2.00         |
| 291                                                   | S291P   | 0.75 | 0.72            | 0.88           | 1.18         | 1.19         |
| 291                                                   | S291T   | 1.56 | 2.62            | 2.22           | 1.11         | 2.02         |
| 291                                                   | S291V   | 0.99 | 0.61            | 0.24           | 1.25         | 1.24         |
| 291                                                   | S291W   | 1.09 | 2.20            | 1.87           | 1.01         | 1.50         |
| 291                                                   | S291Y   | 0.90 | 1.18            | 0.85           | 0.95         | 1.48         |
| 292                                                   | S292A   | 0.96 | 0.97            | 1.12           | 0.99         | 0.94         |
| 292                                                   | S292C   | 0.44 | 0.53            | 0.61           | 1.35         | 1.67         |
| 292                                                   | S292D   | 0.65 | 0.79            | 0.74           | 0.91         | 1.11         |
| 292                                                   | S292E   | 0.50 | 0.73            | 0.47           | 1.14         | 1.40         |
| 292                                                   | S292F   | 0.74 | 0.87            | 0.48           | 0.86         | 1.05         |
| 292                                                   | S292G   | 0.67 | 0.99            | 0.68           | 0.74         | 0.80         |
| 292                                                   | S292H   | 0.35 | 0.67            | 0.63           | 1.16         | 1.90         |
| 292                                                   | S292I   | 0.41 | 0.79            | 0.46           | 0.62         | 0.83         |
| 292                                                   | S292K   | 0.85 | 0.84            | 0.72           | 0.78         | 0.75         |
| 292                                                   | S292L   | 0.31 | 0.61            | 0.65           | 0.59         | 0.60         |
| 292                                                   | S292M   | 0.75 | 0.99            | 0.74           | 0.72         | 0.87         |
| 292                                                   | S292N   | 0.69 | 0.99            | 0.75           | 0.90         | 1.06         |
| 292                                                   | S292P   | 0.42 | 0.52            | 0.34           | 0.53         | 0.85         |
| 292                                                   | S292Q   | 0.55 | 0.87            | 0.58           | 0.97         | 0.79         |
| 292                                                   | S292R   | 1.12 | 0.98            | 0.45           | 0.84         | 0.85         |
| 292                                                   | S292T   | 0.84 | 1.01            | 0.65           | 0.86         | 0.92         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 292                                                   | S292V   | 0.44 | 0.70            | 0.51           | 0.95         | 1.25         |
| 292                                                   | S292W   | 0.91 | 1.10            | 0.82           | 0.83         | 0.64         |
| 292                                                   | S292Y   | 1.20 | 0.94            | 0.62           | 1.00         | 0.91         |
| 293                                                   | P293A   | 0.34 | 0.93            | 0.85           | 0.94         | 0.76         |
| 293                                                   | P293C   | 0.21 | 0.46            | 0.51           | 0.96         | 0.58         |
| 293                                                   | P293D   | 0.30 | 0.14            | 0.25           | 0.25         | 0.54         |
| 293                                                   | P293E   | 0.13 | 0.14            | 0.23           | 0.94         | 1.08         |
| 293                                                   | P293F   | 0.16 | 0.18            | 0.22           | 0.64         | 0.94         |
| 293                                                   | P293G   | 0.19 | 0.40            | 0.59           | 0.72         | 0.53         |
| 293                                                   | P293H   | 0.17 | 0.14            | 0.14           | 0.62         | 1.01         |
| 293                                                   | P293I   | 0.17 | 0.29            | 0.30           | 0.65         | 0.92         |
| 293                                                   | P293K   | 0.13 | 0.21            | 0.53           | 4.29         | 0.89         |
| 293                                                   | P293L   | 0.16 | 0.27            | 0.21           | 0.80         | 0.51         |
| 293                                                   | P293M   | 0.15 | 0.24            | 0.12           | 5.43         | 0.67         |
| 293                                                   | P293N   | 0.16 | 0.31            | 0.24           | 0.63         | 0.92         |
| 293                                                   | P293Q   | 0.22 | 0.16            | 0.17           | 0.29         | 0.54         |
| 293                                                   | P293R   | 0.15 | 0.31            | 0.62           | 1.31         | 0.86         |
| 293                                                   | P293S   | 0.18 | 0.38            | 0.24           | 1.04         | 0.74         |
| 293                                                   | P293T   | 0.18 | 0.28            | 0.12           | 0.44         | 0.78         |
| 293                                                   | P293V   | 0.17 | 0.77            | 0.60           | 0.93         | 0.73         |
| 293                                                   | P293W   | 0.18 | 0.11            | 0.16           | 2.77         | 9.61         |
| 293                                                   | P293Y   | 0.13 | 0.08            | 0.11           | 0.31         | 1.24         |
| 294                                                   | R294A   | 0.97 | 1.06            | 0.89           | 0.92         | 0.77         |
| 294                                                   | R294C   | 0.70 | 1.15            | 0.74           | 1.01         | 1.16         |
| 294                                                   | R294D   | 0.75 | 0.94            | 0.85           | 0.94         | 1.09         |
| 294                                                   | R294E   | 0.73 | 0.82            | 0.66           | 1.02         | 1.20         |
| 294                                                   | R294F   | 0.37 | 0.52            | 0.54           | 0.90         | 1.56         |
| 294                                                   | R294G   | 1.25 | 0.93            | 0.84           | 0.71         | 0.87         |
| 294                                                   | R294H   | 0.96 | 0.93            | 0.55           | 0.97         | 1.18         |
| 294                                                   | R294I   | 0.77 | 0.84            | 0.72           | 0.98         | 1.01         |
| 294                                                   | R294K   | 1.34 | 0.94            | 0.69           | 1.04         | 1.20         |
| 294                                                   | R294L   | 0.54 | 1.06            | 1.03           | 1.05         | 1.22         |
| 294                                                   | R294M   | 0.34 | 0.76            | 0.78           | 0.81         | 1.78         |
| 294                                                   | R294N   | 0.50 | 0.80            | 0.64           | 1.30         | 1.06         |
| 294                                                   | R294P   | 0.48 | 0.86            | 0.74           | 0.74         | 0.99         |
| 294                                                   | R294Q   | 0.50 | 0.79            | 0.75           | 1.23         | 1.30         |
| 294                                                   | R294S   | 0.64 | 1.00            | 0.57           | 1.32         | 1.47         |
| 294                                                   | R294T   | 1.12 | 1.03            | 0.97           | 1.02         | 1.10         |
| 294                                                   | R294V   | 0.83 | 0.96            | 0.83           | 0.82         | 1.03         |
| 294                                                   | R294W   | 1.08 | 1.01            | 0.57           | 0.56         | 0.58         |
| 294                                                   | R294Y   | 0.26 | 0.43            | 0.31           | 2.39         | 3.19         |
| 303                                                   | V303A   | 0.30 | 0.46            | 0.33           | 0.40         | 0.38         |
| 303                                                   | V303E   | 0.21 | 0.12            | 0.24           | 2.70         | 4.92         |
| 303                                                   | V303F   | 0.22 | 0.14            | 0.16           | 0.70         | 0.93         |
| 303                                                   | V303G   | 0.23 | 0.24            | 0.29           | 0.48         | 0.86         |
| 303                                                   | V303H   | 0.29 | 0.13            | 0.31           | 0.84         | 2.17         |
| 303                                                   | V303I   | 0.85 | 1.07            | 0.68           | 0.67         | 0.37         |
| 303                                                   | V303K   | 0.24 | 0.15            | 0.31           | 1.05         | 1.94         |
| 303                                                   | V303L   | 0.29 | 0.69            | 0.19           | 0.17         | 0.16         |
| 303                                                   | V303M   | 0.18 | 0.29            | 0.13           | 0.47         | 44.68        |
| 303                                                   | V303N   | 0.25 | 0.30            | 0.25           | 0.46         | 0.65         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 303                                                   | V303P   | 0.30 | 0.13            | 0.13           | 1.31         | 2.70         |
| 303                                                   | V303Q   | 0.32 | 0.16            | 0.20           | 1.08         | 1.32         |
| 303                                                   | V303R   | 0.19 | 0.14            | 0.21           | 1.74         | 1.78         |
| 303                                                   | V303S   | 0.26 | 0.37            | 0.14           | 1.00         | 0.83         |
| 303                                                   | V303T   | 0.39 | 0.98            | 0.48           | 0.70         | 0.45         |
| 303                                                   | V303W   | 0.18 | 0.15            | 0.23           | 1.50         | 1.97         |
| 303                                                   | V303Y   | 0.23 | 0.19            | 0.15           | 2.71         | 1.26         |
| 304                                                   | A304C   | 0.76 | 0.47            | 0.36           | 0.62         | 0.39         |
| 304                                                   | A304D   | 1.25 | 0.13            | 0.16           | 15.46        | 16.85        |
| 304                                                   | A304E   | 0.32 | 0.08            | 0.30           | 3.56         | 3.07         |
| 304                                                   | A304F   | 0.49 | 0.08            | 0.15           | 1.43         | 1.58         |
| 304                                                   | A304G   | 1.16 | 0.65            | 0.42           | 0.80         | 0.66         |
| 304                                                   | A304H   | 0.73 | 0.09            | 0.28           | 0.97         | 1.38         |
| 304                                                   | A304I   | 0.66 | 0.15            | 0.39           | 1.93         | 1.79         |
| 304                                                   | A304K   | 0.82 | 0.15            | 0.17           | 1.63         | 1.59         |
| 304                                                   | A304L   | 0.52 | 0.10            | 0.25           | 1.55         | 2.80         |
| 304                                                   | A304M   | 0.82 | 0.11            | 0.12           | 1.89         | 1.39         |
| 304                                                   | A304N   | 0.65 | 0.11            | 0.14           | 5.49         | 17.77        |
| 304                                                   | A304P   | 0.31 | 0.11            | 0.14           | 1.16         | 2.23         |
| 304                                                   | A304Q   | 0.66 | 0.13            | 0.22           | 2.05         | 1.82         |
| 304                                                   | A304R   | 1.17 | 0.14            | 0.22           | 1.45         | 1.27         |
| 304                                                   | A304S   | 0.42 | 0.83            | 0.82           | 0.83         | 0.67         |
| 304                                                   | A304T   | 0.58 | 0.19            | 0.22           | 1.08         | 1.33         |
| 304                                                   | A304V   | 0.53 | 0.20            | 0.31           | 0.80         | 1.47         |
| 304                                                   | A304W   | 0.50 | 0.12            | 0.23           | 1.41         | 2.07         |
| 304                                                   | A304Y   | 0.54 | 0.05            | 0.32           | 3.22         | 4.73         |
| 306                                                   | Y306A   | 0.35 | 0.77            | 0.39           | 0.10         | 0.15         |
| 306                                                   | Y306C   | 0.16 | 0.49            | 0.27           | 0.21         | 0.91         |
| 306                                                   | Y306D   | 0.18 | 0.39            | 0.31           | 0.15         | 0.30         |
| 306                                                   | Y306E   | 0.15 | 0.45            | 0.22           | 0.15         | 0.46         |
| 306                                                   | Y306G   | 0.16 | 0.45            | 0.18           | 0.32         | 0.59         |
| 306                                                   | Y306H   | 0.32 | 0.60            | 0.19           | 0.07         | 0.39         |
| 306                                                   | Y306I   | 0.34 | 0.81            | 0.23           | 0.09         | 0.17         |
| 306                                                   | Y306K   | 0.19 | 0.17            | 0.28           | 0.41         | 0.58         |
| 306                                                   | Y306L   | 0.16 | 0.55            | 0.29           | 0.16         | 0.47         |
| 306                                                   | Y306N   | 0.22 | 0.60            | 0.13           | 0.08         | 0.21         |
| 306                                                   | Y306P   | 0.21 | 0.28            | 0.22           | 0.34         | 0.74         |
| 306                                                   | Y306Q   | 0.23 | 0.70            | 0.17           | 0.11         | 0.25         |
| 306                                                   | Y306R   | 0.20 | 0.51            | 0.23           | 0.18         | 0.35         |
| 306                                                   | Y306S   | 0.35 | 0.80            | 0.26           | 0.06         | 0.15         |
| 306                                                   | Y306T   | 0.60 | 0.91            | 0.17           | 0.09         | 0.13         |
| 306                                                   | Y306V   | 0.35 | 0.82            | 0.17           | 0.09         | 0.12         |
| 306                                                   | Y306W   | 0.50 | 0.78            | 0.68           | 0.56         | 0.75         |
| 307                                                   | N307A   | 0.13 | 0.23            | 0.25           | 0.54         | 0.73         |
| 307                                                   | N307C   | 0.17 | 0.34            | 0.22           | 0.19         | 0.38         |
| 307                                                   | N307D   | 0.20 | 0.77            | 0.57           | 0.15         | 0.24         |
| 307                                                   | N307E   | 0.11 | 0.56            | 0.16           | 0.53         | 0.82         |
| 307                                                   | N307F   | 0.13 | 0.24            | 0.20           | 0.43         | 0.88         |
| 307                                                   | N307G   | 0.25 | 0.59            | 0.25           | 0.20         | 0.52         |
| 307                                                   | N307H   | 0.15 | 0.22            | 0.23           | 0.40         | 0.61         |
| 307                                                   | N307I   | 0.23 | 0.42            | 0.20           | 0.15         | 0.33         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 307                                                   | N307K   | 0.14 | 0.21            | 0.26           | 0.38         | 1.07         |
| 307                                                   | N307L   | 0.12 | 0.30            | 0.21           | 0.34         | 0.69         |
| 307                                                   | N307M   | 0.15 | 0.33            | 0.17           | 0.33         | 0.77         |
| 307                                                   | N307P   | 0.14 | 0.15            | 0.23           | 0.83         | 2.18         |
| 307                                                   | N307Q   | 0.17 | 0.44            | 0.25           | 0.19         | 0.59         |
| 307                                                   | N307R   | 0.18 | 0.21            | 0.17           | 0.56         | 0.85         |
| 307                                                   | N307S   | 0.27 | 0.70            | 0.24           | 0.14         | 0.25         |
| 307                                                   | N307T   | 0.14 | 0.31            | 0.12           | 0.53         | 0.74         |
| 307                                                   | N307V   | 0.15 | 0.29            | 0.25           | 0.27         | 0.82         |
| 307                                                   | N307W   | 0.20 | 0.21            | 0.11           | 0.61         | 0.99         |
| 307                                                   | N307Y   | 0.20 | 0.18            | 0.19           | 0.30         | 1.06         |
| 312                                                   | T312A   | 1.35 | 2.81            | 1.85           | 0.20         | 0.23         |
| 312                                                   | T312C   | 0.80 | 0.73            | 0.48           | 0.67         | 0.88         |
| 312                                                   | T312D   | 1.09 | 1.51            | 0.93           | 0.22         | 0.28         |
| 312                                                   | T312E   | 1.39 | 2.50            | 1.93           | 0.31         | 0.13         |
| 312                                                   | T312F   | 1.07 | 1.90            | 0.98           | 0.25         | 0.05         |
| 312                                                   | T312G   | 1.17 | 2.33            | 0.47           | 0.16         | 0.27         |
| 312                                                   | T312H   | 0.98 | 1.48            | 0.64           | 0.70         | 0.46         |
| 312                                                   | T312I   | 1.21 | 2.10            | 0.21           | 0.20         | 0.06         |
| 312                                                   | T312K   | 0.78 | 2.31            | 0.87           | 0.36         | 0.24         |
| 312                                                   | T312L   | 0.76 | 0.69            | 0.23           | 1.03         | 0.05         |
| 312                                                   | T312N   | 0.66 | 0.82            | 0.30           | 0.80         | 0.22         |
| 312                                                   | T312P   | 1.18 | 2.16            | 0.42           | 0.29         | 0.14         |
| 312                                                   | T312Q   | 0.81 | 1.81            | 0.48           | 0.43         | 0.25         |
| 312                                                   | T312R   | 1.20 | 2.32            | 0.76           | 0.32         | 0.32         |
| 312                                                   | T312S   | 1.32 | 2.02            | 1.79           | 0.92         | 1.28         |
| 312                                                   | T312V   | 0.35 | 0.31            | 0.10           | 1.17         | 2.92         |
| 312                                                   | T312W   | 1.11 | 1.34            | 0.32           | 0.19         | 0.38         |
| 312                                                   | T312Y   | 1.05 | 1.62            | 0.71           | 0.24         | 0.13         |
| 313                                                   | S313A   | 0.34 | 1.08            | 0.85           | 0.77         | 0.60         |
| 313                                                   | S313C   | 0.34 | 0.94            | 0.58           | 0.79         | 0.84         |
| 313                                                   | S313D   | 0.43 | 1.04            | 1.32           | 1.01         | 0.99         |
| 313                                                   | S313E   | 0.46 | 1.20            | 0.90           | 0.99         | 0.88         |
| 313                                                   | S313F   | 0.59 | 1.02            | 0.54           | 0.82         | 0.78         |
| 313                                                   | S313G   | 0.73 | 1.05            | 0.30           | 0.30         | 0.13         |
| 313                                                   | S313I   | 0.69 | 1.06            | 0.69           | 0.90         | 0.90         |
| 313                                                   | S313K   | 1.64 | 1.07            | 0.53           | 1.04         | 1.16         |
| 313                                                   | S313L   | 0.86 | 1.08            | 0.55           | 0.62         | 0.29         |
| 313                                                   | S313M   | 0.50 | 1.12            | 0.72           | 0.74         | 0.57         |
| 313                                                   | S313P   | 1.00 | 0.95            | 0.55           | 0.56         | 0.31         |
| 313                                                   | S313Q   | 0.74 | 1.04            | 0.81           | 0.93         | 1.01         |
| 313                                                   | S313R   | 0.55 | 1.05            | 0.37           | 0.83         | 0.76         |
| 313                                                   | S313T   | 0.98 | 1.15            | 1.21           | 1.00         | 1.16         |
| 313                                                   | S313V   | 0.53 | 1.05            | 0.81           | 0.87         | 0.82         |
| 313                                                   | S313W   | 0.70 | 1.00            | 0.63           | 0.78         | 0.46         |
| 313                                                   | S313Y   | 0.52 | 0.97            | 0.49           | 0.67         | 0.31         |
| 316                                                   | S316A   | 0.59 | 0.96            | 1.36           | 1.49         | 1.15         |
| 316                                                   | S316C   | 0.77 | 0.87            | 1.10           | 1.43         | 1.15         |
| 316                                                   | S316D   | 0.85 | 0.98            | 1.18           | 0.63         | 0.46         |
| 316                                                   | S316E   | 0.13 | 0.12            | 0.05           | 1.22         | 1.85         |
| 316                                                   | S316F   | 0.65 | 0.99            | 0.68           | 1.06         | 0.83         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 316                                                   | S316G   | 0.69 | 0.97            | 0.60           | 0.71         | 0.79         |
| 316                                                   | S316H   | 0.63 | 1.05            | 0.48           | 1.28         | 0.80         |
| 316                                                   | S316I   | 0.50 | 0.90            | 0.51           | 1.62         | 0.91         |
| 316                                                   | S316K   | 0.78 | 0.99            | 0.66           | 0.97         | 0.85         |
| 316                                                   | S316L   | 1.27 | 1.00            | 1.19           | 0.99         | 0.88         |
| 316                                                   | S316M   | 0.15 | 0.07            | 0.15           | 14.10        | 22.76        |
| 316                                                   | S316N   | 0.73 | 1.06            | 0.92           | 0.76         | 0.70         |
| 316                                                   | S316P   | 0.98 | 0.95            | 1.46           | 1.74         | 1.48         |
| 316                                                   | S316Q   | 0.82 | 0.97            | 0.91           | 0.85         | 0.74         |
| 316                                                   | S316R   | 1.18 | 0.93            | 0.78           | 1.20         | 0.76         |
| 316                                                   | S316T   | 0.92 | 0.92            | 0.89           | 0.89         | 0.68         |
| 316                                                   | S316V   | 0.76 | 0.98            | 0.88           | 1.12         | 0.94         |
| 316                                                   | S316W   | 1.13 | 0.92            | 1.16           | 1.54         | 1.39         |
| 316                                                   | S316Y   | 0.85 | 0.93            | 0.60           | 0.56         | 0.87         |
| 319                                                   | Q319A   | 0.55 | 1.03            | 0.96           | 1.06         | 1.00         |
| 319                                                   | Q319C   | 0.52 | 0.95            | 0.76           | 1.04         | 0.91         |
| 319                                                   | Q319D   | 0.78 | 1.01            | 0.86           | 0.95         | 0.80         |
| 319                                                   | Q319E   | 0.83 | 1.08            | 0.92           | 1.03         | 0.88         |
| 319                                                   | Q319F   | 0.71 | 0.98            | 0.40           | 1.06         | 0.94         |
| 319                                                   | Q319G   | 0.81 | 0.98            | 0.41           | 0.78         | 0.56         |
| 319                                                   | Q319I   | 0.71 | 1.04            | 0.60           | 1.03         | 1.01         |
| 319                                                   | Q319K   | 0.84 | 1.00            | 0.45           | 1.04         | 1.12         |
| 319                                                   | Q319L   | 0.48 | 1.03            | 0.61           | 1.10         | 1.13         |
| 319                                                   | Q319M   | 0.52 | 1.03            | 0.89           | 0.96         | 0.98         |
| 319                                                   | Q319N   | 0.81 | 1.05            | 0.70           | 0.96         | 0.92         |
| 319                                                   | Q319P   | 0.44 | 0.91            | 0.05           | 0.26         | 0.30         |
| 319                                                   | Q319R   | 0.71 | 1.01            | 0.49           | 1.06         | 1.14         |
| 319                                                   | Q319S   | 1.06 | 1.03            | 0.69           | 0.99         | 1.00         |
| 319                                                   | Q319T   | 0.93 | 1.06            | 0.77           | 0.97         | 0.95         |
| 319                                                   | Q319V   | 0.65 | 1.02            | 0.69           | 1.00         | 1.02         |
| 319                                                   | Q319W   | 0.50 | 0.85            | 0.59           | 1.06         | 0.99         |
| 319                                                   | Q319Y   | 1.02 | 1.01            | 0.81           | 1.01         | 1.05         |
| 322                                                   | A322C   | 0.52 | 0.98            | 0.98           | 0.95         | 0.85         |
| 322                                                   | A322E   | 0.56 | 1.02            | 1.05           | 0.99         | 0.98         |
| 322                                                   | A322F   | 0.58 | 0.99            | 0.64           | 0.81         | 0.69         |
| 322                                                   | A322G   | 0.68 | 0.94            | 0.70           | 0.97         | 0.78         |
| 322                                                   | A322H   | 0.48 | 0.97            | 0.50           | 0.91         | 0.84         |
| 322                                                   | A322I   | 0.58 | 0.98            | 0.79           | 0.96         | 0.94         |
| 322                                                   | A322K   | 0.56 | 1.00            | 0.56           | 0.95         | 1.02         |
| 322                                                   | A322L   | 0.57 | 1.00            | 0.57           | 0.94         | 0.93         |
| 322                                                   | A322M   | 0.81 | 1.05            | 0.97           | 1.00         | 0.96         |
| 322                                                   | A322N   | 0.45 | 0.98            | 0.73           | 0.92         | 0.96         |
| 322                                                   | A322P   | 0.88 | 1.02            | 1.06           | 1.09         | 1.21         |
| 322                                                   | A322Q   | 0.05 | 0.05            | 0.05           | 0.05         | 0.05         |
| 322                                                   | A322R   | 0.51 | 1.01            | 0.33           | 1.01         | 1.01         |
| 322                                                   | A322S   | 0.53 | 1.00            | 1.00           | 1.03         | 1.01         |
| 322                                                   | A322T   | 0.91 | 1.03            | 0.91           | 0.99         | 1.01         |
| 322                                                   | A322V   | 0.62 | 1.02            | 0.93           | 0.93         | 1.00         |
| 322                                                   | A322W   | 0.47 | 0.91            | 0.44           | 0.87         | 0.76         |
| 322                                                   | A322Y   | 0.45 | 0.93            | 0.56           | 0.90         | 0.82         |
| 323                                                   | V323A   | 0.89 | 1.01            | 0.80           | 0.61         | 0.62         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 323                                                   | V323C   | 0.73 | 1.05            | 1.21           | 1.51         | 0.84         |
| 323                                                   | V323D   | 0.48 | 0.79            | 0.46           | 0.88         | 0.81         |
| 323                                                   | V323E   | 0.49 | 0.76            | 0.59           | 0.80         | 0.79         |
| 323                                                   | V323F   | 0.68 | 0.92            | 0.86           | 1.09         | 0.85         |
| 323                                                   | V323G   | 0.30 | 0.84            | 0.27           | 1.23         | 1.27         |
| 323                                                   | V323H   | 0.67 | 0.90            | 0.27           | 0.66         | 0.56         |
| 323                                                   | V323I   | 0.70 | 1.07            | 1.04           | 0.53         | 0.73         |
| 323                                                   | V323K   | 0.38 | 0.93            | 0.51           | 1.34         | 1.11         |
| 323                                                   | V323L   | 0.57 | 0.99            | 0.22           | 0.60         | 0.68         |
| 323                                                   | V323N   | 0.47 | 1.01            | 0.38           | 0.88         | 1.04         |
| 323                                                   | V323Q   | 0.42 | 0.97            | 0.47           | 0.72         | 1.05         |
| 323                                                   | V323R   | 0.23 | 0.69            | 0.26           | 2.20         | 2.25         |
| 323                                                   | V323S   | 0.30 | 0.80            | 0.09           | 1.00         | 1.42         |
| 323                                                   | V323T   | 0.60 | 0.80            | 0.05           | 0.66         | 1.05         |
| 323                                                   | V323W   | 0.45 | 0.90            | 0.20           | 0.71         | 1.06         |
| 323                                                   | V323Y   | 0.35 | 1.00            | 0.39           | 0.82         | 1.16         |
| 327                                                   | K327A   | 0.86 | 1.03            | 0.98           | 0.76         | 0.76         |
| 327                                                   | K327C   | 0.42 | 0.93            | 1.25           | 1.28         | 1.02         |
| 327                                                   | K327D   | 0.31 | 0.73            | 0.76           | 1.13         | 1.81         |
| 327                                                   | K327E   | 0.73 | 0.84            | 0.85           | 1.05         | 0.87         |
| 327                                                   | K327F   | 0.24 | 0.61            | 0.44           | 1.91         | 2.14         |
| 327                                                   | K327G   | 0.24 | 0.83            | 0.16           | 1.65         | 1.30         |
| 327                                                   | K327H   | 0.58 | 1.05            | 1.22           | 0.66         | 0.80         |
| 327                                                   | K327I   | 0.31 | 0.74            | 0.23           | 1.44         | 1.37         |
| 327                                                   | K327L   | 0.26 | 0.94            | 0.60           | 0.89         | 1.30         |
| 327                                                   | K327M   | 0.41 | 0.90            | 0.90           | 0.87         | 1.44         |
| 327                                                   | K327N   | 0.41 | 0.90            | 1.04           | 0.74         | 1.33         |
| 327                                                   | K327P   | 0.21 | 0.58            | 0.05           | 1.65         | 2.77         |
| 327                                                   | K327Q   | 1.32 | 1.06            | 1.42           | 1.15         | 0.77         |
| 327                                                   | K327R   | 0.56 | 0.85            | 0.89           | 1.07         | 1.16         |
| 327                                                   | K327S   | 0.20 | 0.63            | 0.58           | 2.39         | 2.92         |
| 327                                                   | K327T   | 0.21 | 0.29            | 0.09           | 3.69         | 7.57         |
| 327                                                   | K327V   | 0.28 | 0.86            | 0.58           | 0.66         | 1.26         |
| 327                                                   | K327W   | 0.28 | 1.05            | 0.74           | 0.93         | 1.57         |
| 327                                                   | K327Y   | 0.93 | 1.04            | 1.08           | 0.49         | 0.60         |
| 328                                                   | L328A   | 0.91 | 1.11            | 0.89           | 0.49         | 0.18         |
| 328                                                   | L328C   | 0.05 | 0.05            | 0.05           | 0.05         | 0.05         |
| 328                                                   | L328D   | 0.95 | 1.01            | 0.55           | 0.21         | 0.06         |
| 328                                                   | L328E   | 0.97 | 0.97            | 0.54           | 0.31         | 0.10         |
| 328                                                   | L328F   | 0.53 | 0.92            | 0.27           | 0.47         | 0.21         |
| 328                                                   | L328G   | 0.68 | 0.96            | 0.55           | 0.61         | 0.30         |
| 328                                                   | L328H   | 0.62 | 1.05            | 0.27           | 0.22         | 0.16         |
| 328                                                   | L328I   | 0.67 | 1.01            | 0.72           | 0.82         | 0.58         |
| 328                                                   | L328K   | 0.91 | 1.02            | 0.45           | 0.88         | 0.77         |
| 328                                                   | L328M   | 0.91 | 0.95            | 0.61           | 0.89         | 0.82         |
| 328                                                   | L328N   | 0.86 | 0.90            | 0.44           | 0.49         | 0.22         |
| 328                                                   | L328P   | 0.25 | 0.58            | 0.17           | 0.24         | 0.29         |
| 328                                                   | L328Q   | 2.73 | 1.07            | 0.86           | 0.85         | 0.76         |
| 328                                                   | L328R   | 0.74 | 0.92            | 0.34           | 0.81         | 0.62         |
| 328                                                   | L328S   | 0.40 | 0.90            | 0.42           | 0.38         | 0.18         |
| 328                                                   | L328T   | 0.57 | 0.93            | 0.50           | 0.30         | 0.13         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 328                                                   | L328V   | 0.40 | 0.74            | 0.20           | 0.14         | 0.10         |
| 328                                                   | L328W   | 0.45 | 0.88            | 0.27           | 0.12         | 0.14         |
| 328                                                   | L328Y   | 0.05 | 0.05            | 0.05           | 0.05         | 0.05         |
| 331                                                   | H331A   | 0.31 | 0.58            | 0.60           | 0.52         | 0.18         |
| 331                                                   | H331C   | 0.36 | 0.17            | 0.32           | 0.70         | 0.09         |
| 331                                                   | H331D   | 0.46 | 0.79            | 0.66           | 0.50         | 0.15         |
| 331                                                   | H331E   | 0.34 | 0.66            | 0.60           | 0.64         | 0.36         |
| 331                                                   | H331F   | 0.29 | 0.23            | 0.13           | 0.33         | 0.05         |
| 331                                                   | H331G   | 0.29 | 0.68            | 0.51           | 0.57         | 0.24         |
| 331                                                   | H331I   | 0.25 | 0.14            | 0.26           | 0.06         | 12.66        |
| 331                                                   | H331K   | 0.39 | 0.79            | 0.60           | 0.50         | 0.14         |
| 331                                                   | H331L   | 0.24 | 0.36            | 0.31           | 0.37         | 0.20         |
| 331                                                   | H331M   | 0.25 | 0.41            | 0.29           | 0.18         | 0.07         |
| 331                                                   | H331N   | 0.58 | 0.98            | 1.06           | 0.82         | 0.64         |
| 331                                                   | H331P   | 0.25 | 0.09            | 0.30           | 0.26         | 1.41         |
| 331                                                   | H331Q   | 0.40 | 0.84            | 0.92           | 0.62         | 0.35         |
| 331                                                   | H331R   | 0.38 | 0.67            | 0.43           | 0.53         | 0.21         |
| 331                                                   | H331S   | 0.33 | 0.56            | 0.55           | 0.74         | 0.35         |
| 331                                                   | H331T   | 0.24 | 0.64            | 0.58           | 0.57         | 0.27         |
| 331                                                   | H331V   | 0.22 | 0.25            | 0.30           | 0.16         | 0.22         |
| 331                                                   | H331W   | 0.32 | 0.69            | 0.69           | 0.66         | 0.44         |
| 331                                                   | H331Y   | 0.58 | 0.81            | 0.72           | 0.68         | 0.36         |
| 338                                                   | A338C   | 0.79 | 0.94            | 0.81           | 0.93         | 0.91         |
| 338                                                   | A338D   | 1.11 | 0.97            | 0.90           | 1.03         | 1.06         |
| 338                                                   | A338E   | 0.71 | 0.97            | 0.74           | 0.95         | 0.93         |
| 338                                                   | A338F   | 0.56 | 0.90            | 0.72           | 0.92         | 0.88         |
| 338                                                   | A338G   | 0.71 | 0.98            | 0.75           | 0.93         | 0.92         |
| 338                                                   | A338H   | 0.85 | 1.05            | 0.57           | 0.94         | 1.02         |
| 338                                                   | A338I   | 0.74 | 0.97            | 0.87           | 1.00         | 1.14         |
| 338                                                   | A338K   | 0.90 | 1.05            | 0.67           | 0.98         | 1.02         |
| 338                                                   | A338L   | 0.36 | 0.68            | 0.71           | 1.02         | 0.97         |
| 338                                                   | A338M   | 0.72 | 0.88            | 0.84           | 1.10         | 1.15         |
| 338                                                   | A338N   | 0.87 | 1.02            | 0.81           | 0.97         | 1.04         |
| 338                                                   | A338P   | 0.48 | 0.88            | 0.85           | 0.97         | 1.03         |
| 338                                                   | A338Q   | 0.34 | 0.66            | 0.57           | 0.92         | 0.86         |
| 338                                                   | A338R   | 0.70 | 1.09            | 0.79           | 0.95         | 0.97         |
| 338                                                   | A338S   | 0.70 | 0.94            | 0.86           | 1.00         | 1.05         |
| 338                                                   | A338T   | 0.21 | 0.68            | 0.68           | 0.83         | 0.88         |
| 338                                                   | A338V   | 0.48 | 1.00            | 1.01           | 0.92         | 1.01         |
| 338                                                   | A338W   | 0.30 | 0.83            | 0.75           | 0.87         | 0.94         |
| 339                                                   | N339A   | 0.67 | 1.03            | 1.24           | 1.06         | 1.10         |
| 339                                                   | N339C   | 0.60 | 0.98            | 1.09           | 0.96         | 0.96         |
| 339                                                   | N339D   | 0.92 | 1.04            | 1.05           | 1.04         | 1.10         |
| 339                                                   | N339E   | 0.94 | 1.03            | 0.83           | 1.08         | 1.13         |
| 339                                                   | N339F   | 0.53 | 1.02            | 0.56           | 1.08         | 1.12         |
| 339                                                   | N339G   | 0.57 | 1.01            | 0.63           | 1.09         | 1.00         |
| 339                                                   | N339H   | 0.66 | 1.05            | 0.58           | 1.00         | 1.11         |
| 339                                                   | N339I   | 0.66 | 1.09            | 0.77           | 1.05         | 1.13         |
| 339                                                   | N339K   | 0.69 | 1.03            | 0.65           | 1.06         | 1.11         |
| 339                                                   | N339L   | 0.61 | 1.13            | 0.74           | 1.05         | 1.15         |
| 339                                                   | N339M   | 0.47 | 1.03            | 0.51           | 1.05         | 1.09         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 339                                                   | N339P   | 0.19 | 0.90            | 0.64           | 0.84         | 0.74         |
| 339                                                   | N339Q   | 0.36 | 1.06            | 0.69           | 1.06         | 0.84         |
| 339                                                   | N339R   | 0.97 | 1.01            | 0.58           | 1.07         | 1.10         |
| 339                                                   | N339S   | 0.74 | 1.04            | 0.85           | 0.97         | 1.02         |
| 339                                                   | N339T   | 0.71 | 1.03            | 0.91           | 0.98         | 1.00         |
| 339                                                   | N339V   | 0.75 | 1.08            | 0.65           | 1.04         | 1.08         |
| 339                                                   | N339W   | 0.17 | 0.46            | 0.44           | 0.83         | 1.26         |
| 339                                                   | N339Y   | 0.59 | 0.97            | 0.73           | 0.88         | 0.81         |
| 340                                                   | H340A   | 0.25 | 0.18            | 0.44           | 0.23         | 0.05         |
| 340                                                   | H340C   | 0.39 | 0.69            | 0.72           | 0.65         | 0.37         |
| 340                                                   | H340D   | 0.32 | 0.45            | 0.78           | 0.41         | 0.28         |
| 340                                                   | H340E   | 0.39 | 0.73            | 0.79           | 0.80         | 0.51         |
| 340                                                   | H340F   | 0.68 | 1.08            | 0.91           | 0.77         | 0.64         |
| 340                                                   | H340G   | 0.35 | 0.44            | 0.50           | 0.47         | 0.24         |
| 340                                                   | H340I   | 0.28 | 0.29            | 0.44           | 0.51         | 0.14         |
| 340                                                   | H340K   | 0.71 | 1.03            | 1.00           | 0.83         | 0.69         |
| 340                                                   | H340L   | 0.36 | 0.84            | 0.70           | 0.63         | 0.42         |
| 340                                                   | H340M   | 0.36 | 0.73            | 0.68           | 0.81         | 0.52         |
| 340                                                   | H340N   | 0.76 | 0.92            | 1.02           | 0.92         | 0.81         |
| 340                                                   | H340P   | 0.22 | 0.17            | 0.21           | 0.16         | 0.39         |
| 340                                                   | H340Q   | 0.33 | 0.69            | 0.49           | 0.66         | 0.42         |
| 340                                                   | H340R   | 0.22 | 0.12            | 0.36           | 0.13         | 0.13         |
| 340                                                   | H340S   | 0.36 | 0.81            | 0.83           | 0.65         | 0.46         |
| 340                                                   | H340T   | 0.24 | 0.36            | 0.31           | 0.28         | 0.14         |
| 340                                                   | H340V   | 0.31 | 0.48            | 0.60           | 0.52         | 0.26         |
| 340                                                   | H340W   | 1.02 | 1.03            | 0.97           | 0.88         | 0.80         |
| 340                                                   | H340Y   | 0.38 | 0.85            | 1.01           | 0.83         | 0.66         |
| 343                                                   | S343A   | 1.40 | 2.48            | 2.75           | 0.94         | 1.77         |
| 343                                                   | S343C   | 3.71 | 2.52            | 3.51           | 1.02         | 2.16         |
| 343                                                   | S343D   | 1.37 | 2.87            | 3.53           | 0.95         | 1.67         |
| 343                                                   | S343E   | 4.68 | 2.37            | 3.30           | 1.09         | 2.31         |
| 343                                                   | S343F   | 1.52 | 2.15            | 1.82           | 0.95         | 1.59         |
| 343                                                   | S343G   | 0.51 | 0.48            | 0.19           | 3.20         | 5.39         |
| 343                                                   | S343H   | 0.66 | 0.73            | 0.33           | 0.97         | 2.31         |
| 343                                                   | S343I   | 1.19 | 2.45            | 1.98           | 1.09         | 1.76         |
| 343                                                   | S343K   | 2.34 | 2.56            | 3.03           | 1.19         | 2.04         |
| 343                                                   | S343L   | 1.32 | 2.65            | 2.59           | 1.00         | 1.83         |
| 343                                                   | S343M   | 0.18 | 0.57            | 0.73           | 6.24         | 57.92        |
| 343                                                   | S343P   | 2.09 | 2.50            | 2.38           | 1.01         | 2.03         |
| 343                                                   | S343Q   | 3.31 | 2.39            | 2.38           | 1.12         | 2.29         |
| 343                                                   | S343R   | 1.02 | 2.73            | 1.69           | 1.22         | 1.75         |
| 343                                                   | S343T   | 4.19 | 2.37            | 3.08           | 1.15         | 2.26         |
| 343                                                   | S343V   | 0.47 | 0.49            | 0.17           | 1.38         | 2.43         |
| 343                                                   | S343W   | 2.83 | 2.47            | 2.53           | 1.12         | 2.19         |
| 343                                                   | S343Y   | 2.20 | 2.50            | 2.89           | 1.07         | 2.01         |
| 344                                                   | N344A   | 0.58 | 1.02            | 1.17           | 1.13         | 1.12         |
| 344                                                   | N344C   | 0.46 | 0.96            | 1.31           | 0.84         | 0.96         |
| 344                                                   | N344D   | 0.29 | 0.85            | 1.10           | 1.43         | 1.31         |
| 344                                                   | N344F   | 0.31 | 0.87            | 0.42           | 1.03         | 1.08         |
| 344                                                   | N344G   | 0.73 | 1.02            | 1.25           | 1.17         | 1.25         |
| 344                                                   | N344H   | 0.64 | 0.96            | 0.36           | 0.81         | 0.90         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 344                                                   | N344I   | 0.26 | 0.78            | 0.60           | 1.33         | 1.49         |
| 344                                                   | N344K   | 0.89 | 1.04            | 0.91           | 0.90         | 0.72         |
| 344                                                   | N344L   | 0.23 | 0.69            | 0.40           | 1.49         | 2.90         |
| 344                                                   | N344M   | 0.53 | 0.99            | 0.79           | 0.60         | 0.75         |
| 344                                                   | N344P   | 0.33 | 0.87            | 0.61           | 1.62         | 1.82         |
| 344                                                   | N344Q   | 0.69 | 1.01            | 0.68           | 1.07         | 1.06         |
| 344                                                   | N344R   | 0.28 | 0.87            | 0.30           | 1.45         | 1.62         |
| 344                                                   | N344S   | 0.84 | 0.94            | 0.77           | 0.98         | 0.92         |
| 344                                                   | N344T   | 0.27 | 0.67            | 0.39           | 1.51         | 2.53         |
| 344                                                   | N344V   | 0.26 | 0.82            | 0.42           | 1.44         | 1.84         |
| 344                                                   | N344W   | 0.22 | 0.64            | 0.05           | 1.86         | 2.16         |
| 344                                                   | N344Y   | 0.32 | 0.91            | 0.43           | 0.89         | 1.49         |
| 346                                                   | F346A   | 0.70 | 1.02            | 1.25           | 0.95         | 0.99         |
| 346                                                   | F346C   | 0.48 | 0.90            | 0.99           | 0.82         | 0.87         |
| 346                                                   | F346D   | 0.18 | 0.26            | 0.35           | 1.22         | 2.13         |
| 346                                                   | F346E   | 0.21 | 0.31            | 0.30           | 1.53         | 2.18         |
| 346                                                   | F346G   | 0.60 | 1.01            | 0.72           | 0.97         | 1.10         |
| 346                                                   | F346H   | 1.05 | 1.02            | 0.91           | 1.06         | 1.22         |
| 346                                                   | F346I   | 0.20 | 0.62            | 0.31           | 1.42         | 1.75         |
| 346                                                   | F346K   | 1.01 | 0.98            | 0.78           | 1.00         | 1.21         |
| 346                                                   | F346L   | 0.28 | 0.66            | 0.63           | 0.96         | 1.13         |
| 346                                                   | F346M   | 0.63 | 0.98            | 0.77           | 1.03         | 1.04         |
| 346                                                   | F346N   | 0.43 | 0.82            | 0.61           | 0.92         | 1.14         |
| 346                                                   | F346P   | 0.31 | 0.24            | 0.22           | 1.16         | 2.49         |
| 346                                                   | F346Q   | 0.89 | 0.93            | 0.71           | 1.26         | 1.70         |
| 346                                                   | F346R   | 1.02 | 0.94            | 0.69           | 1.07         | 1.30         |
| 346                                                   | F346S   | 0.78 | 0.95            | 0.94           | 1.01         | 0.99         |
| 346                                                   | F346T   | 0.27 | 0.85            | 0.58           | 0.76         | 0.98         |
| 346                                                   | F346V   | 0.30 | 0.69            | 0.60           | 0.89         | 1.09         |
| 346                                                   | F346W   | 0.41 | 0.91            | 0.87           | 0.96         | 1.00         |
| 346                                                   | F346Y   | 0.91 | 1.02            | 0.85           | 1.10         | 1.31         |
| 356                                                   | K356A   | 1.22 | 1.03            | 0.88           | 0.23         | 0.25         |
| 356                                                   | K356C   | 0.62 | 0.92            | 0.98           | 0.29         | 0.45         |
| 356                                                   | K356D   | 0.42 | 0.87            | 0.57           | 0.22         | 0.42         |
| 356                                                   | K356E   | 0.43 | 0.98            | 0.71           | 0.28         | 0.43         |
| 356                                                   | K356F   | 0.69 | 0.99            | 0.45           | 0.15         | 0.39         |
| 356                                                   | K356G   | 0.97 | 0.98            | 0.20           | 0.20         | 0.40         |
| 356                                                   | K356H   | 0.70 | 0.88            | 0.63           | 0.52         | 0.52         |
| 356                                                   | K356I   | 0.76 | 0.96            | 0.60           | 0.26         | 0.46         |
| 356                                                   | K356L   | 0.67 | 1.00            | 0.68           | 0.45         | 0.39         |
| 356                                                   | K356M   | 0.68 | 0.99            | 0.88           | 0.54         | 0.53         |
| 356                                                   | K356N   | 0.98 | 1.04            | 0.72           | 0.19         | 0.37         |
| 356                                                   | K356P   | 0.23 | 0.52            | 0.33           | 1.09         | 0.99         |
| 356                                                   | K356Q   | 0.77 | 0.99            | 0.77           | 0.41         | 0.43         |
| 356                                                   | K356R   | 0.70 | 0.98            | 0.62           | 0.95         | 0.76         |
| 356                                                   | K356S   | 0.58 | 0.88            | 0.48           | 0.22         | 0.41         |
| 356                                                   | K356T   | 0.63 | 1.00            | 0.49           | 0.28         | 0.43         |
| 356                                                   | K356V   | 0.91 | 0.90            | 0.75           | 0.35         | 0.31         |
| 356                                                   | K356W   | 0.39 | 0.90            | 0.42           | 0.24         | 0.47         |
| 356                                                   | K356Y   | 0.52 | 0.96            | 0.47           | 0.20         | 0.51         |
| 360                                                   | G360A   | 0.56 | 0.89            | 0.47           | 0.08         | 0.05         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 360                                                   | G360E   | 0.62 | 0.88            | 0.45           | 0.05         | 0.05         |
| 360                                                   | G360F   | 0.24 | 0.45            | 0.08           | 0.08         | 0.05         |
| 360                                                   | G360H   | 0.51 | 0.84            | 0.21           | 0.07         | 0.05         |
| 360                                                   | G360I   | 0.41 | 0.83            | 0.19           | 0.07         | 0.05         |
| 360                                                   | G360K   | 0.19 | 0.46            | 0.07           | 0.05         | 0.08         |
| 360                                                   | G360L   | 0.69 | 0.93            | 0.33           | 0.05         | 0.05         |
| 360                                                   | G360M   | 0.84 | 1.06            | 0.47           | 0.07         | 0.05         |
| 360                                                   | G360N   | 0.60 | 0.93            | 0.23           | 0.05         | 0.05         |
| 360                                                   | G360Q   | 0.68 | 1.01            | 0.58           | 0.05         | 0.05         |
| 360                                                   | G360R   | 0.77 | 1.01            | 0.29           | 0.05         | 0.05         |
| 360                                                   | G360S   | 0.73 | 0.96            | 0.47           | 0.05         | 0.05         |
| 360                                                   | G360T   | 0.58 | 0.97            | 0.13           | 0.05         | 0.05         |
| 360                                                   | G360V   | 0.10 | 0.07            | 0.14           | 4.46         | 16.21        |
| 360                                                   | G360W   | 0.61 | 0.92            | 0.27           | 0.05         | 0.07         |
| 360                                                   | G360Y   | 0.11 | 0.05            | 0.06           | 0.78         | 6.25         |
| 361                                                   | Q361A   | 0.93 | 1.02            | 0.18           | 0.13         | 0.14         |
| 361                                                   | Q361C   | 0.45 | 0.91            | 0.18           | 0.21         | 0.20         |
| 361                                                   | Q361D   | 0.43 | 0.65            | 0.10           | 0.19         | 0.29         |
| 361                                                   | Q361E   | 0.07 | 1.37            | 0.05           | 1.01         | 0.93         |
| 361                                                   | Q361F   | 0.34 | 0.40            | 0.05           | 0.31         | 0.49         |
| 361                                                   | Q361G   | 0.33 | 1.01            | 0.10           | 0.23         | 0.24         |
| 361                                                   | Q361H   | 0.25 | 0.87            | 0.20           | 0.74         | 0.59         |
| 361                                                   | Q361I   | 0.19 | 0.49            | 0.19           | 0.91         | 1.00         |
| 361                                                   | Q361K   | 0.66 | 0.73            | 0.07           | 0.27         | 0.34         |
| 361                                                   | Q361L   | 0.86 | 0.86            | 0.21           | 0.20         | 0.19         |
| 361                                                   | Q361M   | 0.59 | 0.86            | 0.10           | 0.27         | 0.25         |
| 361                                                   | Q361N   | 0.06 | 0.05            | 0.05           | 0.36         | 4.82         |
| 361                                                   | Q361P   | 0.26 | 0.21            | 0.05           | 0.80         | 0.86         |
| 361                                                   | Q361R   | 0.69 | 0.75            | 0.10           | 0.13         | 0.18         |
| 361                                                   | Q361S   | 0.55 | 0.90            | 0.13           | 0.27         | 0.22         |
| 361                                                   | Q361T   | 0.57 | 0.83            | 0.12           | 0.25         | 0.26         |
| 361                                                   | Q361V   | 0.44 | 0.80            | 0.09           | 0.08         | 0.12         |
| 361                                                   | Q361W   | 0.49 | 0.66            | 0.27           | 0.41         | 0.38         |
| 361                                                   | Q361Y   | 0.31 | 0.39            | 0.22           | 0.79         | 0.58         |
| 362                                                   | Q362A   | 0.81 | 0.98            | 0.90           | 0.83         | 0.69         |
| 362                                                   | Q362C   | 0.68 | 0.95            | 0.83           | 0.90         | 0.98         |
| 362                                                   | Q362D   | 1.15 | 1.02            | 0.95           | 0.85         | 0.78         |
| 362                                                   | Q362E   | 0.93 | 0.95            | 0.70           | 0.86         | 0.81         |
| 362                                                   | Q362F   | 0.73 | 0.97            | 0.54           | 0.75         | 0.66         |
| 362                                                   | Q362G   | 0.70 | 0.99            | 0.34           | 0.31         | 0.44         |
| 362                                                   | Q362H   | 1.00 | 0.96            | 0.41           | 0.96         | 0.96         |
| 362                                                   | Q362I   | 0.86 | 0.99            | 1.04           | 1.30         | 1.81         |
| 362                                                   | Q362K   | 0.17 | 0.44            | 0.28           | 0.64         | 3.22         |
| 362                                                   | Q362L   | 0.71 | 0.96            | 0.89           | 1.29         | 1.86         |
| 362                                                   | Q362M   | 0.75 | 0.96            | 0.88           | 1.10         | 1.45         |
| 362                                                   | Q362N   | 0.78 | 0.98            | 0.74           | 0.96         | 0.99         |
| 362                                                   | Q362P   | 0.55 | 0.86            | 0.71           | 0.90         | 0.90         |
| 362                                                   | Q362R   | 0.96 | 0.96            | 0.53           | 1.03         | 1.15         |
| 362                                                   | Q362S   | 0.68 | 0.98            | 0.61           | 0.78         | 0.80         |
| 362                                                   | Q362T   | 0.73 | 0.99            | 0.60           | 0.85         | 0.73         |
| 362                                                   | Q362V   | 1.05 | 0.96            | 0.77           | 0.98         | 1.00         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 362                                                   | Q362W   | 0.94 | 0.97            | 0.54           | 0.75         | 0.60         |
| 362                                                   | Q362Y   | 0.78 | 1.05            | 0.76           | 0.71         | 0.61         |
| 363                                                   | Q363A   | 1.26 | 1.00            | 0.95           | 1.07         | 1.25         |
| 363                                                   | Q363C   | 0.27 | 0.69            | 0.50           | 0.50         | 1.16         |
| 363                                                   | Q363D   | 0.30 | 0.83            | 0.94           | 0.98         | 1.30         |
| 363                                                   | Q363E   | 0.91 | 1.00            | 0.87           | 0.97         | 1.03         |
| 363                                                   | Q363F   | 1.37 | 0.95            | 0.72           | 0.56         | 1.15         |
| 363                                                   | Q363G   | 0.17 | 0.31            | 0.23           | 1.14         | 2.30         |
| 363                                                   | Q363H   | 0.39 | 0.92            | 0.39           | 1.05         | 1.40         |
| 363                                                   | Q363I   | 0.43 | 0.92            | 0.28           | 0.31         | 0.56         |
| 363                                                   | Q363K   | 0.44 | 0.95            | 0.60           | 0.63         | 1.22         |
| 363                                                   | Q363L   | 0.21 | 0.80            | 0.38           | 0.93         | 0.93         |
| 363                                                   | Q363M   | 0.65 | 0.95            | 0.62           | 0.90         | 0.84         |
| 363                                                   | Q363N   | 3.15 | 0.98            | 1.15           | 1.31         | 1.70         |
| 363                                                   | Q363P   | 0.37 | 0.63            | 0.20           | 0.48         | 0.82         |
| 363                                                   | Q363R   | 0.94 | 0.94            | 0.55           | 1.11         | 1.22         |
| 363                                                   | Q363S   | 0.62 | 1.00            | 0.85           | 1.05         | 1.40         |
| 363                                                   | Q363T   | 1.10 | 0.98            | 0.74           | 0.99         | 0.86         |
| 363                                                   | Q363V   | 0.18 | 0.88            | 0.50           | 0.86         | 1.24         |
| 363                                                   | Q363W   | 0.70 | 0.88            | 0.45           | 0.68         | 0.61         |
| 363                                                   | Q363Y   | 0.75 | 0.91            | 0.75           | 1.05         | 1.08         |
| 364                                                   | W364A   | 0.95 | 0.85            | 0.19           | 0.13         | 0.18         |
| 364                                                   | W364C   | 0.81 | 0.69            | 0.50           | 0.77         | 0.65         |
| 364                                                   | W364D   | 1.65 | 0.88            | 0.31           | 0.11         | 0.11         |
| 364                                                   | W364E   | 0.69 | 0.93            | 0.27           | 0.18         | 0.22         |
| 364                                                   | W364F   | 0.54 | 0.77            | 0.08           | 0.28         | 0.30         |
| 364                                                   | W364G   | 0.70 | 0.83            | 0.21           | 0.18         | 0.13         |
| 364                                                   | W364I   | 0.46 | 0.82            | 0.18           | 0.14         | 0.19         |
| 364                                                   | W364K   | 0.69 | 0.50            | 0.12           | 0.21         | 0.09         |
| 364                                                   | W364L   | 0.35 | 0.83            | 0.13           | 0.10         | 0.19         |
| 364                                                   | W364M   | 0.63 | 0.93            | 0.17           | 0.19         | 0.12         |
| 364                                                   | W364N   | 0.54 | 0.83            | 0.23           | 0.11         | 0.11         |
| 364                                                   | W364P   | 0.72 | 0.81            | 0.08           | 0.20         | 0.23         |
| 364                                                   | W364Q   | 0.55 | 0.92            | 0.16           | 0.17         | 0.20         |
| 364                                                   | W364R   | 1.01 | 0.61            | 0.09           | 0.14         | 0.16         |
| 364                                                   | W364S   | 0.65 | 0.82            | 0.10           | 0.20         | 0.24         |
| 364                                                   | W364T   | 0.83 | 0.79            | 0.11           | 0.06         | 0.13         |
| 364                                                   | W364V   | 0.52 | 0.85            | 0.51           | 0.12         | 0.20         |
| 364                                                   | W364Y   | 0.66 | 0.68            | 0.14           | 0.21         | 0.29         |
| 365                                                   | G365C   | 0.48 | 0.59            | 0.37           | 0.87         | 0.88         |
| 365                                                   | G365E   | 0.96 | 0.83            | 0.57           | 0.92         | 0.92         |
| 365                                                   | G365F   | 0.38 | 0.66            | 0.18           | 0.64         | 0.53         |
| 365                                                   | G365H   | 0.47 | 0.72            | 0.21           | 0.74         | 0.66         |
| 365                                                   | G365I   | 0.38 | 0.52            | 0.09           | 0.68         | 0.61         |
| 365                                                   | G365K   | 0.74 | 0.72            | 0.28           | 0.85         | 0.79         |
| 365                                                   | G365L   | 0.40 | 0.57            | 0.29           | 0.86         | 0.86         |
| 365                                                   | G365M   | 0.62 | 0.66            | 0.39           | 0.97         | 1.08         |
| 365                                                   | G365N   | 0.30 | 0.66            | 0.29           | 0.84         | 0.86         |
| 365                                                   | G365P   | 0.37 | 0.64            | 0.23           | 0.53         | 0.26         |
| 365                                                   | G365Q   | 0.25 | 0.15            | 0.21           | 0.82         | 1.38         |
| 365                                                   | G365R   | 0.64 | 0.72            | 0.26           | 0.83         | 0.86         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 365                                                   | G365S   | 0.71 | 0.80            | 0.74           | 1.01         | 1.14         |
| 365                                                   | G365T   | 0.85 | 0.66            | 0.36           | 0.94         | 1.11         |
| 365                                                   | G365V   | 0.65 | 0.55            | 0.17           | 0.68         | 0.78         |
| 365                                                   | G365W   | 0.80 | 0.77            | 0.36           | 0.66         | 0.58         |
| 365                                                   | G365Y   | 0.06 | 1.15            | 0.15           | 0.99         | 1.15         |
| 371                                                   | I371A   | 0.34 | 1.05            | 1.25           | 0.75         | 0.57         |
| 371                                                   | I371C   | 0.34 | 0.87            | 0.84           | 0.88         | 0.59         |
| 371                                                   | I371D   | 0.62 | 0.99            | 0.88           | 0.90         | 0.79         |
| 371                                                   | I371E   | 0.36 | 0.98            | 0.73           | 0.81         | 0.54         |
| 371                                                   | I371F   | 0.21 | 0.24            | 0.07           | 0.70         | 0.49         |
| 371                                                   | I371G   | 0.54 | 0.98            | 0.57           | 0.88         | 0.70         |
| 371                                                   | I371H   | 0.24 | 0.08            | 0.05           | 0.63         | 0.94         |
| 371                                                   | I371K   | 0.11 | 0.07            | 0.05           | 0.86         | 1.24         |
| 371                                                   | I371L   | 0.29 | 0.64            | 0.55           | 0.54         | 0.28         |
| 371                                                   | I371M   | 0.28 | 0.87            | 0.53           | 0.68         | 0.40         |
| 371                                                   | I371N   | 0.64 | 1.06            | 0.74           | 0.93         | 0.82         |
| 371                                                   | I371P   | 0.21 | 0.18            | 0.05           | 1.78         | 1.31         |
| 371                                                   | I371Q   | 0.31 | 0.81            | 0.67           | 0.77         | 0.57         |
| 371                                                   | I371R   | 0.21 | 0.43            | 0.13           | 0.63         | 0.55         |
| 371                                                   | I371S   | 1.05 | 1.00            | 1.10           | 1.14         | 1.12         |
| 371                                                   | I371T   | 0.95 | 1.00            | 0.80           | 1.08         | 1.00         |
| 371                                                   | I371V   | 0.28 | 0.59            | 0.37           | 0.73         | 0.52         |
| 371                                                   | I371W   | 0.23 | 0.18            | 0.10           | 0.74         | 0.73         |
| 371                                                   | I371Y   | 0.24 | 0.42            | 0.27           | 0.56         | 0.45         |
| 378                                                   | R378A   | 0.21 | 0.76            | 0.79           | 0.25         | 0.54         |
| 378                                                   | R378C   | 0.25 | 0.34            | 0.42           | 0.74         | 1.01         |
| 378                                                   | R378D   | 0.19 | 0.23            | 0.33           | 0.49         | 0.58         |
| 378                                                   | R378E   | 0.17 | 0.18            | 0.30           | 0.92         | 0.94         |
| 378                                                   | R378F   | 0.13 | 0.39            | 1.34           | 0.12         | 0.38         |
| 378                                                   | R378G   | 0.08 | 0.22            | 1.48           | 0.19         | 0.82         |
| 378                                                   | R378H   | 0.13 | 0.22            | 0.82           | 0.62         | 1.22         |
| 378                                                   | R378I   | 0.19 | 0.42            | 0.33           | 0.38         | 0.69         |
| 378                                                   | R378K   | 0.15 | 0.45            | 0.27           | 0.61         | 0.98         |
| 378                                                   | R378L   | 0.21 | 0.75            | 0.79           | 0.33         | 0.53         |
| 378                                                   | R378M   | 0.14 | 0.52            | 0.77           | 0.37         | 0.47         |
| 378                                                   | R378N   | 0.12 | 0.19            | 0.48           | 0.38         | 0.81         |
| 378                                                   | R378P   | 0.17 | 0.65            | 0.54           | 0.48         | 0.62         |
| 378                                                   | R378Q   | 0.20 | 0.82            | 0.94           | 0.56         | 0.59         |
| 378                                                   | R378S   | 0.18 | 0.65            | 0.47           | 0.45         | 0.64         |
| 378                                                   | R378T   | 0.18 | 0.52            | 1.03           | 0.25         | 0.43         |
| 378                                                   | R378V   | 0.36 | 1.01            | 0.98           | 1.06         | 1.19         |
| 378                                                   | R378W   | 0.09 | 0.10            | 0.64           | 0.34         | 0.30         |
| 378                                                   | R378Y   | 0.24 | 0.60            | 0.62           | 0.23         | 0.53         |
| 380                                                   | S380A   | 0.95 | 0.99            | 0.48           | 0.31         | 0.10         |
| 380                                                   | S380C   | 0.52 | 1.03            | 0.63           | 0.57         | 0.37         |
| 380                                                   | S380D   | 0.61 | 1.12            | 0.45           | 0.12         | 0.16         |
| 380                                                   | S380E   | 0.52 | 0.94            | 0.29           | 0.41         | 0.20         |
| 380                                                   | S380F   | 0.60 | 1.05            | 0.22           | 0.08         | 0.10         |
| 380                                                   | S380G   | 0.68 | 1.05            | 0.20           | 0.17         | 0.21         |
| 380                                                   | S380H   | 0.68 | 1.09            | 0.37           | 0.42         | 0.14         |
| 380                                                   | S380I   | 0.73 | 1.06            | 0.66           | 0.99         | 0.96         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 380                                                   | S380K   | 0.80 | 1.08            | 0.42           | 0.94         | 0.94         |
| 380                                                   | S380L   | 1.04 | 1.07            | 0.57           | 0.78         | 0.60         |
| 380                                                   | S380M   | 1.02 | 1.03            | 0.43           | 0.85         | 0.69         |
| 380                                                   | S380N   | 0.81 | 1.09            | 0.58           | 0.95         | 0.89         |
| 380                                                   | S380P   | 0.73 | 1.04            | 0.08           | 0.07         | 0.11         |
| 380                                                   | S380Q   | 0.69 | 1.02            | 0.44           | 0.45         | 0.17         |
| 380                                                   | S380R   | 0.83 | 1.04            | 0.38           | 0.86         | 0.73         |
| 380                                                   | S380T   | 0.30 | 0.70            | 0.17           | 0.56         | 0.35         |
| 380                                                   | S380V   | 0.98 | 1.02            | 0.79           | 0.99         | 0.96         |
| 380                                                   | S380W   | 0.85 | 1.00            | 0.11           | 0.05         | 0.06         |
| 380                                                   | S380Y   | 1.01 | 1.01            | 0.25           | 0.14         | 0.10         |
| 381                                                   | A381C   | 0.67 | 1.05            | 0.94           | 0.89         | 0.83         |
| 381                                                   | A381D   | 0.67 | 1.05            | 0.96           | 0.90         | 0.79         |
| 381                                                   | A381E   | 0.52 | 1.08            | 0.84           | 0.85         | 0.75         |
| 381                                                   | A381F   | 0.69 | 1.07            | 0.66           | 0.89         | 0.82         |
| 381                                                   | A381G   | 0.36 | 0.98            | 0.76           | 0.76         | 0.65         |
| 381                                                   | A381H   | 0.14 | 0.15            | 0.05           | 1.39         | 0.95         |
| 381                                                   | A381I   | 1.12 | 1.09            | 0.84           | 0.89         | 0.83         |
| 381                                                   | A381K   | 0.13 | 0.11            | 0.05           | 0.27         | 1.00         |
| 381                                                   | A381L   | 0.52 | 0.95            | 0.62           | 0.78         | 0.62         |
| 381                                                   | A381M   | 0.47 | 1.05            | 0.68           | 0.82         | 0.75         |
| 381                                                   | A381N   | 0.38 | 0.89            | 0.62           | 0.84         | 0.76         |
| 381                                                   | A381P   | 0.54 | 1.00            | 0.72           | 0.94         | 1.02         |
| 381                                                   | A381Q   | 0.48 | 1.01            | 0.60           | 0.87         | 0.75         |
| 381                                                   | A381R   | 0.39 | 0.95            | 0.59           | 0.80         | 0.75         |
| 381                                                   | A381S   | 1.20 | 1.05            | 0.78           | 1.04         | 1.06         |
| 381                                                   | A381T   | 0.93 | 0.97            | 0.73           | 0.97         | 1.01         |
| 381                                                   | A381V   | 0.54 | 0.97            | 0.59           | 0.90         | 0.81         |
| 381                                                   | A381W   | 0.54 | 0.87            | 0.64           | 0.97         | 0.93         |
| 381                                                   | A381Y   | 0.61 | 0.92            | 0.60           | 0.92         | 0.87         |
| 382                                                   | N382A   | 1.22 | 0.70            |                | 2.07         | 0.91         |
| 382                                                   | N382C   | 1.11 | 0.57            |                | 1.52         | 1.54         |
| 382                                                   | N382D   | 0.71 | 0.81            |                | 1.17         | 1.15         |
| 382                                                   | N382E   | 2.21 | 0.16            |                | 6.19         | 5.56         |
| 382                                                   | N382F   | 0.91 | 1.23            |                | 1.03         | 0.66         |
| 382                                                   | N382G   | 0.78 | 0.56            |                | 1.73         | 1.31         |
| 382                                                   | N382H   | 0.81 | 1.01            |                | 1.23         | 1.08         |
| 382                                                   | N382I   | 0.48 | 0.80            |                | 1.63         | 1.35         |
| 382                                                   | N382K   | 1.66 | 0.50            |                | 2.10         | 1.85         |
| 382                                                   | N382L   | 0.56 | 0.74            |                | 1.58         | 1.31         |
| 382                                                   | N382M   | 0.50 | 0.66            |                | 1.75         | 1.57         |
| 382                                                   | N382Q   | 1.08 | 0.65            |                | 1.45         | 1.46         |
| 382                                                   | N382T   | 0.50 | 1.04            |                | 1.31         | 0.99         |
| 382                                                   | N382V   | 1.53 | 0.58            |                | 1.57         | 1.66         |
| 382                                                   | N382Y   | 0.49 | 0.62            |                | 1.67         | 1.55         |
| 384                                                   | G384A   | 0.58 | 0.72            | 0.68           | 1.26         | 0.79         |
| 384                                                   | G384C   | 0.65 | 1.06            | 1.22           | 0.88         | 0.91         |
| 384                                                   | G384D   | 1.36 | 1.06            | 1.31           | 0.84         | 0.73         |
| 384                                                   | G384E   | 0.52 | 0.92            | 1.05           | 1.33         | 0.96         |
| 384                                                   | G384F   | 0.75 | 1.00            | 0.87           | 1.16         | 0.96         |
| 384                                                   | G384H   | 0.48 | 0.73            | 0.86           | 1.02         | 1.01         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 384                                                   | G384K   | 0.50 | 0.60            |                | 1.41         | 1.56         |
| 384                                                   | G384L   | 0.41 | 0.76            | 1.03           | 0.95         | 0.90         |
| 384                                                   | G384M   | 0.61 | 0.70            | 1.01           | 1.19         | 1.24         |
| 384                                                   | G384N   | 0.76 | 0.53            | 0.61           | 1.03         | 1.43         |
| 384                                                   | G384Q   | 0.57 | 1.15            | 1.19           | 0.92         | 0.85         |
| 384                                                   | G384R   | 0.67 | 0.62            | 0.55           | 1.20         | 1.70         |
| 384                                                   | G384S   | 0.16 | 0.17            | 0.51           | 0.59         | 1.21         |
| 384                                                   | G384T   | 0.87 | 0.06            |                | 4.44         | 3.08         |
| 384                                                   | G384V   | 0.38 | 0.80            | 0.53           | 0.72         | 0.80         |
| 384                                                   | G384W   | 0.69 | 1.12            | 0.71           | 0.97         | 0.95         |
| 384                                                   | G384Y   | 0.45 | 0.50            |                | 1.83         | 2.73         |
| 386                                                   | S386A   | 1.89 | 2.69            | 3.12           | 0.99         | 1.88         |
| 386                                                   | S386C   | 2.63 | 2.50            | 3.75           | 1.03         | 2.09         |
| 386                                                   | S386D   | 1.57 | 2.52            | 2.56           | 0.92         | 1.79         |
| 386                                                   | S386E   | 6.64 | 2.19            | 4.12           | 1.05         | 2.33         |
| 386                                                   | S386F   | 2.11 | 3.11            | 2.96           | 1.05         | 2.19         |
| 386                                                   | S386G   | 0.45 | 0.51            | 0.43           | 1.78         | 3.74         |
| 386                                                   | S386H   | 2.52 | 2.56            | 2.50           | 1.11         | 2.13         |
| 386                                                   | S386I   | 2.44 | 2.52            | 2.64           | 1.08         | 2.03         |
| 386                                                   | S386K   | 0.93 | 0.36            | 0.30           | 2.91         | 2.66         |
| 386                                                   | S386L   | 1.33 | 2.29            | 1.43           | 0.92         | 1.65         |
| 386                                                   | S386M   | 0.43 | 0.26            | 0.05           | 1.20         | 7.13         |
| 386                                                   | S386P   | 4.61 | 2.34            | 2.31           | 1.10         | 2.27         |
| 386                                                   | S386Q   | 0.57 | 2.58            | 0.27           | 0.38         | 0.71         |
| 386                                                   | S386R   | 1.16 | 2.27            | 0.76           | 0.91         | 1.58         |
| 386                                                   | S386T   | 1.05 | 2.01            | 1.88           | 0.96         | 1.70         |
| 386                                                   | S386V   | 3.71 | 2.47            | 3.11           | 1.04         | 2.15         |
| 386                                                   | S386W   | 0.88 | 2.31            | 1.52           | 0.97         | 1.32         |
| 394                                                   | V394A   | 0.53 | 0.99            | 0.74           | 0.67         | 0.40         |
| 394                                                   | V394C   | 0.32 | 0.87            | 0.81           | 0.80         | 0.52         |
| 394                                                   | V394D   | 0.11 | 0.18            | 0.31           | 0.98         | 0.85         |
| 394                                                   | V394E   | 0.06 | 0.43            | 0.05           | 0.34         | 0.64         |
| 394                                                   | V394F   | 0.06 | 0.42            | 0.17           | 0.46         | 0.56         |
| 394                                                   | V394G   | 0.27 | 0.76            | 0.56           | 0.48         | 0.28         |
| 394                                                   | V394H   | 0.09 | 0.18            | 0.07           | 0.68         | 1.18         |
| 394                                                   | V394I   | 0.51 | 1.01            | 0.76           | 0.89         | 0.80         |
| 394                                                   | V394K   | 0.07 | 0.23            | 0.73           | 1.04         | 0.86         |
| 394                                                   | V394L   | 0.17 | 0.87            | 0.23           | 0.09         | 0.24         |
| 394                                                   | V394M   | 0.71 | 0.94            | 0.31           | 0.11         | 0.11         |
| 394                                                   | V394N   | 0.09 | 0.30            | 0.18           | 0.79         | 0.67         |
| 394                                                   | V394P   | 0.11 | 0.18            | 0.08           | 0.73         | 1.42         |
| 394                                                   | V394Q   | 0.11 | 0.22            | 0.11           | 0.87         | 1.30         |
| 394                                                   | V394R   | 0.06 | 0.28            | 0.23           | 0.87         | 1.11         |
| 394                                                   | V394S   | 0.12 | 0.58            | 0.19           | 0.21         | 0.37         |
| 394                                                   | V394T   | 0.22 | 0.79            | 0.29           | 0.15         | 0.21         |
| 394                                                   | V394W   | 0.09 | 0.15            | 0.31           | 0.15         | 1.55         |
| 394                                                   | V394Y   | 0.11 | 0.17            | 0.05           | 0.54         | 1.12         |
| 396                                                   | P396A   | 0.52 | 0.68            | 1.01           | 0.81         | 0.51         |
| 396                                                   | P396C   | 0.29 | 0.24            | 0.73           | 0.76         | 0.73         |
| 396                                                   | P396D   | 0.09 | 0.56            | 0.88           | 0.44         | 0.76         |
| 396                                                   | P396E   | 0.31 | 0.58            | 0.90           | 0.48         | 0.44         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 396                                                   | P396F   | 0.29 | 0.48            | 0.48           | 0.29         | 0.30         |
| 396                                                   | P396G   | 0.49 | 0.63            | 0.82           | 0.44         | 0.25         |
| 396                                                   | P396H   | 0.19 | 0.33            | 0.58           | 0.50         | 0.75         |
| 396                                                   | P396I   | 0.34 | 0.57            | 0.52           | 0.11         | 0.35         |
| 396                                                   | P396K   | 0.34 | 0.29            | 0.40           | 0.43         | 0.80         |
| 396                                                   | P396L   | 0.48 | 0.51            | 0.52           | 0.16         | 0.30         |
| 396                                                   | P396M   | 0.40 | 0.66            | 0.52           | 0.12         | 0.31         |
| 396                                                   | P396N   | 0.12 | 0.47            | 0.58           | 0.79         | 0.98         |
| 396                                                   | P396Q   | 0.06 | 0.05            | 0.32           | 0.65         | 1.26         |
| 396                                                   | P396R   | 0.29 | 0.31            | 0.46           | 0.24         | 0.56         |
| 396                                                   | P396S   | 0.16 | 0.14            | 0.43           | 0.35         | 1.18         |
| 396                                                   | P396T   | 0.48 | 0.60            | 0.50           | 0.22         | 0.26         |
| 396                                                   | P396V   | 0.40 | 0.59            | 0.51           | 0.42         | 0.27         |
| 396                                                   | P396W   | 0.26 | 0.25            | 0.56           | 0.21         | 0.30         |
| 396                                                   | P396Y   | 0.27 | 0.32            | 0.42           | 0.37         | 0.37         |
| 399                                                   | E399A   | 0.68 | 0.36            | 0.39           | 0.09         | 0.08         |
| 399                                                   | E399C   | 0.31 | 0.11            | 0.26           | 0.24         | 0.19         |
| 399                                                   | E399D   | 0.15 | 0.31            | 0.21           | 0.32         | 0.39         |
| 399                                                   | E399F   | 1.02 | 0.30            | 0.25           | 0.11         | 0.05         |
| 399                                                   | E399G   | 0.75 | 0.32            | 0.20           | 0.13         | 0.05         |
| 399                                                   | E399H   | 0.86 | 0.29            | 0.33           | 0.18         | 0.16         |
| 399                                                   | E399I   | 0.54 | 0.18            | 0.16           | 0.80         | 0.33         |
| 399                                                   | E399K   | 0.43 | 0.21            | 0.21           | 0.35         | 0.22         |
| 399                                                   | E399L   | 0.68 | 0.19            | 0.23           | 0.32         | 0.24         |
| 399                                                   | E399M   | 0.56 | 0.22            | 0.21           | 0.31         | 0.28         |
| 399                                                   | E399N   | 0.50 | 0.33            | 0.33           | 0.20         | 0.19         |
| 399                                                   | E399P   | 0.80 | 0.23            | 0.27           | 0.15         | 0.17         |
| 399                                                   | E399Q   | 0.69 | 0.68            | 0.40           | 0.30         | 0.09         |
| 399                                                   | E399R   | 0.69 | 0.13            | 0.15           | 0.62         | 0.49         |
| 399                                                   | E399S   | 0.64 | 0.39            | 0.23           | 0.13         | 0.14         |
| 399                                                   | E399T   | 0.12 | 0.31            | 0.05           | 1.16         | 1.26         |
| 399                                                   | E399V   | 0.49 | 0.20            | 0.25           | 0.53         | 0.54         |
| 399                                                   | E399W   | 1.00 | 0.30            | 0.31           | 0.55         | 0.30         |
| 399                                                   | E399Y   | 0.82 | 0.30            | 0.26           | 0.22         | 0.19         |
| 400                                                   | C400A   | 1.11 | 0.98            | 0.72           | 0.89         | 0.74         |
| 400                                                   | C400D   | 0.42 | 0.76            | 0.48           | 0.48         | 0.48         |
| 400                                                   | C400E   | 0.32 | 0.35            | 0.05           | 0.47         | 0.28         |
| 400                                                   | C400F   | 0.57 | 0.27            | 0.05           | 0.50         | 0.22         |
| 400                                                   | C400G   | 0.75 | 1.01            | 0.47           | 0.32         | 0.11         |
| 400                                                   | C400H   | 0.24 | 0.11            | 0.09           | 1.53         | 1.07         |
| 400                                                   | C400I   | 0.65 | 0.40            | 0.10           | 0.51         | 0.11         |
| 400                                                   | C400K   | 0.63 | 0.36            | 0.08           | 0.32         | 0.33         |
| 400                                                   | C400L   | 0.62 | 0.36            | 0.05           | 0.60         | 0.49         |
| 400                                                   | C400M   | 0.69 | 0.46            | 0.05           | 0.22         | 0.29         |
| 400                                                   | C400N   | 0.11 | 0.05            | 0.09           | 5.30         | 3.48         |
| 400                                                   | C400P   | 0.48 | 0.38            | 0.05           | 0.45         | 0.36         |
| 400                                                   | C400Q   | 0.45 | 0.21            | 0.05           | 0.87         | 0.61         |
| 400                                                   | C400R   | 0.61 | 0.20            | 0.07           | 0.57         | 0.73         |
| 400                                                   | C400S   | 0.88 | 0.90            | 1.02           | 1.04         | 1.51         |
| 400                                                   | C400T   | 0.93 | 0.75            | 0.14           | 0.20         | 0.21         |
| 400                                                   | C400V   | 1.18 | 0.55            | 0.25           | 0.23         | 0.14         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 400                                                   | C400W   | 0.32 | 0.09            | 0.08           | 0.27         | 0.22         |
| 400                                                   | C400Y   | 0.57 | 0.20            | 0.05           | 0.40         | 0.22         |
| 405                                                   | D405A   | 1.75 | 0.82            | 0.33           | 0.32         | 0.60         |
| 405                                                   | D405C   | 0.45 | 0.63            | 0.57           | 0.72         | 0.97         |
| 405                                                   | D405E   | 0.85 | 0.81            | 0.63           | 0.50         | 0.65         |
| 405                                                   | D405F   | 0.79 | 0.95            | 0.24           | 0.56         | 0.82         |
| 405                                                   | D405G   | 0.77 | 0.87            | 0.16           | 0.56         | 0.86         |
| 405                                                   | D405H   | 0.27 | 0.38            | 0.25           | 3.35         | 3.59         |
| 405                                                   | D405I   | 0.62 | 1.04            | 0.49           | 0.83         | 1.02         |
| 405                                                   | D405K   | 0.63 | 0.65            | 0.24           | 0.43         | 0.72         |
| 405                                                   | D405L   | 0.41 | 0.95            | 0.76           | 0.38         | 0.43         |
| 405                                                   | D405M   | 0.52 | 1.31            | 0.58           | 0.59         | 0.69         |
| 405                                                   | D405N   | 0.74 | 1.04            | 0.78           | 1.34         | 1.32         |
| 405                                                   | D405P   | 0.77 | 0.80            | 0.19           | 0.99         | 1.02         |
| 405                                                   | D405Q   | 0.71 | 1.12            | 0.39           | 1.10         | 1.21         |
| 405                                                   | D405R   | 0.93 | 1.20            | 0.36           | 0.56         | 0.61         |
| 405                                                   | D405S   | 0.95 | 1.24            | 0.40           | 0.75         | 0.96         |
| 405                                                   | D405T   | 0.74 | 1.17            | 0.32           | 0.64         | 0.83         |
| 405                                                   | D405V   | 0.39 | 1.05            | 0.60           | 1.32         | 1.24         |
| 405                                                   | D405W   | 1.12 | 1.17            | 0.40           | 0.42         | 0.48         |
| 405                                                   | D405Y   | 1.56 | 1.07            | 0.39           | 0.31         | 0.07         |
| 406                                                   | S406A   | 1.28 | 1.05            | 1.70           | 1.01         | 0.99         |
| 406                                                   | S406C   | 0.57 | 0.86            | 1.05           | 0.66         | 0.71         |
| 406                                                   | S406D   | 0.10 | 0.09            | 0.07           | 0.34         | 1.33         |
| 406                                                   | S406E   | 1.24 | 1.04            | 0.99           | 0.94         | 0.90         |
| 406                                                   | S406F   | 1.01 | 1.04            | 0.72           | 0.56         | 0.38         |
| 406                                                   | S406G   | 0.84 | 1.00            | 0.67           | 0.49         | 0.39         |
| 406                                                   | S406H   | 0.62 | 0.99            | 0.78           | 0.85         | 0.79         |
| 406                                                   | S406I   | 2.11 | 1.06            | 0.70           | 0.83         | 0.63         |
| 406                                                   | S406K   | 0.15 | 0.11            | 0.05           | 1.18         | 0.67         |
| 406                                                   | S406L   | 0.18 | 0.22            | 0.32           | 0.26         | 0.42         |
| 406                                                   | S406M   | 0.89 | 1.01            | 0.78           | 0.85         | 0.75         |
| 406                                                   | S406N   | 0.73 | 1.02            | 0.79           | 0.80         | 0.63         |
| 406                                                   | S406P   | 1.07 | 1.02            | 1.10           | 1.16         | 1.41         |
| 406                                                   | S406Q   | 1.01 | 0.99            | 0.77           | 0.90         | 0.79         |
| 406                                                   | S406R   | 1.26 | 0.99            | 0.73           | 1.13         | 1.28         |
| 406                                                   | S406T   | 0.83 | 0.98            | 0.77           | 0.80         | 0.72         |
| 406                                                   | S406V   | 0.95 | 1.00            | 0.61           | 0.76         | 0.57         |
| 406                                                   | S406W   | 0.72 | 0.96            | 1.15           | 1.03         | 1.21         |
| 406                                                   | S406Y   | 0.86 | 0.98            | 0.77           | 0.59         | 0.39         |
| 407                                                   | S407A   | 0.92 | 1.00            | 1.15           | 0.89         | 0.86         |
| 407                                                   | S407C   | 0.55 | 1.03            | 1.19           | 0.80         | 0.87         |
| 407                                                   | S407D   | 0.93 | 1.00            | 0.91           | 0.71         | 0.61         |
| 407                                                   | S407E   | 0.79 | 1.00            | 1.03           | 0.83         | 0.75         |
| 407                                                   | S407F   | 1.19 | 1.05            | 0.57           | 0.61         | 0.39         |
| 407                                                   | S407G   | 0.90 | 1.03            | 0.63           | 0.80         | 0.63         |
| 407                                                   | S407H   | 1.17 | 1.01            | 0.61           | 1.09         | 1.27         |
| 407                                                   | S407I   | 0.23 | 0.13            | 0.21           | 0.57         | 1.16         |
| 407                                                   | S407K   | 0.90 | 1.04            | 0.49           | 1.07         | 1.29         |
| 407                                                   | S407L   | 1.02 | 1.08            | 0.66           | 0.73         | 0.56         |
| 407                                                   | S407M   | 0.11 | 0.13            | 0.09           | 0.37         | 1.24         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 407                                                   | S407N   | 1.09 | 1.02            | 0.70           | 1.06         | 1.12         |
| 407                                                   | S407P   | 0.56 | 0.94            | 0.64           | 0.19         | 0.39         |
| 407                                                   | S407Q   | 0.09 | 0.13            | 0.28           | 0.40         | 1.29         |
| 407                                                   | S407R   | 1.07 | 1.02            | 0.67           | 1.08         | 1.17         |
| 407                                                   | S407T   | 1.26 | 1.06            | 0.98           | 1.11         | 1.21         |
| 407                                                   | S407V   | 0.91 | 0.99            | 0.39           | 0.38         | 0.38         |
| 407                                                   | S407W   | 0.79 | 0.99            | 0.47           | 0.37         | 0.41         |
| 407                                                   | S407Y   | 1.01 | 0.99            | 0.49           | 0.55         | 0.45         |
| 410                                                   | R410A   | 0.84 | 0.71            | 0.52           | 0.99         | 1.09         |
| 410                                                   | R410C   | 0.43 | 0.74            | 0.99           | 1.09         | 1.15         |
| 410                                                   | R410D   | 1.27 | 0.78            | 0.90           | 1.21         | 1.52         |
| 410                                                   | R410E   | 0.65 | 0.74            | 0.47           | 1.10         | 1.21         |
| 410                                                   | R410F   | 0.54 | 0.65            | 0.43           | 1.04         | 1.23         |
| 410                                                   | R410G   | 0.69 | 0.73            | 0.67           | 1.19         | 1.61         |
| 410                                                   | R410H   | 0.64 | 0.74            | 0.85           | 1.18         | 1.60         |
| 410                                                   | R410I   | 0.49 | 0.53            | 0.38           | 0.77         | 0.82         |
| 410                                                   | R410K   | 0.73 | 0.76            | 0.90           | 1.23         | 1.20         |
| 410                                                   | R410L   | 0.89 | 0.73            | 0.65           | 0.87         | 0.84         |
| 410                                                   | R410M   | 0.53 | 0.71            | 0.70           | 0.90         | 1.03         |
| 410                                                   | R410N   | 0.77 | 0.76            | 0.97           | 1.22         | 1.58         |
| 410                                                   | R410P   | 0.49 | 0.56            | 0.42           | 0.45         | 0.75         |
| 410                                                   | R410Q   | 0.70 | 0.78            | 0.86           | 1.05         | 1.30         |
| 410                                                   | R410S   | 0.63 | 1.51            | 1.83           | 1.14         | 1.38         |
| 410                                                   | R410T   | 0.68 | 0.61            | 0.57           | 1.00         | 1.29         |
| 410                                                   | R410V   | 0.57 | 0.58            | 0.56           | 0.87         | 1.19         |
| 410                                                   | R410W   | 0.72 | 0.66            | 0.36           | 0.84         | 0.86         |
| 410                                                   | R410Y   | 0.71 | 0.63            | 0.68           | 1.11         | 1.40         |
| 413                                                   | S413A   | 0.57 | 1.15            | 1.04           | 1.21         | 1.01         |
| 413                                                   | S413C   | 0.58 | 1.03            | 0.72           | 0.98         | 1.05         |
| 413                                                   | S413D   | 0.28 | 0.90            | 0.90           | 0.64         | 0.72         |
| 413                                                   | S413E   | 0.48 | 0.89            | 0.51           | 0.83         | 1.22         |
| 413                                                   | S413F   | 0.96 | 0.80            | 0.61           | 1.71         | 1.87         |
| 413                                                   | S413G   | 0.35 | 0.81            | 0.61           | 1.26         | 1.07         |
| 413                                                   | S413H   | 0.71 | 0.81            | 0.45           | 1.10         | 1.02         |
| 413                                                   | S413I   | 0.70 | 1.18            | 0.72           | 1.06         | 1.23         |
| 413                                                   | S413K   | 0.64 | 0.86            | 0.61           | 0.99         | 0.82         |
| 413                                                   | S413L   | 1.00 | 1.05            | 0.72           | 1.10         | 1.46         |
| 413                                                   | S413M   | 0.83 | 0.87            | 0.59           | 1.31         | 1.48         |
| 413                                                   | S413N   | 0.54 | 1.03            | 0.81           | 0.73         | 0.69         |
| 413                                                   | S413P   | 0.27 | 0.76            | 0.62           | 1.71         | 2.11         |
| 413                                                   | S413Q   | 0.45 | 1.03            | 0.89           | 0.96         | 1.07         |
| 413                                                   | S413R   | 0.58 | 1.07            | 0.56           | 1.12         | 0.90         |
| 413                                                   | S413T   | 0.63 | 1.08            | 0.85           | 0.92         | 0.87         |
| 413                                                   | S413V   | 1.18 | 0.98            | 0.92           | 0.98         | 1.26         |
| 413                                                   | S413W   | 0.40 | 0.96            | 0.78           | 1.61         | 1.47         |
| 413                                                   | S413Y   | 0.71 | 0.88            | 0.48           | 1.68         | 1.49         |
| 414                                                   | H414A   | 1.38 | 0.98            | 0.99           | 1.04         | 1.08         |
| 414                                                   | H414C   | 0.25 | 0.05            | 0.37           | 4.50         | 4.02         |
| 414                                                   | H414D   | 0.85 | 0.82            | 0.92           | 1.01         | 0.88         |
| 414                                                   | H414E   | 0.50 | 0.91            | 0.43           | 0.05         | 0.11         |
| 414                                                   | H414F   | 0.78 | 0.97            | 0.73           | 0.86         | 0.57         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 414                                                   | H414G   | 0.97 | 0.89            | 0.61           | 0.51         | 0.20         |
| 414                                                   | H414I   | 0.57 | 0.94            | 0.56           | 0.34         | 0.20         |
| 414                                                   | H414K   | 0.82 | 1.04            | 0.94           | 0.89         | 0.77         |
| 414                                                   | H414L   | 0.78 | 0.93            | 0.45           | 0.27         | 0.17         |
| 414                                                   | H414M   | 0.46 | 0.97            | 1.09           | 0.95         | 1.06         |
| 414                                                   | H414N   | 0.10 | 0.13            | 0.13           | 1.62         | 2.06         |
| 414                                                   | H414P   | 0.21 | 0.05            | 0.05           | 3.19         | 0.30         |
| 414                                                   | H414Q   | 0.73 | 0.95            | 0.72           | 0.78         | 0.64         |
| 414                                                   | H414R   | 0.22 | 0.07            | 0.09           | 1.93         | 2.69         |
| 414                                                   | H414S   | 0.67 | 0.92            | 0.93           | 0.83         | 0.84         |
| 414                                                   | H414T   | 0.61 | 1.01            | 0.72           | 0.76         | 0.50         |
| 414                                                   | H414V   | 0.54 | 0.90            | 0.65           | 0.61         | 0.38         |
| 414                                                   | H414W   | 0.28 | 0.48            | 0.47           | 0.58         | 0.24         |
| 414                                                   | H414Y   | 1.00 | 0.98            | 0.78           | 0.86         | 0.67         |
| 416                                                   | A416C   | 0.49 | 0.73            | 0.49           | 0.58         | 0.23         |
| 416                                                   | A416D   | 0.25 | 0.47            | 0.38           | 0.16         | 0.05         |
| 416                                                   | A416E   | 0.37 | 0.98            | 0.69           | 0.34         | 0.05         |
| 416                                                   | A416F   | 0.38 | 0.97            | 0.28           | 0.05         | 0.10         |
| 416                                                   | A416G   | 0.49 | 0.61            | 0.40           | 0.52         | 0.25         |
| 416                                                   | A416H   | 0.43 | 0.87            | 0.23           | 0.10         | 0.05         |
| 416                                                   | A416I   | 0.59 | 0.98            | 0.68           | 0.67         | 0.29         |
| 416                                                   | A416K   | 0.58 | 0.99            | 0.48           | 0.61         | 0.41         |
| 416                                                   | A416L   | 0.22 | 0.46            | 0.12           | 0.19         | 0.17         |
| 416                                                   | A416M   | 0.32 | 0.92            | 0.49           | 0.42         | 0.19         |
| 416                                                   | A416P   | 0.27 | 0.30            | 0.12           | 0.14         | 0.05         |
| 416                                                   | A416Q   | 0.37 | 0.98            | 0.50           | 0.56         | 0.33         |
| 416                                                   | A416R   | 0.58 | 0.95            | 0.20           | 0.59         | 0.31         |
| 416                                                   | A416S   | 0.24 | 0.77            | 0.62           | 0.68         | 0.58         |
| 416                                                   | A416T   | 0.58 | 0.98            | 0.68           | 0.76         | 0.59         |
| 416                                                   | A416V   | 0.59 | 1.10            | 0.56           | 0.66         | 0.33         |
| 416                                                   | A416W   | 0.42 | 0.05            | 0.15           | 0.50         | 1.05         |
| 416                                                   | A416Y   | 0.38 | 0.84            | 0.17           | 0.07         | 0.05         |
| 417                                                   | L417A   | 0.51 | 0.95            | 0.90           | 0.75         | 0.50         |
| 417                                                   | L417C   | 0.36 | 0.81            | 0.57           | 0.35         | 0.05         |
| 417                                                   | L417D   | 0.49 | 0.90            | 0.49           | 0.10         | 0.05         |
| 417                                                   | L417E   | 0.22 | 0.62            | 0.50           | 0.36         | 0.11         |
| 417                                                   | L417F   | 0.45 | 0.96            | 0.74           | 0.67         | 0.49         |
| 417                                                   | L417G   | 0.57 | 0.99            | 0.42           | 0.54         | 0.21         |
| 417                                                   | L417H   | 0.76 | 1.02            | 0.24           | 0.12         | 0.10         |
| 417                                                   | L417I   | 0.48 | 0.89            | 0.30           | 0.20         | 0.05         |
| 417                                                   | L417K   | 0.51 | 1.01            | 0.59           | 0.82         | 0.81         |
| 417                                                   | L417M   | 0.53 | 1.02            | 0.73           | 0.78         | 0.59         |
| 417                                                   | L417P   | 0.23 | 0.62            | 0.28           | 0.07         | 0.07         |
| 417                                                   | L417R   | 0.81 | 1.01            | 0.46           | 0.80         | 0.68         |
| 417                                                   | L417S   | 0.80 | 1.01            | 0.74           | 0.61         | 0.39         |
| 417                                                   | L417T   | 0.66 | 1.01            | 0.51           | 0.41         | 0.12         |
| 417                                                   | L417V   | 0.50 | 0.89            | 0.45           | 0.23         | 0.05         |
| 417                                                   | L417W   | 0.80 | 0.98            | 0.28           | 0.05         | 0.05         |
| 417                                                   | L417Y   | 0.33 | 0.70            | 0.58           | 0.80         | 0.48         |
| 422                                                   | Q422A   | 1.39 | 0.93            | 0.75           | 0.66         | 0.68         |
| 422                                                   | Q422C   | 0.84 | 0.94            | 0.40           | 1.04         | 0.86         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 422                                                   | Q422D   | 0.31 | 1.58            | 0.05           | 0.43         | 0.90         |
| 422                                                   | Q422E   | 0.95 | 0.05            | 0.05           | 53.56        | 10.85        |
| 422                                                   | Q422G   | 0.33 | 1.32            | 0.10           | 0.89         | 1.23         |
| 422                                                   | Q422H   | 1.35 | 0.75            | 0.09           | 0.96         | 1.14         |
| 422                                                   | Q422I   | 0.93 | 1.12            | 0.60           | 0.47         | 0.87         |
| 422                                                   | Q422K   | 0.64 | 1.08            | 0.55           | 1.07         | 1.09         |
| 422                                                   | Q422L   | 0.93 | 0.89            | 0.20           | 1.13         | 0.94         |
| 422                                                   | Q422M   | 0.41 | 0.83            | 0.43           | 2.12         | 1.83         |
| 422                                                   | Q422N   | 1.12 | 0.37            | 0.05           | 2.24         | 2.42         |
| 422                                                   | Q422P   | 0.33 | 1.01            | 0.07           | 1.52         | 1.20         |
| 422                                                   | Q422R   | 0.64 | 0.93            | 0.35           | 1.34         | 1.04         |
| 422                                                   | Q422S   | 0.58 | 1.20            | 0.19           | 0.79         | 0.75         |
| 422                                                   | Q422T   | 0.57 | 1.22            | 0.91           | 0.73         | 0.93         |
| 422                                                   | Q422V   | 0.51 | 1.27            | 0.94           | 1.17         | 0.97         |
| 422                                                   | Q422W   | 0.32 | 0.05            | 0.11           | 6.62         | 0.06         |
| 422                                                   | Q422Y   | 0.71 | 0.70            | 0.11           | 1.26         | 1.39         |
| 426                                                   | Q426A   | 0.30 | 0.53            | 0.69           | 0.68         | 0.57         |
| 426                                                   | Q426C   | 0.83 | 0.83            | 0.81           | 0.78         | 0.78         |
| 426                                                   | Q426D   | 0.90 | 0.93            | 1.02           | 0.97         | 0.91         |
| 426                                                   | Q426E   | 0.05 | 0.36            | 0.20           | 1.40         | 1.97         |
| 426                                                   | Q426F   | 0.47 | 0.76            | 0.56           | 0.58         | 0.31         |
| 426                                                   | Q426G   | 0.75 | 0.82            | 0.69           | 0.87         | 0.66         |
| 426                                                   | Q426H   | 0.82 | 0.81            | 0.46           | 0.70         | 0.38         |
| 426                                                   | Q426I   | 0.59 | 0.72            | 0.75           | 1.01         | 0.89         |
| 426                                                   | Q426K   | 0.63 | 0.65            | 0.44           | 1.29         | 1.23         |
| 426                                                   | Q426L   | 0.86 | 0.87            | 0.56           | 0.74         | 0.54         |
| 426                                                   | Q426M   | 0.52 | 0.71            | 0.67           | 0.96         | 1.00         |
| 426                                                   | Q426N   | 0.51 | 0.77            | 0.49           | 1.06         | 0.98         |
| 426                                                   | Q426P   | 0.60 | 0.85            | 0.76           | 0.98         | 1.02         |
| 426                                                   | Q426R   | 0.51 | 0.87            | 0.43           | 0.79         | 0.79         |
| 426                                                   | Q426S   | 0.67 | 0.84            | 0.75           | 0.88         | 0.73         |
| 426                                                   | Q426T   | 0.63 | 0.88            | 0.71           | 0.81         | 0.64         |
| 426                                                   | Q426V   | 0.51 | 0.91            | 0.44           | 0.53         | 0.40         |
| 426                                                   | Q426W   | 0.38 | 0.72            | 0.44           | 0.49         | 0.23         |
| 426                                                   | Q426Y   | 0.44 | 0.72            | 0.64           | 0.67         | 0.33         |
| 427                                                   | A427C   | 1.03 | 0.36            | 0.31           | 0.88         | 0.54         |
| 427                                                   | A427D   | 1.06 | 0.48            | 0.31           | 0.16         | 0.23         |
| 427                                                   | A427F   | 0.65 | 0.80            | 0.52           | 1.08         | 0.97         |
| 427                                                   | A427G   | 0.84 | 0.78            | 0.44           | 0.37         | 0.29         |
| 427                                                   | A427H   | 0.92 | 0.39            | 0.14           | 0.59         | 0.36         |
| 427                                                   | A427I   | 0.92 | 0.33            | 0.14           | 0.62         | 0.50         |
| 427                                                   | A427L   | 0.62 | 0.20            | 0.16           | 0.76         | 0.40         |
| 427                                                   | A427M   | 0.70 | 0.12            | 0.08           | 1.76         | 1.63         |
| 427                                                   | A427N   | 1.34 | 0.77            | 0.42           | 0.36         | 0.16         |
| 427                                                   | A427P   | 0.79 | 0.59            | 0.35           | 0.60         | 0.51         |
| 427                                                   | A427Q   | 0.14 | 0.10            | 0.05           | 10.42        | 3.80         |
| 427                                                   | A427R   | 0.96 | 0.16            | 0.23           | 1.03         | 1.68         |
| 427                                                   | A427S   | 0.88 | 1.00            | 0.47           | 0.66         | 0.46         |
| 427                                                   | A427T   | 0.67 | 0.75            | 0.61           | 0.58         | 0.47         |
| 427                                                   | A427V   | 1.14 | 0.59            | 0.46           | 0.83         | 0.61         |
| 427                                                   | A427W   | 0.95 | 0.07            | 0.08           | 1.67         | 2.16         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                 |                 |              |
|-------------------------------------------------------|---------|------|-----------------|-----------------|-----------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac.  | Res.<br>EtOH    | Res.<br>Heat |
| 427                                                   | A427Y   | 0.11 | 0.43            | <del>0.05</del> | 0.40            | 0.97         |
| 429                                                   | A429C   | 0.39 | 0.69            | 0.69            | 0.88            | 0.82         |
| 429                                                   | A429D   | 0.70 | 0.88            | 1.07            | 1.07            | 0.90         |
| 429                                                   | A429E   | 1.11 | 0.90            | 0.90            | 1.10            | 1.11         |
| 429                                                   | A429F   | 0.54 | 1.08            | 0.65            | 0.72            | 0.45         |
| 429                                                   | A429G   | 0.62 | 0.88            | 0.61            | 0.66            | 0.41         |
| 429                                                   | A429H   | 0.41 | 0.76            | 0.26            | 0.19            | 0.41         |
| 429                                                   | A429I   | 0.59 | 0.92            | 0.55            | 0.61            | 0.34         |
| 429                                                   | A429K   | 0.89 | 1.03            | 0.69            | 0.83            | 0.84         |
| 429                                                   | A429L   | 0.46 | 0.91            | 0.74            | 0.92            | 0.67         |
| 429                                                   | A429M   | 0.99 | 1.07            | 1.05            | 0.93            | 0.97         |
| 429                                                   | A429N   | 0.13 | 0.18            | 0.22            | 0.38            | 1.99         |
| 429                                                   | A429P   | 0.46 | 0.48            | 0.24            | 0.17            | 0.48         |
| 429                                                   | A429Q   | 0.36 | 1.10            | 0.60            | 0.82            | 0.73         |
| 429                                                   | A429R   | 0.75 | 1.04            | 0.54            | 0.87            | 0.75         |
| 429                                                   | A429S   | 1.34 | 1.04            | 0.85            | 0.90            | 0.84         |
| 429                                                   | A429T   | 0.66 | 1.03            | 0.74            | 0.87            | 0.81         |
| 429                                                   | A429V   | 0.20 | 0.32            | 0.42            | 0.53            | 0.53         |
| 429                                                   | A429W   | 0.29 | 0.87            | 0.32            | <del>0.05</del> | 0.16         |
| 429                                                   | A429Y   | 0.66 | 1.04            | 0.74            | 0.76            | 0.61         |
| 431                                                   | F431A   | 0.40 | 0.97            | 0.35            | 0.22            | 0.18         |
| 431                                                   | F431C   | 0.10 | 3.60            | 0.09            | 1.05            | 1.36         |
| 431                                                   | F431D   | 0.52 | 0.94            | 0.41            | 0.13            | 0.14         |
| 431                                                   | F431E   | 0.33 | 0.59            | 0.37            | 0.23            | 0.28         |
| 431                                                   | F431G   | 0.33 | 0.37            | 0.61            | 0.44            | 0.59         |
| 431                                                   | F431H   | 0.71 | 0.91            | 0.43            | 0.17            | 0.21         |
| 431                                                   | F431I   | 0.93 | 0.92            | 0.36            | 0.06            | 0.18         |
| 431                                                   | F431K   | 0.25 | 0.22            | 0.29            | 0.99            | 1.10         |
| 431                                                   | F431L   | 0.61 | 1.04            | 0.32            | 0.09            | 0.13         |
| 431                                                   | F431M   | 0.46 | 0.94            | 0.53            | 0.12            | 0.17         |
| 431                                                   | F431P   | 0.16 | 0.20            | 0.36            | 1.11            | 1.82         |
| 431                                                   | F431Q   | 0.33 | 1.06            | 0.45            | 0.12            | 0.26         |
| 431                                                   | F431S   | 0.30 | 0.97            | 0.28            | 0.19            | 0.27         |
| 431                                                   | F431T   | 0.10 | 1.39            | 0.30            | 0.27            | 0.36         |
| 431                                                   | F431V   | 0.38 | 1.04            | 0.58            | 0.20            | 0.18         |
| 431                                                   | F431W   | 0.36 | 0.92            | 0.15            | 0.10            | 0.22         |
| 431                                                   | F431Y   | 0.57 | 0.98            | 0.40            | 0.48            | 0.14         |
| 433                                                   | A433C   | 0.42 | 0.96            | 0.69            | 0.55            | 0.33         |
| 433                                                   | A433D   | 1.23 | 1.06            | 0.93            | 0.78            | 0.63         |
| 433                                                   | A433E   | 0.35 | 1.00            | <del>0.05</del> | 0.10            | 0.15         |
| 433                                                   | A433F   | 0.57 | 1.01            | 0.13            | 0.10            | 0.09         |
| 433                                                   | A433G   | 1.08 | 1.08            | 0.84            | 0.56            | 0.25         |
| 433                                                   | A433H   | 0.10 | 0.09            | <del>0.05</del> | 1.01            | 4.13         |
| 433                                                   | A433I   | 0.40 | 0.98            | 0.49            | 0.27            | 0.10         |
| 433                                                   | A433K   | 0.53 | 0.98            | 0.70            | 0.82            | 0.66         |
| 433                                                   | A433L   | 0.46 | 1.07            | 0.57            | 0.71            | 0.51         |
| 433                                                   | A433M   | 0.70 | 1.00            | 0.72            | 0.71            | 0.54         |
| 433                                                   | A433N   | 0.60 | 0.93            | 0.86            | 0.69            | 0.49         |
| 433                                                   | A433P   | 0.52 | 1.00            | 0.66            | 0.71            | 0.50         |
| 433                                                   | A433Q   | 0.85 | 1.06            | 0.71            | 0.81            | 0.68         |
| 433                                                   | A433R   | 1.25 | 1.00            | 0.82            | 0.85            | 0.67         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 433                                                   | A433S   | 0.81 | 1.03            | 0.70           | 0.72         | 0.54         |
| 433                                                   | A433T   | 0.57 | 1.01            | 0.65           | 0.50         | 0.28         |
| 433                                                   | A433V   | 0.46 | 0.94            | 0.90           | 0.21         | 0.14         |
| 433                                                   | A433W   | 0.28 | 0.55            | 0.28           | 0.16         | 0.20         |
| 433                                                   | A433Y   | 0.59 | 0.90            | 0.52           | 0.08         | 0.09         |
| 436                                                   | V436A   | 0.87 | 1.02            | 0.91           | 0.41         | 0.17         |
| 436                                                   | V436C   | 0.73 | 0.96            | 0.76           | 0.73         | 0.48         |
| 436                                                   | V436D   | 0.98 | 0.99            | 0.77           | 0.35         | 0.14         |
| 436                                                   | V436E   | 1.11 | 1.04            | 0.75           | 0.76         | 0.58         |
| 436                                                   | V436F   | 0.64 | 0.99            | 0.53           | 0.45         | 0.15         |
| 436                                                   | V436G   | 0.37 | 0.93            | 0.26           | 0.15         | 0.20         |
| 436                                                   | V436H   | 0.55 | 0.99            | 0.45           | 0.10         | 0.09         |
| 436                                                   | V436I   | 0.58 | 1.02            | 0.76           | 0.93         | 0.87         |
| 436                                                   | V436K   | 0.29 | 0.67            | 0.15           | 0.35         | 0.29         |
| 436                                                   | V436L   | 0.66 | 1.02            | 0.77           | 0.80         | 0.68         |
| 436                                                   | V436M   | 0.53 | 0.99            | 0.74           | 0.69         | 0.42         |
| 436                                                   | V436N   | 0.30 | 0.76            | 0.31           | 0.29         | 0.14         |
| 436                                                   | V436P   | 0.28 | 0.18            | 0.05           | 0.59         | 0.99         |
| 436                                                   | V436Q   | 0.53 | 0.90            | 0.49           | 0.47         | 0.20         |
| 436                                                   | V436R   | 0.87 | 0.98            | 0.47           | 0.28         | 0.10         |
| 436                                                   | V436S   | 0.47 | 0.49            | 0.13           | 0.28         | 0.14         |
| 436                                                   | V436T   | 0.74 | 1.03            | 0.68           | 0.53         | 0.26         |
| 436                                                   | V436W   | 0.59 | 0.94            | 0.56           | 0.11         | 0.13         |
| 436                                                   | V436Y   | 0.05 | 0.05            | 0.05           | 0.05         | 0.05         |
| 440                                                   | T440A   | 1.17 | 1.02            | 0.84           | 0.99         | 0.92         |
| 440                                                   | T440C   | 0.60 | 0.93            | 0.92           | 1.08         | 1.07         |
| 440                                                   | T440D   | 0.86 | 0.97            | 0.85           | 0.90         | 0.79         |
| 440                                                   | T440E   | 0.77 | 1.01            | 0.85           | 1.04         | 1.12         |
| 440                                                   | T440F   | 0.65 | 1.01            | 0.50           | 0.91         | 0.82         |
| 440                                                   | T440G   | 0.40 | 0.91            | 0.51           | 0.66         | 0.51         |
| 440                                                   | T440H   | 1.01 | 1.06            | 0.52           | 0.85         | 0.80         |
| 440                                                   | T440I   | 0.43 | 1.00            | 0.68           | 1.07         | 1.12         |
| 440                                                   | T440K   | 0.82 | 1.02            | 0.64           | 1.01         | 1.07         |
| 440                                                   | T440L   | 0.56 | 0.75            | 0.48           | 1.06         | 1.08         |
| 440                                                   | T440M   | 0.62 | 1.03            | 0.72           | 1.02         | 1.06         |
| 440                                                   | T440N   | 0.68 | 0.91            | 0.24           | 0.11         | 0.13         |
| 440                                                   | T440P   | 0.25 | 0.26            | 0.10           | 0.72         | 0.77         |
| 440                                                   | T440Q   | 1.26 | 1.02            | 0.82           | 1.05         | 1.12         |
| 440                                                   | T440R   | 0.40 | 0.73            | 0.15           | 0.86         | 0.89         |
| 440                                                   | T440S   | 0.88 | 1.01            | 0.91           | 0.94         | 0.93         |
| 440                                                   | T440V   | 0.27 | 0.40            | 0.05           | 0.28         | 0.27         |
| 440                                                   | T440W   | 0.78 | 0.89            | 0.47           | 0.95         | 0.90         |
| 440                                                   | T440Y   | 1.22 | 1.01            | 0.93           | 0.90         | 0.91         |
| 441                                                   | N441A   | 0.16 | 0.07            | 0.71           | 0.26         | 1.16         |
| 441                                                   | N441C   | 0.28 | 0.78            | 1.16           | 0.27         | 0.12         |
| 441                                                   | N441D   | 0.09 | 0.05            | 0.21           | 1.89         | 3.43         |
| 441                                                   | N441E   | 0.48 | 0.89            | 0.96           | 0.44         | 0.10         |
| 441                                                   | N441F   | 0.42 | 0.86            | 0.91           | 0.88         | 0.61         |
| 441                                                   | N441G   | 0.42 | 0.87            | 0.79           | 0.33         | 0.09         |
| 441                                                   | N441H   | 0.11 | 0.06            | 0.15           | 3.38         | 1.88         |
| 441                                                   | N441I   | 0.17 | 0.39            | 0.52           | 0.10         | 0.10         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                |              |              |
|-------------------------------------------------------|---------|------|-----------------|----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac. | Res.<br>EtOH | Res.<br>Heat |
| 441                                                   | N441K   | 0.29 | 0.84            | 0.72           | 0.30         | 0.21         |
| 441                                                   | N441L   | 0.46 | 0.89            | 0.92           | 0.83         | 0.67         |
| 441                                                   | N441M   | 0.38 | 0.87            | 0.66           | 0.41         | 0.10         |
| 441                                                   | N441P   | 0.20 | 0.37            | 0.40           | 0.17         | 0.28         |
| 441                                                   | N441Q   | 0.37 | 0.90            | 0.71           | 0.28         | 0.14         |
| 441                                                   | N441R   | 0.28 | 0.81            | 0.57           | 0.26         | 0.18         |
| 441                                                   | N441S   | 0.39 | 0.89            | 0.88           | 0.52         | 0.31         |
| 441                                                   | N441T   | 0.26 | 0.70            | 0.55           | 0.05         | 0.14         |
| 441                                                   | N441V   | 0.18 | 0.55            | 0.49           | 0.15         | 0.23         |
| 441                                                   | N441W   | 0.31 | 0.79            | 0.72           | 0.70         | 0.36         |
| 441                                                   | N441Y   | 0.33 | 0.83            | 0.88           | 0.80         | 0.68         |
| 443                                                   | N443C   | 0.34 | 1.74            | 1.69           | 1.22         | 0.94         |
| 443                                                   | N443D   | 0.24 | 1.85            | 1.21           | 1.01         | 0.73         |
| 443                                                   | N443E   | 0.30 | 1.75            | 0.76           | 1.19         | 0.89         |
| 443                                                   | N443F   | 0.79 | 0.68            | 0.42           | 1.14         | 1.02         |
| 443                                                   | N443G   | 0.26 | 1.00            | 0.23           | 1.15         | 1.43         |
| 443                                                   | N443I   | 0.38 | 1.07            | 0.53           | 1.40         | 1.20         |
| 443                                                   | N443L   | 0.83 | 0.37            | 0.40           | 1.88         | 2.31         |
| 443                                                   | N443M   | 0.49 | 0.96            | 0.62           | 1.13         | 1.29         |
| 443                                                   | N443P   | 0.48 | 0.16            | 0.13           | 1.56         | 4.87         |
| 443                                                   | N443Q   | 0.34 | 1.36            | 0.89           | 0.46         | 1.02         |
| 443                                                   | N443R   | 0.31 | 1.34            | 0.40           | 0.50         | 0.91         |
| 443                                                   | N443S   | 0.51 | 1.21            | 0.77           | 0.93         | 0.76         |
| 443                                                   | N443T   | 0.54 | 0.33            | 0.12           | 3.61         | 2.60         |
| 443                                                   | N443V   | 0.47 | 1.22            | 0.43           | 1.20         | 0.86         |
| 443                                                   | N443W   | 0.97 | 0.05            | 0.10           | 2.57         | 0.94         |
| 443                                                   | N443Y   | 0.60 | 0.05            | 0.05           | 0.33         | 0.05         |
| 444                                                   | P444A   | 0.85 | 0.40            | 0.34           | 2.29         | 1.44         |
| 444                                                   | P444C   | 0.42 | 0.61            | 0.74           | 2.47         | 2.22         |
| 444                                                   | P444D   | 2.18 | 0.53            | 0.54           | 0.82         | 1.78         |
| 444                                                   | P444E   | 0.93 | 0.53            | 0.29           | 12.61        | 4.67         |
| 444                                                   | P444F   | 0.42 | 0.58            | 0.52           | 3.38         | 2.94         |
| 444                                                   | P444G   | 0.48 | 0.53            | 0.42           | 2.38         | 1.50         |
| 444                                                   | P444H   | 0.27 | 0.63            | 0.25           | 1.19         | 1.66         |
| 444                                                   | P444I   | 0.25 | 0.54            | 0.40           | 1.39         | 1.77         |
| 444                                                   | P444K   | 0.53 | 0.47            | 0.51           | 1.53         | 1.73         |
| 444                                                   | P444L   | 0.20 | 0.58            | 0.45           | 1.57         | 1.57         |
| 444                                                   | P444M   | 0.33 | 0.39            | 0.45           | 2.24         | 2.88         |
| 444                                                   | P444N   | 0.33 | 0.42            | 0.43           | 1.63         | 1.88         |
| 444                                                   | P444Q   | 0.40 | 0.66            | 0.49           | 1.74         | 1.66         |
| 444                                                   | P444R   | 0.38 | 0.43            | 0.54           | 1.98         | 1.85         |
| 444                                                   | P444S   | 0.98 | 0.49            | 0.33           | 1.47         | 2.17         |
| 444                                                   | P444T   | 0.78 | 0.65            | 0.52           | 0.68         | 1.08         |
| 444                                                   | P444V   | 0.33 | 0.54            | 0.41           | 1.80         | 1.60         |
| 444                                                   | P444W   | 0.75 | 0.49            | 0.31           | 1.85         | 2.13         |
| 444                                                   | P444Y   | 0.33 | 0.84            | 0.59           | 0.50         | 0.69         |
| 445                                                   | S445A   | 0.27 | 0.68            | 0.52           | 0.85         | 0.73         |
| 445                                                   | S445D   | 0.35 | 0.88            | 0.90           | 0.57         | 0.22         |
| 445                                                   | S445E   | 0.49 | 0.95            | 0.74           | 0.75         | 0.54         |
| 445                                                   | S445F   | 0.40 | 0.54            | 0.33           | 0.24         | 0.17         |
| 445                                                   | S445G   | 0.37 | 0.67            | 0.61           | 0.76         | 0.64         |

| Table 3-1: Performance Index Values for CBH2 Variants |         |      |                 |                 |              |              |
|-------------------------------------------------------|---------|------|-----------------|-----------------|--------------|--------------|
| Position                                              | Variant | HPLC | PASC<br>Sp. Ac. | PCS<br>Sp. Ac.  | Res.<br>EtOH | Res.<br>Heat |
| 445                                                   | S445H   | 0.07 | 0.16            | <del>0.05</del> | 0.71         | 1.15         |
| 445                                                   | S445I   | 0.41 | 0.61            | 0.34            | 0.34         | 0.20         |
| 445                                                   | S445L   | 0.51 | 0.68            | 0.35            | 0.36         | 0.27         |
| 445                                                   | S445N   | 0.58 | 1.03            | 0.77            | 0.72         | 0.51         |
| 445                                                   | S445P   | 0.65 | 1.00            | 0.67            | 1.01         | 1.09         |
| 445                                                   | S445Q   | 0.32 | 0.83            | 0.57            | 0.78         | 0.60         |
| 445                                                   | S445R   | 0.75 | 1.01            | 0.85            | 0.86         | 0.83         |
| 445                                                   | S445T   | 0.41 | 0.90            | 0.74            | 0.85         | 0.82         |
| 445                                                   | S445V   | 0.53 | 0.83            | 0.62            | 0.57         | 0.38         |
| 445                                                   | S445W   | 0.32 | 0.55            | 0.14            | 0.17         | 0.26         |
| 445                                                   | S445Y   | 0.33 | 0.70            | 0.29            | 0.27         | 0.13         |
| 447                                                   | L447A   | 0.46 | 0.92            | 0.97            | 0.67         | 0.39         |
| 447                                                   | L447C   | 0.47 | 0.94            | 0.96            | 0.84         | 0.70         |
| 447                                                   | L447D   | 0.51 | 0.96            | 1.27            | 0.64         | 0.40         |
| 447                                                   | L447E   | 0.45 | 0.92            | 0.98            | 0.77         | 0.57         |
| 447                                                   | L447F   | 0.40 | 0.91            | 0.92            | 0.99         | 1.10         |
| 447                                                   | L447G   | 0.37 | 0.83            | 0.94            | 0.71         | 0.42         |
| 447                                                   | L447H   | 0.11 | 0.09            | 0.14            | 1.66         | 2.62         |
| 447                                                   | L447I   | 0.29 | 0.39            | 0.37            | 0.57         | 0.59         |
| 447                                                   | L447K   | 0.34 | 0.93            | 0.94            | 0.63         | 0.45         |
| 447                                                   | L447M   | 0.76 | 1.02            | 0.99            | 0.93         | 0.97         |
| 447                                                   | L447N   | 0.58 | 0.97            | 0.86            | 0.80         | 0.52         |
| 447                                                   | L447P   | 0.44 | 0.97            | 0.98            | 0.74         | 0.50         |
| 447                                                   | L447Q   | 0.49 | 0.97            | 0.68            | 0.72         | 0.51         |
| 447                                                   | L447R   | 0.41 | 0.95            | 1.02            | 0.64         | 0.43         |
| 447                                                   | L447S   | 0.50 | 0.95            | 0.59            | 0.70         | 0.42         |
| 447                                                   | L447T   | 0.51 | 0.99            | 0.93            | 0.78         | 0.59         |
| 447                                                   | L447V   | 0.72 | 0.99            | 1.02            | 0.86         | 0.76         |
| 447                                                   | L447W   | 0.60 | 0.96            | 0.85            | 0.89         | 0.79         |
| 447                                                   | L447Y   | 0.69 | 0.97            | 0.89            | 0.92         | 0.89         |

[232] Non Combinable variants are those for which all Pi values are  $\leq 0.05$ . For *Hypocrea jecorina* CBH2, of the 2,828 variants, 6 are non-combinable. They are A121Q, L243H, A322Q, L328C, L328Y, V436Y. Any CBH2 which has one of the above substitutions relative to *Hypocrea jecorina* CBH2 can be improved by mutating that amino acid to one of the combinable substitutions at that position, or to the amino acid present in *Hypocrea jecorina* CBH2 at position 121, 243, 322, 328, or 463.

| Table 3-2: CBH2 Combinatorial Variant | HPLC | Act.<br>CC | Act.<br>PASC | Act.<br>PCS | EtOH<br>ratio | Heat<br>ratio |
|---------------------------------------|------|------------|--------------|-------------|---------------|---------------|
| CBH2.L111S/L144W/T154C/Y162N          | 0.17 | 0.61       | 0.67         | 1.45        | 1.05          | 1.16          |
| CBH2.L111S/L144W/T154V/Y162N/S413Y    | 0.39 | 0.39       | 0.73         | 0.97        | 0.97          | 1.05          |
| CBH2.L144W/T154C/S413Y                | 0.39 | 0.32       | 0.88         | 1.07        | 1.02          | 1.09          |
| CBH2.L111S/T154V                      | 0.10 | 0.72       | 0.49         | 0.92        | 1.06          | 1.12          |
| CBH2.T154C                            | 0.47 | 0.44       | 1.02         | 1.05        | 0.92          | 0.89          |
| CBH2.L144Q/T154V/Y162N/R410S/S413Y    | 0.15 | 0.31       | 0.50         | 0.81        | 0.95          | 0.96          |

| <b>Table 3-2: CBH2 Combinatorial Variant</b> | <b>HPLC</b> | <b>Act.<br/>CC</b> | <b>Act.<br/>PASC</b> | <b>Act.<br/>PCS</b> | <b>EtOH<br/>ratio</b> | <b>Heat<br/>ratio</b> |
|----------------------------------------------|-------------|--------------------|----------------------|---------------------|-----------------------|-----------------------|
| CBH2.L111S/T154C/Y162N                       | 0.13        | 5.44               | 1.11                 | 6.50                | 0.91                  | 0.82                  |
| CBH2.L111S/L144W/Y162N/R410S/S413W           | 0.12        | 1.03               | 0.50                 | 0.94                | 0.97                  | 1.07                  |
| CBH2.L111S/L144W/T154C/R410S/S413Y           | 0.08        | 0.79               | 0.48                 | 0.85                | 0.88                  | 0.85                  |
| CBH2.L144W/T154C/Y162N/R410S/S413W           | 0.12        | 1.52               | 0.43                 | 0.23                | 0.95                  | 0.55                  |
| CBH2.Y162N/R410S/S413Y                       | 0.57        | 0.55               | 0.77                 | 0.89                | 1.09                  | 1.42                  |
| CBH2.L144W/T154C/R410S/S413Y                 | 0.52        | 0.72               | 0.85                 | 0.97                | 1.05                  | 1.32                  |
| CBH2.L144Q                                   | 0.61        | 0.41               | 0.75                 | 0.98                | 1.10                  | 1.39                  |
| CBH2.L111S/L144W/R410S/S413Y                 | 0.66        | 0.46               | 0.70                 | 0.67                | 1.11                  | 1.43                  |
| No Sequence                                  | 0.20        | 0.23               | 0.54                 | 1.06                | 1.07                  | 1.19                  |
| CBH2.L144Q/Y162N/R410S/S413Y                 | 0.20        | 0.62               | 0.69                 | 0.84                | 1.02                  | 1.42                  |
| CBH2.L144W/T154V/R410S/S413Y                 |             |                    |                      |                     |                       |                       |
| CBH2.L144W/T154C/S413W                       | 0.14        | 1.09               | 0.44                 | 0.78                | 1.17                  | 1.23                  |
| CBH2.R410S/S413Y                             | 0.12        | 0.92               | 0.68                 | 1.10                | 0.89                  | 1.06                  |
| CBH2.L111S/L144Q/T154V/Y162N/R410S/S413Y     | 0.12        | 1.23               | 0.54                 | 1.02                | 0.95                  | 1.20                  |
| CBH2.L111S/T154C/R410S/S413W                 | 0.39        | 0.64               | 0.54                 | 0.64                | 1.15                  | 1.13                  |
| CBH2.L144W/R410S/S413Y                       | 0.27        | 0.99               | 0.63                 | 0.74                | 1.03                  | 1.39                  |
| CBH2.L144Q/T154C/Y162N/S413Y                 | 0.09        | 1.72               | 0.54                 | 1.35                | 1.01                  | 1.21                  |
| CBH2.L144Q/Y162N/R410S/S413W                 | 0.13        | 1.65               | 0.64                 | 1.27                | 1.04                  | 1.20                  |
| CBH2.L111S/L144W/T154C/Y162N/R410S/S413Y     | 0.11        | 1.15               | 0.46                 | 0.91                | 1.16                  | 1.15                  |
| CBH2.L144W/T154V/Y162N                       | 0.19        | 0.95               | 0.80                 | 1.00                | 0.94                  | 0.72                  |
| CBH2.L111S/T154C/Y162N/R410S/S413Y           | 0.23        | 0.50               | 0.56                 | 0.79                | 1.03                  | 1.31                  |
| CBH2.L144Q/T154C/S413W                       | 0.32        | 0.95               | 0.75                 | 0.79                | 1.06                  | 1.32                  |
| CBH2.L144W/T154V/S413Y                       | 0.20        | 1.18               | 0.73                 | 0.72                | 0.84                  | 0.53                  |
| CBH2.T154V/Y162N/R410S/S413Y                 | 0.65        | 0.69               | 0.78                 | 0.96                | 1.15                  | 1.51                  |
| CBH2.L111S                                   | 0.61        | 1.25               | 0.95                 | 1.02                | 1.00                  | 1.14                  |
| CBH2.T154V/R410S/S413W                       | 0.17        | 1.28               | 0.44                 | 0.75                | 1.06                  | 1.28                  |
| CBH2.L111S/L144W/T154V/R410S/S413W           | 0.15        | 3.33               | 0.52                 | 0.68                | 0.93                  | 1.45                  |
| CBH2.T154C/Y162N/S413Y                       | 0.15        | 1.01               | 0.70                 | 1.12                | 1.09                  | 1.16                  |
| CBH2.L111S/L144W/R410S/S413W                 | 0.08        | 1.75               | 0.43                 | 0.94                | 0.99                  | 1.16                  |
| CBH2.L144W/T154C/Y162N/S413Y                 | 0.12        | 2.38               | 0.67                 | 1.43                | 0.92                  | 0.96                  |
| CBH2.L144W/Y162N                             | 0.16        | 0.80               | 0.83                 | 1.13                | 0.93                  | 0.72                  |
| CBH2.T154C/Y162N/R410S/S413W                 | 0.25        | 1.88               | 0.59                 | 0.96                | 1.10                  | 1.43                  |
| CBH2.L111S/L144Q/T154C/R410S/S413Y           | 0.12        | 0.00               | 0.09                 | 0.36                | 0.43                  | 0.36                  |
| CBH2.L111S/L144W/Y162N/R410S                 |             |                    |                      |                     |                       |                       |
| CBH2.L144W/T154C                             | 0.13        | 2.35               | 0.56                 | 0.99                | 0.94                  | 1.13                  |
| CBH2.L111S/L144W/T154C/S413Y                 | 0.54        | 0.95               | 0.89                 | 0.99                | 1.08                  | 1.26                  |
| CBH2.L111S/L144W/Y162N/R410S/S413Y           | 0.20        | 1.01               | 0.57                 | 0.88                | 1.12                  | 1.31                  |
| CBH2.L111S/R410S/S413W                       | 0.62        | 0.43               | 0.69                 | 0.66                | 1.12                  | 1.39                  |
| CBH2.L111S/L144Q/T154V/R410S/S413Y           | 0.50        | 0.74               | 0.68                 | 0.75                | 1.15                  | 1.45                  |
| CBH2.L111S/L144Q/R410S                       | 0.61        | 1.00               | 0.76                 | 0.83                | 0.99                  | 1.03                  |
| CBH2.L111S/L144Q/T154C/S413Y                 | 0.54        | 0.88               | 0.81                 | 0.89                | 1.07                  | 1.29                  |
| CBH2.L111S/L144Q/T154V/R410S                 |             |                    |                      |                     |                       |                       |
| CBH2.L111S/L144W/Y162N/S413Y                 | 0.18        | 3.03               | 0.63                 | 1.01                | 1.03                  | 1.18                  |
| CBH2.L111S/L144Q/T154C/Y162N/R410S           | 0.13        | 4.55               | 0.55                 | 0.79                | 0.65                  | 0.75                  |
| CBH2.T154V/R410S/S413Y                       | 0.51        | 0.88               | 0.71                 | 0.82                | 1.10                  | 1.38                  |
| CBH2.L144W/T154V/Y162N/R410S/S413Y           | 0.22        | 2.00               | 0.58                 | 0.85                | 1.15                  | 1.33                  |
| CBH2.L144W/T154V/Y162N/S413Y                 | 0.02        | 4.80               | 0.12                 | 0.13                | 1.26                  | 1.41                  |
| CBH2.L111S/L144Q/R410S/S413W                 | 0.45        | 0.42               | 0.66                 | 0.85                | 1.12                  | 1.30                  |
| CBH2.L111S/L144W/T154V/R410S/S413Y           | 0.70        | 0.58               | 0.75                 | 0.82                | 1.10                  | 1.43                  |
| CBH2.L111S/L144W/Y162N                       | 0.13        | 2.62               | 0.64                 | 0.58                | 0.92                  | 0.80                  |
| CBH2.L144Q/T154C/Y162N/R410S/S413Y           |             |                    |                      |                     |                       |                       |
| CBH2.L111S/T154V/S413Y                       | 0.59        | 0.66               | 0.90                 | 1.00                | 1.10                  | 1.43                  |

| <b>Table 3-2: CBH2 Combinatorial Variant</b> | <b>HPLC</b> | <b>Act.<br/>CC</b> | <b>Act.<br/>PASC</b> | <b>Act.<br/>PCS</b> | <b>EtOH<br/>ratio</b> | <b>Heat<br/>ratio</b> |
|----------------------------------------------|-------------|--------------------|----------------------|---------------------|-----------------------|-----------------------|
| CBH2.L111S/T154V/Y162N/S413W                 | 0.09        | 5.39               | 0.46                 | 0.80                | 0.83                  | 1.58                  |
| CBH2.L111S/Y162N/S413W                       | 0.15        | 2.13               | 0.48                 | 0.77                | 0.95                  | 1.12                  |
| CBH2.L111S/L144W/T154C                       | 0.57        | 1.70               | 0.94                 | 0.97                | 0.92                  | 1.02                  |
| CBH2.L111S/L144Q/Y162N/R410S                 | 0.17        | 2.48               | 0.54                 | 0.93                | 0.96                  | 0.95                  |
| CBH2.L111S/T154V/Y162N                       | 0.29        | 1.41               | 0.90                 | 0.96                | 0.89                  | 0.97                  |
| CBH2.L111S/L144W/S413Y                       | 0.80        | 0.78               | 0.90                 | 0.86                | 1.07                  | 1.43                  |
| CBH2.T154V/Y162N                             |             |                    |                      |                     |                       |                       |
| CBH2.L111S/T154C/S413Y                       | 0.65        | 0.89               | 0.89                 | 1.19                | 1.16                  | 1.36                  |
| CBH2.L111S/L144W/T154V/S413Y                 | 0.60        | 0.98               | 0.90                 | 0.93                | 1.11                  | 1.32                  |
| CBH2.L111S/L144W/T154V/R410S                 | 0.52        | 0.88               | 0.74                 | 0.81                | 1.04                  | 1.27                  |
| CBH2.L111S/L144W/T154C/S413W                 | 0.42        | 0.81               | 0.76                 | 0.78                | 1.10                  | 1.39                  |
| CBH2.L111S/L144W/T154V/Y162N/R410S           | 0.24        | 1.65               | 0.65                 | 0.95                | 0.95                  | 1.09                  |
| CBH2.L111S/L144Q                             | 0.74        | 1.29               | 0.95                 | 0.95                | 0.93                  | 0.92                  |
| CBH2.L111S/L144Q/T154V/S413Y                 | 0.54        | 1.49               | 0.90                 | 1.01                | 1.06                  | 1.24                  |
| CBH2.L111S/L144Q/S413Y                       | 0.77        | 1.20               | 0.92                 | 0.89                | 1.01                  | 1.17                  |
| CBH2.L144Q/R410S/S413Y                       | 0.32        | 0.44               | 0.83                 | 1.29                | 1.10                  | 1.26                  |
| CBH2.L111F/L144W/T154V/Y162N/S413Y           | 0.12        | 0.88               | 0.59                 | 1.30                | 0.96                  | 1.19                  |
| CBH2.L111S/L144Q/T154C/Y162N/S413W           | 0.12        | 0.04               | 0.26                 | 0.82                | 1.05                  | 1.05                  |
| CBH2.S313T/S316P/G384Q                       | 1.01        |                    | 1.08                 | 1.22                | 1.12                  | 1.41                  |
| CBH2.P98L/S316P/G384Q                        | 0.48        |                    | 1.01                 | 1.31                | 1.15                  | 1.56                  |
| CBH2.P98L/S313T/G384Q/N443I                  | 0.14        |                    | 0.94                 | 1.08                | 0.90                  | 0.31                  |
| CBH2.K194E/S313T/S316P/G384C                 | 0.27        |                    | 0.90                 | 0.83                | 1.11                  | 1.60                  |
| CBH2.P98L/K194E/S313T/S316F/G384Q            | 0.12        |                    | 1.25                 | 1.77                | 1.03                  | 1.36                  |
| CBH2.P98L/K194C/G384Q                        |             |                    |                      |                     |                       |                       |
| CBH2.K194C/S316P                             | 0.23        |                    | 1.12                 | 1.14                | 0.96                  | 1.16                  |
| CBH2.K194E                                   | 0.41        |                    | 1.05                 | 0.97                | 0.92                  | 0.91                  |
| CBH2.P98L/K194C/S313T/G384Q/N443I            |             |                    |                      |                     |                       |                       |
| CBH2.K194C/S313T/S316P/G384Q/N443I           | 0.08        |                    | 1.08                 | 1.37                | 0.53                  | 0.50                  |
| CBH2.P98L/G384C                              | 0.36        |                    | 1.18                 | 0.95                | 0.98                  | 1.17                  |
| CBH2.K194C                                   | 0.27        |                    | 1.16                 | 1.00                | 0.76                  | 0.80                  |
| CBH2.K194E/G384C                             | 0.21        |                    | 0.79                 | 0.78                | 1.05                  | 1.10                  |
| CBH2.P98L/K194E/S313T/S316P/G384C/N443I      |             |                    |                      |                     |                       |                       |
| CBH2.P98L/S313T/S316P/G384Q                  | 0.50        |                    | 1.01                 | 1.11                | 1.21                  | 1.61                  |
| CBH2.P98L/K194E/S316P/G384C/N443I            |             |                    |                      |                     |                       |                       |
| CBH2.P98L/K194E/S316P                        | 0.27        |                    | 1.15                 | 1.14                | 1.11                  | 1.39                  |
| CBH2.S316P/G384Q/N443I                       | 0.16        |                    | 0.89                 | 0.72                | 0.66                  | 0.42                  |
| CBH2.P98L/K194E/S316P/G384Q                  | 0.15        |                    | 1.14                 | 1.56                | 1.05                  | 1.37                  |
| CBH2.P98L/S313T/N443I                        | 0.19        |                    | 0.99                 | 1.11                | 0.71                  | 0.29                  |
| CBH2.K194E/G384C/N443I                       | 0.05        |                    | 0.63                 | 0.91                | 0.10                  | 0.73                  |
| CBH2.P98L/K194C/G384C/N443I                  |             |                    |                      |                     |                       |                       |
| CBH2.K194E/S313T                             | 0.38        |                    | 1.12                 | 0.99                | 0.87                  | 1.04                  |
| CBH2.P98L/K194E/G384C                        | 0.08        |                    | 1.14                 | 1.75                | 0.87                  | 1.21                  |
| CBH2.K194E/S313T/S316P/G384Q                 | 0.47        |                    | 0.98                 | 1.37                | 1.14                  | 1.55                  |
| CBH2.K194C/G384C/N443I                       |             |                    |                      |                     |                       |                       |
| CBH2.K194E/S313T/S316P/G384Q/N443I           | 0.05        |                    | 0.78                 | 0.78                | 0.07                  | 0.32                  |
| CBH2.P98L/K194E/S313T/G384Q/N443I            | 0.05        |                    | 0.83                 | 1.08                | 0.50                  | 0.53                  |
| CBH2.K194C/S313T/N443I                       | 0.09        |                    | 1.07                 | 0.72                | 0.44                  | 0.09                  |
| CBH2.P98L/K194C/S316P/N443I                  | 0.07        |                    | 1.12                 | 0.99                | 0.61                  | 0.85                  |
| CBH2.P98L/K194C/S313T/G384C/N443I            |             |                    |                      |                     |                       |                       |
| CBH2.P98L/S316P/G384C                        | 0.33        |                    | 1.10                 | 1.20                | 1.05                  | 1.35                  |
| CBH2.P98L/K194C/S316P/G384Q/N443I            |             |                    |                      |                     |                       |                       |
| CBH2.K194E/S313T/G384Q/N443I                 | 0.08        |                    | 0.83                 | 1.07                | 0.20                  | 0.34                  |

| <b>Table 3-2: CBH2 Combinatorial Variant</b> | <b>HPLC</b> | <b>Act.<br/>CC</b> | <b>Act.<br/>PASC</b> | <b>Act.<br/>PCS</b> | <b>EtOH<br/>ratio</b> | <b>Heat<br/>ratio</b> |
|----------------------------------------------|-------------|--------------------|----------------------|---------------------|-----------------------|-----------------------|
| CBH2.K194C/S313T/S316P/N443I                 | 0.11        |                    | 1.15                 | 0.92                | 0.52                  | 0.53                  |
| CBH2.P98L/K194E/N443I                        | 0.08        |                    | 1.37                 | 1.40                | 0.25                  | 0.48                  |
| CBH2.P98L/G384Q                              | 0.54        |                    | 0.99                 | 1.14                | 1.12                  | 1.36                  |
| CBH2.P98L/S313T/S316P/G384Q/N443I            | 0.19        |                    | 0.99                 | 1.23                | 1.04                  | 1.03                  |
| CBH2.S316P/G384C/N443I                       | 0.07        |                    | 0.63                 | 0.83                | 0.63                  | 0.42                  |
| CBH2.K194E/N443I                             |             |                    |                      |                     |                       |                       |
| CBH2.P98L/K194E/S313T/N443I                  | 0.08        |                    | 1.17                 | 1.46                | 0.56                  | 0.44                  |
| CBH2.P98L/K194E/S316P/N443I                  | 0.11        |                    | 1.04                 | 1.26                | 0.84                  | 0.94                  |
| CBH2.G384Q/N443I                             | 0.16        |                    | 0.99                 | 0.80                | 0.42                  | 0.27                  |
| CBH2.P98L/K194E/S313T/S316P                  |             |                    |                      |                     |                       |                       |
| CBH2.K194C/S313T/G384Q/N443I                 | 0.06        |                    | 0.87                 | 0.91                | 0.18                  | 0.36                  |
| CBH2.P98L/G384Q/N443I                        | 0.13        |                    | 1.15                 | 1.18                | 0.41                  | 0.37                  |
| CBH2.G384C/N443I                             | 0.06        |                    | 0.75                 | 0.72                | 0.23                  | 0.44                  |
| CBH2.K194E/S316P/G384Q/N443I                 | 0.10        |                    | 0.88                 | 1.30                | 0.75                  | 0.65                  |
| CBH2.K194E/S316P/G384C/N443I                 | 0.05        |                    | 0.69                 | 0.83                | 0.59                  | 0.45                  |
| CBH2.P98L/K194C/N443I                        | 0.07        |                    | 0.90                 | 1.19                | 0.42                  | 0.40                  |
| CBH2.K194E/S316P/G384Q                       | 0.33        |                    | 1.06                 | 1.40                | 1.07                  | 1.37                  |
| CBH2.K194E/S313T/S316P/N443I                 | 0.16        |                    | 1.09                 | 1.18                | 0.78                  | 0.54                  |
| CBH2.K194C/S316P/N443I                       | 0.09        |                    | 0.99                 | 1.11                | 0.60                  | 0.40                  |
| CBH2.K194E/S313T/N443I                       | 0.13        |                    | 1.10                 | 1.10                | 0.40                  | 0.06                  |
| CBH2.K194E/S313T/G384Q                       | 0.54        |                    | 1.12                 | 1.16                | 0.97                  | 1.06                  |
| CBH2.K194C/S313T/G384Q                       | 0.28        |                    | 0.99                 | 0.85                | 0.86                  | 1.01                  |
| CBH2.P98L/S316P/G384C/N443I                  |             |                    |                      |                     |                       |                       |
| CBH2.P98L/K194C/S313T/G384C                  | 0.06        |                    | 1.14                 | 1.49                | 0.92                  | 1.17                  |
| CBH2.S316P                                   | 1.26        |                    | 0.94                 | 1.13                | 1.19                  | 1.39                  |
| CBH2.P98L/K194C/G384Q/N443I                  |             |                    |                      |                     |                       |                       |
| CBH2.P98L/K194E/S313T/S316P/G384Q/N443I      | 0.07        |                    | 0.94                 | 1.21                | 1.03                  | 1.03                  |
| CBH2.K194E/S313T/G384C/N443I                 | 0.05        |                    | 0.65                 | 1.37                | 0.30                  | 0.35                  |
| CBH2.K194E/S316P/N443I                       | 0.17        |                    | 1.02                 | 1.07                | 0.74                  | 0.49                  |
| CBH2.P98L/S313T/S316P/G384C                  | 0.37        |                    | 1.00                 | 1.43                | 1.09                  | 1.50                  |
| CBH2.S313T/S316P                             |             |                    |                      |                     |                       |                       |
| CBH2.P98L/K194E/S313T/S316P/G384Q            | 0.11        |                    | 1.01                 | 1.54                | 0.98                  | 1.40                  |
| CBH2.K194C/G384Q/N443I                       | 0.06        |                    | 0.92                 | 1.13                | 0.24                  | 0.57                  |
| CBH2.P98L/K194C/S313T/S316P/G384Q            | 0.08        |                    | 1.29                 | 1.45                | 1.01                  | 1.52                  |
| CBH2.P98L/K194C/S313T/G384Q                  | 0.10        |                    | 1.37                 | 1.77                | 0.86                  | 1.17                  |
| CBH2.P98L/K194E/S313T/S316P/N443I            | 0.14        |                    | 1.02                 | 1.44                | 1.04                  | 1.15                  |
| CBH2.G384Q                                   |             |                    |                      |                     |                       |                       |
| CBH2.P98L                                    | 0.68        |                    | 1.03                 | 1.29                | 1.08                  | 1.27                  |
| CBH2.P98L/S313T/S316P/N443I                  | 0.29        |                    | 1.02                 | 1.10                | 0.94                  | 0.88                  |
| CBH2.K194C/S313T/S316P/G384Q                 | 0.37        |                    | 0.99                 | 1.15                | 1.08                  | 1.40                  |
| CBH2.P98L/K194E/S313T/G384Q                  | 0.22        |                    | 1.19                 | 1.28                | 0.93                  | 1.18                  |
| CBH2.P98L/K194C/S316P                        | 0.19        |                    | 0.98                 | 1.09                | 1.04                  | 1.42                  |
| CBH2.R153Q/N161W/Q422V                       | 0.05        | 3.70               | 0.66                 | 1.20                | 0.39                  | 0.66                  |
| CBH2.N161W/P233D/Q422V                       | 0.06        | 0.57               | 0.87                 | 0.93                | 0.42                  | 0.41                  |
| CBH2.R203H/Q422V/P444Q                       |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/N161A/R203H/P444Q                 |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/R203H/P444Q                       |             |                    |                      |                     |                       |                       |
| CBH2.N161W/R203H/P233D/Q422V/P444Q           |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/N161A/P444Q                       |             |                    |                      |                     |                       |                       |
| CBH2.N161W/R203H/P233D/P444Q                 |             |                    |                      |                     |                       |                       |
| CBH2.N161A/R203H/Q422V/P444Q                 |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/N161A/R203H/P233D/Q422V/P444Q     |             |                    |                      |                     |                       |                       |

| <b>Table 3-2: CBH2 Combinatorial Variant</b> | <b>HPLC</b> | <b>Act.<br/>CC</b> | <b>Act.<br/>PASC</b> | <b>Act.<br/>PCS</b> | <b>EtOH<br/>ratio</b> | <b>Heat<br/>ratio</b> |
|----------------------------------------------|-------------|--------------------|----------------------|---------------------|-----------------------|-----------------------|
| CBH2.R153Q/R203H/Q422V                       | 0.16        | 1.07               | 0.75                 | 0.70                | 0.45                  | 0.42                  |
| CBH2.R153Q/P233D/P444Q                       |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/N161A/R203H/P233D/P444Q           |             |                    |                      |                     |                       |                       |
| CBH2.N161W/R203H/P233D/Q422V                 |             |                    |                      |                     |                       |                       |
| CBH2.N161W/R203H/P444Q                       |             |                    |                      |                     |                       |                       |
| CBH2.N161A/P233D/Q422V/P444Q                 |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/R203H/P233D/Q422V/P444Q           |             |                    |                      |                     |                       |                       |
| CBH2.N161A/Q422V/P444Q                       |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/N161A/R203H/P233D                 |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/N161W/R203H/P233D/Q422V           |             |                    |                      |                     |                       |                       |
| CBH2.N161W/P233D/Q422V/P444Q                 |             |                    |                      |                     |                       |                       |
| CBH2.R203H/P444Q                             |             |                    |                      |                     |                       |                       |
| CBH2.N161A/Q422V                             | 0.58        | 0.73               | 1.04                 | 0.65                | 0.60                  | 0.27                  |
| CBH2.R203H/P233D/P444Q                       |             |                    |                      |                     |                       |                       |
| CBH2.N161A/R203H/P444Q                       |             |                    |                      |                     |                       |                       |
| CBH2.N161A/R203H/P233D/Q422V                 | 0.18        | 0.51               | 0.73                 | 0.57                | 0.24                  | 0.26                  |
| CBH2.N161W/P233D                             | 0.21        | 0.69               | 0.86                 | 1.02                | 0.87                  | 0.72                  |
| CBH2.R153Q/N161W/R203H/Q422V/P444Q           |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/N161A/P233D                       | 0.15        | 0.81               | 0.65                 | 0.73                | 0.80                  | 0.75                  |
| CBH2.R153Q/N161A/R203H/P233D/Q422V           |             |                    |                      |                     |                       |                       |
| CBH2.N161W/R203H/P233D                       |             |                    |                      |                     |                       |                       |
| CBH2.P233D/P444Q                             |             |                    |                      |                     |                       |                       |
| CBH2.P233D                                   | 0.84        | 0.49               | 1.00                 | 0.82                | 1.01                  | 1.00                  |
| CBH2.R153Q/P233D/Q422V/P444Q                 |             |                    |                      |                     |                       |                       |
| CBH2.N161A/P233D/Q422V                       | 0.28        | 1.19               | 0.87                 | 0.48                | 0.37                  | 0.23                  |
| CBH2.N161A                                   | 0.83        | 1.01               | 1.05                 | 1.05                | 1.16                  | 1.13                  |
| CBH2.N161W/P444Q                             |             |                    |                      |                     |                       |                       |
| CBH2.N161W/Q422F/P444Q                       |             |                    |                      |                     |                       |                       |
| CBH2.N161A/P233D                             | 0.45        | 0.97               | 0.96                 | 0.90                | 0.95                  | 0.89                  |
| CBH2.Q422V/P444Q                             |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/N161A/R203H/Q422V                 |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/N161A/R203H/Q422V/P444Q           |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/N161A/P233D/Q422V                 | 0.12        | 0.00               | 0.50                 | 0.20                | 0.24                  | 0.62                  |
| CBH2.Q422V                                   | 0.79        | 1.00               | 1.02                 | 0.61                | 0.67                  | 0.37                  |
| CBH2.R153Q/N161A/R203H                       | 0.15        | 1.28               | 0.59                 | 0.48                | 0.79                  | 0.81                  |
| CBH2.R153Q/R203H/P233D/P444Q                 |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/N161W/P233D/Q422V/P444Q           |             |                    |                      |                     |                       |                       |
| CBH2.N161A/R203H/P233D/P444Q                 |             |                    |                      |                     |                       |                       |
| CBH2.N161A/R203H/P233D                       | 0.23        | 0.57               | 0.79                 | 0.82                | 0.86                  | 0.68                  |
| CBH2.R203H/Q422V                             |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/N161A/Q422V/P444Q                 |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/R203H/P233D                       | 0.10        | 1.11               | 0.52                 | 0.87                | 0.81                  | 0.88                  |
| CBH2.N161W/P233D/P444Q                       |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/R203H/P233D/Q422V                 | 0.13        | 0.65               | 0.42                 | 0.35                | 0.40                  | 0.38                  |
| CBH2.R203H/P233D/Q422V/P444Q                 |             |                    |                      |                     |                       |                       |
| CBH2.R153Q/N161W/R203H                       |             |                    |                      |                     |                       |                       |
| CBH2.N161A/P233D/P444Q                       |             |                    |                      |                     |                       |                       |
| CBH2.R203H/P233D                             | 0.46        | 0.94               | 0.91                 | 0.78                | 0.98                  | 0.79                  |
| CBH2.P233D/Q422V/P444Q                       |             |                    |                      |                     |                       |                       |
| CBH2.R203H/P233D/Q422V                       |             |                    |                      |                     |                       |                       |
| CBH2.R203H                                   | 0.59        | 1.02               | 0.92                 | 0.86                | 1.05                  | 1.07                  |
| CBH2.N161A/P444Q                             |             |                    |                      |                     |                       |                       |

| <b>Table 3-2: CBH2 Combinatorial Variant</b> | <b>HPLC</b> | <b>Act.<br/>CC</b> | <b>Act.<br/>PASC</b> | <b>Act.<br/>PCS</b> | <b>EtOH<br/>ratio</b> | <b>Heat<br/>ratio</b> |
|----------------------------------------------|-------------|--------------------|----------------------|---------------------|-----------------------|-----------------------|
| CBH2.R153Q/N161W/R203H/P233D                 |             |                    |                      |                     |                       |                       |
| CBH2.N161A/R203H/Q422V                       | 0.26        | 1.17               | 0.94                 | 0.80                | 0.46                  | 0.26                  |
| CBH2.P233D/Q422V                             | 0.56        | 1.36               | 0.91                 | 0.58                | 0.22                  | 0.19                  |
| CBH2.R153Q/N161A/Q422V                       |             |                    |                      |                     |                       |                       |
| CBH2.P444Q                                   |             |                    |                      |                     |                       |                       |
| CBH2.N161W/R203H                             | 0.22        | 0.53               | 0.74                 | 0.90                | 0.90                  | 0.76                  |
| CBH2.N161W/Q422V                             | 0.37        | 1.16               | 0.92                 | 0.69                | 0.48                  | 0.22                  |
| CBH2.R153Q/P233D                             |             |                    |                      |                     |                       |                       |
| CBH2.N161W/R203H/Q422V/P444Q                 |             |                    |                      |                     |                       |                       |
| CBH2.P98L/S413W                              | 0.22        | 1.15               | 0.56                 | 0.74                | 0.16                  | 0.23                  |
| CBH2.L144W/S313T/S316P                       | 1.30        | 0.70               | 0.96                 | 0.99                | 1.40                  | 1.12                  |
| CBH2.P98L/L144W/S316P/Q422V                  | 0.51        | 0.67               | 0.97                 | 0.94                | 0.75                  | 0.82                  |
| CBH2.L111S/L144W/S316P                       | 1.23        | 0.96               | 0.93                 | 1.01                | 1.31                  | 1.12                  |
| CBH2.P98L/L111S/S313T/S316P                  |             |                    |                      |                     |                       |                       |
| CBH2.L111S/S316P/S413W                       | 0.99        | 1.08               | 0.88                 | 0.85                | 1.51                  | 1.11                  |
| CBH2.L111S/L144W/S313T/S316P/S413W/Q422V     | 0.66        | 1.31               | 0.89                 | 0.92                | 1.31                  | 1.04                  |
| CBH2.P98L/L144W/S313T/S316P                  | 0.66        | 1.53               | 1.06                 | 1.32                | 1.30                  | 1.12                  |
| CBH2.L144W/S313T/Q422V                       | 0.63        | 1.50               | 0.97                 | 0.97                | 0.27                  | 0.59                  |
| CBH2.L111S/S313T/Q422V                       | 0.72        | 1.71               | 0.98                 | 0.92                | 0.44                  | 0.61                  |
| CBH2.P98L/L111S/L144W/S313T/S316P/S413W      | 0.40        | 1.80               | 1.03                 | 1.58                | 1.38                  | 1.01                  |
| CBH2.S313T/S316P/S413W/Q422V                 |             |                    |                      |                     |                       |                       |
| CBH2.P98L/L111S/S316P                        | 0.08        | 6.48               | 1.20                 | 5.02                | 1.39                  | 1.09                  |
| CBH2.L111S/S313T                             |             |                    |                      |                     |                       |                       |
| CBH2.P98L/L111S/S316P/S413W                  | 0.11        | 2.45               | 0.92                 | 3.57                | 1.47                  | 1.06                  |
| CBH2.L144W/S313T/S413W/Q422V                 | 0.50        | 0.95               | 0.97                 | 1.13                | 0.94                  | 0.79                  |
| CBH2.L111S/L144W/S316P/Q422V                 | 0.70        | 1.37               | 0.90                 | 0.89                | 0.72                  | 0.87                  |
| CBH2.L111S/L144W/S413W/Q422V                 |             |                    |                      |                     |                       |                       |
| CBH2.Q422V                                   | 0.55        | 1.20               | 1.10                 | 0.97                | 0.28                  | 0.54                  |
| CBH2.P98L/L111S/L144W/S313T/S413W/Q422V      |             |                    |                      |                     |                       |                       |
| CBH2.P98L/L111S/L144W/S413W                  | 0.21        | 0.42               | 0.55                 | 0.40                | 0.26                  | 0.25                  |
| CBH2.P98L/L111S/S313T                        | 0.56        | 1.68               | 1.09                 | 1.51                | 1.31                  | 1.05                  |
| CBH2.P98L/L111S/L144W/S413W/Q422V            | 0.18        | 2.34               | 1.07                 | 1.55                | 0.67                  | 0.67                  |
| CBH2.L111S/L144W/S313T/S413W/Q422V           | 0.33        | 1.64               | 1.08                 | 1.22                | 0.80                  | 0.74                  |
| CBH2.S313T                                   | 1.18        | 0.49               | 0.97                 | 0.94                | 1.13                  | 1.07                  |
| CBH2.L111S/Q422V                             |             |                    |                      |                     |                       |                       |
| CBH2.P98L/L111S/L144W/S313T/Q422V            |             |                    |                      |                     |                       |                       |
| CBH2.P98L/L111S/S313T/S413W                  | 0.42        | 1.17               | 0.89                 | 1.21                | 1.52                  | 1.07                  |
| CBH2.S313T/S413W                             |             |                    |                      |                     |                       |                       |
| CBH2.P98L/S313T/S413W                        | 0.35        | 0.80               | 0.92                 | 1.25                | 1.37                  | 1.07                  |
| CBH2.P98L/L111S/S413W/Q422V                  | 0.06        | 6.43               | 0.93                 | 3.08                | 0.82                  | 0.77                  |
| CBH2.P98L/L111S/S313T/Q422V                  |             |                    |                      |                     |                       |                       |
| CBH2.S316P                                   | 0.92        | 1.17               | 1.02                 | 1.15                | 1.34                  | 1.12                  |
| CBH2.P98L/L144W                              | 0.54        | 1.54               | 1.07                 | 1.19                | 1.11                  | 0.94                  |
| CBH2.L144W/S413W/Q422V                       | 0.33        | 1.57               | 0.92                 | 0.86                | 0.53                  | 0.70                  |
| CBH2.L111S/L144W/S313T/S316P/S413W           | 0.54        | 1.63               | 1.05                 | 1.39                | 1.18                  | 1.02                  |
| CBH2.L111S                                   | 0.34        | 1.56               | 0.54                 | 0.34                | 0.33                  | 0.13                  |
| CBH2.P98L/L111S                              | 0.69        | 1.36               | 1.04                 | 1.38                | 1.30                  | 1.03                  |
| CBH2.P98L/L111S/L144W                        | 0.49        | 1.37               | 1.02                 | 1.45                | 1.12                  | 1.05                  |
| CBH2.P98L/L111S/S313T/S316P/Q422V            | 0.54        | 1.38               | 1.03                 | 1.22                | 1.07                  | 1.00                  |
| CBH2.P98L/S316P/S413W                        |             |                    |                      |                     |                       |                       |
| CBH2.P98L/S313T/S413W/Q422V                  | 0.06        | 5.98               | 0.95                 | 3.57                | 0.89                  | 0.78                  |
| CBH2.P98L/L144W/S313T/Q422V                  | 0.05        | 7.12               | 0.98                 | 3.45                | 0.30                  | 0.42                  |

| <b>Table 3-2: CBH2 Combinatorial Variant</b>  | <b>HPLC</b> | <b>Act.<br/>CC</b> | <b>Act.<br/>PASC</b> | <b>Act.<br/>PCS</b> | <b>EtOH<br/>ratio</b> | <b>Heat<br/>ratio</b> |
|-----------------------------------------------|-------------|--------------------|----------------------|---------------------|-----------------------|-----------------------|
| CBH2.P98L/S313T/S316P/Q422V                   | 0.14        | 3.62               | 1.14                 | 2.12                | 0.96                  | 0.82                  |
| CBH2.L111S/L144W/S313T                        | 0.76        | 0.78               | 0.98                 | 1.06                | 1.01                  | 0.96                  |
| CBH2.L111S/L144W/S316P/S413W/Q422V            | 0.81        | 0.82               | 0.79                 | 0.77                | 0.93                  | 0.85                  |
| CBH2.P98L/L111S/L144W/S316P/S413W/Q422V       | 0.38        | 1.47               | 0.95                 | 1.01                | 0.50                  | 0.41                  |
| CBH2.L111S/S313T/S316P/Q422V                  |             |                    |                      |                     |                       |                       |
| CBH2.P98L/L111S/Q422V                         |             |                    |                      |                     |                       |                       |
| CBH2.P98L/L111S/L144W/S313T/S316P/Q422V       | 0.07        | 3.16               | 1.06                 | 3.78                | 0.75                  | 0.75                  |
| CBH2.P98L/L111S/L144W/S313T                   | 0.42        | 1.68               | 0.95                 | 1.42                | 1.31                  | 1.11                  |
| CBH2.L111S/S316P                              | 0.56        | 2.08               | 0.90                 | 1.21                | 1.23                  | 1.01                  |
| CBH2.P98L/L111S/L144W/S313T/S316P             | 0.59        | 1.47               | 0.97                 | 1.38                | 1.46                  | 1.15                  |
| CBH2.L111S/L144W                              | 0.22        | 0.96               | 0.53                 | 0.35                | 0.16                  | 0.10                  |
| CBH2.L111S/L144W/S413W                        | 0.77        | 1.22               | 0.82                 | 0.86                | 1.36                  | 1.12                  |
| CBH2.L144W/S313T/S316P/S413W                  |             |                    |                      |                     |                       |                       |
| CBH2.P98L/L111S/L144W/S313T/S316P/S413W/Q422V | 0.49        | 0.30               | 0.83                 | 0.78                | 1.00                  | 0.85                  |
| CBH2.P98L/L111S/L144W/S316P/S413W             | 0.49        | 1.26               | 0.97                 | 1.13                | 1.29                  | 0.97                  |
| CBH2.L111S/S313T/S413W/Q422V                  | 0.27        | 2.43               | 1.01                 | 1.84                | 0.96                  | 0.85                  |
| CBH2.P98L/Q422V                               | 0.56        | 1.65               | 1.06                 | 1.05                | 0.59                  | 0.79                  |
| CBH2.P98L/L144W/S313T/S413W                   | 0.26        | 1.01               | 0.69                 | 0.55                | 0.33                  | 0.13                  |
| CBH2.L111S/L144W/Q422V                        | 0.70        | 1.40               | 0.94                 | 0.88                | 0.28                  | 0.61                  |
| CBH2.S313T/Q422V                              | 0.97        | 0.93               | 1.04                 | 0.76                | 0.38                  | 0.70                  |
| CBH2.P98L/S316P/Q422V                         | 0.49        | 1.19               | 1.05                 | 1.38                | 1.04                  | 1.03                  |
| CBH2.S313T/S413W/Q422V                        | 0.16        | 3.56               | 1.05                 | 1.87                | 0.77                  | 0.67                  |
| CBH2.P98L/L111S/L144W/S316P                   | 0.43        | 1.36               | 0.83                 | 0.52                | 0.12                  | 0.00                  |
| CBH2.P98L/S316P                               | 0.79        | 1.30               | 1.12                 | 1.24                | 1.38                  | 1.03                  |
| CBH2.P98L/L144W/S413W/Q422V                   | 0.36        | 1.64               | 0.94                 | 0.98                | 0.35                  | 0.46                  |
| CBH2.L111S/L144W/S313T/S413W                  | 0.40        | 1.30               | 0.91                 | 0.51                | 0.86                  | 0.74                  |
| CBH2.P98L/L111S/L144W/Q422V                   |             |                    |                      |                     |                       |                       |
| CBH2.P98L/L111S/S316P/Q422V                   |             |                    |                      |                     |                       |                       |
| CBH2.P98L/S413W/Q422V                         | 0.32        | 1.50               | 1.06                 | 1.55                | 1.14                  | 0.93                  |
| CBH2.P98L/S313T/Q422V                         | 0.27        | 0.65               | 0.88                 | 0.23                | 0.08                  | 0.08                  |
| CBH2.P98L                                     | 0.79        | 1.21               | 1.10                 | 1.37                | 1.16                  | 1.00                  |
| CBH2.L144W/S316P/S413W/Q422V                  | 0.65        | 1.38               | 0.86                 | 0.90                | 1.07                  | 0.91                  |
| CBH2.L111S/L144W/S313T/S316P                  | 1.00        | 0.91               | 0.98                 | 1.11                | 1.36                  | 1.09                  |
| CBH2.S413W                                    | 0.45        | 0.48               | 0.32                 | 0.17                | 0.69                  | 0.31                  |
| CBH2.L144W/S316P/S413W                        | 0.55        | 1.44               | 1.05                 | 1.18                | 1.20                  | 1.00                  |

## EXAMPLE 4

### Classification of CBH2 Positions

[233] As described throughout, functionality of cellulase variants was quantified as a performance index (PI), which is the ratio of performance of a variant to a parent or reference cellulase. 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 = 0.05; combinable mutations are those mutations for which the variant has Performance index values = 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 performance indices for one or more desired properties. 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.

- 5 [234] As shown in Table 4-1, for the 162 positions tested in CBH2, all 162 are Non-restrictive. 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.

| Table 4-1: Non-restrictive Positions of CBH2 |       |               |                  |                          |                        |                       |                       |
|----------------------------------------------|-------|---------------|------------------|--------------------------|------------------------|-----------------------|-----------------------|
| Position                                     | WT AA | # at Position | HPLC PI % > 0.50 | PASC Sp. Ac. PI % > 0.50 | PCS Sp Ac. PI % > 0.50 | Res. EtOH PI % > 0.50 | Res. Heat PI % > 0.50 |
| 5                                            | S     | 19            | 47%              | 100%                     | 84%                    | 100%                  | 100%                  |
| 18                                           | P     | 19            | 58%              | 84%                      | 63%                    | 95%                   | 95%                   |
| 19                                           | T     | 17            | 71%              | 82%                      | 76%                    | 100%                  | 100%                  |
| 28                                           | V     | 19            | 74%              | 89%                      | 89%                    | 100%                  | 100%                  |
| 30                                           | S     | 19            | 53%              | 89%                      | 63%                    | 100%                  | 100%                  |
| 32                                           | D     | 19            | 32%              | 100%                     | 89%                    | 95%                   | 95%                   |
| 35                                           | S     | 15            | 67%              | 93%                      | 100%                   | 100%                  | 100%                  |
| 38                                           | L     | 5             | 60%              | 80%                      | 80%                    | 100%                  | 100%                  |
| 63                                           | R     | 19            | 84%              | 95%                      | 95%                    | 95%                   | 100%                  |
| 77                                           | R     | 4             | 25%              | 100%                     | 75%                    | 100%                  | 100%                  |
| 79                                           | P     | 14            | 50%              | 100%                     | 100%                   | 100%                  | 100%                  |
| 80                                           | P     | 14            | 57%              | 100%                     | 100%                   | 100%                  | 100%                  |
| 89                                           | S     | 19            | 58%              | 100%                     | 100%                   | 100%                  | 100%                  |
| 94                                           | V     | 11            | 27%              | 91%                      | 64%                    | 91%                   | 100%                  |
| 98                                           | P     | 19            | 42%              | 63%                      | 47%                    | 100%                  | 100%                  |
| 100                                          | A     | 16            | 6%               | 75%                      | 56%                    | 81%                   | 44%                   |
| 102                                          | A     | 19            | 58%              | 84%                      | 84%                    | 100%                  | 95%                   |
| 103                                          | Y     | 18            | 72%              | 89%                      | 67%                    | 67%                   | 67%                   |
| 104                                          | Y     | 19            | 11%              | 58%                      | 11%                    | 26%                   | 21%                   |
| 105                                          | A     | 7             | 0%               | 71%                      | 71%                    | 57%                   | 43%                   |
| 107                                          | E     | 12            | 58%              | 75%                      | 33%                    | 17%                   | 0%                    |
| 111                                          | L     | 19            | 74%              | 95%                      | 89%                    | 95%                   | 68%                   |
| 117                                          | T     | 18            | 83%              | 94%                      | 100%                   | 100%                  | 94%                   |
| 119                                          | A     | 19            | 42%              | 100%                     | 79%                    | 100%                  | 100%                  |
| 120                                          | M     | 19            | 32%              | 84%                      | 47%                    | 84%                   | 74%                   |
| 121                                          | A     | 19            | 5%               | 63%                      | 37%                    | 63%                   | 53%                   |
| 125                                          | A     | 19            | 37%              | 89%                      | 26%                    | 68%                   | 63%                   |
| 126                                          | A     | 18            | 61%              | 83%                      | 89%                    | 89%                   | 89%                   |
| 129                                          | K     | 8             | 13%              | 100%                     | 88%                    | 100%                  | 75%                   |
| 133                                          | F     | 12            | 0%               | 17%                      | 0%                     | 67%                   | 83%                   |
| 134                                          | M     | 19            | 79%              | 95%                      | 89%                    | 100%                  | 100%                  |
| 137                                          | D     | 19            | 16%              | 37%                      | 16%                    | 100%                  | 100%                  |
| 138                                          | T     | 19            | 74%              | 84%                      | 74%                    | 100%                  | 100%                  |
| 139                                          | L     | 19            | 42%              | 89%                      | 79%                    | 100%                  | 95%                   |
| 140                                          | D     | 19            | 21%              | 89%                      | 74%                    | 100%                  | 100%                  |
| 141                                          | K     | 16            | 75%              | 75%                      | 0%                     | 100%                  | 94%                   |

| <b>Table 4-1: Non-restrictive Positions of CBH2</b> |              |                      |                            |                                    |                                  |                                 |                                 |
|-----------------------------------------------------|--------------|----------------------|----------------------------|------------------------------------|----------------------------------|---------------------------------|---------------------------------|
| <b>Position</b>                                     | <b>WT AA</b> | <b># at Position</b> | <b>HPLC PI % &gt; 0.50</b> | <b>PASC Sp. Ac. PI % &gt; 0.50</b> | <b>PCS Sp Ac. PI % &gt; 0.50</b> | <b>Res. EtOH PI % &gt; 0.50</b> | <b>Res. Heat PI % &gt; 0.50</b> |
| 143                                                 | P            | 19                   | 63%                        | 89%                                | 89%                              | 100%                            | 100%                            |
| 144                                                 | L            | 18                   | 33%                        | 94%                                | 89%                              | 100%                            | 100%                            |
| 147                                                 | Q            | 16                   | 25%                        | 94%                                | 81%                              | 100%                            | 100%                            |
| 150                                                 | A            | 19                   | 47%                        | 89%                                | 79%                              | 100%                            | 100%                            |
| 153                                                 | R            | 19                   | 0%                         | 53%                                | 79%                              | 95%                             | 95%                             |
| 154                                                 | T            | 19                   | 26%                        | 89%                                | 89%                              | 100%                            | 95%                             |
| 157                                                 | K            | 16                   | 44%                        | 100%                               | 94%                              | 100%                            | 100%                            |
| 158                                                 | N            | 18                   | 33%                        | 94%                                | 83%                              | 89%                             | 94%                             |
| 161                                                 | N            | 18                   | 72%                        | 100%                               | 94%                              | 94%                             | 94%                             |
| 162                                                 | Y            | 19                   | 16%                        | 84%                                | 79%                              | 100%                            | 100%                            |
| 177                                                 | A            | 19                   | 37%                        | 68%                                | 42%                              | 100%                            | 100%                            |
| 178                                                 | A            | 19                   | 58%                        | 32%                                | 16%                              | 100%                            | 100%                            |
| 179                                                 | L            | 18                   | 50%                        | 83%                                | 72%                              | 94%                             | 100%                            |
| 180                                                 | A            | 19                   | 84%                        | 26%                                | 5%                               | 89%                             | 89%                             |
| 181                                                 | S            | 17                   | 82%                        | 6%                                 | 0%                               | 94%                             | 100%                            |
| 182                                                 | N            | 18                   | 89%                        | 89%                                | 72%                              | 100%                            | 100%                            |
| 185                                                 | Y            | 17                   | 24%                        | 35%                                | 24%                              | 59%                             | 65%                             |
| 186                                                 | S            | 19                   | 63%                        | 95%                                | 79%                              | 100%                            | 100%                            |
| 188                                                 | A            | 18                   | 56%                        | 83%                                | 72%                              | 100%                            | 100%                            |
| 189                                                 | D            | 18                   | 11%                        | 89%                                | 72%                              | 94%                             | 100%                            |
| 190                                                 | G            | 18                   | 39%                        | 83%                                | 61%                              | 89%                             | 100%                            |
| 191                                                 | G            | 14                   | 0%                         | 7%                                 | 7%                               | 71%                             | 93%                             |
| 192                                                 | V            | 16                   | 25%                        | 75%                                | 31%                              | 81%                             | 94%                             |
| 193                                                 | A            | 18                   | 6%                         | 94%                                | 89%                              | 100%                            | 100%                            |
| 194                                                 | K            | 19                   | 47%                        | 74%                                | 89%                              | 89%                             | 95%                             |
| 196                                                 | K            | 9                    | 44%                        | 100%                               | 78%                              | 100%                            | 100%                            |
| 197                                                 | N            | 17                   | 47%                        | 100%                               | 88%                              | 100%                            | 100%                            |
| 201                                                 | T            | 19                   | 5%                         | 100%                               | 79%                              | 100%                            | 100%                            |
| 203                                                 | R            | 16                   | 69%                        | 94%                                | 94%                              | 100%                            | 100%                            |
| 204                                                 | Q            | 18                   | 72%                        | 94%                                | 94%                              | 94%                             | 94%                             |
| 206                                                 | V            | 15                   | 33%                        | 67%                                | 40%                              | 100%                            | 100%                            |
| 207                                                 | V            | 17                   | 82%                        | 82%                                | 82%                              | 100%                            | 100%                            |
| 210                                                 | S            | 14                   | 21%                        | 100%                               | 93%                              | 100%                            | 100%                            |
| 214                                                 | T            | 18                   | 44%                        | 83%                                | 61%                              | 94%                             | 94%                             |
| 225                                                 | N            | 19                   | 0%                         | 11%                                | 5%                               | 100%                            | 58%                             |
| 226                                                 | L            | 15                   | 7%                         | 33%                                | 7%                               | 93%                             | 100%                            |
| 228                                                 | T            | 15                   | 7%                         | 13%                                | 33%                              | 60%                             | 80%                             |
| 229                                                 | N            | 19                   | 26%                        | 79%                                | 68%                              | 100%                            | 100%                            |
| 230                                                 | L            | 19                   | 16%                        | 89%                                | 26%                              | 95%                             | 74%                             |
| 231                                                 | G            | 18                   | 39%                        | 67%                                | 50%                              | 100%                            | 94%                             |
| 232                                                 | T            | 14                   | 21%                        | 79%                                | 93%                              | 93%                             | 100%                            |
| 233                                                 | P            | 19                   | 11%                        | 100%                               | 95%                              | 100%                            | 100%                            |
| 234                                                 | K            | 13                   | 54%                        | 100%                               | 31%                              | 100%                            | 100%                            |
| 236                                                 | A            | 19                   | 11%                        | 89%                                | 79%                              | 95%                             | 95%                             |
| 237                                                 | N            | 16                   | 75%                        | 100%                               | 44%                              | 100%                            | 100%                            |
| 239                                                 | Q            | 18                   | 89%                        | 100%                               | 89%                              | 94%                             | 100%                            |
| 240                                                 | S            | 19                   | 74%                        | 89%                                | 63%                              | 100%                            | 100%                            |
| 243                                                 | L            | 19                   | 53%                        | 58%                                | 47%                              | 95%                             | 95%                             |
| 245                                                 | C            | 18                   | 0%                         | 22%                                | 17%                              | 78%                             | 100%                            |
| 247                                                 | N            | 18                   | 83%                        | 83%                                | 39%                              | 100%                            | 100%                            |

| <b>Table 4-1: Non-restrictive Positions of CBH2</b> |              |                      |                            |                                    |                                  |                                 |                                 |
|-----------------------------------------------------|--------------|----------------------|----------------------------|------------------------------------|----------------------------------|---------------------------------|---------------------------------|
| <b>Position</b>                                     | <b>WT AA</b> | <b># at Position</b> | <b>HPLC PI % &gt; 0.50</b> | <b>PASC Sp. Ac. PI % &gt; 0.50</b> | <b>PCS Sp Ac. PI % &gt; 0.50</b> | <b>Res. EtOH PI % &gt; 0.50</b> | <b>Res. Heat PI % &gt; 0.50</b> |
| 251                                                 | T            | 19                   | 26%                        | 89%                                | 89%                              | 100%                            | 100%                            |
| 252                                                 | Q            | 19                   | 47%                        | 95%                                | 84%                              | 100%                            | 100%                            |
| 254                                                 | N            | 17                   | 6%                         | 29%                                | 24%                              | 88%                             | 100%                            |
| 258                                                 | V            | 19                   | 16%                        | 37%                                | 42%                              | 89%                             | 100%                            |
| 266                                                 | H            | 16                   | 0%                         | 6%                                 | 0%                               | 88%                             | 94%                             |
| 267                                                 | A            | 18                   | 17%                        | 33%                                | 11%                              | 78%                             | 67%                             |
| 268                                                 | G            | 19                   | 95%                        | 32%                                | 21%                              | 89%                             | 89%                             |
| 272                                                 | W            | 19                   | 89%                        | 58%                                | 0%                               | 63%                             | 11%                             |
| 274                                                 | A            | 19                   | 11%                        | 89%                                | 42%                              | 100%                            | 84%                             |
| 275                                                 | N            | 16                   | 75%                        | 100%                               | 75%                              | 94%                             | 94%                             |
| 281                                                 | Q            | 11                   | 100%                       | 100%                               | 36%                              | 100%                            | 82%                             |
| 285                                                 | N            | 14                   | 21%                        | 93%                                | 43%                              | 100%                            | 100%                            |
| 288                                                 | K            | 16                   | 13%                        | 75%                                | 31%                              | 100%                            | 100%                            |
| 289                                                 | N            | 18                   | 6%                         | 56%                                | 50%                              | 100%                            | 100%                            |
| 291                                                 | S            | 17                   | 88%                        | 82%                                | 76%                              | 100%                            | 100%                            |
| 292                                                 | S            | 19                   | 63%                        | 100%                               | 74%                              | 100%                            | 100%                            |
| 293                                                 | P            | 19                   | 0%                         | 11%                                | 32%                              | 79%                             | 100%                            |
| 294                                                 | R            | 19                   | 79%                        | 95%                                | 95%                              | 100%                            | 100%                            |
| 303                                                 | V            | 17                   | 6%                         | 18%                                | 6%                               | 71%                             | 76%                             |
| 304                                                 | A            | 19                   | 79%                        | 11%                                | 5%                               | 100%                            | 95%                             |
| 306                                                 | Y            | 17                   | 6%                         | 65%                                | 6%                               | 6%                              | 29%                             |
| 307                                                 | N            | 19                   | 0%                         | 21%                                | 5%                               | 32%                             | 79%                             |
| 312                                                 | T            | 18                   | 94%                        | 94%                                | 50%                              | 33%                             | 17%                             |
| 313                                                 | S            | 17                   | 71%                        | 100%                               | 82%                              | 94%                             | 71%                             |
| 316                                                 | S            | 19                   | 84%                        | 89%                                | 84%                              | 100%                            | 95%                             |
| 319                                                 | Q            | 18                   | 89%                        | 100%                               | 72%                              | 94%                             | 94%                             |
| 322                                                 | A            | 18                   | 72%                        | 94%                                | 83%                              | 94%                             | 94%                             |
| 323                                                 | V            | 17                   | 41%                        | 100%                               | 35%                              | 100%                            | 100%                            |
| 327                                                 | K            | 19                   | 32%                        | 95%                                | 74%                              | 95%                             | 100%                            |
| 328                                                 | L            | 19                   | 68%                        | 89%                                | 37%                              | 32%                             | 26%                             |
| 331                                                 | H            | 19                   | 11%                        | 63%                                | 58%                              | 68%                             | 16%                             |
| 338                                                 | A            | 18                   | 67%                        | 100%                               | 100%                             | 100%                            | 100%                            |
| 339                                                 | N            | 19                   | 79%                        | 95%                                | 95%                              | 100%                            | 100%                            |
| 340                                                 | H            | 19                   | 21%                        | 58%                                | 68%                              | 68%                             | 37%                             |
| 343                                                 | S            | 18                   | 89%                        | 89%                                | 83%                              | 100%                            | 100%                            |
| 344                                                 | N            | 18                   | 39%                        | 100%                               | 56%                              | 100%                            | 100%                            |
| 346                                                 | F            | 19                   | 47%                        | 84%                                | 79%                              | 100%                            | 100%                            |
| 356                                                 | K            | 19                   | 79%                        | 100%                               | 63%                              | 21%                             | 26%                             |
| 360                                                 | G            | 16                   | 69%                        | 75%                                | 6%                               | 13%                             | 13%                             |
| 361                                                 | Q            | 19                   | 37%                        | 74%                                | 0%                               | 26%                             | 32%                             |
| 362                                                 | Q            | 19                   | 95%                        | 95%                                | 84%                              | 95%                             | 95%                             |
| 363                                                 | Q            | 19                   | 53%                        | 95%                                | 63%                              | 89%                             | 100%                            |
| 364                                                 | W            | 18                   | 89%                        | 94%                                | 6%                               | 6%                              | 6%                              |
| 365                                                 | G            | 17                   | 47%                        | 94%                                | 12%                              | 100%                            | 94%                             |
| 371                                                 | I            | 19                   | 26%                        | 63%                                | 58%                              | 100%                            | 79%                             |
| 378                                                 | R            | 19                   | 0%                         | 47%                                | 63%                              | 32%                             | 79%                             |
| 380                                                 | S            | 19                   | 95%                        | 100%                               | 26%                              | 47%                             | 37%                             |
| 381                                                 | A            | 19                   | 63%                        | 89%                                | 89%                              | 95%                             | 100%                            |
| 382                                                 | N            | 15                   | 80%                        | 87%                                | 0%                               | 100%                            | 100%                            |
| 384                                                 | G            | 17                   | 65%                        | 88%                                | 82%                              | 100%                            | 100%                            |

| Table 4-1: Non-restrictive Positions of CBH2 |       |               |                  |                          |                        |                       |                       |
|----------------------------------------------|-------|---------------|------------------|--------------------------|------------------------|-----------------------|-----------------------|
| Position                                     | WT AA | # at Position | HPLC PI % > 0.50 | PASC Sp. Ac. PI % > 0.50 | PCS Sp Ac. PI % > 0.50 | Res. EtOH PI % > 0.50 | Res. Heat PI % > 0.50 |
| 386                                          | S     | 17            | 88%              | 88%                      | 76%                    | 94%                   | 100%                  |
| 394                                          | V     | 19            | 16%              | 42%                      | 26%                    | 58%                   | 68%                   |
| 396                                          | P     | 19            | 5%               | 47%                      | 68%                    | 26%                   | 47%                   |
| 399                                          | E     | 19            | 74%              | 5%                       | 0%                     | 26%                   | 11%                   |
| 400                                          | C     | 19            | 63%              | 32%                      | 11%                    | 47%                   | 32%                   |
| 405                                          | D     | 19            | 79%              | 95%                      | 32%                    | 74%                   | 84%                   |
| 406                                          | S     | 19            | 84%              | 84%                      | 84%                    | 84%                   | 79%                   |
| 407                                          | S     | 19            | 84%              | 84%                      | 63%                    | 74%                   | 74%                   |
| 410                                          | R     | 19            | 84%              | 100%                     | 74%                    | 95%                   | 100%                  |
| 413                                          | S     | 19            | 68%              | 100%                     | 89%                    | 100%                  | 100%                  |
| 414                                          | H     | 19            | 63%              | 74%                      | 63%                    | 84%                   | 63%                   |
| 416                                          | A     | 18            | 28%              | 78%                      | 33%                    | 50%                   | 17%                   |
| 417                                          | L     | 17            | 59%              | 100%                     | 47%                    | 47%                   | 18%                   |
| 422                                          | Q     | 18            | 72%              | 83%                      | 28%                    | 89%                   | 94%                   |
| 426                                          | Q     | 19            | 74%              | 95%                      | 63%                    | 95%                   | 74%                   |
| 427                                          | A     | 17            | 88%              | 41%                      | 12%                    | 76%                   | 53%                   |
| 429                                          | A     | 19            | 58%              | 84%                      | 74%                    | 79%                   | 68%                   |
| 431                                          | F     | 17            | 29%              | 82%                      | 18%                    | 18%                   | 24%                   |
| 433                                          | A     | 19            | 63%              | 95%                      | 74%                    | 63%                   | 47%                   |
| 436                                          | V     | 19            | 68%              | 84%                      | 53%                    | 37%                   | 21%                   |
| 440                                          | T     | 19            | 74%              | 89%                      | 63%                    | 89%                   | 89%                   |
| 441                                          | N     | 19            | 0%               | 74%                      | 79%                    | 37%                   | 32%                   |
| 443                                          | N     | 16            | 38%              | 69%                      | 44%                    | 88%                   | 94%                   |
| 444                                          | P     | 19            | 37%              | 63%                      | 37%                    | 95%                   | 100%                  |
| 445                                          | S     | 16            | 31%              | 94%                      | 63%                    | 69%                   | 56%                   |
| 447                                          | L     | 19            | 37%              | 89%                      | 89%                    | 100%                  | 68%                   |

EXAMPLE 5

Effect of Charge Change on the Activity of CBH2 Variants

5 [236] In this example, the effect of charge change on the activity of CBH2 in PCS and PASC assays was assessed. Briefly, the number of PCS and PASC winners in the CBH2 SELs was determined as a property of net charge change. In Tables 5-1 and 5-2, the ratio of observed to expected (o/e) winners was determined in PCS and PASC assays respectively. Values in bold italics are significantly different from the average of 10 random distributions plus or minus the number of standard deviations (sd) listed in the respective columns.

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| Table 5-1: Charge Effect on Activity of CBH2 Variants in a PCS Assay |                    |                 |                 |                                   |
|----------------------------------------------------------------------|--------------------|-----------------|-----------------|-----------------------------------|
| PCS                                                                  | o/e 1 sd           | o/e 2 sd        | o/e 3 sd        | Results                           |
| Charge change                                                        |                    |                 |                 |                                   |
| -2.00                                                                | <b><i>1.20</i></b> | <del>1.20</del> | <del>1.20</del> | >90% confident more than expected |

| <b>Table 5-1: Charge Effect on Activity of CBH2 Variants in a PCS Assay</b> |                 |                 |                 |                                   |
|-----------------------------------------------------------------------------|-----------------|-----------------|-----------------|-----------------------------------|
| -1.00                                                                       | <b>1.40</b>     | <b>1.40</b>     | <b>1.40</b>     | >99% confident more than expected |
| 0.00                                                                        | <del>1.03</del> | <del>1.03</del> | <del>1.03</del> | as expected                       |
| 1.00                                                                        | <b>0.46</b>     | <b>0.46</b>     | <del>0.46</del> | >95% confident less than expected |
| 2.00                                                                        | <del>0.00</del> | <del>0.00</del> | <del>0.00</del> | as expected                       |

| <b>Table 5-2: Charge Effect on Activity of CBH2 Variants in a PASC Assay</b> |                 |                 |                 |                                    |
|------------------------------------------------------------------------------|-----------------|-----------------|-----------------|------------------------------------|
| PASC                                                                         | o/e 1 sd        | o/e 2 sd        | o/e 3 sd        | Results                            |
| Charge change                                                                |                 |                 |                 |                                    |
| -2.00                                                                        | <b>0.13</b>     | <b>0.13</b>     | <b>0.13</b>     | >99% confident less than expected  |
| -1.00                                                                        | <b>0.82</b>     | <del>0.82</del> | <del>0.82</del> | >90% confident less than expected  |
| 0.00                                                                         | <b>1.06</b>     | <b>1.06</b>     | <del>1.06</del> | > 95% confident more than expected |
| 1.00                                                                         | <del>1.03</del> | <del>1.03</del> | <del>1.03</del> | as expected                        |
| 2.00                                                                         | <b>0.40</b>     | <b>0.40</b>     | <del>0.40</del> | > 95% confident less than expected |

[237] As shown in Table 5-1 and Figure 2A, decreasing charge (e.g., -1, -2) results in a significantly higher frequency of CBH2 winners in the PCS assay, while increasing charge (e.g., +1) results in a significantly lower frequency of CBH2 winners in the PCS assay. In contrast, as shown in Table 5-2 and Figure 2B, a significantly higher frequency of CBH2 winners is observed for variants without a charge change (e.g., 0), while a significantly lower frequency of CBH2 winners is observed for charge changes (e.g., -2, -1, +2).

[238] In conclusion, CBH2 activity on PCS correlates with decrease in charge. CBH2 activity on PASC however, shows that this trend does not correlate with cellulose hydrolyzing activity, but is specific for PCS material.

## EXAMPLE 6

### Creation of *Trichoderma reesei* Screening Strains

[239] Improved screening strains were created to increase the consistency of CBH2 variant expression in the presence of factors unrelated to the amino acid sequences of the enzyme variants. In particular, *T. reesei* screening strains were developed in combination with a targeting vector to force integration of *cbh2* variant genes (e.g., coding region in operable combination with a regulatory sequence). The new strains prepared during development of

the present disclosure, combine several mutations that are advantageous for screening variant libraries. A schematic of the genetic engineering steps is shown in Figure 4.

[240] Deletion of *ku80* from the *T. reesei* quad deleted derivative strain. A single orthologue of MUS52, the *N. crassa* orthologue of the human KU80, was identified by

5 TBLASTN search in the genome sequence of *H. jecorina* QM6a (*Trichoderma reesei*) and was consequently named *T. reesei ku80*. protein id 58213; <http://genome.jgi-psf.org/Trire2/Trire2.home.html> The nucleotide sequence of the *T. reesei ku80* gene is provided as SEQ ID NO:23 :

```

10 ATGGCGGACAAGGAAGCAACCGTCTTCATCATCGACCTCGGCGCGTCCATGGCA
   GCTGTCAATGGGGGTTCGAGAAGAATCCGACCTTGATTGGAGCATGAGCTACGTC
   TGGGACAAGATCAGCAACGTCGTGGCCTCGAATCGCAAGACGCTGTGCGTTGGC
   GTCGTGGGGTTCAGAACCGACGAGACAAACCACACGCTGAGCGAGGATGGGTAC
   GAGAACATCTCCATATTGCAGCCCCCTGGGGCCGATGAGCATGTCCAGCCTCAAG
   GCTCTTCAGCCCAAGGTGAAGCCGAGCAGGACGGTGGAAGGCGATGCCATCTCG
15 GCGATTGTCATTGCCGTCGACATGATTGACAAGTACACGAAGAAGAACAATGG
   AAGCGGCAGATTGTTCTCATTACCGACGGCCAAGGCGAGATTGATCCAGATGAT
   ATTGGCGACATTGCTAGAAAGATGCGCGACTCGAATATTGAATTGACAGTCTTGT
   GAGTTGGCGAGACCGTTTGGCGGACGGTAATGGTGCTGACGGTGATGCAAGGGG
   CGTCGACTTTGATGCTCCCGATTACGGCTTCAAAGAGGAGGACAAACCTTCAGTC
20 AAGGTACTCCATATGTTCACTTCTTTTCTTTTCTTTTCTTTTATTTTCTTTTCTTTTGA
   GCTTTCATTAACCTCTTCGTTAGAAGCAAAACGAAGAGACCCTAAAAAAGCTCGT
   GGATGGCTGTGGCGACGACTCAAGGTTTCGCCTCCATGGTCGAGGCCATTGACGA
   CTTGAATGAGCCACGAGCAAAGTCGGTCAAGCCTTACAAAACGTACGAAGGTCT
   CTTGACCTTGGGAGATCCGAAAAACGCTCCCGCAGTGGTGGAATCCGCGTCGA
25 GAGATACTTCAAGACCCATCTAGCCAGGCCACCTGCCGCCAGCACCGTGGTGGTC
   AAGGAGGAGCAAGCTGGGGCCGTCTCAGGCAGACGAGGACGAACAGATGGACGG
   AGCGGAACCTACAGCTGTGAGGCAGGCCAGGACATACAAGGTCAATGATCCAGA
   TGCCCCCTGGCGGTAAGCGTGACGTTGAGTTTGAGTCTCTGGCCAAAGGGTACGAG
   TACGGCAGGACGGCAGTCCACATCAGCGAGTCTGATCAAAACGTACACCAAGCTC
30 GCGACAGAAAAGAGCTTCAAGATCATCGGCTTCGTCCAGAAAAGAAAAGGTATTG
   GCTTGGCTCTCAGCATTTGACCCGTTGCTCTTGGCTAACCCTTGTTTAGTATGAAA
   TGCTCCTTAATCTTGGCGAAACCTGCGTTACCGTTGCATCCAAGTACGATGAAAA
   GTCTGAGCTGGCTTTTAGCTCTCTGGTGTGGGCGCTCTCGGAGCTCGACGCCTAC
   GCCGTGGCCCGCCTAGTAACCTAAGGACCAAAAGGACCCCATGCTGGTGTACTG
35 ATGCCGTATATGGAGCCTGATTATGTTTGTCTCTATGATGTGCCTCTGCCTTTTCGC
   AGAGGACATCAGGACGTACCAGTTTCCTCCCTTGGACAGAGTCGTTACCGTCAGT
   GGCCAAACGCTACCAACCATCGCCTATTGCCATCCGACGAGCTCAACCAAGCG
   ATGAGCGACTACGTAGATGCCATGGACATTTCAAGTTATGGTATCGATGAAGATG
   GGTGAGTATAGAAGATGATTGTTCAAATCTTTCACCTTCTAAGCATTGCTTCTGATC
40 TAGGCAACCGGCTGAATATGCCACCATCGATGAGTTATACAACCCTGCGATACAT
   CGCATAGGCCATGCGATCAAACAACGAGCGATCCACCCAGAGAAACCCGTGCCC
   GAGATCCCCCAGTCTTGCTTAGATTTCGCAGCACCCCCGACAGAACTCGTCGAGA
   CTGTGCAGCCTCATATCGATGCACTGATTCACGCTGCAGACGTGAAGAAAGGTAC
   TGATTCCATTACATATGCTTCTCTGCACACTGATGTTTGATTTGTGCTAACGCCCC
45 CCTTAGTGCCGCCCAAGGCCAAGGGCAAGCGCCAAAGAGAAACAGTTAAACCCA
   TCTCGGGACTGGATGTGGATGCCCTTCTGGGAGAAGAGCAGAAAGGTTCCATTA

```

GTCCGGAGAATGCCATTCCGGACTTCAAACGAGCCCTCAACTCGTCCGAAGAAG  
 TCGAGCAGATTGCCGACGCCACAAAACAAATGGGGGCCATTGTGCGGTCTCTCA  
 TTACGGACAGCTTCGGGGATAGCAAATATGCCCAGGCAATGGAAGGCATTGGTG  
 CGATGCGTGAGGAGCTGATCAACCTGGAAGAGCCTGGCCTGTACAACGACTTTG  
 5 TGCGCGACTTGAAGAAAAGTTTGCTATCTGGAGCCTTGGGTGGTGACAGGCGAG  
 ATTTCTGGTTCAAGATGAGGTGGGCGAAGCTGGGCCTGATTGACAAGAAACAGT  
 CGGAGGTGTCTTCGGTCACTCTTGAGGAGGCGGACGAGGTGAGTGGTGCAGCAT  
 GCTGTCGGATTATACGGACGTTGTTTGCTAACTTGTGGGATAGTTTTACAAGTCG  
 AGGTGAGGTATCTACGTTGACCAAGAATGGGACCATGTATATGAGCGGTGTAAC  
 10 AACAGAATCCTGTGCTTTGAGCATTGTATGA

[241] The *T. reesei ku80* gene was deleted from the quad deleted derivative strain using standard methods of the art (WO 2005/001036). Briefly, a *ku80* deletion cassette was utilized that employed a selectable marker flanked between 1.3 kb of 5' *ku80* sequence and 2.3 kb of 3' *ku80* sequence, as schematically shown in figure 5. The variant *T. reesei als*, which confers resistance to the herbicide chlorimuron ethyl, was used as selectable marker as described in WO 2008/039370. The nucleotide sequence of the *ku80* knockout cassette is 7685 base pairs in length: bases 1-1271 correspond to the 5' *ku80* homologous region; bases 1280-7685 correspond to the *als*-chlorimuron ethyl resistant variant (A190D); and bases 5381-7685 correspond to the 3' *ku80* homologous region. The nucleotide sequence of the *ku80* knockout cassette is provided as SEQ ID NO:14:

ggccgcctcaacacccacactcgaggcacacgagttcatcggcggcttccccacaagctctcggccaacctgctaccggctctctc  
 gcgagacttcccaaagcctacaaacgaggtcgacgtcaaggaggccctcgagcgccagccccggcagatggagcctccagggcca  
 gatcaaggccaacaacatgagagcccagagcgccgcactccggctcgacgacaaggagggcaaggcgagagcctttgaggagg  
 ccaagcgcgagctactggcgtatcaccacagcgccctgcggaagccttccggcgcaagataatgagcttgatcgcaatgacgagttc  
 25 acgtacgctttgcatattgttgttgccttttggctcctacatgtacggcgcatgtgttgggaggatataccacggagagtgtccgagt  
 ggcttctgggatttagagcgtcattagcaggatagagatggttggccaggggaatggaattgacttttactacaaggaactgttcactc  
 tgggtgttgattcccattgcgtgactggtagtagggaggaatgctttactttgtgccactagaccgcagagaagggttggtgcaagcgg  
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 gcgtgaagccgtacgccaccagggcgacgaactcgacaagggtgacgagggaggagatgccgtgcagcatgcccacttctgttg  
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 tacttctgctgcttgctggcgctgctctcggttatgcgtgtcaaggaccagggtgtgttcgcatcggttttccagcgccgattaccgg  
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cgagcgagagcgcaatgcttcacaatacgcaggaatttcttcatttggcaggttaagggcaaaggggctgacgtgg.

[242] Creation of the Archy2 strain from the *T. reesei*  $\Delta ku80$  quad deleted derivative strain.

- 5 The *pyr2* gene was deleted from the *ku80* knockout strain. The *pyr2* deletion cassette contains the *T. reesei* *cbh1* promoter, a hygromycin resistance gene and a partial *amdS* selectable marker flanked by 5' and 3' *pyr2* sequences, schematically shown in figure 6. Use of this vector permits screening for resistance to hygromycin and fluoroorotic acid of *pyr2* knockout transformants. The partial *amdS* gene contains the 3' portion of the gene, but lacks
- 10 a promoter and the amino-terminal portion of the coding region, and is consequently non-functional. The nucleotide sequence of the *pyr2* knockout cassette is 9259 base pairs in length: bases 1-1994 correspond to the *pyr2* 3' homologous region; bases 2002-3497 correspond to the *T. reesei* *cbh1* promoter; bases 3563-5449 correspond to the hygromycin resistance selectable marker; bases 5450-7440 correspond to the *A. nidulans* *amdS* 3' partial
- 15 marker; and bases 7441-9259 correspond to the *pyr2* 5' homologous region. The nucleotide sequence of the *pyr2* knockout cassette is provided as SEQ ID NO:15:

atcacgccctcgcataaaagaccctcaagagtccatgtgccctatctgcctgatcttctaacccttatttaacattggccctatcacacc  
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 gctacatctcatggctaaacaagtacgacgaaggggactcgggtctgacaaccatgctccgcaaagccggtgccgtcttctacgtcaa  
 15 gacctctgtcccgcagaccctgatggtctgcgagacagtcaacaacatcatcgggcgcaccgtcaacccacgcaacaagaactggtc  
 gtgcggcggcagttctggtggtgaggggtcgatcggttggtggtcgtggtggcgatcggtgtaggaacggatatcggtggctcgatt  
 cgagtgcgggcgcggttcaacttctgtacgggttaaggccgagtcagggcggtgccgtatgcaaagatggcgaacagcatgga  
 gggtcaggagacgggtgcacagcgttgctggggccgattacgcactctgttgagggtgagtccttcgctcttcttcttcttctgctctata  
 ccaggcctccactgtcctccttcttctgtttttatactatatacagagaccggcagtcactgatgaagtatgtagacctccgcctcttccaca  
 20 aatccgtctcggtcaggagccatggaaatacgaactcaaggtcatccccatgccctggcgccagtcaggagtcggacattattgcctc  
 caagatcaagaacggcgggctcaatatcggtactacaacttcgacggcaatgtccttccacacctcctatctcgcgcggcgtggaa  
 accaccgtcgccgcactcgccaaagccggtcacaccgtgaccccggtgacgccatacaagcacgatttcggccacgatctcatctcc  
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 ctgaacccgaacatcaaagctgttaacatgaacgagctctgggacacgcactctccagaagtggaaattaccagatggagtaccttgaga  
 25 aatggcgggagggtgaagaaaaggccgggaagggaactggacgccatcatcgcgccgattacgcctaccgctgcggtacggcatga  
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 ataagaagaatgagagtttcaaggcgggttagtgagcttgatgcctcgtgcaggaagagtatgatccggaggcgtaccatggggcac  
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 gtggtgactccatagctaataagtgtcagatagcaatttgacaagaatcaataccagcaactgtaaataagcgtgaagtgacctg  
 30 ccatgctacgaaagagcagaaaaaacctgccgtagaacgaagagatatgacacgcttccatctctcaaaggaagaatcccttcag  
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 atgatctgcgatccagatgtgcttctggcctcactctgccttcagggtgatgttacttaattggtgacgaattcagctgatttgctgcagt  
 atgctttgtgttggttcttccagggttggtgccagccatgagcgctttgagagcatgttgctacttataaactcgagtaacggccacatattg

[243] Creation of the Archy3 strain from the Archy2 *T. reesei* strain. The Archy 2 strain was transformed with a vector to integrate at the same *pyr2* locus and replace the hygromycin resistance gene with the coding region of the *pyr2* gene. The hygromycin deletion cassette is shown in figure 7. This re-introduction of the *pyr2* gene back into the *pyr2* locus placed it between the *T. reesei* *cbh1* promoter and the partial *amdS* selectable marker. This strain could be selected for uridine prototrophy and sensitivity to hygromycin. The nucleotide sequence of the *hygR* knockout cassette is 9088 base pairs in length: bases 1-1994 correspond to the *pyr2* 3' homologous region; bases 1995-3497 correspond to the *T. reesei* *cbh1* promoter; bases 3564-5137 correspond to the *pyr2* selectable marker; bases 5280-7270 correspond to the *A. nidulans* *amdS* 3' partial marker; bases 7271-9088 correspond to the *pyr2* 5' homologous region. The nucleotide sequence of the *hygR* knockout cassette is provided as SEQ ID NO:16:

agtcagatgaagaagcaagaagacgccatggtagccaccgcttgtcagggctccttagcaacgaacaactctagcttggggacttgtc  
 gatgtgtcgtttccttccctacccatcagcaccaacgatgagttcgatatagacgaggacctcatggaagtagagaccattgggttcgaca  
 ggatctctcagtttcacttctatgaggtctgtcgtcggatgacttttgaggagcttccccctctgcttcaaccccaactctcttctgaa  
 accgcagcacgttggcacggccgtgttgcaggagcagtttgcttcgagcactctcagcgtggttcagcagcccactggtagtggc  
 5 ctcctttgacgtccacaccttgctcctgtcgcacgtatctggtgggaacgactgtccaaggaggattgctaacgaggtttaggccc  
 aatatcgcatcagattctccggaaccttagctacggcctctcaacatctgtgacatgacggagcgcaagtactggtaggtggcgacca  
 agatgcgaggctggaacatcgacggctgccccgaagacgtcaggagactcatgtttgtcacatcatcgccaccctgggatgcagcc  
 ccgtcgtgacggatgaagacatggactacccaagaactgggcggcaattctccacggtagagacagatatccgagtgaacctgtgg  
 gccaccggcctcatgggcgcaccatctgcctccactcggtagggcgtctgcctcgtctccagggttgggtctcggtactgcgactct  
 10 gaagtcgtatgtgcagcgcatgaacagcctcggcgccgaggaccgtgttgcctcgtttgccgcaagcccagacgagatttttgaa  
 agatgcgggcttcaggaacagcggccggagtagtatcaagactctggtcggcgaatactacaacatggtagtggttccacatcgactt  
 ggccagactctatacgattttcaaacctcgtatagctcatattgacttgtttcttaggtcttcgatttgccccgggccccaaagactttatcga  
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 tggcggctctttgtataccttgggacacggcagacgcatgtctatgtgaagaaaacgttcacggcgctgtttgcatcaggaatatgatca  
 15 ttaaacatggagcgtaatggtattaatgatcaactagaaaaatggtatggaagggcgagagggcgatcaacaaagcagccccggggc  
 atagtctggaagcagcaggaattggaagggaaaaggaagctgcacaatgaagggatatcgtgagcggagtggctcacgagagtatc  
 aacagactggcgaaagcaagcaattgccaacgccggctattagccataagatggcctgttgtagtcccagttgcacgtatccccat  
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 ggagaacacatgggcaaccgggcaatacacgcgatgtgagacgagttcaacggcgaatggaaaatctgaaaaacaaaataaaata  
 20 actgccctccatacgggtatcaaattcaagcagttgtacggaggctagatagagttgtgaagtcggtaatcccgtgtatagtaatacga  
 gtcgcacataaatactccgaagctgtgcgaaccgggagaaatcgagatgtgctggaagcttctagcagcggctaaattagcatgaa  
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 tggggctgttcgaagcccgaatgtaggattgtatccgaactctgctcgtagaggcatgttgatgtgtcgggcaggacacgcctc  
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 30 ccccaattgggtcgttgtttgtccggtgaagtgaagaagacagaggtagaatgtctgactcggagcgtttgcatacaaccaag  
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 gctcggcgagctggcgccccagaacctggaccgcgtctcctactcgtttgaccgcaaggaggccaaggaccacggcgagggcg  
 15 caacatcgtcggcgcttcgctcaagggaagagggtcctgattgtcgacgacgtcatcaccgcccgcaccgccaagagggaacgccc  
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 5 ggcattgaccagttccggtactatgggtatgcctctgtgatcaacctgctggatttcacgagcgtggtgttcggttacctttgcggataa  
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 10 ccttcagggttgctttccagtagtgattttaccgctgatgaaatgactggactccctcctcctgctcttatacgaaaaattgcctgactctg  
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 gcaatccatgatctgcgatccagatgtgcttctggcctcactctgccttcagggtgatgttcaactaattggtgacgaattcagctgatttg  
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 acatattgttcaacttgaatcacatacctaatttgatagaattgacatgtttaagagctgaggtagctttaatgcctctgaagtattgtga  
 15 cacagcttctcacagagtgagaatgaaaagttggactccccctaataagtaaaagtctctgaacgggtgaagagcatagatccg  
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 cactgggggtccatggtgcgtgtatcgtccccccacaattctatgccatggtacctccagcttatcaatgccccgctaacaagtcgcctc  
 20 tttgccttgatagcttatcgataaaacttttttccgccagaaaggctccgccacagacaagaaaaaaattcaccgcctagcctttggc  
 cccggcatttggttaaactcgagcctctctcccgtcttggggatcaggaagaaaagaaaaaaatccatcgccaagggtgttttggc  
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 caggagtttctggacattgtgctgagcaggaccagcgcaactgccactcagatccgtgccggcttcaagattagcagaattcgag  
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 25 ccttctacactcgaaaggtaagttcaccaggagacgttttccgaaaagttcgctccatcctcgacagcttccctcgctccaggaca  
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 ctggtcgagaccatctcgcgcgactacgtcctgtcttgaatacgeccagtcgctctaccagtgaagcagctcaagcgggcccgtc  
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 ccggtattctggaccacctcttgaggagatgaacactatcgaaatgcagaggtatgtggcgcggct.

[244] Creation of the A5D strain from the Archy3 *T. reesei* strain. Native *T. reesei* *bglI* was deleted from the Archy 3 strain using a double recombination vector known in the art.

Hygromycin resistance was used as the selectable marker for *bglI* deletion. In addition, the hygromycin resistance marker was flanked by loxP sites. The deletion cassette is shown in figure 8. Subsequent hygromycin resistant transformants were analyzed for *bglI* deletion. A strain confirmed for deletion of *bglI* was next transformed with a telomeric vector encoding cre recombinase and a functional *amdS* for selection of transformants to facilitate removal of the hygromycin by cre recombinase expression and loop out of the loxP sites. The telomeric vector encoding the cre recombinase is schematically shown in figure 10. Transformants were first obtained on acetamide media, then transferred to potato dextrose agar and replica plated onto hygromycin media to screen for hygromycin sensitivity. Strains sensitive to hygromycin, were again transferred to potato dextrose media and replica plated to acetamide media. A strain was selected which lost the ability to grow on acetamide, indicative of a loss of the telomeric vector. The nucleotide sequence of the *bglI* knockout cassette is provided as SEQ ID NO:17:

aatgtaggaatgctgggatataaggctctgtgctggcaagttgatggatcctcgaatgaggccgccctgcaaggggaacatcagagat  
 ctaccattgcctccttgcccacatccactatcatacctacctcatgatcattcctgcgaaggtctaccagtaaataattcctcgtcccggtgtt  
 catcatgtccagaacctcatctcgccaaattgactttgccacagtgtctggagctgggtaagcagcgtgccaaggaattgtgtcagtc  
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 tcaggtgctcagggcatagcgttctctgcaaatagaggaaagagaatagcactagtgaagtgatgaatcacaatgaagaggaggtgt  
 tgccggaatgctttgagcagcgtcaaagtgaactgaagctatcacaattgcagggtaaagtacatgttggtgccagtttgacagcac  
 agtgcgcggagcggaggatgtcgcggaagaggcgcgacgctaaccgggccttctctcagtgagcagaactcctgctgcaagagt  
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 tacctcctcaattcacttctgcttcggccgtcttcattcatgttactgctctgatgcctatcctgaagattgtattcctgcagtattcacgcc  
 atcccaccttcggtcctcactcacaggtcaaccgccttcacctcctcgcatgatgtcggcaatctggtggatcaatgtgcgg  
 ttgagggccgcccgtagtgaggatgggcatggggaacgaggtcgcccatcgcccacagataactcgtatagcatacattatacgaag  
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 aattttacatcattttcttttctcagtaacggccacctttgtaaagcgtaaccagcaaaacaaattgcaattggcccgtagcaaggtagt  
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142

ttgccggtgtaccagatgtaagtctgtccaggggttgactttccatcgggtcggagggtcatgtcattctgggggaattggtgaacatactc  
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 gagtgccatcgcttctgagcaggacgatgcctcaacagcagcctcgtagagatgtt.

10 [245] The nucleotide sequence of the telomeric vector, pTTT-cre, is provided as SEQ ID  
 NO:18:

ttgtacaaagtggatgcgcgcgcgcgcagctccgtgcgaaagcctgacgcaccggtagattcttggtgagcccgtatcatgacg  
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 30 ctgagaccggcgccatggtccgactcctcgaggacggcgactaaaccagcttct.

[246] Creation of the MAD6 strain from A5D *T. reesei* strain. Native *egl3* was deleted from the A5D strain using the method previously described for *bgl1* deletion. A schematic of the deletion cassette is shown in figure 9. Hygromycin resistance was used as the selectable

marker for egl3 deletion. The hygromycin resistance marker was flanked by loxP sites. A transformant was confirmed with a deletion of the egl3. The hygromycin marker was removed from this strain as described for creation of the A5D strain. The nucleotide sequence of the egl3 deletion cassette is provided as SEQ ID NO:19:

5    gggaggtaggcgcagatacggatgcatgggacccgaacccgtaaccggaacacgaccttatcagccctccaactcacaccctctcgc  
      ctatcactatcctagatagttcatcggccaactcatgtaacctagctacctacctacctggtaagaatgcgggctatcatgtctcacggcg  
      cggatcatgtcggatctcgtcgttccccgcaggttgacgtcggaaatccatgcaagtactccctgaaatcgagacgacagagagaac  
      aaccaacgcgcttaaacgcttcatgttcatctaagaggcacattcgaagaactagcttaacacactagacctggcttttcgacccctcc  
      gcagaaagccgttttctcctcaatcctccccgggcttggtttgtcagtcctgacttgcgtacacagagcttggacgcagcgtttgc  
 10    gcatcagcttgcaggcgggtcacgggactaggacaacaggggatgtgacaggccggatagtaattatgggttatccggggtaagca  
      gggaaattacgaggccgctttacgtgggggaacagccacttgcggggggaagaggagtagtaggcgactcggtcgatgagctcga  
      ggtgtctggttgacttgactgcagagcgtaggttaattgagatcgggcaacattatcgggtgtcggctcgggtatggccgagttgcgact  
      gcttggtcattcggcgaagctgatgtcgtggtatcctgaagcatcgatcggaaacctgatggtcagtctatctgacgtgtcgggtga  
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      ggacgtcactcccccaacctacaggaagagcaaaatgggagcagtggtctgcaatgagctatataatagatcgctcgatctcatacaaa  
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      cgtatagcctagaaagcgacgccatcttcatcttttacgcgattagcctcatggctatttgtgccgaagtgggagttgtatggtagcagtg  
      aggagattgtggctacgacacaggcgggttctcttgagcggcttacatctccgattaggcctgcgtacgatccagatcatgggaaact  
      ttacaatggcttactcgttttatctcaacactgagcttccaattcactctatgcattgattaacacgtttggtcatgtggttctcagctgtaaat  
      cttcagcttcccaagaattgcaacctcgctgattgctaatagtgttgcatgcgttgcatcctgggtgcggcagtgcaaaggagagtgcaaag  
 25    tagccggcagattaatttaagcttatatcactcaggggttaaacagccgtaaaggacctttgatctaactgccgatgtgtatgtagatca  
      cgcaatgccaccatatcttggcagtcagatttgcctgtggcgccgaagtataacttcgtataatgtatgctatacgaagtatcgggccg  
      gcgtattgggtgttacggagcattcactaggcaacctggttactattgtatacccatcttagtaggaatgatttgcagggtttatacctacg  
      atgaatgtgtgtcctgtaggcttgagagttcaaggaagaaacagtgcattatctttgcgaaccaggggctggtgacggaattttcata  
      gtcaagctatcagagtaaagaagaggagcatgtcaaagtacaattagagacaaatatatagtcgctggagccaagagcggattcctc  
 30    agtctcgtaggtctcttgacgaccgttgatctgcttgatctcgtctcccgaaaatgaaaatagactctgctaagctattcttctgcttcgccg  
      gagcctgaaggggcgtactagggttgcgaggtccaatgcattaatgcattgcagatgagctgtatctggaagaggtaaacccgaaacg  
      cgttttattcttgttgacatggagctattaaatcactagaaggcactctttgctgcttgacaaatgaacgtatcttatcgagatcctgaacac  
      catttctcaactccggagctgacatcgacaccaacgatcttatatccagattcgtcaagctgttgatgatttcagtaacgttaagtggat  
      cccggtcggcatctactctattcctttgccctcggacgagtgctggggcgctcggtttccactatcggcgagtgacttctacacagccatcg

gtccagacggccgcgttctgcgggagatttgtgtacgcccagacagtcgggctccggatcggacgattgcgtcgcacgacctgc  
 gccaagctgcacatcgaaattgccgtcaaccaagctctgatagagttggtaagaccaatgcggagcatatacgcccggagccgc  
 ggcatcctgcaagctccggatgcctccgctcgaagtagcgcgtctgctgctccatacaagccaaccacggcctccagaagaagatg  
 ttggcgacctcgtattgggaatccccgaacatgcctcgtccagtcgaatgaccgctgttatgcggccattgtccgtcaggacattgtg  
 5 gagecgaaatccgcgtgcacgaggtgccggacttcggggcagtcctcggcccaaagcatcagtcacgagagcctgcgcgacgg  
 acgcactgacgggtgctgcatcacagtttgccagtatacacatggggatcagcaatcgcgcatatgaaatcacgcatgtagtgtatt  
 gaccgattccttgcgggtccgaatggggcgaacccgctcgtctggctaagatcgccgcagcgcacatccatggcctccgcgaccg  
 gctgcagaacagcgggagttcggtttcaggcaggtcttgcaacgtgacacctgtgcacggcgggagatgcaataggtcaggctct  
 cgctgaattccccaatgtcaagcacttcgggaatcgggagcgcggccgatgcaaagtgccgataaacataacgatctttgtagaacc  
 10 atcggcgcagctatttaccgcaggacatatccacgcccctctacatcgaagctgaaagcacgagattcttcgcccctccgagagctgc  
 atcaggctggagacgctgtcgaacttttcgatcagaaactctcagacagacgtcgcggtgagttcaggcttttcatatcgattgtgatgtg  
 atggagttgagatggaggtgaggagatggatgatgggaaaggaagatggactgaggatggaagaagagaagaagagagagaga  
 gaaagtcttcaggagagaaaggggaaccgaagaaaaatggggaggaaaccggccctagcacctaaatacgtctcccgcttaatttc  
 gccctttttcaccaaaccctccgcgtctttcgtgcgctagctgtcttggggggtgtgtaaaacttgggaacaaccctacgccgaacctcc  
 15 cgtacgaagcccgtacagtgtattctatccctggctttcccagcctggtaagtcgggtcccttcgggacggggccaaggagactgagtt  
 tccgggttaaccaataatgccgccagccgtggagcgggtctgagctgtctatcgtgaatccgtgacgctgaattgctcagtcgaagtcgg  
 agacgctggaatccaccgggtgctccagccacggcgaagaatccacttaccttccgggcttccgccagctggcaacataattttgaggc  
 tgatatagccttctcctccatcacgataagccctgactaccttgctacggggccaattgcaatttggttgctggttacgctttacaaaagggtg  
 ccgttactgagcaaaaagaaaatgatgtaaaatttgcgagtggtgcccatagctggatgggtccgataaaatggtactgccccactta  
 20 gtggcagctcgcgaccagtcacaagcccaggataacttcgtataatgtatgctatacgaagttatctgtgggcgttatgaataatagact  
 ggaaccggggccctttgattgacgactccataatttttagatgtagcaactcggcaagagcattatgtgcaatacatttgttaccatacaa  
 ggcagctgccagacgacttgattgcgtacaattctcacggcaagctttccagggtgttatgcattatgcgcaaatgcttgatgcttaccgc  
 aggattaatctcggaagaagcgtgcaagctatatgggtgtagtagatatgtagatgtaccaaccaatgaagaacatttatggctagaa  
 cgtagtgtgaaggttttgagtaatttgatcaagtaagacgatatttgatataataccaagcataattcatgataaattacttgaacca  
 25 cccttgcgtccggcctcacgagccttctactgccgggctcgaaggagccactggaggcctgtccacccttggtatgcgatttctgca  
 ccttttcttgggcctgcacgtcgattagacatgattcaaatcgagatcttggaaatcttacatgctggcgaagccaccgggtgtggctgg  
 actgtccgcccttctgcgcaatgctttgaacctcctccttggggctgtgtagaaagggtttgtagcaacattactacaactctcaggactcg  
 gtggtcgtaccgggtggcgaagtttccgggggtatcgttgccagacattgtgtgattatttgggtgtgcaaatgtgtgctatgtgtgttgtgt  
 gttggtgatgatgctgaagctgttgaaagcaggctggttctgtgggagagacttgggatatttatccaaagttcggctcgtgttctctg  
 30 gaagctcttctactccatacaatcatccaaagttgtcgtcattgagcgttgatcagtagtagcctctgaggctacccatgatccttccg  
 gccaacagtcggcactcatcaacagcaacaatcagccgccacaaacataggtacagtaaggagttagatatcatgtagtcgtcgagta  
 ctcgacatcatgacgtacaagctttgccagtgtcggttaggtgcaagtatgatgatcgtatccgccgttggttcgatcgaacagagtgcggt  
 cagattcacggtttctcacttgaacattggatgcaattggattgatccacaatcctggagaatggcttcaagctcactgctccagtcgc  
 aagcttcagagcctattactaagggttagagctacctatgtcaagagttttcaaggtaacctaagctacatgtgatagtcggcaagccattt

gaacgcagaccgtgaacgggtgatgtaaaccgggatagacgccaagcgtgccgtgtcaatgacgctagatacacctcgatttacgta  
gagtgaatgccagccaatggagtcatgcacataacccgcttagactctgctcggggcgatacccgatcgagaggcagagccgctta  
aacgcgatcgcggtaacctgtaatcagagccagcgctcgatgaattgcatcatggaagccattgatgtggaatgttgagcgtataaaa  
cacgaattgaagacgacattgacttgcttcaagtgaaggagaattgccgggcagacaagataggtaggctcttggtgcgctgtcacat  
5 caatccattccttttctctgttcaatctctatgttgacattctgatagggatcattggatgccaatgcaaagaacatgagagtgtggtctgc  
attcaagtatcctgggtcgtgaagctgtggccatggggcgctgcggtcaaggtaacgcgatgactaatcagtcctcggtgactctggggcg  
gtagaggcagtgctgtgaaccaaagcttgagccgagggcaaaaacaacggcgcatcaacaatcaacgaaagcatcgtaacagt  
gtctcttcccagtcgaattacttcgaaaaccttctcgatagaaccttcagacgatgaacaggccacgcaaccgtcagccgcgcccccc  
aggacagactcagcgccccgggaggcagatcgacacaccttggtcgacgagctc.

10

### EXAMPLE 7

#### Analysis of Select Singly-Substituted CBH2 Variants in Activity and Thermostability Assays

[247] Protoplasts of a six-fold deleted *T. reesei* strain ( $\Delta egl1$ ,  $\Delta egl2$ ,  $\Delta egl3$ ,  $\Delta cbh1$ ,  $\Delta cbh2$ ,  
15  $\Delta bgl1$ ) were transformed with selected CBH2 SEL variants encoded on the pTTTpyrG  
vector as described (WO 2009/048488). Spores were harvested, replated on acetamide agar,  
and incubated at 28°C for 7 days. Next, CBH2 variants were produced by inoculating 25 ml  
of YEG culture (5 g/L yeast extract, 20 g/L glucose) with a sporulating *Trichoderma* strain on  
agar, followed by a two day incubation period at 28°C, 200 rpm. Five ml of the YEG culture  
20 was used to inoculate 45 ml of glycine medium containing a 2% glucose sophorose mixture.  
The culture was dispensed over a 6-well microtiterplate and incubated stationary for five days  
at 28°C in an oxygen-enriched atmosphere. Cells were removed by filtration through a 0.2  
µm filter. Supernatants were subsequently concentrated using Vivaspin spin cells with a 10  
kD molecular weight cutoff. The variants were tested for their ability to hydrolyze  
25 phosphoric acid swollen cellulose (PASC) at 50°C, dilute acid pretreated corn stover (PCS) at  
50°C, dilute ammonia pretreated corncob (CC) at 50°C and 57°C. In addition, the variants  
were tested for thermostability.

[248] PASC 50°C. Enzyme activity on phosphoric acid swollen cellulose was examined  
essentially as described in Example 1, Section C, with the following changes. To each well,  
30 10 µl was added containing 4.9 µg protein in supernatant from a CBH2 deleted strain ( $\Delta egl1$ ,  
 $\Delta egl2$ ,  $\Delta cbh1$ ,  $\Delta cbh2$ ). The plate was incubated at 50°C while shaking at 200 rpm. After 2  
hours the plate was put on ice for 5 min and 100 µl of 100 mM glycine pH 10.0 was added.  
After mixing, the plate was centrifuged at 3000 rpm for 5 min. A volume of 40 µl

supernatant was diluted in 160  $\mu$ l water. Ten  $\mu$ l of the diluted solution was transferred to a new 96-well microtiterplate (Costar Flat Bottom PS) containing 100  $\mu$ l ABTS glucose assay mixture (2.74 mg/ml 2,2' azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid, 1 U/ml horseradish peroxidase type VI, 1 U/ml glucose oxidase) and increase in  $A_{420}$  was recorded in a microtiterplate spectrophotometer (Spectramax Plus 384, Molecular Devices). A range of glucose concentrations was included as a standard on each plate (0; 0.008; 0.016; 0.031; 0.063; 0.125; 0.25; 0.5; 1 mg/ml). Assays were done in duplicate. A dose response curve was generated for the wild-type CBH2 by fitting the data with a Temkin isotherm equation ( $y = a + b(\ln(1 + c \cdot x))$ ) and the activities of the CBH2 variants were divided by a calculated activity of wild-type CBH2 of the same plate to yield a performance index.

[249] *PCS 50°C*. Enzyme activity on washed dilute acid pretreated cornstover was examined essentially as described in Example 1, Section D, with the following changes. To each well, 10  $\mu$ l was added containing 49  $\mu$ g protein in supernatant from a CBH2 deleted strain ( $\Delta$ egl1,  $\Delta$ egl2,  $\Delta$ cbh1,  $\Delta$ cbh2). The plate was incubated at 50°C while shaking at 200 rpm. After 2 days the plate was put on ice for 5 min and 100  $\mu$ l of 100 mM glycine pH 10.0 was added. After mixing, the plate was centrifuged at 3000 rpm for 5 min. A volume of 10  $\mu$ l supernatant was diluted in 190  $\mu$ l water. Ten  $\mu$ l of the diluted solution was transferred to a new 96-well microtiter plate (Costar Flat Bottom PS) containing 100  $\mu$ l ABTS glucose assay mixture and assayed and analyzed as described above for the PASC assay.

[250] *Corncob 50°C*. Enzyme activity on corncob at 50°C was performed essentially as described in Example 1, Section E, with the following changes. To each well 10  $\mu$ l solution was added containing 46.55  $\mu$ g protein of supernatant from a CBH2 deleted strain ( $\Delta$ egl1,  $\Delta$ egl2,  $\Delta$ cbh1,  $\Delta$ cbh2), supplemented with 4.90  $\mu$ g *T. reesei* CBH1, 6.84  $\mu$ g *T. reesei* Xyn2 Y5 (Xiong et al, Extremophiles 8:393-400, 2004), 2.28  $\mu$ g *Fusarium verticillioides* (Fv) 51A, 5.32  $\mu$ g Fv3A, 0.76  $\mu$ g Fv43D, and 2.45  $\mu$ g *T. reesei* BGL1. The *Fusarium verticillioides* enzymes have been described in US 61/245269 (herein incorporated by reference for the teaching of this method). Different volumes of supernatant of a transformed *T. reesei* strain expressing a CBH2 variant were added as described in Example 1. The plates were incubated for two days at 50°C with shaking at 200 rpm.

[251] *Corncob 57°C*. Corn cob was ground to pass through a 0.9 mm screen then pretreated as described in Example 4 of WO 2006/110901 (herein incorporated by reference for the teaching of this method). Pretreated corn cob was used as a 7% cellulose suspension in 50 mM sodium acetate pH 5.0. Seventy microliters of the suspension was added per well to a

96-well microtiterplate (Nunc Flat Bottom PS). To each well 10 µl solution containing 46.55 µg protein of supernatant from a CBH2 deleted strain ( $\Delta$ egl1,  $\Delta$ egl2,  $\Delta$ cbh1,  $\Delta$ cbh2), 4.90 µg CBH1 variant

(S8P/T41I/N89D/S92T/S113N/S196T/P227L/D249K/T255P/S278P/E295K/T296P/T332Y/

5 V403D/S411F/T462I), 6.84 µg *T. reesei* Xyn2 Y5 (Xiong et al, Extremophiles 8:393-400, 2004), 2.28 µg Fv51A, 5.32 µg Fv3A, 0.76 µg Fv43D, 2.45 ug *Talaromyces emersonii* beta-glucosidase were added. Up to twenty microliters of supernatant from *H. jecorina* cells expressing either wild-type CBH2 or a CBH2 variant was added. Compensating volumes of acetate buffer were added to make up for differences in total volume. The plate was  
10 incubated at 57°C while shaking at 200 rpm. After 2 days the plate was put on ice for 5 min and 100 µl of 100 mM glycine pH 10.0 was added. After mixing, the plate was centrifuged at 3000 rpm for 5 min. A volume of 10 µl supernatant was diluted in 190 µl water. Ten µl of the diluted solution was transferred to a new 96-well microtiterplate (Costar Flat Bottom PS) containing 100 µl ABTS glucose assay mixture and assayed and analyzed as described above.

15 [252] *CBH2 Thermostability Assay 2*. To test the thermostability of the CBH2 variants, 50 µl of supernatant was incubated in a PCR machine across a temperature range of 50-70°C.

The remaining activity was determined by incubating 20 µl supernatant with 80 µl 0.625 mg/ml celotriose in 50 mM sodium acetate buffer pH5.0 with 0.0025% Tween80. After 1 hour, 40 µl of 100 mM glycine pH10 was added. Twenty µl was added to 80 µl ABTS

20 reagent (see above) and color development at OD420 was recorded for 5 min. A pseudo melting temperature ( $T_m$ ) was calculated by fitting the remaining activity at each temperature with the formula:

$$y = 1/(1+\exp(-(a*(1-b/c-\ln(c/b))+d*((1/c)-(1/b)))/e))$$

in which a is the heat capacity (kcal\*mol<sup>-1</sup>\*K<sup>-1</sup>), b is the melting temperature (K), c is the  
25 assay temperature (K), d is the enthalpy change (kcal\*mol<sup>-1</sup>\*K<sup>-1</sup>), and e is the gas constant (kcal\*mol<sup>-1</sup>\*K<sup>-1</sup>).

**Table 7-1. Performance Index and  $T_m$  Values for Select CBH2 Variants**

| Variant | PASC 50°C | PCS 50°C | CC 50°C | CC 57°C | $T_m$ (°C) <sup>1</sup> |
|---------|-----------|----------|---------|---------|-------------------------|
| T19K    | 0.93      | 0.86     | 0.99    | 0.88    | 55.7                    |
| R63L    | 0.95      | 0.64     | 1.05    | 0.92    | 56.4                    |
| V94A    | 0.81      | 0.92     | 0.89    | 0.79    | 56.8                    |
| P98Q    | 1.19      | 1.07     | 1.06    | 0.98    | 58.8                    |

| <b>Table 7-1. Performance Index and Tm Values for Select CBH2 Variants</b> |                  |                 |                |                |                            |
|----------------------------------------------------------------------------|------------------|-----------------|----------------|----------------|----------------------------|
| <b>Variant</b>                                                             | <b>PASC 50°C</b> | <b>PCS 50°C</b> | <b>CC 50°C</b> | <b>CC 57°C</b> | <b>Tm (°C)<sup>1</sup></b> |
| P98L                                                                       | 0.96             | 0.52            | 0.80           | 0.77           | 58.3                       |
| L111S                                                                      | 0.97             | 0.96            | 0.98           | 1.03           | 57.8                       |
| T138S                                                                      | 1.09             | 1.02            | 1.03           | 1.05           | 56.5                       |
| T138C                                                                      | 1.13             | 1.09            | 1.07           | 1.08           | 56.3                       |
| T138A                                                                      | 0.90             | 0.93            | 1.03           | 0.75           | 55.6                       |
| P143S                                                                      | 0.76             | 0.69            | 0.92           | 0.68           | 56.5                       |
| L144W                                                                      | 0.88             | 1.07            | 1.03           | 0.90           | 56.5                       |
| A150H                                                                      | 1.01             | 0.95            | 1.04           | 1.02           | 56.9                       |
| T154D                                                                      | 0.96             | 0.97            | 0.97           | 0.82           | 55.9                       |
| N161P                                                                      | 0.93             | 0.88            | 1.00           | 0.87           | 57.0                       |
| A177N                                                                      | 0.81             | 0.81            | 0.94           | 0.74           | 57.3                       |
| A177S                                                                      | 0.94             | 1.41            | 1.07           | 0.89           | 57.1                       |
| N182A                                                                      | 0.89             | 0.80            | 0.94           | 0.79           | 57.4                       |
| N182M                                                                      | 1.02             | 0.97            | 1.08           | 1.11           | 57.5                       |
| N182W                                                                      | 0.92             | 0.33            | 0.70           | 0.57           | 58.8                       |
| A188S                                                                      | 1.16             | 1.30            | 1.20           | 1.05           | 56.4                       |
| N197A                                                                      | 1.03             | 1.16            | 1.16           | 1.18           | 56.1                       |
| K288N                                                                      | 0.90             | 0.80            | 0.93           | 0.96           | 57.4                       |
| S291A                                                                      | 1.01             | 1.03            | 1.06           | 1.15           | 57.1                       |
| S291T                                                                      | 0.88             | 0.73            | 0.94           | 0.92           | 57.0                       |
| S291C                                                                      | 1.53             | 0.95            | 1.02           | 1.06           | 56.3                       |
| S291H                                                                      | 1.07             | 0.87            | 1.11           | 1.00           | 56.6                       |
| S291E                                                                      | 0.85             | 0.61            | 0.84           | 0.95           | 58.0                       |
| T312S                                                                      | 1.08             | 1.07            | 1.09           | 1.11           | 56.7                       |
| S313T                                                                      | 1.01             | 1.08            | 1.20           | 1.21           | 56.4                       |
| S316A                                                                      | 1.03             | 1.03            | 1.17           | 1.04           | 57.0                       |
| S316P                                                                      | 1.05             | 1.09            | 1.03           | 1.06           | 57.1                       |
| S316W                                                                      | 1.02             | 0.94            | 1.11           | 1.05           | 57.0                       |
| A322P                                                                      | 1.04             | 1.05            | 1.16           | 1.13           | 56.7                       |
| S343W                                                                      | 0.80             | 0.60            | 0.92           | 1.00           | 57.1                       |

| <b>Table 7-1. Performance Index and T<sub>m</sub> Values for Select CBH2 Variants</b> |                  |                 |                |                |                                       |
|---------------------------------------------------------------------------------------|------------------|-----------------|----------------|----------------|---------------------------------------|
| <b>Variant</b>                                                                        | <b>PASC 50°C</b> | <b>PCS 50°C</b> | <b>CC 50°C</b> | <b>CC 57°C</b> | <b>T<sub>m</sub> (°C)<sup>1</sup></b> |
| S343Q                                                                                 | 0.94             | 0.94            | 1.06           | 1.16           | 56.5                                  |
| S343T                                                                                 | 1.09             | 1.02            | 1.04           | 0.82           | 57.0                                  |
| S343E                                                                                 | 1.10             | 1.13            | 1.18           | 1.12           | 56.4                                  |
| F346A                                                                                 | 1.04             | 1.07            | 1.18           | 1.14           | 57.0                                  |
| F346Y                                                                                 | 1.06             | 1.15            | 1.06           | 1.05           | 56.6                                  |
| F346H                                                                                 | 1.04             | 0.91            | 1.04           | 1.05           | 57.0                                  |
| Q362L                                                                                 | 1.24             | 0.99            | 0.95           | 1.14           | 58.1                                  |
| Q362I                                                                                 | 1.02             | 0.94            | 0.98           | 0.93           | 58.3                                  |
| Q363S                                                                                 | 1.00             | 1.02            | 1.08           | 1.08           | 57.1                                  |
| S386D                                                                                 | 0.99             | 0.96            | 1.00           | 1.03           | 55.9                                  |
| S386A                                                                                 | 1.03             | 1.00            | 1.06           | 1.08           | 55.9                                  |
| S386F                                                                                 | 1.16             | 0.63            | 0.94           | 0.79           | 52.8                                  |
| S386I                                                                                 | 0.91             | 0.86            | 0.98           | 0.78           | 56.3                                  |
| S386C                                                                                 | 1.64             | 1.33            | 1.10           | 1.24           | 56.1                                  |
| S386V                                                                                 | 1.09             | 0.97            | 1.04           | 1.08           | 56.6                                  |
| C400S                                                                                 | 0.95             | 0.87            | 0.93           | 0.95           | 64.6                                  |
| S406P                                                                                 | 1.34             | 1.10            | 1.06           | 1.11           | 57.4                                  |
| S406A                                                                                 | 1.00             | 1.00            | 1.02           | 1.02           | 57.1                                  |
| S407T                                                                                 | 1.16             | 1.05            | 1.08           | 1.05           | 57.0                                  |
| S413W                                                                                 | 0.88             | 0.27            | 0.66           | 0.53           | 59.4                                  |
| S413Y                                                                                 | 0.98             | 0.91            | 0.94           | 1.01           | 58.0                                  |
| Q422V                                                                                 | 0.97             | 0.87            | 1.03           | 0.93           | 55.1                                  |
| Q422T                                                                                 | 1.03             | 1.00            | 1.08           | 1.04           | 55.2                                  |

<sup>1</sup> As determined by thermostability assay 2. The T<sub>m</sub> of the reference CBH2 of SEQ ID NO:3 was 57.5°C under the test conditions.

[253] A category of mutations was devised, that is highly combinable mutations, to encompass mutations that have a PI > 0.75 for at least one property, and >0.05 for all properties. As illustrated in the succeeding examples, some variants having two highly combinable mutations, have activity and/or stability that is greater than the reference CBH2 enzyme. An accumulation of highly combinable mutations can be used to make a beneficial

variant where each individual mutation is not sufficient to confer superior activity, thermostability and/or expression levels to a variant enzyme.

### EXAMPLE 8

#### CBH2 Combinatorial Library Variants and Activities Thereof

- 5 [254] A synthetic CBH2 combinatorial library was produced by GeneOracle (Mountain View, CA). Table 8-1 lists the possible substitutions of members of the CBH2 combinatorial library (numbered according to the CBH2 mature amino acid sequence). This library was created by combining substitutions classified as up mutations and/or highly combinable mutations on the basis of their T<sub>m</sub> and performance as listed in Table 7-1.

10

| Table 8-1: CBH2 Combinatorial Library Design |                   |              |
|----------------------------------------------|-------------------|--------------|
| Targeted Position                            | Wild-Type Residue | Substitution |
| 98                                           | P                 | P,Q,L        |
| 111                                          | L                 | L,S          |
| 182                                          | N                 | N, W         |
| 291                                          | S                 | S, E         |
| 316                                          | S                 | S,P          |
| 362                                          | Q                 | Q,I,L        |
| 400                                          | C                 | C,S          |

The library was received from the above-mentioned provider as purified PCR products in which primers GACCGGACGTGTTTTGCCCTTCAT (SEQ ID NO:20) and GTGTGACCGGCTTTGGCGAGTG (SEQ ID NO:21) were used to amplify the *cbh2* gene flanked upstream by about 1.1 kb of the *cbh1* promoter and downstream by about 1.85 kb of the *amdS* marker for forced integration in the *pyr2* locus in the *H. jecorina* host strain. A schematic of the homologous recombination of the expression cassette into the screening strain is shown in figure 11. The nucleotide sequence of a PCR fragment (partial *cbh1* promoter, *cbh2* gene, and partial *amdS* gene) amplified from pTTTpyrG-CBH2 using the primers of SEQ ID NO:20 and SEQ ID NO:21, is provided below as SEQ ID NO:22:

20

gaccggacgtgttttgcccttcatttgagaaataatgcattgcgatgtgtaattgcctgcttgaccgactggggctgttcgaagcccg  
aatgtaggattgttatccgaactctgctcgtagaggcatgttgtaatctgtgtcgggcaggacacgcctcgaagggtcacggcaaggg  
aaaccaccgatagcagtgtctagtagcaacctgtaaagccgcaatgcagcatcactggaaaatacaaaccaatggctaaaagtacata  
agttaatgcctaaagaagtcataaccagcggctaataattgtacaatcaagtggtacgtaccgtaatttgccaacggcttggtgggt  
25 tgcagaagcaacggcaaagccccacttccccacgtttgtttcttcaactcagccaatctcagctgggtgatccccaattgggtcgttgttt

gttccggtgaagtgaaagaagacagaggtgaagaatgtctgactcggagcgtttgcatacaaccaagggcagtgatggaagacagt  
 aaatgttgacattcaaggagtatttagccagggatgcttgagtgtatcgtgtaaggagggttgctgccgatacgacgaatactgtatagt  
 cacttctgatgaagtggccatattgaaatgtaaagtcggcactgaacaggcaaaagattgagttgaaactgcctaagatctcgggccct  
 cgggcccttcggcccttgggtgtacatgtttgtgctccgggcaaatgcaaagtgtggtaggatcgaacacactgctgcctttaccaagca  
 5 gctgagggtatgtgataggcaaatgttcaggggccactgcatggttcgaatagaaagagaagcttagccaagaacaatagccgataa  
 agatagcctcattaaacggaatgagctagtaggcaaaagtcagcgaatgtgtatatataaagggttcgagggtccgtgcctccctcatgctct  
 ccccatctactcatcaactcagatcctccaggagactgtacaccatctttgaggcacagaaaccaatagtcacccatcacaaagtgtgt  
 aaaaaaagcaggctccgcggccgcccccttaccaccatgattgtcggcattctcaccacgctggctacgctggccacactcgca  
 gctagtgtgcctctagaggagcggcaagcttgcctcaagcgtctggttaattatgtgaaccctctcaagagacccaaatactgagatatgtc  
 10 aagggggcaaatgtggtggccagaattggcgggtccgacttgctgtgcttccggaagcacatgcgtctactccaacgactattactccc  
 agtgtcttcccgcgctgcaagctcaagctcgtccacgcgcgcgcgctcgacgacttctcgagtatccccacaacatcccggtcga  
 gctccgcgacgcctccacctggttctactactaccagagtacctccagtcggatcgggaaccgctacgtattcaggcaaccctttgttg  
 gggtcactccttgggccaatgcatactacgcctctgaagttagcagcctcgtattcctagcttgactggagccatggccactgctgcag  
 cagctgtcgcaaagggttccctctttatgtggctgtaggtcctcccggaaccaaggcaatctgttactgaaggctcatcattcactgcaga  
 15 gatactcttgacaagacccctctcatggagcaaaccttggccgacatccgcaccgccaacaagaatggcggtaactatgccggacag  
 tttgtggtgtatgacttgccggatcgcgattgcgctgcccttgctcgaatggcgaatactctattgccgatggtggcgctcgccaaatata  
 agaactatategacaccattcgtcaaattgctgtggaatatccgatatccggaccctcctggttattggTAtgagtttaaacacctgcct  
 ccccccccccttcccttcttcccgccggcatcttgctgtgtgctaactattgttccctcttccagagcctgactctcttgccaacctgggtg  
 accaacctcgggtactccaaagtgtgccaatgctcagtcagcctaccttgagtgcataactacgccgtcacacagctgaaccttccaaa  
 20 tgttgcatgtatttggacgctggccatgcaggatggcttggctggccggcaaaccaagacccggccgctcagctatttgcaaatgttt  
 acaagaatgcatcgtctccgagagctcttcgcggattggcaaccaatgtcgccaactacaacgggtggaacattaccagccccccatc  
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 ttggtattcgcccatccgcaaacactggggactcgttgctggattcgtttgtctgggtcaagccaggcggcgagtgtagcggcaccag  
 25 cgacagcagtgccgacgatttgactcccactgtgcgctcccagatgccttgcaaccggcgccctcaagctggtgcttgggtccaagcc  
 tactttgtgcagcttctcacaacgcaaacccatcgcttctgtaaAagggtgggcgcgcccagccagcttcttgtaaaagtgggtgatc  
 gcgcgcgcgcgagctccgtgcgaaagcctgacgcaccggtagattcttggtgagcccgtatcatgacggcgggcgaggctacatg  
 gccccgggtgatttattttttgtatctactctgaccttttcaaatatacgggtcaactcatcttactggagatgcggcctgcttggattg  
 cgatgttgtagcttggcaaatgtggcttctgaaaacacaaaacgattccttagtagccatgcattttaagataacggaatagaagaaag  
 30 aggaaattaaaaaaaaaaaaaaaaaaaaacacatcccggttcataaccgtagaatcgccgctcttcgtgtatcccagtagcagtttatttga  
 tagctcgcccgctggagagcactctgaatgcaagtaacaaccgtagaggctgacacggcaggtgttgctaggagcgtcgtgtteta  
 caaggccagacgtcttcgcggttgatatatatgtatgtttgactgcaggctgctcagcgacgacagtcgaagttcgccctcgtgcttgtg  
 caataatcgagtggggaagccacaccgtgactcccatcttccagtaaagctctgttgggtgttatcagcaatacacgtaatttaaacctgt  
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accagctaggcaccagctaaaccctataattagctcttattcaacaccatccgctccccgggatcaatgaggagaatgagggggatg  
 cggggctaaagaagcctacataaccctcatgccaaactcccagtttacactcgtcgagccaacatcctgactataagctaacacagaatg  
 cctcaatcctgggaagaactggccgctgataagcgcgcccgcctcgcaaaaaccatccctgatgaatggaaagtccagacgctgcct  
 gcggaagacagcggtattgatttcccaaagaaatcggggatcctttcagaggccgaactgaagatcacagaggcctccgctgcagatc  
 5 ttgtgtccaagctggcggccggagagttgacctcgggtggaagttacgctagcattctgtaaacgggcagcaatcgcccagcagttagt  
 aggggtcccctctacctctcaggagatgtaacaacgccaccttatgggactatcaagctgacgctggcttctgtgcagacaaactgcgc  
 ccacgagttcttccctgacgccgctctcgcgcaggcaagggaactcgatgaatactacgcaaagcacaagagacccgttggtccact  
 ccatggcctccccatctctctcaaagaccagcttcgagtcaaggtacaccgttgcccctaagtcgttagatgtccctttttgtcagctaaca  
 tatgccaccagggtctacgaaacatcaatgggctacatctcatggctaaacaagtacgacgaaggggactcggttctgacaacctatgct  
 10 ccgcaaagccggtgccgttctctacgtcaagacctctgtcccgcagaccctgatggctcgcgagacagtcacaacatcatcggggcg  
 caccgtcaaccacgcaacaagaactggctcgtgcggcggcagttctgggtggtgagggtgcgacggttgggattcgtggtggcgtcat  
 cggtgtaggaacggatcgggtggctcgattcgagtgcgggccggttcaacttctgtacgggtctaaggccgagtcagggcggtcgt  
 ccgtatgcaaagatggcgaacagcatggagggtcaggagacgggtgcacagcggttgcgggccgattacgcactctgttgagggtga  
 gtccttcgctcttcttcttcttctgctctataaccaggcctccactgtcctccttcttcttcttatactatatacagagaccggcagtcactga  
 15 tgaagtatgttagacctccgcctcttcaccaaaccgctcctcggtcaggagccatggaaatacgactccaaggtcatccccatgccttg  
 gcgccagtccgagtcggacattattgcctccaagatcaagaacggcgggctcaatatcggtactacaacttcgacggcaatgtccttc  
 cacacctcctatcctgcgcggcggtggaaaccaccgtcgccgcactcgccaaagccggtcacacc.

[255] Protoplasts of the AD5 *H. jecorina* strain ( $\Delta egl1$ ,  $\Delta egl2$ ,  $\Delta cbh1$ ,  $\Delta cbh2$ ,  $\Delta bgl1$ )  
 20 described in Example 6 were transformed with the linear DNA library as described (US  
 2006/0094080) and grown on selective agar containing acetamide at 28°C for 7 days (0.6 g/L  
 acetamide, 1.68 g/L CsCl, 20 g/L glucose, 6 g/L KH<sub>2</sub>PO<sub>4</sub>, 0.6 g/L MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.6 g/L  
 CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.5 g/L uridine, trace element salts, 10 g/L low melting point agarose). After  
 24 hours the agar was overlaid with selective agar supplemented with 1.2 g/L fluoroorotic  
 25 acid (FOA). A total of 380 colonies were transferred to potato dextrose agar plates  
 containing 1.2 g/L FOA and incubated at 28°C for 4-5 days. Spores were transferred to fresh  
 potato dextrose agar plates, which were incubated at 28°C for 3 days. Alternatively,  
 protoplasts of the MAD6 strain described in Example 6 can be employed instead of AD5 for  
 expression of variant library members. Likewise, protoplasts of derivatives of the MAD6  
 30 strain in which additional cellulases have been inactivated can be used for this purpose. Such  
 derivatives would exhibit even less background cellulase activity.

[256] For CBH2 variant protein production, spores were transferred using a 96-pin  
 replicator to 200  $\mu$ l glycine minimal medium supplemented with 2% glucose/sophorose

mixture in a PVDF filter plate: 6.0 g/L glycine, 4.7 g/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; 5.0 g/L KH<sub>2</sub>PO<sub>4</sub>; 1.0 g/L MgSO<sub>4</sub>·7H<sub>2</sub>O; 33.0 g/L PIPPS; pH 5.5; with sterile addition of a 2% glucose/sophorose mixture as the carbon source, 10 ml/L of 100g/L of CaCl<sub>2</sub>, 2.5 ml/L of *T. reesei* trace elements (400X): 175g/L Citric acid anhydrous; 200g/L FeSO<sub>4</sub>·7H<sub>2</sub>O; 16g/L ZnSO<sub>4</sub>·7H<sub>2</sub>O; 3.2 g/L CuSO<sub>4</sub>·5H<sub>2</sub>O; 1.4 g/L MnSO<sub>4</sub>·H<sub>2</sub>O; 0.8 g/L H<sub>3</sub>BO<sub>3</sub>. Each CBH2 variant was grown in quadruplicate. After sealing the plate with an oxygen permeable membrane, the plates were incubated at 28°C for 6 days, while shaking at 200 rpm. Supernatant was harvested by transferring the culture medium to a microtiter plate under low pressure.

[257] A total of ten variants that showed improved activity on corn cob at 57°C were isolated. Genomic DNA of these strains was isolated and their *cbh2* gene sequences determined. The CBH2 variants were tested for properties of interest. The substitutions and activities of combinatorial library members on corncob and corn stover is shown in Table 8-2. The specific activities for washed dilute acid pretreated cornstover (PCS 50°C), for corncob at 50°C (CC 50°C), and for corncob at 57°C (CC 57°C) were determined as described in Example 7.

| <b>Table 8-2: CBH2 Combinatorial Variants with Improved Activity on Corncob at 57°C.</b> |                |                |                 |
|------------------------------------------------------------------------------------------|----------------|----------------|-----------------|
| <b>Variant</b>                                                                           | <b>CC 57°C</b> | <b>CC 50°C</b> | <b>PCS 50°C</b> |
| CBH2.S291E/Q362I                                                                         | 1.2            | 0.97           | 1.13            |
| CBH2.P98Q/S316P/Q362L/L439P                                                              | 1.52           | 0.97           | 0.1             |
| CBH2.P98Q/N182W/S291E/S316P/C400S                                                        | 1.26           | 0.97           | 0.75            |
| CBH2.P98Q/N182W/S291E/S316P/C400S                                                        | 1.24           | 0.97           | 1.02            |
| CBH2.P98L/N182W/S291E/Q362I/C400S                                                        | 1.26           | 0.97           | 1.05            |
| CBH2.P98L/N182W/S291E/Q362I/C400S                                                        | 1.28           | 0.97           | 1.14            |
| CBH2.T74S/P98L/N182W/S291E                                                               | 1.31           | 0.97           | 1.13            |
| CBH2.P98L/N182W/S291E/S316P/Q362I                                                        | 1.26           | 0.97           | 1.22            |
| CBH2.N182W/S291E/Q362L/C400S                                                             | 1.3            | 0.97           | 1.08            |
| CBH2.S291E/Q362L                                                                         | 1.29           | 0.97           | 1.18            |

## EXAMPLE 9

### CBH2 Singly and Doubly Substituted Variants and Activities Thereof

[258] Synthetic genes encoding CBH2 variants were constructed by GeneOracle (Mountain View, CA) in pTTTpyrG. Table 9-1 lists the single and double variants that were constructed (numbered according to the CBH2 mature amino acid sequence).

| Table 9-1: Singly and Doubly Substituted CBH2 Variants |                 |     |                 |
|--------------------------------------------------------|-----------------|-----|-----------------|
| #                                                      | Substitution(s) | #   | Substitution(s) |
| 1.                                                     | P98Q            | 19. | T138C/S386C     |
| 2.                                                     | T138C           | 20. | T138C/C400S     |
| 3.                                                     | S316P           | 21. | T138C/S406P     |
| 4.                                                     | S343Q           | 22. | S316P/S343Q     |
| 5.                                                     | Q362I           | 23. | S316P/Q362I     |
| 6.                                                     | S386C           | 24. | S316P/S386C     |
| 7.                                                     | C400S           | 25. | S316P/C400S     |
| 8.                                                     | S406P           | 26. | S316P/S406P     |
| 9.                                                     | P98Q/T138C      | 27. | S343Q/Q362I     |
| 10.                                                    | P98Q/S316P      | 28. | S343Q/S386C     |
| 11.                                                    | P98Q/S343Q      | 29. | S343Q/C400S     |
| 12.                                                    | P98Q/Q362I      | 30. | S343Q/S406P     |
| 13.                                                    | P98Q/S386C      | 31. | Q362I/S386C     |
| 14.                                                    | P98Q/C400S      | 32. | Q362I/C400S     |
| 15.                                                    | P98Q/S406P      | 33. | Q362I/S406P     |
| 16.                                                    | T138C/S316P     | 34. | S386C/C400S     |
| 17.                                                    | T138C/S343Q     | 35. | S386C/S406P     |
| 18.                                                    | T138C/Q362I     | 36. | C400S/S406P     |

5

[259] The *cbh2* variant genes were received from the above-mentioned provider as purified PCR products in which primers GACCGGACGTGTTTTGCCCTTCAT (SEQ ID NO:20) and GTGTGACCGGCTTTGGCGAGTG (SEQ ID NO:21) were used to amplify the *cbh2* gene flanked upstream by about 1000 bp of the *cbh1* promoter and downstream by about 1000 bp of the *amdS* marker for forced integration in the *pyr2* locus in the *H. jecorina* host strain. The nucleotide sequence of a PCR fragment (partial *cbh1* promoter, *cbh2* gene, and partial *amdS* gene) amplified from pTTTpyrG-CBH2 using the primers of SEQ ID NO:20 and SEQ ID NO:21, is provided above in Example 8 as SEQ ID NO:2q22.

10

[260] Protoplasts of the MAD6 *H. jecorina* strain ( $\Delta egl1$ ,  $\Delta egl2$ ,  $\Delta egl3$ ,  $\Delta cbh1$ ,  $\Delta cbh2$ ,  $\Delta bgl1$ ) described in Example 6, were transformed with the linear DNA fragments as described (US 2006/0094080) and grown on selective agar containing acetamide at 28°C for 7 days as described. After 24 hours the agar was overlain with selective agar supplemented  
5 with 1.2 g/L fluoro-orotic acid (FOA). Colonies were transferred to potato dextrose agar plates containing 1.2 g/L FOA and incubated at 28°C for 4-5 days. Spores were transferred to potato dextrose agar plates, which were incubated at 28°C for 3 days.

[261] The variants were grown in microtiterplates as described in Example 8. For growth in shake flasks, 25 ml of YEG culture (5 g/L yeast extract, 20 g/L glucose) was inoculated with  
10 a sporulating *Trichoderma* strain on agar and incubated at 28°C at 200 rpm for 2 days. Next, 5 ml of the YEG culture was used to inoculate 45 ml of glycine medium containing a 2% glucose sophorose mixture in a shake flask. Following inoculation, the cultures were incubated in a resonant acoustic incubator (Applikon) at 28°C for 3 days. Cells were removed by filtration through a 0.2  $\mu$ m filter. Supernatants were concentrated using Vivaspin  
15 spin cells with a 10 kD molecular weight cutoff.

[262] Samples grown in microtiter plate were analyzed for CBH2 production levels by HPLC as described in Example 1. Both microtiter plate and shake flask grown samples were tested for properties of interest. Table 9-2 lists the performance of the CBH2 variants for multiple properties of interest.

20

| Table 9-2: Performance of Singly and Doubly Substituted CBH2 Variants. |      |      |      |      |      |                      |                 |
|------------------------------------------------------------------------|------|------|------|------|------|----------------------|-----------------|
| Variant                                                                | HPLC | PASC | PCS  | CC50 | CC57 | Tm <sup>1</sup> (°C) | IR <sup>2</sup> |
| 1                                                                      | 0.53 | 1.04 | 1.03 | 0.98 | 1.04 | 57.4                 | 1.11            |
| 2                                                                      | 0.76 | 1.00 | 1.06 | 1.03 | 1.10 | 50.1                 | 1.06            |
| 3                                                                      | 1.01 | 0.98 | 1.04 | 0.99 | 1.00 | 55.2                 | 1.40            |
| 4                                                                      | 0.98 | 0.91 | 0.99 | 0.94 | 0.94 | 56.9                 | 1.05            |
| 5                                                                      | 0.97 | 0.93 | 1.02 | 1.03 | 1.08 | 56.3                 | 1.20            |
| 6                                                                      | 0.48 | 1.01 | 1.01 | 1.02 | 1.05 | 54.2                 | 0.89            |
| 7                                                                      | 1.07 | 0.94 | 0.96 | 0.95 | 0.97 | 64.9                 | 2.29            |
| 8                                                                      | 1.02 | 0.95 | 1.05 | 0.99 | 1.07 | 58.6                 | 1.22            |
| 9                                                                      | 0.53 | 1.01 | 1.03 | 1.00 | 1.03 | 59.3                 | 1.19            |
| 10                                                                     | 0.63 | 0.94 | 0.97 | 0.96 | 1.06 | 59.5                 | 1.50            |

| <b>Table 9-2: Performance of Singly and Doubly Substituted CBH2 Variants.</b> |      |      |      |      |      |      |      |
|-------------------------------------------------------------------------------|------|------|------|------|------|------|------|
| 11                                                                            | 0.56 | 0.98 | 0.94 | 1.05 | 0.95 | 57.6 | 1.12 |
| 12                                                                            | 0.50 | 0.99 | 1.01 | 1.15 | 1.07 | 58.7 | 1.39 |
| 13                                                                            | 0.19 | 1.19 | 0.87 | 1.02 | 1.12 | 50.3 | 1.21 |
| 14                                                                            | 0.96 | 0.97 | 1.01 | 0.97 | 1.11 | 59.3 | 2.39 |
| 15                                                                            | 0.66 | 0.97 | 0.97 | 1.00 | 1.10 | 57.0 | 1.40 |
| 16                                                                            | 0.84 | 0.99 | 1.03 | 1.03 | 1.15 | 59.4 | 1.60 |
| 17                                                                            | 0.78 | 1.00 | 1.00 | 1.04 | 1.04 | 52.0 | 1.45 |
| 18                                                                            | 0.72 | 1.02 | 1.00 | 0.98 | 1.03 | 57.6 | 1.28 |
| 19                                                                            | 0.40 | 1.02 | 1.05 | 1.01 | 1.08 | 56.6 | 1.08 |
| 20                                                                            | 0.94 | 0.96 | 1.02 | 0.92 | 1.10 | 59.0 | 2.30 |
| 21                                                                            | 0.83 | 0.94 | 0.99 | 0.96 | 1.04 | 54.4 | 1.37 |
| 22                                                                            | 1.04 | 0.88 | 0.96 | 0.92 | 0.99 | 47.3 | 1.26 |
| 23                                                                            | 1.00 | 0.91 | 0.96 | 1.00 | 0.97 | 60.4 | 1.48 |
| 24                                                                            | 0.55 | 1.02 | 1.04 | 1.07 | 1.04 | 58.7 | 1.30 |
| 25                                                                            | 1.15 | 0.96 | 0.94 | 0.87 | 0.93 | 64.6 | 3.19 |
| 26                                                                            | 0.89 | 0.94 | 0.98 | 0.98 | 0.99 | 59.2 | 1.67 |
| 27                                                                            | 0.95 | 0.89 | 0.93 | 0.95 | 0.96 | 55.8 | 1.33 |
| 28                                                                            | 0.47 | 0.98 | 0.98 | 1.00 | 1.12 | 56.9 | 1.27 |
| 29                                                                            | 1.12 | 0.93 | 1.02 | 0.93 | 1.01 | 62.3 | 2.57 |
| 30                                                                            | 1.00 | 0.96 | 0.91 | 0.95 | 0.98 | 60.6 | 1.23 |
| 31                                                                            | 0.47 | 1.03 | 1.00 | 1.00 | 1.07 | 60.1 | 1.37 |
| 32                                                                            | 1.06 | 0.92 | 0.89 | 0.96 | 0.98 | 65.9 | 2.90 |
| 33                                                                            | 0.99 | 0.88 | 0.96 | 0.97 | 0.95 | 60.1 | 1.60 |
| 34                                                                            | 0.88 | 0.88 | 0.94 | 0.98 | 1.08 | 58.1 | 2.41 |
| 35                                                                            | 0.55 | 0.98 | 1.08 | 1.03 | 1.10 | 59.2 | 1.24 |
| 36                                                                            | 1.09 | 0.89 | 0.96 | 1.00 | 0.99 | 65.5 | 3.19 |
| Wt                                                                            | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 57.6 | 1.00 |

1 T<sub>m</sub> = pseudo melting point temperature (°C) was determined by thermostability assay 2.

2 IR = Performance index for stability as determined by thermostability assay 3.

[263] *CBH2 Thermostability Assay 3.* To analyze the thermostability of the CBH2 variants, 40  $\mu$ l of supernatant was incubated in a PCR machine 58°C in triplicate and samples were removed after 30, 60 and 120 min of incubation. The remaining activity was determined by incubating 10  $\mu$ l supernatant with 50  $\mu$ l of 1 mg/ml cellotriose in 50 mM sodium acetate buffer pH 5.0 with 0.0025% Tween20. After one hour, 40  $\mu$ l of 100 mM glycine pH 10 was added. Fifty  $\mu$ l of 3X concentrated ABTS reagent (see above) was added and color development at OD420 was recorded for 3 min. The residual activity in time was fitted with a formula for exponential decay:  $y = A_0 \cdot \exp(-k \cdot t)$ , where  $A_0$  is activity at  $t=0$ ,  $t$  is time, and  $k$  is the decay constant. Performance indici (PI) were calculated by the following formula:

10  $PI = -\log(k_{\text{variant}}) / -\log(k_{\text{wt}})$ .

## CLAIMS

We claim:

1. A cellulase variant, wherein the variant is a mature form having cellulase  
5 activity and comprising a substitution at one or more positions selected from the group  
consisting of: 5, 18, 19, 28, 30, 32, 35, 38, 79, 80, 89, 100, 102, 103, 104, 105, 111, 117,  
119, 121, 125, 126, 133, 137, 138, 139, 140, 141, 143, 150, 158, 162, 177, 180, 181, 182,  
185, 186, 188, 190, 191, 192, 193, 196, 201, 207, 225, 226, 228, 229, 230, 233, 234, 236,  
240, 243, 245, 251, 252, 258, 267, 268, 274, 292, 293, 303, 304, 306, 307, 313, 319, 322,  
10 328, 331, 338, 340, 346, 361, 362, 363, 364, 365, 371, 384, 394, 396, 400, 406, 407, 414,  
417, 422, 427, 431, 433, 436, 440, 441, 443, 444, 445 and 447, wherein the positions are  
numbered by correspondence with the amino acid sequence of a reference cellobiohydrolase  
II (CBH2) set forth as SEQ ID NO:3.
2. The cellulase variant of Claim 1, wherein the variant comprises a further  
15 substitution at one or more further positions selected from:
  - (i) a first group consisting of 63, 77, 129, 146, 147, 151, 153, 157, 161, 189, 194, 197,  
203, 204, 208, 211, 237, 239, 244, 247, 254, 277, 281, 285, 288, 289, 294, 327, 339, 344,  
356, 378, 382 and 405; or
  - (ii) a second group consisting of 94, 98, 107, 120, 134, 144, 154, 178, 179, 206, 210,  
20 214, 231, 232, 266, 272, 275, 291, 312, 316, 323, 343, 360, 380, 381, 386, 399, 410, 413,  
416, 426, and 429,wherein the further positions are numbered by correspondence with the amino acid  
sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3.
3. A cellulase variant, wherein said variant is a mature form having cellulase  
25 activity and comprising a substitution at one or more positions selected from the group  
consisting of: 63, 77, 129, 146, 147, 151, 153, 157, 161, 189, 194, 197, 203, 204, 208, 211,  
237, 239, 244, 247, 254, 277, 281, 285, 288, 289, 294, 327, 339, 344, 356, 378, 382 and 405,  
wherein the positions are numbered by correspondence with the amino acid sequence of a  
reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3, and wherein when the

cellulase variant consists of a single substitution, the single substitution is not a K/E, R/Q, N/D, Q/E, D/N or E/Q substitution.

4. The cellulase variant of Claim 3, wherein the variant comprises a further substitution at one or more further positions selected from:

5 (i) a first group consisting of 5, 18, 19, 28, 30, 32, 35, 38, 79, 80, 89, 100, 102, 103, 104, 105, 111, 117, 119, 121, 125, 126, 133, 137, 138, 139, 140, 141, 143, 150, 158, 162, 177, 180, 181, 182, 185, 186, 188, 190, 191, 192, 193, 196, 201, 207, 225, 226, 228, 229, 230, 233, 234, 236, 240, 243, 245, 251, 252, 258, 267, 268, 274, 292, 293, 303, 304, 306, 307, 313, 319, 322, 328, 331, 338, 340, 346, 361, 362, 363, 364, 365, 371, 384, 394, 396,  
10 400, 406, 407, 414, 417, 422, 427, 431, 433, 436, 440, 441, 443, 444, 445 and 447; or

(ii) a second group consisting of 94, 98, 107, 120, 134, 144, 154, 178, 179, 206, 210, 214, 231, 232, 266, 272, 275, 291, 312, 316, 323, 343, 360, 380, 381, 386, 399, 410, 413, 416, 426, and 429,

wherein the further positions are numbered by correspondence with the amino acid  
15 sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3.

5. A cellulase variant, wherein the variant is a mature form having cellulase activity and comprising a substitution at one or more positions selected from the group consisting of: 94, 98, 107, 120, 134, 144, 154, 178, 179, 206, 210, 214, 231, 232, 266, 272, 275, 291, 312, 316, 323, 343, 360, 380, 381, 386, 399, 410, 413, 416, 426, and 429, wherein  
20 the positions are numbered by correspondence with the amino acid sequence of a reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3, and wherein when the cellulase variant consists of a single substitution, the single substitution is not one of the group consisting of V94E, P98L, E107Q, M120L, M134G, M134L, M134V, L144G, L144R, L144S, T154A, A178V, L179C, V206L, S210L, S210R, T214M, T214Y, G231P, G231I,  
25 G231A, G231N, G231S, G231T, T232V, H266S, H266A, W272A, W272D, W272Y, N275L, S316P, V323L, V323N, V323Y, G360R, S380T, A381T, E399N, E399D, R413Y, R413P, and A416G.

6. The cellulase variant of Claim 5, wherein the variant comprises a further substitution at one or more further positions selected from:

(i) a first group consisting of 5, 18, 19, 28, 30, 32, 35, 38, 79, 80, 89, 100, 102, 103, 104, 105, 111, 117, 119, 121, 125, 126, 133, 137, 138, 139, 140, 141, 143, 150, 158, 162, 177, 180, 181, 182, 185, 186, 188, 190, 191, 192, 193, 196, 201, 207, 225, 226, 228, 229, 230, 233, 234, 236, 240, 243, 245, 251, 252, 258, 267, 268, 274, 292, 293, 303, 304, 306, 307, 313, 319, 322, 328, 331, 338, 340, 346, 361, 362, 363, 364, 365, 371, 384, 394, 396, 400, 406, 407, 414, 417, 422, 427, 431, 433, 436, 440, 441, 443, 444, 445 and 447; or

(ii) a second group consisting of 63, 77, 129, 146, 147, 151, 153, 157, 161, 189, 194, 197, 203, 204, 208, 211, 237, 239, 244, 247, 254, 277, 281, 285, 288, 289, 294, 327, 339, 344, 356, 378, 382 and 405,

wherein the further positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3.

7. The cellulase variant of any one of Claims 1-6, wherein the substitution at one or more positions is selected from the group consisting of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 and 25 positions.

8. A cellulase variant, wherein the variant is a mature form having cellulase activity and comprising a substitution at from one to six positions selected from the group consisting of 111, 144, 154, 162, 410 and 413, wherein the cellulase variant comprises:

leucine or serine at position 111;

leucine, glutamine or tryptophan at position 144;

threonine, cysteine or valine at position 154;

tyrosine or asparagine at position 162;

arginine or serine at position 410; and

serine, tryptophan or tyrosine at position 413;

wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3, and wherein when the cellulase variant consists of a single substitution, the single substitution is not a S413Y substitution.

9. A cellulase variant, wherein the variant is a mature form having cellulase activity and comprising a substitution at from one to six positions selected from the group consisting of 98, 194, 313, 316, 384 and 443, wherein the cellulase variant comprises:

proline or leucine at position 98;

lysine, cysteine or glutamic acid at position 194;

serine or threonine at position 313;

serine or proline at position 316;

glycine, cysteine or glutamine at position 384;

5 and asparagine or isoleucine at position 443;

wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3, and wherein when the cellulase variant consists of a single substitution, the single substitution is not one of the group selected from a P98L substitution, a K194E substitution, and a S316P substitution.

10 10. A cellulase variant, wherein the variant is a mature form having cellulase activity and comprising a substitution at from one to six positions selected from the group consisting of 153, 161, 203, 233, 422 and 444, wherein the cellulase variant comprises:

arginine or glutamine at position 153;

asparagine, alanine or tryptophan at position 161;

15 arginine or histidine at position 203;

proline or aspartic acid at position 233;

glutamine or valine at position 422; and

proline or glutamine at position 443;

wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3, and wherein when the cellulase variant consists of a single substitution, the single substitution is not a R153Q substitution.

11. A cellulase variant, wherein the variant is a mature form having cellulase activity and comprising a substitution at from one to seven positions selected from the group consisting of 98, 111, 144, 313, 316, 413 and 422, wherein the cellulase variant comprises:

25 proline or leucine at position 98;

leucine or serine at position 111;

leucine or tryptophan at position 144;

serine or threonine at position 313;

30 serine or proline at position 316;

serine or tryptophan at position 413; and

glutamine or valine at position 422;

wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3, and wherein when the cellulase variant consists of a single substitution, the single substitution is not one of the group selected from a P98L substitution, and a S316P substitution.

5           12.     A cellulase variant comprising a glutamine at position 98, and a substitution selected from the group consisting of a T138C, a S316P, a S343Q, a Q362I, a S386C, a C400S and a S406P, wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3.

10           13.     A cellulase variant comprising a cysteine at position 138, and a substitution selected from the group consisting of a S316P, a S343Q, a Q362I, a S386C, a C400S and a S406P, wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3.

15           14.     A cellulase variant comprising a proline at position 316, and a substitution selected from the group consisting of a S343Q, a Q362I, a S386C, a C400S and a S406P, wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3.

20           15.     A cellulase variant comprising a glutamine at position 343, and a substitution selected from the group consisting of a Q362I, a S386C, a C400S and a S406P, wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3.

          16.     A cellulase variant comprising an isoleucine at position 362, and a substitution selected from the group consisting of a S386C, a C400S and a S406P, wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3.

25           17.     A cellulase variant comprising a cysteine at position 386, and a substitution selected from the group consisting of a C400S and a S406P, wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3.

18. The cellulase variant of Claim 1, comprising a serine at position 400 and a proline at position 406.

19. A cellulase variant, wherein the variant is a mature form having cellulase activity and comprising a substitution at from one to seven positions selected from the group

5 consisting of 98, 111, 182, 291, 316, 362 and 400, wherein the cellulase variant comprises:

proline, leucine or glutamine at position 98;

leucine or serine at position 111;

asparagine or tryptophan at position 182;

serine or a glutamic acid at position 291;

10 serine or proline at position 316;

glutamine, isoleucine or leucine at position 362; and

cysteine or serine at position 400;

wherein the positions are numbered by correspondence with the amino acid sequence of the reference cellobiohydrolase II (CBH2) set forth as SEQ ID NO:3, and wherein when

15 the cellulase variant consists of a single substitution, the single substitution is not one of the group selected from a P98L substitution, and a S316P substitution.

20. The cellulase variant of Claim 19, wherein the variant further comprises a L439P substitution or a T74S substitution.

21. The cellulase variant of any one of Claims 1-20, wherein the variant is derived

20 from a parent cellulase selected from the group consisting of *Hypocrea jecorina* CBH2, *Hypocrea koningii* CBH2, *Humicola insolens* CBH2, *Acremonium cellulolyticus* CBH2, *Agaricus bisporus* CBH2, *Fusarium osysporum* EG, *Phanerochaete chrysosporium* CBH2, *Talaromyces emersonii* CBH2, *Thermobifida. fusca* 6B/E3 CBH2, *Thermobifida fusca* 6A/E2 EG, and *Cellulomonas fimi* CenA EG.

22. The cellulase variant of any one of Claims 1-21, wherein the variant is derived from a parent cellulase whose amino acid sequence is at least 75% 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a member of the group consisting of SEQ ID NO:3, SEQ ID NO:4, 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, and SEQ ID NO:13.

23. The cellulase variant of any one of Claims 1-22, wherein the variant is isolated.

24. A composition comprising the cellulase variant of any one of Claims 1 to 23.

25. The composition of Claim 24, wherein the composition is enriched in the cellulase variant.

26. An isolated nucleic acid encoding the cellulase variant of any one of Claims 1  
5 to 22.

27. An expression vector comprising the isolated nucleic acid of Claim 26 operably linked to a regulatory sequence.

28. A host cell comprising the expression vector of Claim 27.

29. A method for producing a cellulase variant, comprising culturing the host cell  
10 of Claim 28 in a culture medium, under suitable conditions to produce the cellulase variant.

30. A composition comprising the host cell of Claim 29 and culture medium.

31. A composition comprising the cellulase variant in supernatant of the culture medium of Claim 29.

32. The composition of Claim 24, further comprising at least one additional  
15 enzyme selected from the group consisting of a subtilisin, a neutral metalloprotease, a lipase, a cutinase, an amylase, a carbohydrase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, an oxidase, and a peroxidase.

33. A method of cleaning or fabric care comprising contacting a surface or an article comprising a fabric with the composition of Claim 24 or 32.

20 34. A method for depilling and surface finishing a fabric comprising contacting a surface or an article comprising a fabric with the composition of Claims 24 or 32.

35. A method for converting biomass to sugars comprising contacting the biomass with the composition of Claims 24 or 32.

25 36. A method for producing a fuel comprising: contacting a biomass composition with an enzymatic composition comprising the cellulase variant of any one of Claims 1 to 22 to yield a sugar solution; and culturing the sugar solution with a fermentative microorganism under conditions sufficient to produce a fuel.

## FIG. 1

```

T. reesei CBH #3      -----QACSSVWGQCGGQN----W
H. koningii CBH #4    -----QACSSVWGQCGGQN----W
H. insolens CBH #5    -----APVVEERQNCAPTGWQCGGIG----F
A. cellulolyticus CBH #6 -----MLRYLSIVAATAILTGVEAQQSVWGQCGGQG----W
A. bisporus CBH #7     -----QSPV-----WGQCGGNG----W
F. oxysporum EG #8     -----APVEERQSCSNGVWAQCGGQN----W
P. chrysosporium CBH #9 -----MKSTAFFAALVTLLPAYVAGQASE--WGQCGGIG----W
T. emersonii CBH #10   -----MRNLLALAPAALLVGAAEAQQLWGQCGGSS----W
T. fusc CBH #11        -AGCSVDTYTVN-SWGTGFTANVTITNLGSAINGWTLEWDFPGNQQVTNLW
T. fusc EG #12         -----NDSPFYVNPNMSSAEWVRNPNND-----
C. fimi EG #13         APGCRVDYAVTNQWPGGFGANVTITNLGDPVSSWKLDWTTYTAGQRIQQLW

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T. reesei CBH #3      SGPTCCASGSTCVYSNDYYSQCLP--GA---ASSSSSTRAAST----TSR
H. koningii CBH #4    SGPTCCASGSTCVYSNDYYSQCLP--GA-----ASSSSSTRASSTTA
H. insolens CBH #5    NGPTCCQSGSTCVKQNDWYSQCLP--GSQVTTTSTTSTSSSSST----TSR
A. cellulolyticus CBH #6 SGATSCAAGSTCSTLNPYYAQCI--GT-----ATSTTLV----KTT
A. bisporus CBH #7     TGPTTCASGSTCVKQNDFYQCLP--NN-----Q
F. oxysporum EG #8     SGTPCCTSGNKCCKVKNDFYSQCQP--GS-----AEPS----STA
P. chrysosporium CBH #9 TGPTTCVSGTTCTVLNPYYQCLP--GS-AVTTTSTVITSHSSS----VSS
T. emersonii CBH #10   TGATSCAAGATCSTINPYYAQCV--AT-----ATPTTLT----TTT
T. fusc CBH #11        NGTYTQSGQHVSVSNAFYNASI-PANGTVEFGFNGSYSGSNDIPSSFKNL
T. fusc EG #12         PRTPVIRDRIASVPQGTWFAHNP--GQ-----ITGQVDA----LMS
C. fimi EG #13         NGTASTNGGQVSVTSLPWNGSI-PTGGTASFGFNGSWAGSNPTPASFSLN

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T. reesei CBH #3      VSPTTSR---SSSATPPPGSTTTRVPP-VGSGTATYSGNPFVGVTPWANA
H. koningii CBH #4    RASSTTS---RSSATPPPGSSTTRVPP-VGSGTATYSGNPFVGVTPWANA
H. insolens CBH #5    ATSTTRT---GGVTSITTAPTRTVTIPGGATTTASYNGNPFEGVQLWANN
A. cellulolyticus CBH #6 SSTS VGT-----TSPPTTTTTKAST-TATTTAAASGNPFSGYQLYANP
A. bisporus CBH #7     APPSTTT---QPGTTPPATTTSGGTGP-TSGA-----GNPYTGKT VWLSP
F. oxysporum EG #8     AGPSSTT---ATKTTATGGSSTTAGGS-VTSAPPAASDNPFYAGVDLWANN
P. chrysosporium CBH #9 VSSHSGS---STSTSSPTGPTGTNPPP-PPSA-----NNPWTGFQIFLSP
T. emersonii CBH #10   KPTSTGG---AAPTTPPPTTTGTTTSP-VVTRPASASGNPFEGYQLYANP
T. fusc CBH #11        GVTCDGSDDPDPEPSPSPSPSPPTDPEPGGPTNPPTNPGEKVD---NP
T. fusc EG #12         AAQAAGK---IPILVVYNAPGRDCGNH-SSGGAPSHS-----AYRSWIDE
C. fimi EG #13         GTTCTGT---VPTTSPTPTPTPTPTPT-TPTPTPTPT--PTVTPQPTSGF

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T. reesei CBH #3      YYASEVSSLAIPSLTG-AMATAAAVAVKVPSEFMWLDL-----DKTP-LM
H. koningii CBH #4    YYASEVSSLAIPSLTG-AMATAAAVAVKVPSSMWLDTF-----DKTP-LM
H. insolens CBH #5    YYRSEVHTLAIPQITDPALRAAASAVAEPVSFQWLDRN-----VTVDLTL
A. cellulolyticus CBH #6 YYSSEVHTLAIPSLTG-SLAAAATKAAEIPSFVWLDTA-----AKVP-TM
A. bisporus CBH #7     FYADEVAQ-AAADISNPSLATKAASVAKIPTFVWFDTV-----AKVP-DL
F. oxysporum EG #8     YYRSEVMNLAVPKLSG-AKATAAAKVADVPSFQWMDTY-----DHIS-LM
P. chrysosporium CBH #9 YYANEVAA-AAQITDPTLSSKAASVANIPTFTWLDSV-----AKIP-DL
T. emersonii CBH #10   YYASEVISLAIPSLSS-ELVPKASEVAVKVPSEFVWLDQA-----AKVP-SM
T. fusc CBH #11        FEGAKLYVNPVWSAKA-AAEPGGSANAVANESTAVWLDRIGAIEGNDSPPTTG
T. fusc EG #12         F-AAGLKNRPAYIIVEPDLISLMSSCMQHVQQEVLETM-----AYAGKAL
C. fimi EG #13         YVDPTTQGYRAWQAASGTDKALLEKIALTPQAYWVGW-----ADAS---

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## Figure 1 continued

|                          |                                                     |
|--------------------------|-----------------------------------------------------|
| T. reesei CBH #3         | EQTLAD-IRTANKNGGN--YAGQFVVYDLPDRDCAALASNGEYSIADGGV  |
| H. koningii CBH #4       | EQTLAD-IRTANKNGGN--YAGQFVVYDLPDRDCAALASNGEYSIADGGV  |
| H. insolens CBH #5       | VETLSE-IRAANQAGANPPYAAQIVVYDLPDRDCAAAASNGEWAIANNGA  |
| A. cellulolyticus CBH #6 | GTYLAN-IEAANKAGASPPIAGIFVVYDLPDRDCAAAASNGEYTVANNGV  |
| A. bisporus CBH #7       | GGYLAD-ARSKNQ-----LVQIVVYDLPDRDCAALASNGEFSLANDGL    |
| F. oxysporum EG #8       | EDTLAD-IRKANKAGGK--YAGQFVVYDLPNRDCAAAASNGEYSLDKDGA  |
| P. chrysosporium CBH #9  | GTYLAS--ASALGKSTGTKQLVQIVVIYDLPDRDCAAKASNGEFSIANNGQ |
| T. emersonii CBH #10     | GDYLDK-IQSQNAAGADPPIAGIFVVYDLPDRDCAAAASNGEFSIANNGV  |
| T. fusc CBH #11          | SMGLRDHLEEAVRQSGGDPLTIQVVIYNLPGRDCAALASNGELGP--DEL  |
| T. fusc EG #12           | KAGSSQ-ARIYFDAGHSAWHSPAQMASWL---QQADISNSAHGIA-TNT   |
| C. fimi EG #13           | -HAQAE-VADYTGRAVAAGKTPMLVVYAIPGRDCGSHSGGG-----VSE   |
|                          | : : : ...                                           |

|                          |                                                     |
|--------------------------|-----------------------------------------------------|
| T. reesei CBH #3         | AKYKN-YIDTIRQIVVEYSD---IRTLVIEPDSLNLVTNLGTPK----    |
| H. koningii CBH #4       | DKYKN-YIDTIRQIVVEYSD---IRTLVIEPDSLNLVTNLGTPK----    |
| H. insolens CBH #5       | NNYKG-YINRIREILISFSD---VRTILVIEPDSLNLMTNMNVAK----   |
| A. cellulolyticus CBH #6 | ANYKA-YIDSIVAQLKAYPD---VHTILIEPDSLNLMTNLSTAK----    |
| A. bisporus CBH #7       | NKYKN-YVDQIAAQIKQFPD---VSVVAVIEPDSLNLVTNLNVQK----   |
| F. oxysporum EG #8       | NKYKA-YIAKIKGILQNYSD---TKVILVIEPDSLNLVTNLNVQK----   |
| P. chrysosporium CBH #9  | ANYEN-YIDQIVAQIQFPD---VRVAVIEPDSLNLVTNLNVQK----     |
| T. emersonii CBH #10     | ALYKQ-YIDSIREQLTTYSD---VHTILVIEPDSLNLVVTNLNVQK----  |
| T. fusc CBH #11          | DRYKSEYIDPIADIMWDFADYENLRIVAIIEIDSLPNLVTNLVGGNGGTEL |
| T. fusc EG #12           | SNYRW-TADEVA-----YAK---AVLSAIGNPSLRAVIDTSRNGNG----  |
| C. fimi EG #13           | SEYAR-WVDTVAQGIK-----GNPIVILEPDALAQGLD-----         |
|                          | * : : . . :                                         |

|                          |                                                     |
|--------------------------|-----------------------------------------------------|
| T. reesei CBH #3         | CA--NAQSAYLECINYAVTQL-NLPNVAMYLDAGHAGWLGWPANQDPAAQ  |
| H. koningii CBH #4       | CA--NAQSAYLECINYAVTQL-NLPNVAMYLDAGHAGWLGWPANQDPAAQ  |
| H. insolens CBH #5       | CS--GAASTYRELTIYALKQL-DLPHVAMYMDAGHAGWLGWPANIQPAAE  |
| A. cellulolyticus CBH #6 | CA--EAQSAYYECVNYALINL-NLANVAMYIDAGHAGWLGWSANLSPAAQ  |
| A. bisporus CBH #7       | CA--NAQSAYKEGVYIYAVQKL-NAVGVMTYIDAGHAGWLGWPANLSPAAQ |
| F. oxysporum EG #8       | CA--KAESAYKELTVYAIKEL-NLPNVSMYLDAGHGGWLGWPANIGPAAK  |
| P. chrysosporium CBH #9  | CA--NAKTTYLACVNYALTNL-AKVGVMYMDAGHAGWLGWPANLSPAAQ   |
| T. emersonii CBH #10     | CA--NAQDAYLECINYAITQL-DLPNVAMYLDAGHAGWLGWQANLAPAAQ  |
| T. fusc CBH #11          | CAYMKQNGGYVNGVGYALRKLGEIPNVYNYIDAAHHGWIGWDSNFGPSVD  |
| T. fusc EG #12           | ----PAGNEWCDPSGRAIGTPSTTNTGDPMDAFL--WIKLPGEADGCIA   |
| C. fimi EG #13           | CS---GQGDRVGFLKYAAKSL-TLKGARVYIDAGHAKWLSVDTPVNRLNQ  |
|                          | * : ** * :                                          |

|                          |                                                     |
|--------------------------|-----------------------------------------------------|
| T. reesei CBH #3         | LFANVYKNAS-SPRALRGLATNVANYNGWNIT-----SPPSY          |
| H. koningii CBH #4       | LFANVYKNAS-SPRALRGLATNVANYNGWNIT-----SPPSY          |
| H. insolens CBH #5       | LFakiYEDAG-KPRAVRGLATNVANYNAWSIS-----SPPPY          |
| A. cellulolyticus CBH #6 | LFATVYKNAS-APASLRGLATNVANYNAWSIS-----SPPSY          |
| A. bisporus CBH #7       | LFAQIYRDAG-SPRNLRGIATNVANFNALRAS-----SPDPI          |
| F. oxysporum EG #8       | LYAQIYKDAG-KPSRVRGLVTNVSNYNGWKLS-----TKPDY          |
| P. chrysosporium CBH #9  | LFTQVWQAG-KSPFIKGLATNVANYNALQAA-----SPDPI           |
| T. emersonii CBH #10     | LFASVYKNAS-SPASVRGLATNVANYNAWSIS-----RCPSY          |
| T. fusc CBH #11          | IFYEAAANASGSTVDYVHGFISNTANYSATVEPYLDVNGTVNGQLIRQSKW |
| T. fusc EG #12           | GAGQFVPQAAYEMAIAAGGTNPNNPNNP--TP-----TPTPT          |
| C. fimi EG #13           | V-----GFEYAVGFALNTSNTYQT-----                       |
|                          | * . * .                                             |

## Figure 1 continued

|                          |                                                    |        |
|--------------------------|----------------------------------------------------|--------|
| T. reesei CBH #3         | TQGNNAVYNEKLYIHAIGPLLANHGWSN-AFFITDQGRSGK----      | QPTGQQ |
| H. koningii CBH #4       | TQGNNAVYNEQLYIHAIGPLLANHGWSN-AFFITDQGRSGK----      | QPTGQQ |
| H. insolens CBH #5       | TSPNPNYDEKHYIEAFRPLLEARGFP--AQFIVDQGRSGK----       | QPTGQK |
| A. cellulolyticus CBH #6 | TSGDSNYDEKLYINALSPLLTNSGWPNAHFIMDTSRNGV----        | QPTKQQ |
| A. bisporus CBH #7       | TQGNSNYDEIHYIEALAPMLSNAGFP--AHFIVDQGRSGV----       | QNIRD  |
| F. oxysporum EG #8       | TESNPNYDEQRYINAFAPLLAQEGWSN-VKFIVDQGRSGK----       | QPTGQK |
| P. chrysosporium CBH #9  | TQGNPNYDEIHYINALAPLLQQAGWD--ATFIVDQGRSGV----       | QNIRQ  |
| T. emersonii CBH #10     | TQGDANCDEEDYVNALGPLFQEQGFP--AYFIIDTSRNGV----       | RPTKQS |
| T. fusc CBH #11          | VDWNQYVDELSFVQDLRQALIAKGFRSDIGMLIDTSRNGWGGPNRPTGPS |        |
| T. fusc EG #12           | PTPPPGSSGACTATYTIANEWNDGFGATVTVTANQNITGW-----      | TV     |
| C. fimi EG #13           | -----TADSKAYGQQISQRLGG-----KKFVIDTSRNGN-----       | G      |

. . . \*

|                          |                                                     |
|--------------------------|-----------------------------------------------------|
| T. reesei CBH #3         | QWGD-----WCNVIGTGFGIRPSANTGDSLLDSFVWV               |
| H. koningii CBH #4       | QWGD-----WCNVIGTGFGIRPSANTGDSLLDSFVWI               |
| H. insolens CBH #5       | EWGH-----WCNAIGTGFGMRPTANTGHQYVDAFVWV               |
| A. cellulolyticus CBH #6 | AWGD-----WCNVIGTGFGVQPTTNTGDPLEDAFVWV               |
| A. bisporus CBH #7       | QWGD-----WCNVKGAGFGQRPTTNTGSSSLIDAIVWV              |
| F. oxysporum EG #8       | AQGD-----WCNAKGTGFGLRPSTNTGDALADAFVWV               |
| P. chrysosporium CBH #9  | QWGD-----WCNIKAGAGFGTRPTTNTGSQFIDSIVWV              |
| T. emersonii CBH #10     | QWGD-----WCNVIGTGFGVRPTTDTGNPLEDAFVWV               |
| T. fusc CBH #11          | SSTDNLNTYVDESRIDRRIHPGNWCNQAGAGLGERPTVNPA-PGVDAFVWV |
| T. fusc EG #12           | TWT-----FTDGQITITNAWNADVSTSGSSVTARNVG               |
| C. fimi EG #13           | SNGE-----WCNPRGRALGERPVAVNDGSGLDALLWV               |

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|                          |                                                    |
|--------------------------|----------------------------------------------------|
| T. reesei CBH #3         | KPGGECDDGTSDDS----APRFD SHCA-----LPDALQPAPQAG      |
| H. koningii CBH #4       | KPGGECDDGTSDDS----APRFD SHCA-----LPDALQPAPQAG      |
| H. insolens CBH #5       | KPGGECDDGTSDDT----AARYDYHCG-----LEDALKPAPEAG       |
| A. cellulolyticus CBH #6 | KPGGESDGTSSNSS----ATRYDFHCG-----YSDALQPAPPEAG      |
| A. bisporus CBH #7       | KPGGECDDGTSDNS----SPRFD SHCS-----LSDAHQPAPPEAG     |
| F. oxysporum EG #8       | KPGGESDGTSDTS----AARYDYHCG-----LDDALKPAPEAG        |
| P. chrysosporium CBH #9  | KPGGECDDGTSNSS----SPRYDSTCS-----LPDAAQPAPPEAG      |
| T. emersonii CBH #10     | KPGGESDGTSTNTT----SPRYDYHCG-----LSDALQPAPPEAG      |
| T. fusc CBH #11          | KPPGESDGASEEIPNDEGKGFDRCMDPTYQGNARNGNNPSGALPNAPISG |
| T. fusc EG #12           | HNGTLSQGASTEFG-----GFVG                            |
| C. fimi EG #13           | KLPGESDGACNGG-----PAAG                             |

: . . \* . .

\*

|                          |                     |
|--------------------------|---------------------|
| T. reesei CBH #3         | AWFQAYFVQLLTNANPSFL |
| H. koningii CBH #4       | AWFQAYFVQLLTNANPSFL |
| H. insolens CBH #5       | QWFQAYFEQLLRNANPPF- |
| A. cellulolyticus CBH #6 | TWFQAYFVQLLTNANPALV |
| A. bisporus CBH #7       | TWFQAYFETLVANANPAL- |
| F. oxysporum EG #8       | TWFQAYFEQLLDNANPSFL |
| P. chrysosporium CBH #9  | TWFQAYFQTLVSAANPPL- |
| T. emersonii CBH #10     | TWFQAYFEQLLTNANPLF- |
| T. fusc CBH #11          | HWFSAQFRELLANAYPPL- |
| T. fusc EG #12           | SKGNSNSVPTLTCAAS--- |
| C. fimi EG #13           | QWWQEIALEMARNARW--- |

\*

FIG. 2A

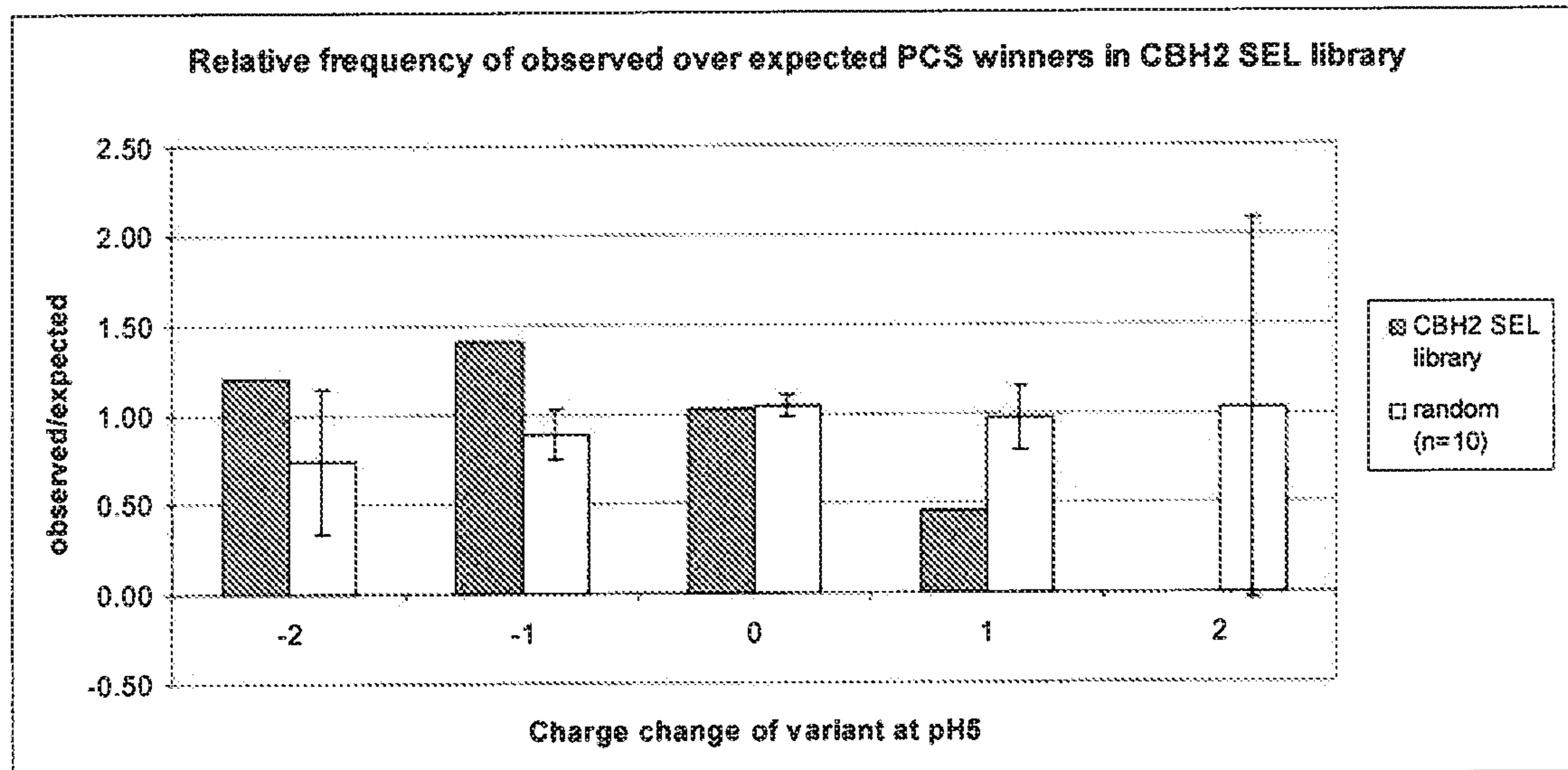


FIG. 2B

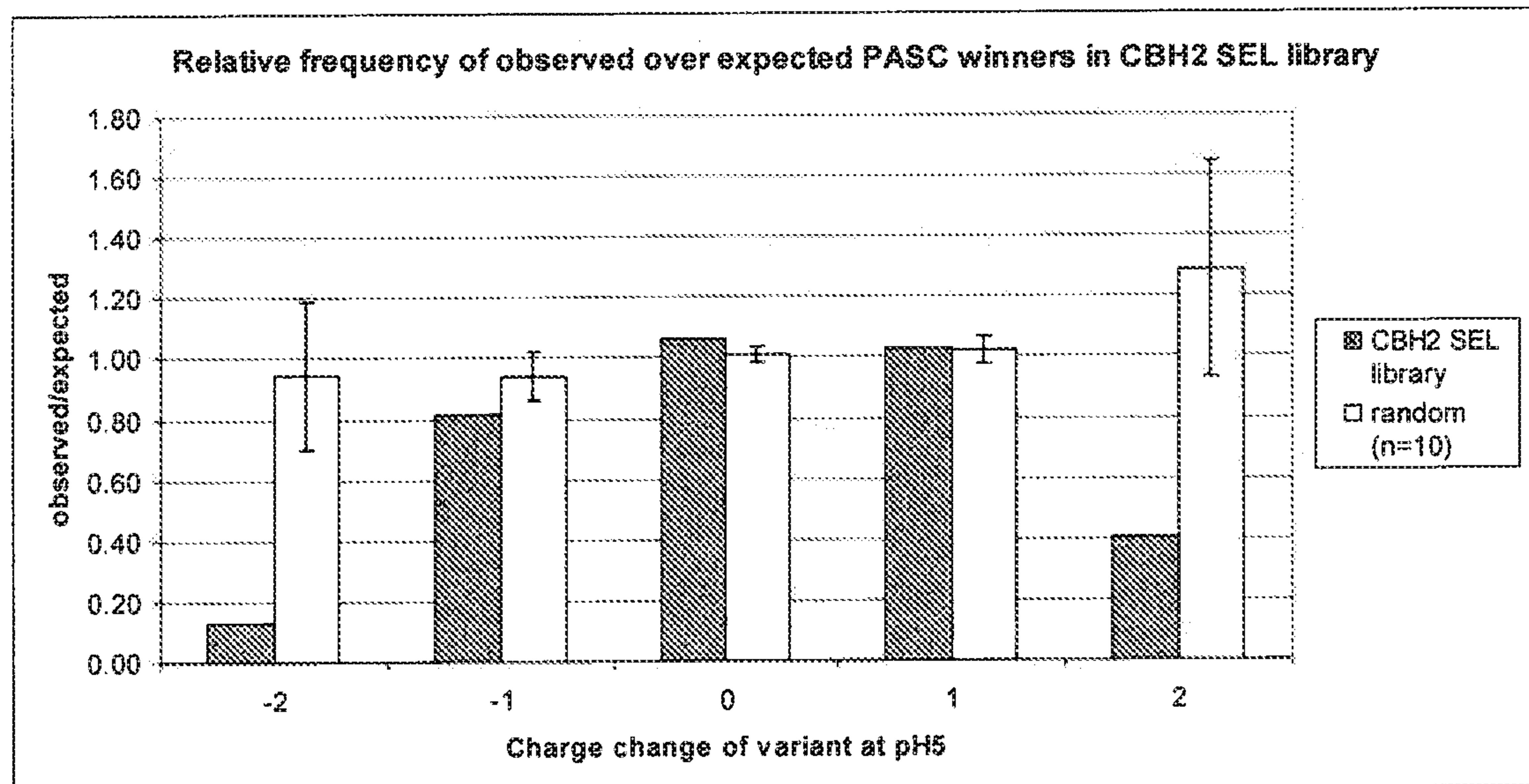
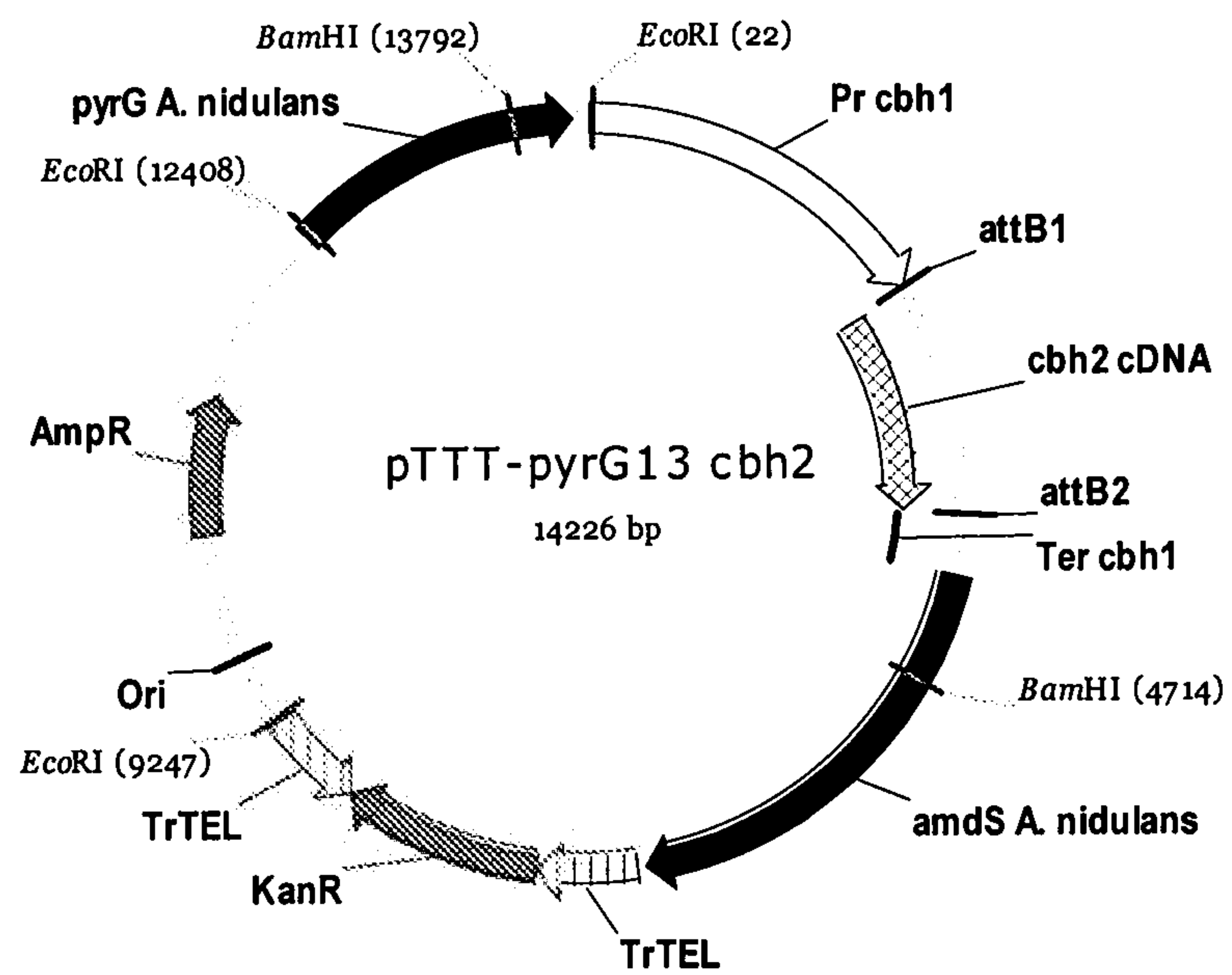


FIG. 3



## Figure 4. Deletion vectors used to create screening strains

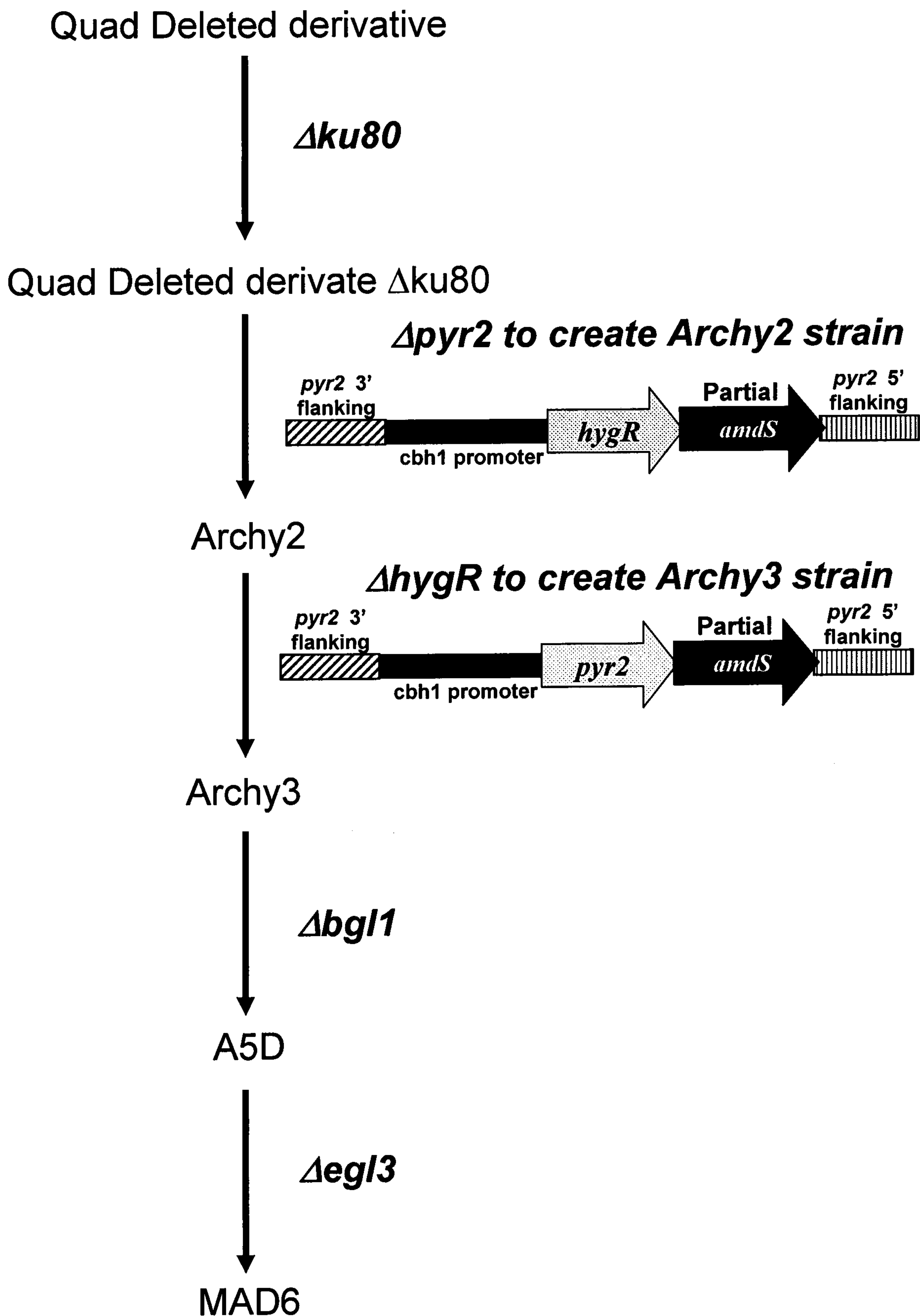
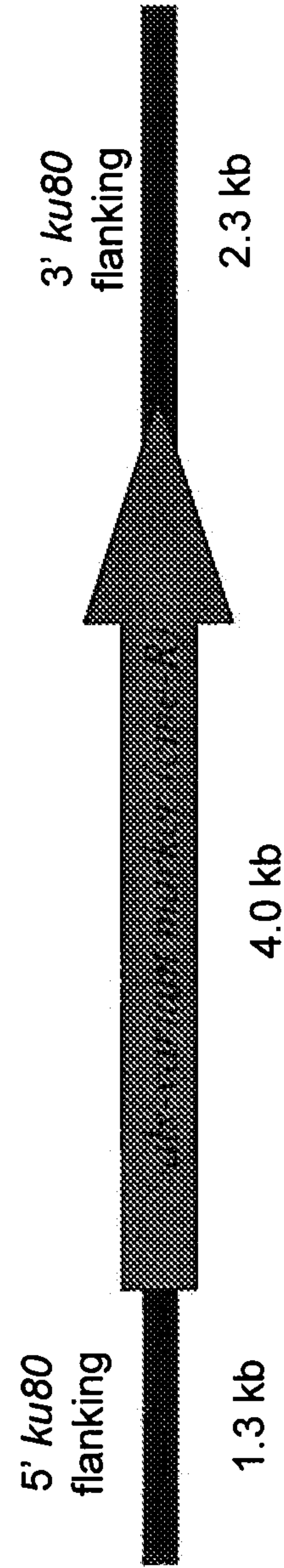
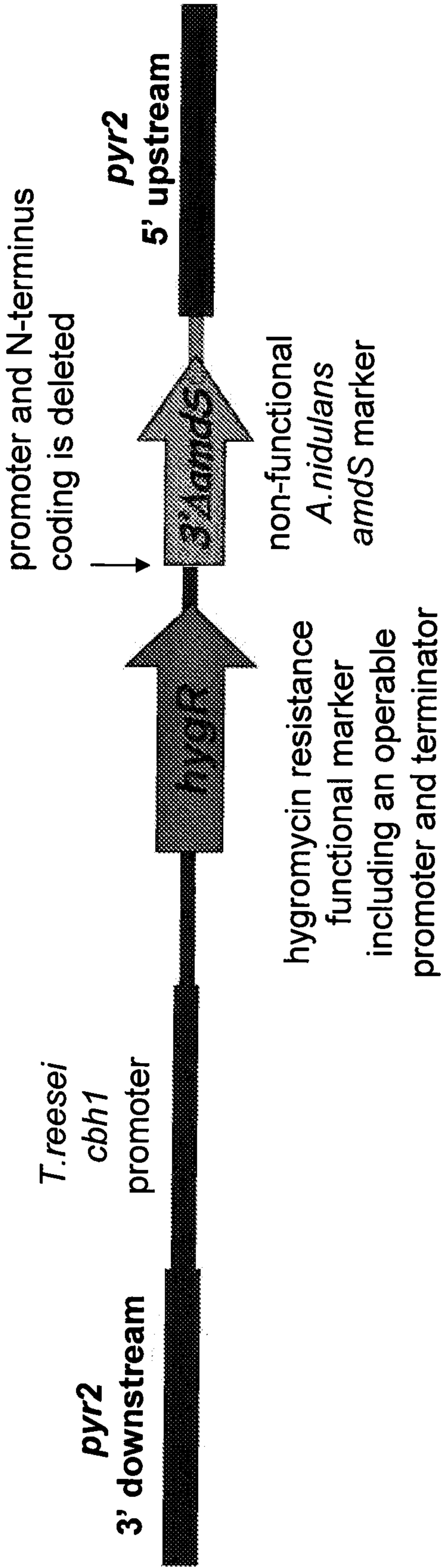


Figure 5.  $\Delta ku80$  deletion cassette



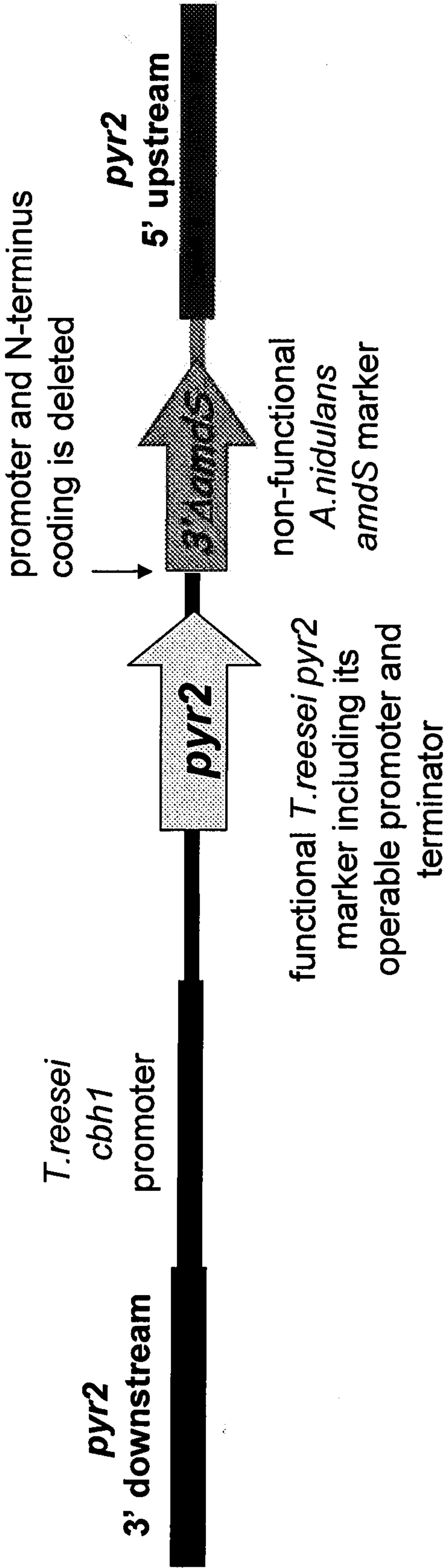
this vector deleted the native *T.reesei ku80*

Figure 6.  $\Delta pyr2$  deletion cassette to create Archy2 strain



this vector deleted the native *T. reesei pyr2*

Figure 7. *ΔhygR* deletion cassette to create Archy3 strain



this vector deleted the hygromycin resistance marker that was formerly inserted into the *pyr2* locus

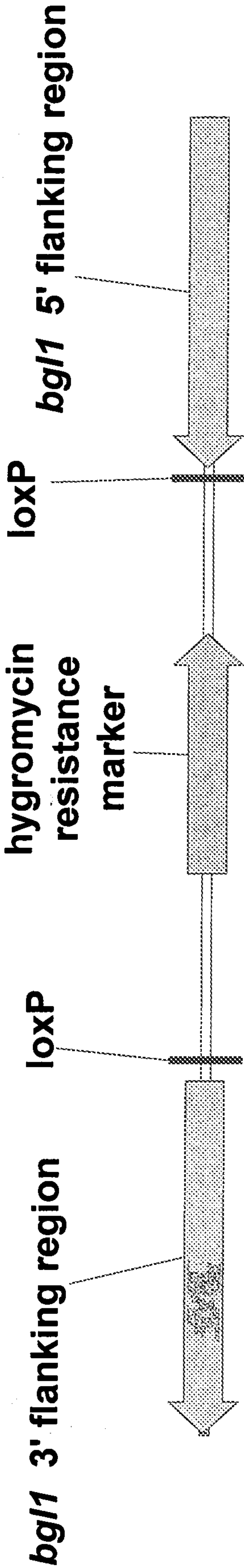


Figure 8.  $\Delta bgl1$  deletion cassette (5610 bp)

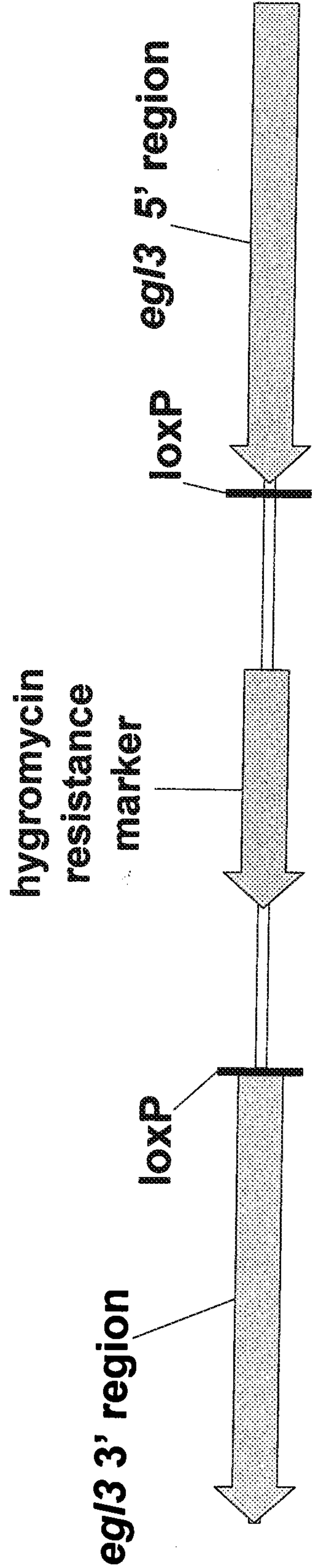


Figure 9.  $\Delta$ egI3 deletion cassette (6511 bp)

Figure 10. telomeric plasmid for expression of cre recombinase

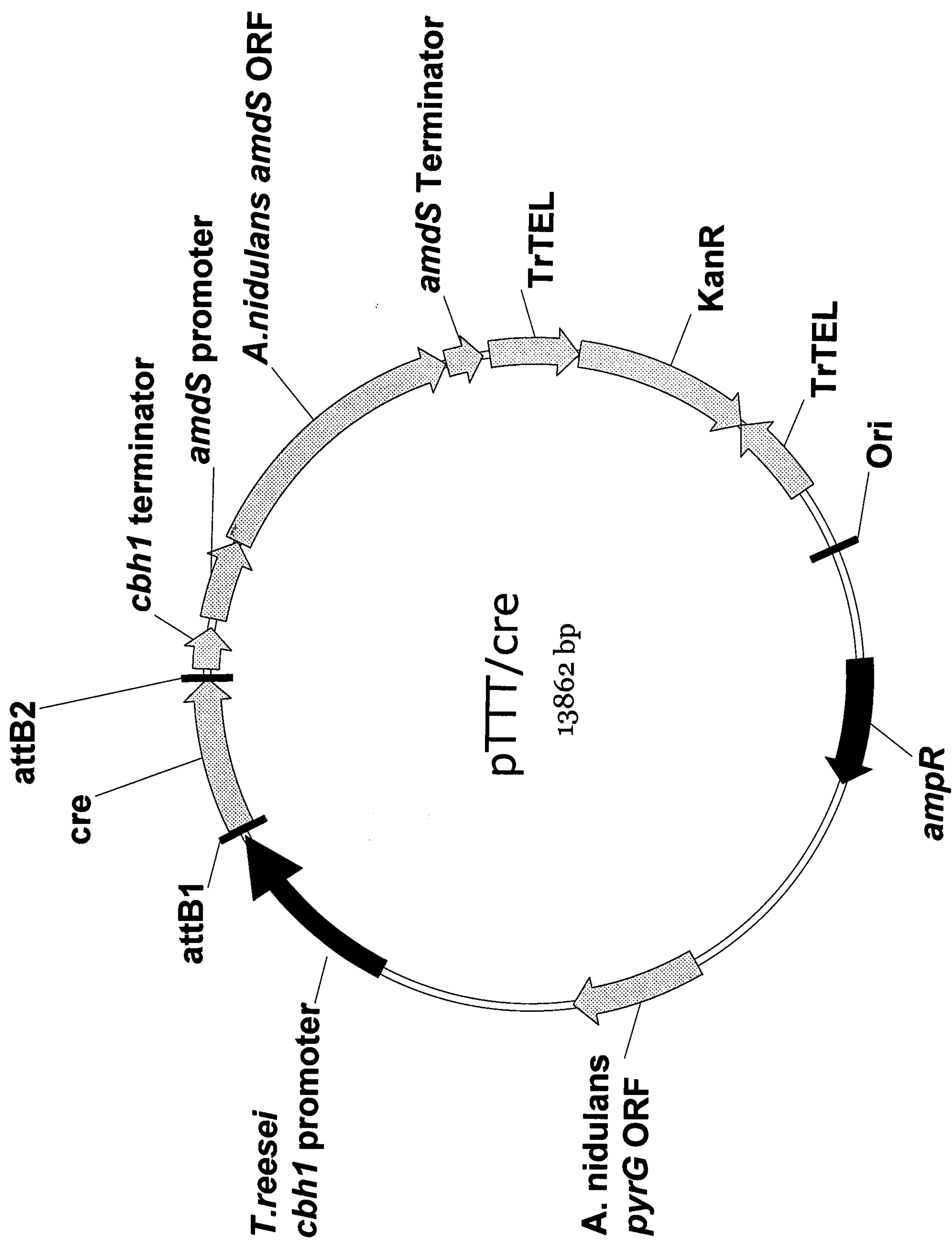


Figure 11. Homologous recombination of expression cassette into targeted locus:

