

METHODS AND COMPOSITIONS FOR TREATING CONGENITAL SUCRASE-ISOMALTASE DEFICIENCY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 63/621,997, filed on January 17, 2024; the disclosure of which is hereby incorporated by reference in its entirety for all purposes.

SEQUENCE LISTING

[0002] This application contains a Sequence Listing XML, which has been submitted electronically and is hereby incorporated by reference in its entirety. The XML copy of the Sequence Listing, created on December 30, 2024, is named ANR-008WO_SL.xml and is 98,561 bytes in size.

FIELD OF THE INVENTION

[0003] The invention relates generally to recombinant mutant invertase (sucrase) enzymes and methods and compositions for treating sucrase-isomaltase deficiency.

BACKGROUND

[0004] Congenital sucrose-isomaltase deficiency (CSID) is a rare autosomal recessive inherited disease of the small intestine resulting from genetic mutations in sucrase-isomaltase, an enzyme complex responsible for catalyzing the hydrolysis of dietary sucrose and starch. Ritz *et al.* GASTROENTEROLOGY 125(6):1678-85. Decreased or absent invertase (sucrase) and/or isomaltase enzymatic activity has been found in patients with CSID, and investigations at the subcellular and molecular levels in intestinal biopsy specimens have led to the description of several phenotypes, differing in transport efficiency, processing, and sorting of the protein, which result in impaired physiologic functions. Ritz *et al.* (2003) GASTROENTEROLOGY 125(6):1678-85, Jacob *et al.* (2000) J. CLIN. INVEST. 106(2):281-7, Alfalah *et al.* (2009) GASTROENTEROLOGY 136(3):883-92, and Ouwendijk (1996) J. CLIN. INVEST. 97(3):633-41. The final step of starch digestion typically takes place in the intestinal lumen by α -glucosidases that are localized on the brush border membrane (BBM) of the intestinal epithelium. Human sucrase-isomaltase (SI) is responsible for almost all sucrase activity and about 60 to 80% of maltase activity in the intestinal lumen. Brunner *et al.* (1979) J. BIOL. CHEM. 254(6):1821-1828. As a result of the location of sucrase-isomaltase deficiency in the BBM of the intestinal lumen, disaccharidase deficiencies are also common where there is inflammation and has been associated with gastrointestinal complaints in

people with gastrointestinal disorders (*e.g.*, abdominal pain, irritable bowel, and Crohn's disease (CD)). Colombo *et al.* (2021) NATURE SCIENTIFIC REPORTS 11:4902, Chumpitazi (2018) GASTROENTEROL CLIN NORTH AM 47(4):715-726, Dbar *et al.* (2021) WORLD J CLIN CASES 9(17): 4178-4187, Friesen *et al.* (2022) GASTROINTEST. DISORD. 4:1-7.

- 5 [0005] Affected subjects may present with osmotic diarrhea, mild steatorrhea, chronic diarrhea, irritability, and vomiting after consuming sucrose. The difficulty in providing adequate nutrition to these subjects can lead to dehydration, metabolic acidosis, hypercalcemia, failure to thrive, and developmental delay. Cohen (2016) MOL. CELL. PEDIATR. 3:5. Failure to absorb dietary disaccharides and starch has implications for the
- 10 absorption of other nutrients and the hormonal regulation of gastrointestinal function.
- [0006] The prevalence of CSID in the European population has been estimated at 1 in 5,000, but it is higher among the indigenous populations of Alaska, Greenland, and Canada. Marcadier *et al.* (2015) CANADIAN MED. ASSOC. J. 187(2):102-107. There is also evidence that heterozygous carriers also experience symptoms of CSID, such as chronic diarrhea,
- 15 abdominal pain, and bloating. It is currently estimated that 2-9% of Americans of European descent may be affected, suggesting that sucrase-isomaltase deficiency has been underrecognized. DeJonge *et al.* (2014) GASTROENTEROLOGY 146:S705. Disaccharidase (DS) deficiencies have been reported in patients with inflammatory bowel disease (IBD) including CD, ulcerative colitis, and indeterminant colitis. Sucrase deficiency is common in
- 20 approximately 14% of subjects. Friesen *et al.* (2022), *supra*.
- [0007] Currently, CSID may be treated by the oral administration of a solution of sacrosidase (SUCRAID®). However, sacrosidase does not fully replace the enzymatic activity of the endogenous sucrase-isomaltase enzyme complex. The liquid formulation of sacrosidase in 50% glycerol is thermally unstable and unstable under acidic conditions. As a result,
- 25 sacrosidase solution must be discarded four weeks after opening and cannot be used in conjunction with warm beverages and infant formula or fruit juices, negatively impacting ease of use and patient compliance.
- [0008] Although developments have been made to date, there is still an ongoing need for new and effective pharmaceutical compositions and therapies for treating and managing sucrase-
- 30 isomaltase deficiency, such as CSID or acquired sucrase-isomaltase deficiency (ASID).

SUMMARY

[0009] The disclosure is based, in part, upon the discovery of recombinant mutant invertase (sucrase) enzymes that have greater stability and/or activity than naturally occurring enzymes. In particular, the recombinant mutant enzymes described herein exhibit improved pH stability, thermal stability, and/or stability against proteolytic digestion compared to naturally occurring versions of the enzyme. Furthermore, the recombinant mutant enzymes of the invention may have greater specific activity than a wild-type invertase (sucrase) enzyme under physiological conditions, for example, in the stomach and small intestine. Furthermore, it is contemplated that the recombinant mutant enzymes described herein, given their enhanced stability, may be suitable for oral administration and potentially safer, more tolerable, and/or more active than commercially available invertase (sucrase) enzymes. Also provided are pharmaceutical compositions, *e.g.*, solid pharmaceutical compositions, comprising an invertase (sucrase) enzyme described herein and an isomaltase enzyme that are stable at room temperature, allowing for easier distribution, storage, and administration to subjects relative to compositions that are unstable at room temperature and/or require refrigeration. The recombinant enzymes and compositions can be used, among other things, to treat sucrase-isomaltase deficiency (*e.g.*, congenital sucrase-isomaltase deficiency (CSID) or acquired sucrase-isomaltase deficiency (ASID)).

[0010] Accordingly, in one aspect, the disclosure provides a recombinant mutant *S. cerevisiae* invertase enzyme including one or more amino acid substitutions that promote improved pH stability, thermal stability, and/or stability against proteolytic digestion. A recombinant mutant *S. cerevisiae* invertase enzyme of the disclosure can include one or more amino acid substitutions selected from the group consisting of:

- (a) a substitution of a T residue at a position corresponding to position 51 of SEQ ID NO: 3;
- (b) a substitution of an F residue at a position corresponding to position 83 of SEQ ID NO: 3;
- (c) a substitution of an E residue at a position corresponding to position 293 of SEQ ID NO: 3;
- (d) a substitution of an N residue at a position corresponding to position 340 of SEQ ID NO: 3;
- (e) a substitution of a P residue at a position corresponding to position 390 of SEQ ID

NO: 3; and

- (f) a substitution of an A residue at a position corresponding to position 412 of SEQ ID NO: 3; or

a combination of any of the foregoing substitutions, and is catalytically active to digest sucrose into glucose and fructose under physiological conditions of the stomach and/or small intestine. For example, in one embodiment, the invertase includes a combination of four of the foregoing substitutions. In another embodiment, the invertase includes a combination of five of the foregoing substitutions. In another embodiment, the invertase comprises all six of the foregoing substitutions..

10 **[0011]** In certain embodiments, in the invertase,

- (a) the T residue at a position corresponding to position 51 of SEQ ID NO: 3 is substituted by L (T51L);
- (b) the F residue at a position corresponding to position 83 of SEQ ID NO: 3 is substituted by Y (F83Y);
- 15 (c) the E residue at a position corresponding to position 293 of SEQ ID NO: 3 is substituted by Q (E293Q);
- (d) the N residue at a position corresponding to position 340 of SEQ ID NO: 3 is substituted by A (N340A);
- (e) the P residue at a position corresponding to position 390 of SEQ ID NO: 3 is substituted by A (P390A);
- 20 (f) the A residue at a position corresponding to position 412 of SEQ ID NO: 3 is substituted by N (A412N); or

or the invertase includes a combination of any of the foregoing substitutions. For example, in one embodiment, the invertase includes a combination of four of the foregoing substitutions.

25 In another embodiment, the invertase includes a combination of five of the foregoing substitutions. In another embodiment, the invertase comprises all six of the foregoing substitutions.

[0012] The invertase can include additional substitutions. For example, an invertase of the disclosure can further include a substitution at a D residue at a position corresponding to position 183 of SEQ ID NO: 3, *e.g.*, wherein the D residue at a position corresponding to position 183 of SEQ ID NO: 3 is substituted by an N (D183N). The invertase can include a substitution at an E residue at a position corresponding to position 196 of SEQ ID NO: 3,

30

e.g., wherein the E residue at a position corresponding to position 196 of SEQ ID NO: 3 is substituted by an H (E196H).

[0013] The invertase of the disclosure can include four, five, six, seven, eight, nine or ten mutations relative to a corresponding wild-type invertase (*e.g.*, a wild-type invertase of SEQ ID NO: 3). For example, the invertase can include F83Y, N340A, P390A, T51L, E293Q, and A412N substitutions; F83Y, N340A, P390A, T51L, E293Q, A412N, and D183N substitutions; or F83Y, N340A, P390A, T51L, E293Q, A412N, D183N, and E196H substitutions, in each case, relative to SEQ ID NO: 3.

[0014] In certain embodiments, the invertase includes the amino acid sequence of any one of SEQ ID NOs: 51-61 or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 51-61. A recombinant mutant *S. cerevisiae* invertase enzyme of the disclosure can include a substitution or combination of substitutions listed in **TABLE 8** or **TABLE 12**, wherein the invertase enzyme is catalytically active to digest sucrose into glucose and fructose under physiological conditions (*e.g.*, of the stomach and/or small intestine). The invertase can exhibit one or more characteristics indicative of stability or survival in the gastrointestinal tract. For example, the invertase can have a specific activity at about pH 3.5 (*e.g.*, pH 3.6) of at least 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, or 2,000 μmol sucrose consumed per minute per milligram of invertase. In certain embodiments, the invertase has at least 1-fold, 1.5-fold, 2-fold, 2.5-fold, or 3-fold higher activity at about pH 3.5 (*e.g.*, pH 3.6), compared to a corresponding wild-type invertase. In certain embodiments, the invertase has a specific activity at about pH 5.0 (*e.g.*, pH 4.9) of at least 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,100, or 2,200 μmol sucrose consumed per minute per milligram of invertase. In certain embodiments, the invertase has at least 1-fold, 1.5-fold, 2-fold, 2.5-fold, or 3-fold higher activity at about pH 5.0 (*e.g.*, pH 4.9), compared to a corresponding wild-type invertase. In certain embodiments, the invertase has a specific activity at about pH 6.0 or 6.2 of at least 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, or 1,800 μmol sucrose consumed per minute per milligram of invertase. In certain embodiments, the invertase has at least 1-fold, 1.5-fold, 2-fold, 2.5-fold, or 3-fold higher activity at about pH 6.0 or 6.2, compared to a corresponding wild-type invertase. In certain embodiments, the invertase has a specific activity at about pH 7.0 or 7.1 of at least 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, or 1,300 μmol sucrose consumed per minute per milligram of invertase. In certain embodiments, the invertase at

least 1-fold, 1.5-fold, 2-fold, 2.5-fold, or 3-fold higher activity at about pH 7.0 or 7.1, compared to a corresponding wild-type invertase. In certain embodiments, the invertase retains at least 80%, 90%, or 95% of activity following incubation at about pH 2.8 for about 2 hours. In certain embodiments, the invertase retains at least 80%, 90%, or 95% of activity following incubation at about 62 °C for about 1 hour. In certain embodiments, the invertase retains at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% of activity following incubation at about pH 2.5 for about 30 minutes. In certain embodiments, the invertase has at least 1-fold, 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, or 5-fold higher stability at about pH 2.5 compared to a corresponding wild-type invertase. In certain embodiments, the invertase has a T_m of at least 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, or 68 °C. In certain embodiments, the invertase has a T_m that is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 °C higher than a corresponding wild-type invertase. In certain embodiments, the invertase has higher stability in the presence of pancreatin or pepsin compared to a corresponding wild-type invertase.

[0015] In another aspect, the disclosure relates to a nucleic acid encoding an invertase as disclosed herein, an expression vector comprising the nucleic acid, and a cell (*e.g.*, a *P. pastoris* or *S. cerevisiae* cell) comprising the expression vector. In another aspect, the disclosure relates to a method of producing a recombinant mutant *S. cerevisiae* invertase enzyme disclosed herein, the method comprising growing the cell under conditions so that the host cell expresses the invertase, and purifying the invertase.

[0016] In another aspect, the disclosure relates to a pharmaceutical composition comprising an invertase as disclosed herein and a pharmaceutically acceptable carrier and/or an excipient. The pharmaceutical composition can, optionally, include an isomaltase enzyme. The invertase and/or the isomaltase can be dried and, for example, formulated for delivery as a powder or compressed into a tablet.

[0017] The isomaltase can be a microbial isomaltase, or a functional fragment or variant thereof. The isomaltase can be derived from *Saccharomyces cerevisiae*, *e.g.*, can include any one of SEQ ID NOs: 37-41, or a functional fragment or variant thereof. The isomaltase can be a recombinant mutant *Lactobacillus fermentum* isomaltase enzyme (SEQ ID NO: 47), or a functional fragment or variant thereof.

[0018] The isomaltase can include the following substitutions using the numbering in SEQ ID NO: 47:

- (a) K115I, R132K, D226S, E310A, L366M, I421A, A444G, A531D, and F560V substitutions;
- (b) E93K, K115I, R132K, D226S, E310A, L366M, I421A, A444G, A531D, and F560L substitutions;
- 5 (c) E93K, K115I, R132K, D226S, E310A, I421A, A444G, E524Q, A531D, and F560L substitutions;
- (d) E93K, K115I, R132K, D226S, E310A, I421A, A444G, A531D, and F560V substitutions;
- (e) E93K, K115I, R132K, D226S, L366M, I421A, A444G, E524Q, A531D, and F560L substitutions;
- 10 (f) E93K, K115I, R132K, D226S, L366M, I421A, A444G, A531D, and F560V substitutions;
- (g) E93K, K115I, R132K, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions;
- (h) K115I, R132K, D226S, E310A, L366M, I421A, A444G, E524Q, A531D, and F560L substitutions;
- 15 (i) K115I, R132K, D226S, E310A, I421A, A444G, E524Q, A531D, and F560V substitutions;
- (j) K115I, R132K, D226S, L366M, I421A, A444G, E524Q, A531D, and F560V substitutions;
- 20 (k) E93K, K115I, R132K, A211E, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions;
- (l) E93K, K115I, R132K, D226S, L366M, I421A, A444G, E524Q, A531D, and F560V substitutions;
- 25 (m) E93K, K115I, R132K, D226S, E275D, I421A, A444G, E524Q, A531D, and F560V substitutions;
- (n) T89A, E93K, K115I, R132K, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions;
- (o) E93K, K115I, R132K, H182R, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions;
- 30 (p) E93K, K115I, E122D, R132K, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions;
- (q) E93K, K115I, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions;

- (r) E93K, K115I, D226S, I421A, A444G, E524Q, A531D, and F560L substitutions;
- (s) K115I, A211E, D226S, I421A, A444G, A531D, and F560V substitutions;
- (t) K115I, D226S, L366M, I421A, A444G, E524Q, A531D, and F560L substitutions;
- (u) K115I, A211E, D226S, I421A, A444G, E524Q, A531D, and F560L substitutions;
- 5 (v) K115I, R132K, D226S, E310A, L366M, I421A, A444G, A531D, and F560L substitutions;
- (w) E93K, K115I, D226S, E310A, I421A, A444G, A531D, and F560L substitutions;
- (x) K115I, D226S, E310A, I421A, A444G, A531D, and F560V substitutions;
- (y) E93K, K115I, D226S, L366M, I421A, A444G, A531D, and F560L substitutions;
- 10 (z) K115I, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions;
- (aa) K115I, D226S, E310A, I421A, A444G, E524Q, A531D, and F560L substitutions;
- (bb) K115I, A211E, D226S, L366M, I421A, A444G, A531D, and F560L substitutions;
- (cc) K115I, A211E, D226S, E310A, I421A, A444G, A531D, and F560L substitutions;
- 15 (dd) E93K, K115I, D226S, I421A, A444G, A531D, and F560V substitutions;
- (ee) E93K, K115I, A211E, D226S, I421A, A444G, A531D, and F560L substitutions;
- or
- (ff) K115I, D226S, L366M, I421A, A444G, A531D, and F560V substitutions.

[0019] The invertase included in the pharmaceutical composition can include the amino acid
 20 sequence of any one of SEQ ID NOs: 51-61 (*e.g.*, of any one of SEQ ID NOs: 51-53), or a functional fragment thereof.

[0020] The pharmaceutical composition can be formulated as an oral dosage form, for example, as a liquid, powder, sachet, granulate, pellet, micropellet, tablet, or minitab.

[0021] In certain embodiments, the pharmaceutical composition has a shelf-life at room
 25 temperature of at least 3 months, 6 months, 9 months, 12 months, 15 months, 18 months, 21 months, 24 months, 36 months, 48 months, 60 months, 72 months, 84 months, 96 months, 108 months, or 120 months.

[0022] The disclosure further relates to a method of treating sucrase-isomaltase deficiency (*e.g.*, congenital sucrase-isomaltase deficiency (CSID) or acquired sucrase-isomaltase
 30 deficiency (ASID)) in a subject in need thereof. The method can include orally administering to the subject an effective amount of the pharmaceutical composition as disclosed herein.

The pharmaceutical composition can be administered to the subject together with a meal or snack.

[0023] The disclosure further relates to a method of reducing sucrose and branched (1–6 linked) α -limit dextrin concentration in a subject. The method can include orally
5 administering to the subject an effective amount of a pharmaceutical composition disclosed herein. The method can be measured using a hydrogen breath test.

[0024] The disclosure further relates to a method of treating sucrase-isomaltase deficiency (e.g., congenital sucrase-isomaltase deficiency (CSID) or acquired sucrase-isomaltase deficiency (ASID)) in a subject in need thereof, the method comprising orally administering
10 to the subject an invertase enzyme of the disclosure and optionally an isomaltase enzyme as disclosed herein.

[0025] In certain embodiments, the subject is a mammal, e.g., a human.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0026] The invention can be more completely understood with reference to the following
15 drawings.

[0027] **FIG. 1** is a bar chart showing the specific activities of indicated invertase enzymes as measured by consumption of sucrose at pH 3.6, pH 4.9, pH 6.0, and pH 7.2.

[0028] **FIGs. 2A** and **2B** are graphs showing the activities of indicated invertase enzymes following incubation with proteases. **FIG. 2A** shows the activity of indicated invertases
20 following pretreatment with pepsin at pH 3.5 for indicated time periods to reflect the physiological conditions of the stomach. **FIG. 2B** shows the activity of indicated invertases following pretreatment with pancreatin at pH 6.0 for 3 hours to reflect fed state conditions of the small intestine.

[0029] **FIG. 3** is a graph showing the activities of indicated invertase enzymes following
25 incubation at pH 2.8 for indicated time periods to reflect fasted state conditions.

[0030] **FIG. 4** is a graph showing the activities of indicated invertase enzymes following incubation at 62 °C for indicated time periods.

DETAILED DESCRIPTION

[0031] The disclosure is based, in part, upon the discovery of recombinant mutant invertase (sucrase) enzymes that have greater stability and/or activity than naturally occurring enzymes. In particular, the recombinant mutant enzymes described herein exhibit improved
5 pH stability, thermal stability, and/or stability against proteolytic digestion compared to naturally occurring versions of the enzyme. Furthermore, the recombinant mutant enzymes of the invention may have greater specific activity than a wild-type invertase (sucrase) enzyme under physiological conditions, for example, in the stomach and small intestine of a subject, *e.g.*, a human subject. Furthermore, it is contemplated that the recombinant mutant
10 enzymes described herein, given their enhanced stability, may be suitable for oral administration, and potentially safer, more tolerable, and/or more active than commercially available invertase (sucrase) enzymes. Also provided are pharmaceutical compositions, *e.g.*, solid pharmaceutical compositions, comprising an invertase (sucrase) enzyme described herein and an isomaltase enzyme that are stable at room temperature, allowing for easier
15 distribution, storage, and administration to subjects relative to compositions that are unstable at room temperature and/or require refrigeration. The recombinant enzymes and compositions can be used, among other things, to treat sucrase-isomaltase deficiency (*e.g.*, CSID or ASID).

[0032] Various features and aspects of the invention are discussed in detail below.

20 **I. Invertase (Sucrase) Enzymes**

[0033] Among other things, the disclosure provides recombinant mutant invertase (sucrase) enzymes and pharmaceutical compositions comprising recombinant mutant invertase (sucrase) enzymes that, for example, are useful in treating disorders such as sucrase-isomaltase deficiency (for example, congenital sucrase-isomaltase deficiency (CSID) or
25 acquired sucrase-isomaltase deficiency (ASID)). The enzymes described herein were engineered to work under conditions found in the stomach and small intestines of a human subject. Transit through the stomach is typically about 90% complete in 75 minutes, and typically complete in about 90-120 minutes. Transit through the small intestine is typically complete in 4-6 hours (120-260 minutes). Thus, the enzymes were engineered so that, under
30 stomach conditions, at least 75-85% of the enzyme survives typical fed state conditions of stomach, *e.g.*, pH 3.5 and pepsin, for 75 minutes, and, under fed state conditions of the small intestine, 50% of the enzyme survives fed state small intestine conditions for 180 minutes.

The enzymes described herein were also engineered to work briefly under conditions found in the fasted state of the stomach and small intestines.

- [0034]** As used herein, the terms “sucrase” and “invertase” are used interchangeably and refer to any enzyme or a functional fragment thereof, that is capable of catalyzing the hydrolysis of sucrose to fructose and glucose, for example, under physiological conditions, *e.g.*, under the physiological conditions of the stomach and/or small intestine of a subject. Sucrases and invertases are also called, acid invertase, alkaline invertase, β -D-fructofuranosidase, β -fructosidase, β -fructofuranosidase, β -fructofuranoside fructohydrolase, β -fructopyranosidase, β -h-fructosidase, β -invertase, EC 3.2.1.26, exo- β -(2,6)-fructofuraosidase, fructosylinvertase, invertin, glucosucrase, saccharase, and sucrose hydrolase, and, unless indicated otherwise, the terms are used interchangeably herein. The term invertase includes variants having one or more amino acid substitutions, deletions, or insertions relative to a wild-type invertase sequence and/or fusion proteins or conjugates including an invertase.
- [0035]** As used herein, the term “functional fragment” of an invertase refers to fragment of a full-length invertase that retains, for example, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% of the enzymatic activity of the corresponding full-length, naturally occurring invertase. Invertase enzymatic activity may be assayed by any method known in the art. Exemplary invertase activity assays are described in Bacon (1955) METHODS IN ENZYMOLOGY 1: 258-262, Lever (1972) ANALYTICAL BIOCHEM. 47: 273-279, U.S. Patent Application Publication No. 2014/0250942, on the world wide web at sigmaaldrich.com/technical-documents/protocols/biology/enzymatic-assay-of-invertase.html, and in **Example 1** herein.
- [0036]** Exemplary invertase enzymes include invertase enzymes derived from *Saccharomyces cerevisiae*. The amino acid sequence of an exemplary wild-type invertase enzyme derived from *Saccharomyces cerevisiae* (including a native signal sequence) is provided as SEQ ID NO: 1, and a nucleotide sequence encoding an exemplary wild-type invertase enzyme derived from *Saccharomyces cerevisiae* is provided as SEQ ID NO: 35. The amino acid sequence of an exemplary invertase enzyme derived from *Saccharomyces cerevisiae* (including a heterologous FAKS signal sequence, but otherwise wild-type) is provided as SEQ ID NO: 2. The amino acid sequence of an exemplary wild-type invertase

enzyme derived from *Saccharomyces cerevisiae* (without a signal sequence) is provided as SEQ ID NO: 3.

[0037] SEQ ID NO: 1 represents wild-type *S. cerevisiae* invertase with a native signal sequence where the signal sequence is underlined, and the mature protein sequence is italicized:

5 MLLQAFLLFLLAGFAAKISASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYN
PNDTVWGTPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGFFNDTIDPR
QRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMT
AAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISIN
10 *PGAPAGGSFNQYFVGFSFNGTHFEAFDNQSRVVDGKDYALQTFFNTDPTYGSALGIAWASN*
WEYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTL
TKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASAS
SFFLDRGNSKVVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVS
TNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK.

15 **[0038]** SEQ ID NO: 2 represents wild-type *S. cerevisiae* invertase with a heterologous FAKS signal sequence, where the signal sequence is underlined, and the mature protein sequence is italicized:

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLFPFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
20 *WHLYFQYNPNDTVWGTPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGF*
FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
WVMFISINPGAPAGGSFNQYFVGFSFNGTHFEAFDNQSRVVDGKDYALQTFFNTDPTYGSA
LGIAWASNWEYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
25 *RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR*
MGFEASASSFFLDRGNSKVVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
FNDGDVVSNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK.

[0039] SEQ ID NO: 3 represents wild-type *S. cerevisiae* invertase without a signal sequence:
30 *SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGTPLFWGHATSDD*
LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKSW
KLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGFSFNG

THFEAFDNQSRVDFGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVFPDLWLWFKGLEDPPEEYLRMGFEASASSFFLDRGNSKVVKFVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNI LELYFNDGDVVSTNTYFMTTGNALGSVNMTT
 5 GVDNLFFYIDKFQVREVK.

[0040] A contemplated invertase enzyme may comprise the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3 having at least one (*e.g.*, one, two, three, four, five, six, seven, eight, nine, or ten, 2-10, 3-10, 4-10, 5-10, 6-10, 7-10, 8-10, 9-10, 2-9, 3-9, 4-9, 5-9, 6-9, 7-9, 8-9, 2-8, 3-8, 4-8, 5-8, 6-8, 7-8, 2-6, 3-6, 4-6, or 5-6) mutation(s) and/or may
 10 comprise an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

[0041] Additional exemplary invertase enzymes can be found on the world wide web at brenda-enzymes.org/enzyme.php?ecno=3.2.1.26&onlyTable=Sequence. Additional exemplary invertase enzymes are described in U.S. Patent Application Publication No.
 15 2014/0250942.

[0042] Among other things, the disclosure provides recombinant mutant invertases that are useful, for example, in treating disorders associated with a reduced ability to digest or absorb dietary sucrose and starches.

[0043] In certain embodiments, the invertase comprises: (i) increased activity at acidic pH (*e.g.*, about pH 3.5, 3.6, 4.0, 4.5, 4.9, 5.0, 5.5, 6.0, or 6.5) relative to a corresponding wild-type invertase; (ii) increased activity at neutral pH (*e.g.*, about pH 7.0) relative to a corresponding wild-type invertase; (iii) increased stability at acidic pH (*e.g.*, about pH 2.5) relative to a corresponding wild-type invertase, (iv) increased thermal stability relative to a corresponding wild-type invertase, or any combination of features (i), (ii), (iii), or (iv).
 20
 25 Exemplary combinations include (i) and (ii); (i) and (iii); (i) and (iv); (ii) and (iii); (ii) and (iv); (i), (ii), and (iii); (i), (ii), and (iv); (i), (iii), and (iv); (ii), (iii), and (iv); and (i), (ii), (iii), and (iv).

[0044] In certain embodiments, the disclosure provides a recombinant mutant *S. cerevisiae* invertase enzyme comprising one or more amino acid substitutions that promote improved
 30 pH stability, thermal stability, and/or stability against proteolytic digestion. A recombinant mutant *S. cerevisiae* invertase enzyme of the disclosure can comprise one or more amino acid substitutions selected from the group consisting of:

- (a) a substitution of a T residue at a position corresponding to position 51 of SEQ ID NO: 3;
- (b) a substitution of an F residue at a position corresponding to position 83 of SEQ ID NO: 3;
- 5 (c) a substitution of an E residue at a position corresponding to position 293 of SEQ ID NO: 3;
- (d) a substitution of an N residue at a position corresponding to position 340 of SEQ ID NO: 3;
- (e) a substitution of a P residue at a position corresponding to position 390 of SEQ ID NO: 3; and
- 10 (f) a substitution of an A residue at a position corresponding to position 412 of SEQ ID NO: 3; or a combination of any of the foregoing substitutions,

and is catalytically active to digest sucrose into glucose and fructose under physiological conditions of the stomach and/or small intestine. For example, in one embodiment, the invertase includes a combination of four of the foregoing substitutions. In another embodiment, the invertase includes a combination of five of the foregoing substitutions. In another embodiment, the invertase comprises all six of the foregoing substitutions.

15

[0045] In certain embodiments of the foregoing, in the invertase,

- (a) the T residue at a position corresponding to position 51 of SEQ ID NO: 3 is substituted by L (T51L);
- 20 (b) the F residue at a position corresponding to position 83 of SEQ ID NO: 3 is substituted by Y (F83Y);
- (c) the E residue at a position corresponding to position 293 of SEQ ID NO: 3 is substituted by Q (E293Q);
- 25 (d) the N residue at a position corresponding to position 340 of SEQ ID NO: 3 is substituted by A (N340A);
- (e) the P residue at a position corresponding to position 390 of SEQ ID NO: 3 is substituted by A (P390A);
- (f) the A residue at a position corresponding to position 412 of SEQ ID NO: 3 is substituted by N (A412N); or
- 30

the invertase includes a combination of any of the foregoing substitutions. For example, in one embodiment, the invertase includes a combination of four of the foregoing substitutions.

In another embodiment, the invertase includes a combination of five of the foregoing substitutions. In another embodiment, the invertase comprises all six of the foregoing substitutions.

[0046] Depending upon the circumstances, the invertase can include additional substitutions.

- 5 For example, the invertase can further include a substitution at a D residue at a position corresponding to position 183 of SEQ ID NO: 3, *e.g.*, wherein the D residue at a position corresponding to position 183 of SEQ ID NO: 3 is substituted by an N (D183N). The invertase can include a substitution at an E residue at a position corresponding to position 196 of SEQ ID NO: 3, *e.g.*, wherein the E residue at a position corresponding to position 196 of
10 SEQ ID NO: 3 is substituted by an H (E196H).

[0047] In certain embodiments, the recombinant mutant invertase comprises at least one (*e.g.*, one, two, three, four, five, six, seven, eight, nine, or ten, 2-10, 3-10, 4-10, 5-10, 6-10, 7-10, 8-10, 9-10, 2-9, 3-9, 4-9, 5-9, 6-9, 7-9, 8-9, 2-8, 3-8, 4-8, 5-8, 6-8, 7-8, 2-6, 3-6, 4-6, or 5-6) mutation(s) at a position corresponding to wild-type *S. cerevisiae* invertase of SEQ ID NO:

- 15 2. In certain embodiments, at least one mutation is selected from a substitution of a D residue at a position corresponding to position 122 of SEQ ID NO: 2; a substitution of a T residue at a position corresponding to position 140 of SEQ ID NO: 2; a substitution of an E residue at a position corresponding to position 156 of SEQ ID NO: 2; a substitution of an F residue at a position corresponding to position 172 of SEQ ID NO: 2; a substitution of a V residue at a
20 position corresponding to position 178 of SEQ ID NO: 2; a substitution of a K residue at a position corresponding to position 259 of SEQ ID NO: 2; a substitution of a D residue at a position corresponding to position 272 of SEQ ID NO: 2; a substitution of a K residue at a position corresponding to position 277 of SEQ ID NO: 2; a substitution of an E residue at a position corresponding to position 285 of SEQ ID NO: 2; a substitution of an A residue at a
25 position corresponding to position 378 of SEQ ID NO: 2; a substitution of an E residue at a position corresponding to position 382 of SEQ ID NO: 2; a substitution of a V residue at a position corresponding to position 399 of SEQ ID NO: 2; a substitution of a G residue at a position corresponding to position 431 of SEQ ID NO: 2; a substitution of a V residue at a position corresponding to position 477 of SEQ ID NO: 2; a substitution of a P residue at a
30 position corresponding to position 479 of SEQ ID NO: 2; a substitution of an A residue at a position corresponding to position 501 of SEQ ID NO: 2; a substitution of an L residue at a position corresponding to position 549 of SEQ ID NO: 2; a substitution of a V residue at a position corresponding to position 581 of SEQ ID NO: 2; a substitution of a G residue at a

position corresponding to position 586 of SEQ ID NO: 2; or a substitution of an N residue at a position corresponding to position 589 of SEQ ID NO: 2. In certain embodiments, the recombinant mutant invertase comprises a combination of any of the foregoing substitutions. SEQ ID NO: 2 is the amino acid sequence of an exemplary invertase enzyme derived from
5 *Saccharomyces cerevisiae*, that is wild-type but for the inclusion of a heterologous FAKS signal sequence.

[0048] Also contemplated herein are invertases comprising the foregoing substitutions located at the corresponding positions in SEQ ID NO: 1 (wild-type invertase enzyme derived from *Saccharomyces cerevisiae* including a native signal sequence) and in SEQ ID NO: 3
10 (wild-type invertase enzyme derived from *Saccharomyces cerevisiae* without a signal sequence) (see **TABLE 1** below).

[0049] In certain embodiments, the invertase comprises: a substitution of a D residue at a position corresponding to position 52 of SEQ ID NO: 1; a substitution of a T residue at a position corresponding to position 70 of SEQ ID NO: 1; a substitution of an E residue at a
15 position corresponding to position 86 of SEQ ID NO: 1; a substitution of an F residue at a position corresponding to position 102 of SEQ ID NO: 1; a substitution of a V residue at a position corresponding to position 108 of SEQ ID NO: 1; a substitution of a K residue at a position corresponding to position 189 of SEQ ID NO: 1; a substitution of a D residue at a position corresponding to position 202 of SEQ ID NO: 1; a substitution of a K residue at a
20 position corresponding to position 207 of SEQ ID NO: 1; a substitution of an E residue at a position corresponding to position 215 of SEQ ID NO: 1; a substitution of an A residue at a position corresponding to position 308 of SEQ ID NO: 1; a substitution of an E residue at a position corresponding to position 312 of SEQ ID NO: 1; a substitution of a V residue at a position corresponding to position 329 of SEQ ID NO: 1; a substitution of a G residue at a
25 position corresponding to position 361 of SEQ ID NO: 1; a substitution of a V residue at a position corresponding to position 407 of SEQ ID NO: 1; a substitution of a P residue at a position corresponding to position 409 of SEQ ID NO: 1; a substitution of an A residue at a position corresponding to position 431 of SEQ ID NO: 1; a substitution of a L residue at a position corresponding to position 479 of SEQ ID NO: 1; a substitution of a V residue at a
30 position corresponding to position 511 of SEQ ID NO: 1; a substitution of a G residue at a position corresponding to position 516 of SEQ ID NO: 1; or a substitution of an N residue at

a position corresponding to position 519 of SEQ ID NO: 1. In certain embodiments, the recombinant mutant invertase comprises a combination of any of the foregoing substitutions.

- [0050]** In certain embodiments, the invertase comprises: a substitution of a D residue at a position corresponding to position 33 of SEQ ID NO: 3; a substitution of a T residue at a position corresponding to position 51 of SEQ ID NO: 3; a substitution of an E residue at a position corresponding to position 67 of SEQ ID NO: 3; a substitution of an F residue at a position corresponding to position 83 of SEQ ID NO: 3; a substitution of a V residue at a position corresponding to position 89 of SEQ ID NO: 3; a substitution of a K residue at a position corresponding to position 170 of SEQ ID NO: 3; a substitution of a D residue at a position corresponding to position 183 of SEQ ID NO: 3; a substitution of a K residue at a position corresponding to position 188 of SEQ ID NO: 3; a substitution of an E residue at a position corresponding to position 196 of SEQ ID NO: 3; a substitution of an A residue at a position corresponding to position 289 of SEQ ID NO: 3; a substitution of an E residue at a position corresponding to position 293 of SEQ ID NO: 3; a substitution of a V residue at a position corresponding to position 310 of SEQ ID NO: 3; a substitution of a G residue at a position corresponding to position 342 of SEQ ID NO: 3; a substitution of a V residue at a position corresponding to position 388 of SEQ ID NO: 3; a substitution of a P residue at a position corresponding to position 390 of SEQ ID NO: 3; a substitution of an A residue at a position corresponding to position 412 of SEQ ID NO: 3; a substitution of a L residue at a position corresponding to position 460 of SEQ ID NO: 3; a substitution of a V residue at a position corresponding to position 492 of SEQ ID NO: 3; a substitution of a G residue at a position corresponding to position 497 of SEQ ID NO: 3; or a substitution of an N residue at a position corresponding to position 500 of SEQ ID NO: 3. In certain embodiments, the recombinant mutant invertase comprises a combination of any of the foregoing substitutions.
- [0051]** In certain embodiments, the D residue at a position corresponding to position 122 of SEQ ID NO: 2 is substituted by E (D122E); the T residue at a position corresponding to position 140 of SEQ ID NO: 2 is substituted by L (T140L); the E residue at a position corresponding to position 156 of SEQ ID NO: 2 is substituted by Q (E156Q); the F residue at a position corresponding to position 172 of SEQ ID NO: 2 is substituted by Y (F172Y); the V residue at a position corresponding to position 178 of SEQ ID NO: 2 is substituted by I (V178I); the K residue at a position corresponding to position 259 of SEQ ID NO: 2 is substituted by L (K259L); the D residue at a position corresponding to position 272 of SEQ

- ID NO: 2 is substituted by N (D272N); the K residue at a position corresponding to position 277 of SEQ ID NO: 2 is substituted by T (K277T); the E residue at a position corresponding to position 285 of SEQ ID NO: 2 is substituted by H (E285H); the A residue at a position corresponding to position 378 of SEQ ID NO: 2 is substituted by T (A378T); the E residue at a position corresponding to position 382 of SEQ ID NO: 2 is substituted by Q (E382Q); the V residue at a position corresponding to position 399 of SEQ ID NO: 2 is substituted by A (V399A); the G residue at a position corresponding to position 431 of SEQ ID NO: 2 is substituted by R (G431R); the V residue at a position corresponding to position 477 of SEQ ID NO: 2 is substituted by D (V477D); the P residue at a position corresponding to position 479 of SEQ ID NO: 2 is substituted by A (P479A); the A residue at a position corresponding to position 501 of SEQ ID NO: 2 is substituted by N (A501N); the L residue at a position corresponding to position 549 of SEQ ID NO: 2 is substituted by I (L549I); the V residue at a position corresponding to position 581 of SEQ ID NO: 2 is substituted by I (V581I); the G residue at a position corresponding to position 586 of SEQ ID NO: 2 is substituted by S (G586S); or the N residue at a position corresponding to position 589 of SEQ ID NO: 2 is substituted by K (N589K); or the invertase comprises a combination of any of the foregoing substitutions. SEQ ID NO: 2 is the amino acid sequence of an exemplary invertase enzyme derived from *Saccharomyces cerevisiae*, that is wild-type but for the inclusion of a heterologous FAKS signal sequence.
- 20 **[0052]** Also contemplated herein are invertases comprising the foregoing substitutions located at the corresponding positions in SEQ ID NO: 1 (wild-type invertase enzyme derived from *Saccharomyces cerevisiae* including a native signal sequence) and SEQ ID NO: 3 (wild-type invertase enzyme derived from *Saccharomyces cerevisiae* without a signal sequence) (see **TABLE 1** below).
- 25 **[0053]** In certain embodiments, in the invertase: the D residue at a position corresponding to position 52 of SEQ ID NO: 1 is substituted by E (D52E); the A residue at a position corresponding to position 53 of SEQ ID NO: 1 is substituted by G (A53G); the T residue at a position corresponding to position 70 of SEQ ID NO: 1 is substituted by L (T70L); the E residue at a position corresponding to position 86 of SEQ ID NO: 1 is substituted by Q (E86Q); the F residue at a position corresponding to position 102 of SEQ ID NO: 1 is substituted by Y (F102Y); the V residue at a position corresponding to position 108 of SEQ ID NO: 1 is substituted by I (V108I); the K residue at a position corresponding to position 189 of SEQ ID NO: 1 is substituted by L (K189L); the D residue at a position corresponding

to position 202 of SEQ ID NO: 1 is substituted by N (D202N); the K residue at a position corresponding to position 207 of SEQ ID NO: 1 is substituted by T (K207T); the E residue at a position corresponding to position 215 of SEQ ID NO: 1 is substituted by H (E215H); the A residue at a position corresponding to position 308 of SEQ ID NO: 1 is substituted by T (A308T); the E residue at a position corresponding to position 312 of SEQ ID NO: 1 is substituted by Q (E312Q); the V residue at a position corresponding to position 329 of SEQ ID NO: 1 is substituted by A (V329A); the G residue at a position corresponding to position 361 of SEQ ID NO: 1 is substituted by R (G361R); the V residue at a position corresponding to position 407 of SEQ ID NO: 1 is substituted by D (V407D); the P residue at a position corresponding to position 409 of SEQ ID NO: 1 is substituted by A (P409A); the A residue at a position corresponding to position 431 of SEQ ID NO: 1 is substituted by N (A431N); the L residue at a position corresponding to position 479 of SEQ ID NO: 1 is substituted by I (L479I); the V residue at a position corresponding to position 511 of SEQ ID NO: 1 is substituted by I (V511I); the N residue at a position corresponding to position 519 of SEQ ID NO: 1 is substituted by K (N519K); or the invertase comprises a combination of any of the foregoing substitutions.

[0054] In certain embodiments, in the invertase: the D residue at a position corresponding to position 33 of SEQ ID NO: 3 is substituted by E (D33E); the T residue at a position corresponding to position 51 of SEQ ID NO: 3 is substituted by L (T51L); the E residue at a position corresponding to position 67 of SEQ ID NO: 3 is substituted by Q (E67Q); the F residue at a position corresponding to position 83 of SEQ ID NO: 3 is substituted by Y (F83Y); the V residue at a position corresponding to position 89 of SEQ ID NO: 3 is substituted by I (V89I); the K residue at a position corresponding to position 170 of SEQ ID NO: 3 is substituted by L (K170L); the D residue at a position corresponding to position 183 of SEQ ID NO: 3 is substituted by N (D183N); the K residue at a position corresponding to position 188 of SEQ ID NO: 3 is substituted by T (K188T); the E residue at a position corresponding to position 196 of SEQ ID NO: 3 is substituted by H (E196H); the A residue at a position corresponding to position 289 of SEQ ID NO: 3 is substituted by T (A289T); the E residue at a position corresponding to position 293 of SEQ ID NO: 3 is substituted by Q (E293Q); the V residue at a position corresponding to position 310 of SEQ ID NO: 3 is substituted by A (V310A); the G residue at a position corresponding to position 342 of SEQ ID NO: 3 is substituted by R (G342R); the V residue at a position corresponding to position 388 of SEQ ID NO: 3 is substituted by D (V388D); the P residue at a position corresponding

to position 390 of SEQ ID NO: 3 is substituted by A (P390A); the A residue at a position corresponding to position 412 of SEQ ID NO: 3 is substituted by N (A412N); the L residue at a position corresponding to position 460 of SEQ ID NO: 3 is substituted by I (L460I); the V residue at a position corresponding to position 492 of SEQ ID NO: 3 is substituted by I (V492I); the G residue at a position corresponding to position 497 of SEQ ID NO: 3 is substituted by S (G497S); the N residue at a position corresponding to position 500 of SEQ ID NO: 3 is substituted by K (N500K); or the invertase comprises a combination of any of the foregoing substitutions.

[0055] In certain embodiments, one or more mutations may be conservative substitutions relative to wild-type *S. cerevisiae* invertase of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3, whereas in certain other embodiments, one or more mutations may be non-conservative substitutions relative to wild-type *S. cerevisiae* invertase of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. As used herein, the term “conservative substitution” refers to a substitution with a structurally and/or functionally similar amino acid.

[0056] In certain embodiments, the substitution of a given amino acid is with a hydrophobic amino acid (*e.g.*, A, I, L, M, or V), a positively charged amino acid (*e.g.*, K, R, or H), a negatively charged amino acid (*e.g.*, D or E), a polar neutral amino acid (*e.g.*, N, C, Q, S, or T), an aromatic amino acid (*e.g.*, F, Y, or W) or a bulkier amino acid based on side chain volume or a smaller amino acid based on side chain volume. Conservative substitutions may also be defined by the BLAST (Basic Local Alignment Search Tool) algorithm, the BLOSUM substitution matrix (*e.g.*, BLOSUM 62 matrix), or the PAM substitution matrix (*e.g.*, the PAM 250 matrix). Non-conservative substitutions are amino acid substitutions that are not conservative substitutions.

[0057] In certain embodiments, the recombinant mutant invertase enzyme comprises one or more substitutions from **TABLE 1**, wherein the positions of the substitutions are shown relative to SEQ ID NO: 1 (wild-type *Saccharomyces cerevisiae* invertase enzyme, including a native signal sequence) or SEQ ID NO: 2 (*Saccharomyces cerevisiae* invertase enzyme including a heterologous FAKS signal sequence, but otherwise wild-type) or SEQ ID NO: 3 (wild-type *Saccharomyces cerevisiae* invertase enzyme without a signal sequence). The rows in **TABLE 1** correspond to a substitution at the same position, but the numbering is based on whether the protein contains a signal sequence (*e.g.*, SEQ ID NO: 1 or SEQ ID NO: 2) or does not contain a signal sequence (*e.g.*, SEQ ID NO: 3).

TABLE 1

Position relative to SEQ ID NO: 2	Position relative to SEQ ID NO: 1	Position relative to SEQ ID NO: 3	Exemplary Substitutions
E120	E50	E31	A
D122	D52	D33	E
A123	A53	A34	G
T140	T70	T51	L, Q
F143	F73	F54	Y
E156	E86	E67	Q
F172	F102	F83	Y
V178	V108	V89	I
K259	K189	K170	L
D272	D202	D183	N
K277	K207	K188	T
E285	E215	E196	H
L359	L289	L270	F
T365	T295	T276	S
A378	A308	A289	T
E382	E312	E293	Q
N390	N320	N301	D
E415	E345	E326	S
L416	L346	L327	Q
N429	N359	N340	A
L459	L389	L370	F
T470	T400	T381	S
S476	S406	S387	A, N
P479	P409	P390	A
A501	A431	A412	N
N512	N442	N423	G
K514	K444	K425	P
K519	K449	K430	R
S537	S467	S448	D

Position relative to SEQ ID NO: 2	Position relative to SEQ ID NO: 1	Position relative to SEQ ID NO: 3	Exemplary Substitutions
L549	L479	L460	I
T573	T503	T484	S
V581	V511	V492	I
G586	G516	G497	S
N589	N519	N500	K
F596	F526	F507	I
Q597	Q527	Q508	E

[0058] In certain embodiments, the recombinant mutant invertase comprises at least one (e.g., at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, or at least eleven) mutation(s) selected from

5 **TABLE 1.**

[0059] In certain embodiments, the recombinant mutant invertase comprises at least three substitutions selected from: the F172Y, N429A, and P479A substitutions relative to SEQ ID NO: 2; the E120A, D122E, and P479A substitutions relative to SEQ ID NO: 2; the K259L, S476N, and K514P substitutions relative to SEQ ID NO: 2; the T140L, E156Q, and L549I substitutions relative to SEQ ID NO: 2; the S476A, P479A, and Q597E substitutions relative to SEQ ID NO: 2; the K277T, E415S, and V581I substitutions relative to SEQ ID NO: 2; the A123G, T140L, and F143Y substitutions relative to SEQ ID NO: 2; the N390D, S476N, and K519R substitutions relative to SEQ ID NO: 2; the E382Q, T573S, and V581I substitutions relative to SEQ ID NO: 2; the D122E, T470S, and S476N substitutions relative to SEQ ID NO: 2; the T140Q, P479A, and K514P substitutions relative to SEQ ID NO: 2; the E120A, V178I, and L549I substitutions relative to SEQ ID NO: 2; the E382Q, S537D, and N589K substitutions relative to SEQ ID NO: 2; the T140L, G586S, and F596I substitutions relative to SEQ ID NO: 2; or the E382Q, L459F, and K519R substitutions relative to SEQ ID NO: 2. It is understood, the recombinant mutant invertase can comprise the same groups of three substitutions noted above with reference to SEQ ID NO: 2, as well as invertases containing the same groups of three substitutions at the corresponding locations SEQ ID NO: 1 or SEQ ID NO: 3 (see **TABLE 1**).

[0060] In certain embodiments, the recombinant mutant invertase comprises at least three substitutions selected from: the F83Y, N340A, and P390A substitutions relative to SEQ ID

NO: 3; the E31A, D33E, and P390A substitutions relative to SEQ ID NO: 3; the K170L, S387N, and K425P substitutions relative to SEQ ID NO: 3; the T51L, E67Q, and L460I substitutions relative to SEQ ID NO: 3; the S387A, P390A, and Q508E substitutions relative to SEQ ID NO: 3; the K188T, E326S, and V492I substitutions relative to SEQ ID NO: 3; the A34G, T51L, and F54Y substitutions relative to SEQ ID NO: 3; the N301D, S387N, and K430R substitutions relative to SEQ ID NO: 3; the E293Q, T484S, and V492I substitutions relative to SEQ ID NO: 3; the D33E, T381S, and S387N substitutions relative to SEQ ID NO: 3; the T51Q, P390A, and K425P substitutions relative to SEQ ID NO: 3; the E31A, V89I, and L460I substitutions relative to SEQ ID NO: 3; the E293Q, S448D, and N500K substitutions relative to SEQ ID NO: 3; the T51L, G497S, and F507I substitutions relative to SEQ ID NO: 3; or the E293Q, L370F, and K430R substitutions relative to SEQ ID NO: 3.

[0061] In certain embodiments, the invertase comprises the following substitutions at positions corresponding to the amino acids set forth in SEQ ID NO: 1, including: the F102Y, N359A, and P409A substitutions relative to SEQ ID NO: 1; the E50A, D52E, and P409A substitutions relative to SEQ ID NO: 1; the K189L, S406N, and K444P substitutions relative to SEQ ID NO: 1; the T70L, E86Q, and L479I substitutions relative to SEQ ID NO: 1; the S406A, P409A, and Q527E substitutions relative to SEQ ID NO: 1; the K207T, E345S, and V511I substitutions relative to SEQ ID NO: 1; the A53G, T70L, and F73Y substitutions relative to SEQ ID NO: 1; the N320D, S406N, and K449R substitutions relative to SEQ ID NO: 1; the E312Q, T503S, and V511I substitutions relative to SEQ ID NO: 1; the D52E, T400S, and S406N substitutions relative to SEQ ID NO: 1; the T70Q, P409A, and K444P substitutions relative to SEQ ID NO: 1; the E50A, V108I, and L479I substitutions relative to SEQ ID NO: 1; the E312Q, S467D, and N519K substitutions relative to SEQ ID NO: 1; the T70L, G516S, and F526I substitutions relative to SEQ ID NO: 1; or the E312Q, L389F, and K449R substitutions relative to SEQ ID NO: 1.

[0062] In certain embodiments, the recombinant mutant invertase comprises at least three substitutions selected from: the E31A, D33E, and P390A substitutions relative to SEQ ID NO: 3; the T51L, E67Q, and L460I substitutions relative to SEQ ID NO: 3; the K170L, S387N, and K425P substitutions relative to SEQ ID NO: 3; the S387A, P390A, and Q508E substitutions relative to SEQ ID NO: 3; the K188T, E326S, and V492I substitutions relative to SEQ ID NO: 3; the A34G, T51L, and F54Y substitutions relative to SEQ ID NO: 3; the N301D, S387N, and K430R substitutions relative to SEQ ID NO: 3; the E293Q, T484S, and V492I substitutions relative to SEQ ID NO: 3; the D33E, T381S, and S387N substitutions

relative to SEQ ID NO: 3; the T51L, P390A, and K425P substitutions relative to SEQ ID NO: 3; the F83Y, N340A, and P390A substitutions relative to SEQ ID NO: 3; the E31A, V89I, and L460I substitutions relative to SEQ ID NO: 3; the E293Q, S448D, and N500K substitutions relative to SEQ ID NO: 3; the T51L, G497S, and F507I substitutions relative to SEQ ID NO: 3; or the E293Q, L370F, and K430R substitutions relative to SEQ ID NO: 3.

5 [0063] In certain embodiments, the recombinant mutant invertase comprises F172Y, N429A, P479A, and T140L substitutions relative to SEQ ID NO: 2. In certain embodiments, the recombinant mutant invertase comprises F172Y, N429A, P479A, T140L, and D272N substitutions relative to SEQ ID NO: 2. In certain embodiments, the recombinant mutant
10 invertase comprises F172Y, N429A, P479A, T140L, E285H substitutions relative to SEQ ID NO: 2. In certain embodiments, the recombinant mutant invertase comprises F172Y, N429A, P479A, T140L, E382Q substitutions relative to SEQ ID NO: 2.

[0064] In certain embodiments, the recombinant mutant invertase comprises F83Y, N340A, P390A, and T51L substitutions relative to SEQ ID NO: 3. In certain embodiments, the
15 recombinant mutant invertase comprises F83Y, N340A, P390A, and T51L, and D183N substitutions relative to SEQ ID NO: 3. In certain embodiments, the recombinant mutant invertase comprises F83Y, N340A, P390A, and T51L, E196H substitutions relative to SEQ ID NO: 3. In certain embodiments, the recombinant mutant invertase comprises F83Y, N340A, P390A, and T51L, E293Q substitutions relative to SEQ ID NO: 3.

20 [0065] In certain embodiments, the recombinant mutant invertase comprises N429A, P479A, and T140L substitutions relative to SEQ ID NO: 2. In certain embodiments, the recombinant mutant invertase comprises F172Y, P479A, and T140L substitutions relative to SEQ ID NO: 2. In certain embodiments, the recombinant mutant invertase comprises F172Y, N429A, and T140L substitutions relative to SEQ ID NO: 2.

25 [0066] In certain embodiments, the recombinant mutant invertase comprises N340A, P390A, and T51L substitutions relative to SEQ ID NO: 3. In certain embodiments, the recombinant mutant invertase comprises F83Y, P390A, and T51L substitutions relative to SEQ ID NO: 3. In certain embodiments, the recombinant mutant invertase comprises F83Y, N340A, and T51L substitutions relative to SEQ ID NO: 3.

30 [0067] In certain embodiments, the recombinant mutant invertase comprises N429A, P479A, T140L, E382Q, and A501N substitutions relative to SEQ ID NO: 2. In certain embodiments, the recombinant mutant invertase comprises F172Y, P479A, T140L, E382Q, and A501N substitutions relative to SEQ ID NO: 2. In certain embodiments, the recombinant mutant

invertase comprises F172Y, N429A, T140L, E382Q, and A501N substitutions relative to SEQ ID NO: 2. In certain embodiments, the recombinant mutant invertase comprises F172Y, N429A, P479A, E382Q, and A501N substitutions relative to SEQ ID NO: 2. In certain embodiments, the recombinant mutant invertase comprises F172Y, N429A, P479A, T140L, and A501N substitutions relative to SEQ ID NO: 2. In certain embodiments, the recombinant mutant invertase comprises F172Y, N429A, P479A, T140L, and E382Q, and substitutions relative to SEQ ID NO: 2.

[0068] In certain embodiments, the recombinant mutant invertase comprises N340A, P390A, T51L, E293Q, and A412N substitutions relative to SEQ ID NO: 3. In certain embodiments, the recombinant mutant invertase comprises F83Y, P390A, T51L, E293Q, and A412N substitutions relative to SEQ ID NO: 3. In certain embodiments, the recombinant mutant invertase comprises F83Y, N340A, T51L, E293Q, and A412N substitutions relative to SEQ ID NO: 3. In certain embodiments, the recombinant mutant invertase comprises F83Y, N340A, P390A, E293Q, and A412N substitutions relative to SEQ ID NO: 3. In certain embodiments, the recombinant mutant invertase comprises F83Y, N340A, P390A, T51L, and A412N substitutions relative to SEQ ID NO: 3. In certain embodiments, the recombinant mutant invertase comprises F83Y, N340A, P390A, T51L, and E293Q, and substitutions relative to SEQ ID NO: 3.

[0069] In certain embodiments, the recombinant mutant invertase comprises at least six substitutions selected from: the F172Y, N429A, P479A, T140L, E382Q, and A501N substitutions relative to SEQ ID NO: 2; the F172Y, N429A, P479A, T140L, D272N, and E285H substitutions relative to SEQ ID NO: 2; the F172Y, N429A, P479A, T140L, D272N, and L549I substitutions relative to SEQ ID NO: 2; the F172Y, N429A, P479A, T140L, D272N, and V581I substitutions relative to SEQ ID NO: 2; the F172Y, N429A, P479A, T140L, E285H, and V581I substitutions relative to SEQ ID NO: 2; the F172Y, N429A, P479A, T140L, E382Q, and A123G substitutions relative to SEQ ID NO: 2; the F172Y, N429A, P479A, T140L, E382Q, and N512G substitutions relative to SEQ ID NO: 2; the F172Y, N429A, P479A, T140L, E382Q, and V178I substitutions relative to SEQ ID NO: 2; and the F172Y, N429A, P479A, T140L, E382Q, and N390D substitutions relative to SEQ ID NO: 2. In certain embodiments, the recombinant mutant invertase comprises the F172Y, N429A, P479A, T140L, E382Q, A501N, and D272N substitutions relative to SEQ ID NO: 2. In certain embodiments, the recombinant mutant invertase comprises the F172Y, N429A, P479A, T140L, E382Q, A501N, D272N, and E285H substitutions relative to SEQ ID NO: 2.

[0070] In certain embodiments, the recombinant mutant invertase comprises at least six substitutions selected from: the F83Y, N340A, P390A, T51L, E293Q, and A412N substitutions relative to SEQ ID NO: 3; the F83Y, N340A, P390A, T51L, D183N, and E196H substitutions relative to SEQ ID NO: 3; the F83Y, N340A, P390A, T51L, D183N, and L460I substitutions relative to SEQ ID NO: 3; the F83Y, N340A, P390A, T51L, D183N, and V492I substitutions relative to SEQ ID NO: 3; the F83Y, N340A, P390A, T51L, E196H, and V492I substitutions relative to SEQ ID NO: 3; the F83Y, N340A, P390A, T51L, E293Q, and A34G substitutions relative to SEQ ID NO: 3; the F83Y, N340A, P390A, T51L, E293Q, and N423G substitutions relative to SEQ ID NO: 3; the F83Y, N340A, P390A, T51L, E293Q, and V89I substitutions relative to SEQ ID NO: 3; and the F83Y, N340A, P390A, T51L, E293Q, and N301D substitutions relative to SEQ ID NO: 3. In certain embodiments, the recombinant mutant invertase comprises the F83Y, N340A, P390A, T51L, E293Q, A412N, and D183N substitutions relative to SEQ ID NO: 3. In certain embodiments, the recombinant mutant invertase comprises the F83Y, N340A, P390A, T51L, E293Q, A412N, D183N, and E196H substitutions relative to SEQ ID NO: 3.

[0071] In certain embodiments, the recombinant mutant invertase comprises the E120A, D122E, and P479A substitutions (relative to SEQ ID NO: 2). For example, the recombinant mutant invertase may comprise SEQ ID NO: 4, also referred to as V6 herein. In certain embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

[0072] SEQ ID NO: 4

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDAKEAK
 WHLYFQYNPNDTVWGTPLEFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSEFNGTHFEAFDNQSRVVDFGKDYYALQTFNTDPTYGSA
 LGIAWASNWEYSFAVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVFKVKNPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK

[0073] In certain embodiments, the recombinant mutant invertase comprises the T140L, E156Q, and L549I substitutions relative to SEQ ID NO: 2. For example, the recombinant

mutant invertase may comprise SEQ ID NO: 5, also referred to as V9 herein. In certain embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

[0074] SEQ ID NO: 5

MRFPSIFTAVLFAASSALAAPVNTTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 5 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNNDTVWGLPLFWGHATSDDLTHWQDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWE
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSGFNTHFEAFDNQSRVVDFGKDYYALQTFNTDPTYGSA
 10 LGIAWASNWEYSFAVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPPEEYLR
 MGFEASASSFFLDRGNSKVFKVKNPYFTNRMSVNNQPFKSENDLSYYKVYGIILDQNIILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK.

[0075] In certain embodiments, the recombinant mutant invertase comprises the S476A,
 15 P479A, and Q597E substitutions relative to SEQ ID NO: 2. For example, the recombinant
 mutant invertase may comprise SEQ ID NO: 6, also referred to as V14 herein. In certain
 embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

[0076] SEQ ID NO: 6

MRFPSIFTAVLFAASSALAAPVNTTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 20 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNNDTVWGTPPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWE
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSGFNTHFEAFDNQSRVVDFGKDYYALQTFNTDPTYGSA
 25 LGIAWASNWEYSFAVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKAVFADLSLWFKGLEDPPEEYLR
 MGFEASASSFFLDRGNSKVFKVKNPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNIILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKF~~E~~VREVK.

[0077] In certain embodiments, the recombinant mutant invertase comprises the K259L,
 30 S476N, and K514P substitutions (relative to SEQ ID NO: 2). For example, the recombinant
 mutant invertase may comprise SEQ ID NO: 7, also referred to as V22 herein. In certain
 embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

[0078] SEQ ID NO: 7

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNDTVWGTPFLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 5 PSQKWIMTAALSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSGFNHGFHFAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWEYSASFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKNVFPDLSLWFKGLEDPPEEYLR
 MGFEASASSFFLDRGNSPVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNIILELY
 10 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK.

[0079] In certain embodiments, the recombinant mutant invertase comprises the K277T, E415S, and V581I substitutions relative to SEQ ID NO: 2. For example, the recombinant mutant invertase may comprise SEQ ID NO: 8, also referred to as V25 herein. In certain embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

15 **[0080]** SEQ ID NO: 8

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNDTVWGTPFLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 20 PSQKWIMTAAKSQDYKIEIYSSDDLKSWTLESANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSGFNHGFHFAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWEYSASFVPTNPWRSSMSLVRKFSLNTEYQANPETSLINLKAEPILNISNAGPWS
 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPPEEYLR
 MGFEASASSFFLDRGNSKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNIILELY
 25 FNDGDVVSTNTYFMTTGNALGSINMTTGVDNLFYIDKFQVREVK.

[0081] In certain embodiments, the recombinant mutant invertase comprises the A123G, T140L, and F143Y substitutions relative to SEQ ID NO: 2. For example, the recombinant mutant invertase may comprise SEQ ID NO: 9, also referred to as V32 herein. In certain embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

30 **[0082]** SEQ ID NO: 9

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDGK
 WHLYFQYNPNDTVWGLPLYWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGF

FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWE
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSFNGTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWEYSFAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 5 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVKNPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK.

[0083] In certain embodiments, the recombinant mutant invertase comprises the N390D,
 S476N, and K519R substitutions relative to SEQ ID NO: 2. For example, the recombinant
 10 mutant invertase may comprise SEQ ID NO: 10, also referred to as V38 herein. In certain
 embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

[0084] SEQ ID NO: 10

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLFPFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 15 WHLYFQYNPNDTVWGTPFLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWE
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSFNGTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWEYSFAFVPTDPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 20 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKNVFPDLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVRENPNYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK

[0085] In certain embodiments, the recombinant mutant invertase comprises the E382Q,
 T573S, and V581I substitutions relative to SEQ ID NO: 2. For example, the recombinant
 25 mutant invertase may comprise SEQ ID NO: 11, also referred to as V60 herein. In certain
 embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

[0086] SEQ ID NO: 11

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLFPFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 30 WHLYFQYNPNDTVWGTPFLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWE
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSFNGTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA

LGIAWASNWQYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR
MGFEASASSFFLDRGNSKVFKVKNPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
FNDGDVVSTNTYFMS~~T~~GNALGSINMTTGVDNLFYIDKFQVREVK.

- 5 [0087] In certain embodiments, the recombinant mutant invertase comprises the D122E, T470S, and S476N substitutions relative to SEQ ID NO: 2. For example, the recombinant mutant invertase may comprise SEQ ID NO: 12, also referred to as V63 herein. In certain embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

[0088] SEQ ID NO: 12

- 10 MRFPSIFTAVLFAASSALAAPVNTTTTETAQIPAEAVIGYSDLEGDFDVAVLFPFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPKNKGWMNDPNGLWYDEK~~EAK~~
WHLYFQYNPNDTVWGTPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
15 WVMFISINPGAPAGGSFNQYFVGSGFNGTHFEAFDNQSRVVDFGKDYYALQTTFFNTDPTYGSA
LGIAWASNWEYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNT~~S~~QTISK~~N~~VFPDLSLWFKGLEDPEEYLR
MGFEASASSFFLDRGNSKVFKVKNPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK.

- 20 [0089] In certain embodiments, the recombinant mutant invertase comprises the T140Q, P479A, and K514P substitutions relative to SEQ ID NO: 2. For example, the recombinant mutant invertase may comprise SEQ ID NO: 13, also referred to as V70 herein. In certain embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

[0090] SEQ ID NO: 13

- 25 MRFPSIFTAVLFAASSALAAPVNTTTTETAQIPAEAVIGYSDLEGDFDVAVLFPFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPKNKGWMNDPNGLWYDEK~~DAK~~
WHLYFQYNPNDTVWG~~Q~~PLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
30 WVMFISINPGAPAGGSFNQYFVGSGFNGTHFEAFDNQSRVVDFGKDYYALQTTFFNTDPTYGSA
LGIAWASNWEYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVF~~A~~DLSLWFKGLEDPEEYLR

MGFEASASSFFLDGRGNS**P**VKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK.

[0091] In certain embodiments, the recombinant mutant invertase comprises the F172Y,
N429A, and P479A substitutions (relative to SEQ ID NO: 2). For example, the recombinant
5 mutant invertase may comprise SEQ ID NO: 14, also referred to as V78 herein. In certain
embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

[0092] SEQ ID NO: 14

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPKNKGWMNDPNGLWYDEKDAK
10 WHLYFQYNPNDTVWGTPLFWGHATSDDLTHWEDEPIAIAPKRNDSGA**Y**SGSMVVDYNNNTSGF
FNDTIDPRQRCVAIWTYNTPESSEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWE
PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
WVMFISINPGAPAGGSFNQYFVGSGFNGTHFEAFDNQSRVVDFGKDYYALQTFNTDPTYGSA
LGIAWASNWEYSFAFPTNPWRSSMSLVKFSLNTEYQANPETELINLKAEPILNIS**AA**GPWS
15 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVF**AD**LSLWFKGLEDPEEYLR
MGFEASASSFFLDGRGNSKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK.

[0093] In certain embodiments, the recombinant mutant invertase comprises the E120A,
V178I, and L549I substitutions relative to SEQ ID NO: 2. For example, the recombinant
20 mutant invertase may comprise SEQ ID NO: 15, also referred to as V83 herein. In certain
embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

[0094] SEQ ID NO: 15

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPKNKGWMNDPNGLWYD**AK**DAK
25 WHLYFQYNPNDTVWGTPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMV**ID**YNNNTSGF
FNDTIDPRQRCVAIWTYNTPESSEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWE
PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
WVMFISINPGAPAGGSFNQYFVGSGFNGTHFEAFDNQSRVVDFGKDYYALQTFNTDPTYGSA
LGIAWASNWEYSFAFPTNPWRSSMSLVKFSLNTEYQANPETELINLKAEPILNISNAGPWS
30 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDL**SL**WFKGLEDPEEYLR
MGFEASASSFFLDGRGNSKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVY**GILD**QNILELY
FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK.

[0095] In certain embodiments, the recombinant mutant invertase comprises the E382Q, S537D, and N589K substitutions relative to SEQ ID NO: 2. For example, the recombinant mutant invertase may comprise SEQ ID NO: 16, also referred to as V87 herein. In certain embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

5 [0096] SEQ ID NO: 16

MRFPSIFTAVLFAASSALAAPVNTTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNNDTVWGTPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 10 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSGFNGTHFEAFDNQSRVVDFGKDYYALQTFNTDPTYGSA
 LGIAWASNWQYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVKENPYFTNRMSVNNQPFKDENDLSYYKVYGLLDQNILELY
 15 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDKLFYIDKFQVREVK.

[0097] In certain embodiments, the recombinant mutant invertase comprises the T140L, G586S, and F596I substitutions relative to SEQ ID NO: 2. For example, the recombinant mutant invertase may comprise SEQ ID NO: 17, also referred to as V89 herein. In certain embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

20 [0098] SEQ ID NO: 17

MRFPSIFTAVLFAASSALAAPVNTTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNNDTVWGLPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 25 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSGFNGTHFEAFDNQSRVVDFGKDYYALQTFNTDPTYGSA
 LGIAWASNWEYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 30 FNDGDVVSTNTYFMTTGNALGSVNMTTSVDNLFYIDKIQVREVK.

[0099] In certain embodiments, the recombinant mutant invertase comprises the E382Q, L459F, and K519R substitutions relative to SEQ ID NO: 2. For example, the recombinant

mutant invertase may comprise SEQ ID NO: 18, also referred to as V90 herein. In certain embodiments, the recombinant mutant invertase lacks the signal sequence underlined below.

[0100] SEQ ID NO: 18

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 5 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNNDTVWGTPLEFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSFNGTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 10 LGIAWASNWQYSAFVPTNPWRSSMSLVKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTTLTKANSYNVDLSNSTGT**F**EELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFV**R**ENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK.

[0101] In certain embodiments, the recombinant mutant invertase comprises the E31A,
 15 D33E, and P390A substitutions (relative to SEQ ID NO: 3). For example, the recombinant
 mutant invertase may comprise SEQ ID NO: 19, also referred to as V6 herein.

[0102] SEQ ID NO: 19

SMTNETSDRPLVHFTPNKGWMNDPNGLWYD**AKE**AKWHLYFQYNPNNDTVWGTPLEFWGHATSDD
 LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 20 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEYEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSV**F**ADLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
 25 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK.

[0103] In certain embodiments, the recombinant mutant invertase comprises the T51L,
 E67Q, and L460I substitutions relative to SEQ ID NO: 3. For example, the recombinant
 mutant invertase may comprise SEQ ID NO: 20, also referred to as V9 herein.

30 **[0104]** SEQ ID NO: 20

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNNDTVWG**L**PLFWGHATSDD
 LTHW**Q**DEPIAIAPKRNDSGAFSGSMVVDYNNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEYEPSQKWIMTAAKSQDYKIEIYSSDDLKS

WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
 5 FTNRMSVNNQPFKSENDLSYYKVYGI~~LD~~QNI~~LE~~LYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK.

[0105] In certain embodiments, the recombinant mutant invertase comprises the S387A, P390A, and Q508E substitutions relative to SEQ ID NO: 3. For example, the recombinant mutant invertase may comprise SEQ ID NO: 21, also referred to as V14 herein.

10 **[0106]** SEQ ID NO: 21

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
 LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 15 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISK~~AVF~~~~AD~~LSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNI~~LE~~LYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKF~~E~~VREVK.

20 **[0107]** In certain embodiments, the recombinant mutant invertase comprises the K170L, S387N, and K425P substitutions (relative to SEQ ID NO: 3). For example, the recombinant mutant invertase may comprise SEQ ID NO: 22, also referred to as V22 herein.

[0108] SEQ ID NO: 22

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
 25 LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAL~~S~~QDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 30 ELVYAVNTTQTISK~~NV~~F~~PD~~LSLWFKGLEDPEEYLRMGFEASASSFFLDRGNS~~P~~VKFVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNI~~LE~~LYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK

[0109] In certain embodiments, the recombinant mutant invertase comprises the K188T, E326S, and V492I substitutions relative to SEQ ID NO: 3. For example, the recombinant mutant invertase may comprise SEQ ID NO: 23, also referred to as V25 herein.

[0110] SEQ ID NO: 23

5 SMTNETSDRPLVHFTPNKGWMNDP NGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
LTHWEDEPIAIAPKRND SGAFSGSMVVDYNN TSGFFNDTIDPRQRCVAIW TYNTP ESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
WTL ESAFANEGLGYQYECPLIEVPTEQDP SKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVD FGDYALQTF FNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
10 RKFSLNTEYQANPETSLINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKV KFKVPENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSINMTT
GVDNLFYIDKFQVREVK

[0111] In certain embodiments, the recombinant mutant invertase comprises the A34G, T51L, and F54Y substitutions relative to SEQ ID NO: 3. For example, the recombinant mutant invertase may comprise SEQ ID NO: 24, also referred to as V32 herein.

[0112] SEQ ID NO: 24

SMTNETSDRPLVHFTPNKGWMNDP NGLWYDEKDGKWHLYFQYNPNDTVWG LPL YWGHATSDD
LTHWEDEPIAIAPKRND SGAFSGSMVVDYNN TSGFFNDTIDPRQRCVAIW TYNTP ESEEQYI
20 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
WKLESAFANEGLGYQYECPLIEVPTEQDP SKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVD FGDYALQTF FNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKV KFKVPENPY
25 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK.

[0113] In certain embodiments, the recombinant mutant invertase comprises the N301D, S387N, and K430R substitutions relative to SEQ ID NO: 3. For example, the recombinant mutant invertase may comprise SEQ ID NO: 25, also referred to as V38 herein.

30 [0114] SEQ ID NO: 25

SMTNETSDRPLVHFTPNKGWMNDP NGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
LTHWEDEPIAIAPKRND SGAFSGSMVVDYNN TSGFFNDTIDPRQRCVAIW TYNTP ESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS

WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWEYSFAVPTDPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISK**N**VFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFV**R**ENPY
 5 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK.

[0115] In certain embodiments, the recombinant mutant invertase comprises the E293Q, T484S, and V492I substitutions relative to SEQ ID NO: 3. For example, the recombinant mutant invertase may comprise SEQ ID NO: 26, also referred to as V60 herein.

10 **[0116]** SEQ ID NO: 26

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
 LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 15 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASN**W**QYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFM**S**TGNALGS**I**NMTT
 GVDNLFYIDKFQVREVK.

20 **[0117]** In certain embodiments, the recombinant mutant invertase comprises the D33E, T381S, and S387N substitutions relative to SEQ ID NO: 3. For example, the recombinant mutant invertase may comprise SEQ ID NO: 27, also referred to as V63 herein.

[0118] SEQ ID NO: 27

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEK**E**AKWHLYFQYNPNDTVWGTPLEFWGHATSDD
 25 LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWEYSFAVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 30 ELVYAVNT**S**QTISK**N**VFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK.

[0119] In certain embodiments, the recombinant mutant invertase comprises the T51Q, P390A, and K425P substitutions relative to SEQ ID NO: 3. For example, the recombinant mutant invertase may comprise SEQ ID NO: 28, also referred to as V70 herein.

[0120] SEQ ID NO: 28

5 SMTNETSDRPLVHFTPNKGWMNDP NGLWYDEKDAKWHLYFQYNPNDTVWGQPLFWGHATSDD
LTHWEDEPIAIAPKRND SGAFSGSMVVDYNN TSGFFNDTIDPRQRCVAIW TYNTP ESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVF WYEPSQKWIMTAAKSQDYKIEIYSSDDLKS
WKLESAFANEGLGYQYECPLIEVPTEQDP SKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVD FGKDYYALQTF FNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
10 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFADLSLWFKGLEDP EEYLRMGFEASASSFFLDRGNSPVKFKVENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNI LELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK.

[0121] In certain embodiments, the recombinant mutant invertase comprises the F83Y, N340A, and P390A substitutions (relative to SEQ ID NO: 3). For example, the recombinant mutant invertase may comprise SEQ ID NO: 29, also referred to as V78 herein.

[0122] SEQ ID NO: 29

SMTNETSDRPLVHFTPNKGWMNDP NGLWYDEKDAKWHLYFQYNPNDTVWGTP LFWGHATSDD
LTHWEDEPIAIAPKRND SGAYSGSMVVDYNN TSGFFNDTIDPRQRCVAIW TYNTP ESEEQYI
20 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVF WYEPSQKWIMTAAKSQDYKIEIYSSDDLKS
WKLESAFANEGLGYQYECPLIEVPTEQDP SKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVD FGKDYYALQTF FNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISAA GPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFADLSLWFKGLEDP EEYLRMGFEASASSFFLDRGNSKVKFKVENPY
25 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNI LELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK.

[0123] In certain embodiments, the recombinant mutant invertase comprises the E31A, V89I, and L460I substitutions relative to SEQ ID NO: 3. For example, the recombinant mutant invertase may comprise SEQ ID NO: 30, also referred to as V83 herein.

30 [0124] SEQ ID NO: 30

SMTNETSDRPLVHFTPNKGWMNDP NGLWYDAKDAKWHLYFQYNPNDTVWGTP LFWGHATSDD
LTHWEDEPIAIAPKRND SGAFSGSMVIDYNN TSGFFNDTIDPRQRCVAIW TYNTP ESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVF WYEPSQKWIMTAAKSQDYKIEIYSSDDLKS

WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
 5 FTNRMSVNNQPFKSENDLSYYKVYGI~~LD~~QNI~~LE~~LYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK.

[0125] In certain embodiments, the recombinant mutant invertase comprises the E293Q, S448D, and N500K substitutions relative to SEQ ID NO: 3. For example, the recombinant mutant invertase may comprise SEQ ID NO: 31, also referred to as V87 herein.

10 **[0126]** SEQ ID NO: 31

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
 LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 15 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNW~~Q~~YSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
 FTNRMSVNNQPFK~~D~~ENDLSYYKVYGLLDQNI~~LE~~LYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVD~~K~~L~~F~~YIDKFQVREVK.

20 **[0127]** In certain embodiments, the recombinant mutant invertase comprises the T51L, G497S, and F507I substitutions relative to SEQ ID NO: 3. For example, the recombinant mutant invertase may comprise SEQ ID NO: 32, also referred to as V89 herein.

[0128] SEQ ID NO: 32

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGL~~L~~PLFWGHATSDD
 25 LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 30 ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNI~~LE~~LYFNDGDVVSTNTYFMTTGNALGSVNMTT
 SVDNLFYIDK~~I~~QVREVK.

[0129] In certain embodiments, the recombinant mutant invertase comprises the E293Q, L370F, and K430R substitutions relative to SEQ ID NO: 3. For example, the recombinant mutant invertase may comprise SEQ ID NO: 33, also referred to as V90 herein.

[0130] SEQ ID NO: 33

5 SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
LTHWEDEPIAIAIPKRNDSGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWTYNTPESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
10 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTFFEF
ELVYAVNTTQTISKSVFPDLWLWFKGLEDPPEEYLRMGFEASASSFFLDRGNSKVKFVRENPHY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK.

[0131] In certain embodiments, the recombinant mutant invertase comprises a non-wild-type
15 invertase with F172Y and N429A mutations relative to SEQ ID NO: 2, without signal
sequence. For example, the recombinant mutant invertase may comprise the *Sc_S288C*
invertase with F172Y and N429A mutations (SEQ ID NO: 50)

[0132] SEQ ID NO: 50

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
20 LTNWEDQPIAIAIPKRNDSGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWTYNTPESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISAAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
25 ELVYAVNTTQTISKSVFADLSLWFKGLEDPPEEYLRMGFEVSASSFFLDRGNSKVKFVKENPHY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK.

[0133] In certain embodiments, the recombinant mutant invertase comprises a non-wild-type
invertase with F172Y, N429A, P479A, T140L, E382Q, and A501N mutations relative to
30 SEQ ID NO: 2, without signal sequence. For example, the recombinant mutant invertase
may comprise SEQ ID NO: 51, also referred to as VE36 herein.

[0134] SEQ ID NO: 51

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
 LTHWEDEPIAIAIPKRND SGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 5 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISAAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVFADLSLWFKGLEDP EEYL RMGFENSASSFFLDRGNSKV KVFV KENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNI LELYFNDGDVVSTNTYFMTTGNALG SVNMTT
 GVDNLFYIDKFQVREVK.

- 10 **[0135]** In certain embodiments, the recombinant mutant invertase comprises a non-wild-type invertase with F172Y, N429A, P479A, T140L, E382Q, A501N, and D272N mutations relative to SEQ ID NO: 2, without signal sequence. For example, the recombinant mutant invertase may comprise SEQ ID NO: 52, also referred to as VE361 herein.

[0136] SEQ ID NO: 52

- 15 SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
 LTHWEDEPIAIAIPKRND SGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDNLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
 20 RKFSLNTEYQANPETELINLKAEPILNISAAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVFADLSLWFKGLEDP EEYL RMGFENSASSFFLDRGNSKV KVFV KENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNI LELYFNDGDVVSTNTYFMTTGNALG SVNMTT
 GVDNLFYIDKFQVREVK.

- [0137]** In certain embodiments, the recombinant mutant invertase comprises a non-wild-type invertase with F172Y, N429A, P479A, T140L, E382Q, A501N, D272N, and E285H mutations relative to SEQ ID NO: 2, without signal sequence. For example, the recombinant mutant invertase may comprise SEQ ID NO: 53, also referred to as VE362 herein.

[0138] SEQ ID NO: 53

- 30 SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
 LTHWEDEPIAIAIPKRND SGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDNLKS
 WKLESAFANHGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV

RKFSLNTEYQANPETELINLKAEPILNIS**A**AGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVF**A**DL~~SL~~WFKGLEDPEEYLRMGF**E**NSASSFFLDRGNSKV~~K~~FVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNI~~LE~~LYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK.

- 5 **[0139]** In certain embodiments, the recombinant mutant invertase comprises a non-wild-type invertase with F172Y, N429A, P479A, T140L, D272N, and E285H mutations relative to SEQ ID NO: 2, without signal sequence. For example, the recombinant mutant invertase may comprise SEQ ID NO: 54, also referred to as VE01 herein.

[0140] SEQ ID NO: 54

10 SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWG**L**PLFWGHATSDD
 LTHWEDEPIAIAPKRNDSGA**Y**SGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWYEPSQKWIMTAAKSQDYKIEIYSSD**N**LKS
 WKLESAFAN**H**GFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 15 RKFSLNTEYQANPETELINLKAEPILNIS**A**AGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVF**A**DL~~SL~~WFKGLEDPEEYLRMGFEASASSFFLDRGNSKV~~K~~FVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNI~~LE~~LYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK.

- 20 **[0141]** In certain embodiments, the recombinant mutant invertase comprises a non-wild-type invertase with F172Y, N429A, P479A, T140L, D272N, and L549I mutations relative to SEQ ID NO: 2, without signal sequence. For example, the recombinant mutant invertase may comprise SEQ ID NO: 55, also referred to as VE03 herein.

[0142] SEQ ID NO: 55

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWG**L**PLFWGHATSDD
 25 LTHWEDEPIAIAPKRNDSGA**Y**SGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWYEPSQKWIMTAAKSQDYKIEIYSSD**N**LKS
 WKLESAFANE**G**FLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNIS**A**AGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 30 ELVYAVNTTQTISKSVF**A**DL~~SL~~WFKGLEDPEEYLRMGFEASASSFFLDRGNSKV~~K~~FVKENPY
 FTNRMSVNNQPFKSENDLSYYKVY**G**ILDQNI~~LE~~LYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK.

[0143] In certain embodiments, the recombinant mutant invertase comprises a non-wild-type invertase with F172Y, N429A, P479A, T140L, D272N, and V581I mutations relative to SEQ ID NO: 2, without signal sequence. For example, the recombinant mutant invertase may comprise SEQ ID NO: 56, also referred to as VE04 herein.

5 [0144] SEQ ID NO: 56

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
LTHWEDEPIAIAPKRND SGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWTYNTPESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDNLKS
WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
10 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISAAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSINMTT
GVDNLFYIDKFQVREVK.

15 [0145] In certain embodiments, the recombinant mutant invertase comprises a non-wild-type invertase with F172Y, N429A, P479A, T140L, E285H, V581I mutations relative to SEQ ID NO: 2, without signal sequence. For example, the recombinant mutant invertase may comprise SEQ ID NO: 57, also referred to as VE08 herein.

[0146] SEQ ID NO: 57

20 SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
LTHWEDEPIAIAPKRND SGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWTYNTPESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
WKLESAFANHGFGLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
25 RKFSLNTEYQANPETELINLKAEPILNISAAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSINMTT
GVDNLFYIDKFQVREVK.

[0147] In certain embodiments, the recombinant mutant invertase comprises a non-wild-type invertase with F172Y, N429A, P479A, T140L, E382Q, and A123G mutations relative to SEQ ID NO: 2, without signal sequence. For example, the recombinant mutant invertase may comprise SEQ ID NO: 58, also referred to as VE38 herein.

[0148] SEQ ID NO: 58

SMTNETSDRPLVHFTPNKGWMNDP NGLWYDEKDGKWHLYFQYNPNDTVWGLPLFWGHATSDD
 LTHWEDEPIAIAIPKRND SGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVG SFNG
 5 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISAAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVFADLSLWFKGLEDP EEYL RMGFEASASSFFLDRGNSKV KVFV KENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALG SVNMTT
 GVDNLFYIDKFQVREVK.

- 10 **[0149]** In certain embodiments, the recombinant mutant invertase comprises a non-wild-type invertase with F172Y, N429A, P479A, T140L, E382Q, and N512G mutations relative to SEQ ID NO: 2, without signal sequence. For example, the recombinant mutant invertase may comprise SEQ ID NO: 59, also referred to as VE39 herein.

[0150] SEQ ID NO: 59

- 15 SMTNETSDRPLVHFTPNKGWMNDP NGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
 LTHWEDEPIAIAIPKRND SGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVG SFNG
 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
 20 RKFSLNTEYQANPETELINLKAEPILNISAAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVFADLSLWFKGLEDP EEYL RMGFEASASSFFLDRG GSKVKFV KENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALG SVNMTT
 GVDNLFYIDKFQVREVK.

- [0151]** In certain embodiments, the recombinant mutant invertase comprises a non-wild-type invertase with F172Y, N429A, P479A, T140L, E382Q, and V178I mutations relative to SEQ ID NO: 2, without signal sequence. For example, the recombinant mutant invertase may comprise SEQ ID NO: 60, also referred to as VE40 herein.

[0152] SEQ ID NO: 60

- 30 SMTNETSDRPLVHFTPNKGWMNDP NGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
 LTHWEDEPIAIAIPKRND SGAYSGSMVIDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVG SFNG
 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV

RKFSLNTEYQANPETELINLKAEPILNIS**A**AGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVF**A**DLSLWFKGLEDP EEYL RMGF EASASSFFLDRGNSKV K FVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNI L ELYFNDGDVVSTNTYFMTTGNALG SVNMTT
 GVDNLFYIDKFQVREVK.

- 5 **[0153]** In certain embodiments, the recombinant mutant invertase comprises a non-wild-type invertase with F172Y, N429A, P479A, T140L, E382Q, and N390D mutations relative to SEQ ID NO: 2, without signal sequence. For example, the recombinant mutant invertase may comprise SEQ ID NO: 61, also referred to as VE44 herein.

[0154] SEQ ID NO: 61

- 10 SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWG**L**PLFWGHATSDD
 LTHWEDEPIAIAPKRND SG A**Y**SGSMVVDYNN TSGFFNDTIDPRQRCVAIW TYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFN
 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNW**Q**YSAFVPT**D**PWRSSMSLV
 15 RKFSLNTEYQANPETELINLKAEPILNIS**A**AGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVF**A**DLSLWFKGLEDP EEYL RMGF EASASSFFLDRGNSKV K FVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNI L ELYFNDGDVVSTNTYFMTTGNALG SVNMTT
 GVDNLFYIDKFQVREVK.

- [0155]** In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of any one of SEQ ID NOs: 4-18, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 4-18.

- [0156]** In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 4, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 4. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 5. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 6. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 7. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 8, or an amino acid

sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 9, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 9. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 10, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 10. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 11, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 11. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 12, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 12. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 13, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 13. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 14, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 14. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 15, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 15. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 16, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 16. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 17, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 17. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 18 or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 18.

[0157] In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of any one of SEQ ID NOs: 19-33, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 19-33.

[0158] In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 19, or an amino acid sequence that has at least 85%, 90%, 95%,

96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 19. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 20, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 20. In certain embodiments, the recombinant mutant invertase

5 comprises the amino acid sequence of SEQ ID NO: 21, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 21. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 22, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 22. In certain embodiments, the recombinant

10 mutant invertase comprises the amino acid sequence of SEQ ID NO: 23, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 23. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 24, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 24. In certain embodiments,

15 the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 25, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 25. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 26, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 26. In

20 certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 27, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 27. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 28, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ

25 ID NO: 28. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 29, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 29. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 30, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence

30 identity to SEQ ID NO: 30. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 31, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 31. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence

of SEQ ID NO: 32, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 32. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 33 or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 33.

[0159] In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of any one of SEQ ID NOs: 51-61, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 51-53.

[0160] In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 51, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 51. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 51 with up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions.

[0161] In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 52, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 52. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 52 with up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions.

[0162] In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 53, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 53. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 53 with up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions.

[0163] In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 54, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 54. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 54 with up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions.

[0164] In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 55, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 55. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 55 with up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions.

[0165] In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 56, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 56. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 56 with up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions.

[0166] In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 57, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 57. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 57 with up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions.

[0167] In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 58, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 58. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 58 with up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions.

[0168] In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 59, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 59. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 59 with up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions.

[0169] In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 60, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 60. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 60 with up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions.

[0170] In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 61, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 61. In certain embodiments, the recombinant mutant invertase comprises the amino acid sequence of SEQ ID NO: 61 with up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid substitutions.

[0171] As used herein, percent “identity” between a polypeptide sequence and a reference sequence is defined as the percentage of amino acid residues in the polypeptide sequence that are identical to the amino acid residues in the reference sequence, after aligning the

sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Similarly, percent “identity” between a nucleic acid sequence and a reference sequence is defined as the percentage of nucleotides in the nucleic acid sequence that are identical to the nucleotides in the reference sequence, after aligning the sequences and
5 introducing gaps, if necessary, to achieve the maximum percent sequence identity.

Alignment for purposes of determining percent sequence identity (*e.g.*, amino acid sequence identity or nucleic acid sequence identity) can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, MEGALIGN (DNASTAR), CLUSTALW, CLUSTAL OMEGA, or
10 MUSCLE software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0172] In certain embodiments, a recombinant mutant invertase has increased stability at acidic pH (*e.g.*, pH 3.0 or 4.0) relative to a corresponding wild-type invertase enzyme. An
15 increased stability at acidic pH may, in certain conditions, allow the recombinant mutant invertase to survive acidic conditions of the digestive system, especially the stomach. An increased stability at acidic pH may, in certain conditions, also allow the recombinant mutant invertase to survive in certain food or beverages, *e.g.*, fruit juice. An increased stability at acidic pH may, in certain conditions, allow the recombinant mutant invertase to survive in
20 fasting conditions.

[0173] In certain embodiments, the invertase has a specific activity at about pH 3.5 (*e.g.*, 3.6) of at least 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, or 2,000 μmol sucrose consumed per minute per milligram of invertase. In certain
25 embodiments, the invertase has at least 1-fold, 1.5-fold, 2-fold, 2.5-fold, or 3-fold higher activity at about pH 3.5 (*e.g.*, 3.6), compared to a corresponding wild-type invertase. In certain embodiments, the invertase has a specific activity at about pH 5.0 (*e.g.*, pH 4.9) of at least 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,100, or 2,200 μmol sucrose consumed per minute per milligram of invertase. In certain
30 embodiments, the invertase has at least 1-fold, 1.5-fold, 2-fold, 2.5-fold, or 3-fold higher activity at about pH 5.0 (*e.g.*, pH 4.9), compared to a corresponding wild-type invertase. In certain embodiments, the invertase has a specific activity at about pH 6.0 or 6.2 of at least 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, or 1,800 μmol sucrose consumed per minute per milligram of invertase. In certain embodiments, the invertase has at

least 1-fold, 1.5-fold, 2-fold, 2.5-fold, or 3-fold higher activity at about pH 6.0 or 6.2, compared to a corresponding wild-type invertase. In certain embodiments, the invertase has a specific activity at about pH 7.0 or 7.1 of at least 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, or 1,300 μ mol sucrose consumed per minute per milligram of invertase.

- 5 In certain embodiments, the invertase has at least 1-fold, 1.5-fold, 2-fold, 2.5-fold, or 3-fold higher activity at about pH 7.0 or 7.1, compared to a corresponding wild-type invertase. In certain embodiments, the invertase retains at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% of activity following incubation at about pH 2.5 for about 30 minutes. In certain embodiments, the invertase has at least 1-fold, 1.5-fold, 2-fold, 10 2.5-fold, 3-fold, 3.5-fold, 4-fold, or 5-fold higher stability at about pH 2.5 compared to a corresponding wild-type invertase. In certain embodiments, the invertase is deglycosylated. In certain embodiments, the invertase is glycosylated.

- [0174] Methods for testing the stability and activity of an invertase are known in the art and can include, for example, the methods described in Example 1 herein. In certain 15 embodiments, stability of an invertase in low pH is determined using by exposing the invertase to a specific pH, and using the dinitro salicylate (DNS) colorimetric method to monitor reducing sugar formation upon hydrolysis of sucrose.

- [0175] In certain embodiments, a recombinant mutant invertase has increased stability at higher temperature (*e.g.*, T_m of 56-68°C) relative to a corresponding wild-type invertase 20 enzyme. In certain embodiments, the invertase has a T_m of at least 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, or 68 °C. In certain embodiments the invertase has a T_m that is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 °C higher than a corresponding wild-type invertase. Increased thermostability may allow the invertase to be stable when manufactured under conditions, for example, when dried (*e.g.*, spray dried, lyophilized) to form a powder or when compressed 25 under high temperatures as a tablet.

- [0176] Methods for testing the thermostability and activity of an invertase are known in the art and can include, for example, the methods described in Example 1 herein. In certain 30 embodiments, stability and activity of an invertase at high temperature is determined by treatment of invertase samples at high temperature prior to assaying residual activity using the DNS method as described above.

[0177] In certain embodiments, the invertase has increased stability in the presence of a protease (*e.g.*, a serine protease and/or an aspartic protease) relative to the corresponding wild-type invertase enzyme. Increased stability in the presence of a protease may, in certain

conditions, allow a recombinant mutant invertase to survive the physiological conditions of the gastrointestinal lumen (*e.g.*, stomach and/or small intestine) in a fed or fasted state. In certain embodiments, a recombinant mutant invertase has increased stability in the presence of pancreatin or pepsin relative to a corresponding wild-type invertase enzyme. Without wishing to be bound by theory, it is contemplated that the increased stability allows the invertase enzyme to be immediately active to degrade sucrose prior to uptake in the brush borders of the upper small intestine.

[0178] In certain embodiments, a disclosed invertase enzyme retains at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% of activity following incubation with pepsin (*e.g.*, incubation with about 10 mg/ml pepsin at about 37 °C in simulated gastric fluid (SGF) for about 2 hours). In certain embodiments, a disclosed invertase enzyme retains at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% of activity following incubation with pancreatin (*e.g.*, incubation with about 3.4 mg/ml pancreatin at about 37 °C in simulated intestinal fluid (SIF) for about 2 hours).

[0179] Methods for testing the proteolytic stability and activity of an invertase are known in the art and can include, for example, the methods described in Example 1 herein. In certain embodiments, stability and activity of an invertase in the presence of pepsin or pancreatin is determined by incubation of invertase with pepsin or pancreatin prior to assaying residual activity using the DNS method as described above.

II. Isomaltase Enzymes

[0180] Among other things, the invention provides pharmaceutical compositions optionally further comprising an isomaltase enzyme that, for example, are useful in treating disorders such as congenital sucrase-isomaltase deficiency (CSID).

[0181] As used herein, the term “isomaltase” refers to any enzyme, or a functional fragment thereof, that is capable of catalyzing the cleavage of branched (1–6 linked) α -limit dextrins (starches). Isomaltases are also called α -glucosidase, oligo-1,6-glucosidase, and EC 3.2.1.10, and, unless indicated otherwise, the terms are used interchangeably herein. The term isomaltase includes variants having one or more amino acid substitutions, deletions, or insertions relative to a wild-type isomaltase sequence, and/or fusion proteins or conjugates including a isomaltase. As used herein, the term “functional fragment” of a isomaltase refers to fragment of a full-length isomaltase that retains, for example, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% of the enzymatic activity of the corresponding full-length, naturally occurring

isomaltase. Exemplary isomaltase activity assays are described in Noguchi *et al.* (2003) J. BIOCHEM. 134(4):543-50 and Schonert *et al.* (1998) J. BACTERIOL. 180(9):2574-8.

Additionally, because glucose is a product of the enzymatic reactions catalyzed by both invertase and isomaltase, it is understood that an assay based on the detection of glucose may in certain embodiments be used to measure the activity of both an invertase enzyme and an isomaltase enzyme.

[0182] Exemplary isomaltase enzymes include isomaltase enzymes derived from *Saccharomyces cerevisiae*. The amino acid sequences of exemplary wild-type isomaltase enzymes derived from *Saccharomyces cerevisiae* are provided as SEQ ID NOs: 37-41 and nucleotide sequences encoding exemplary wild-type isomaltase enzymes derived from *Saccharomyces cerevisiae* are provided as SEQ ID NOs: 42-46.

[0183] An exemplary wild-type isomaltase enzyme derived from *L. fermentum* is provided as SEQ ID NO: 47.

[0184] SEQ ID NO: 47

MIYTPKWWQNSVVYQVYPRSFQDSNHDGIGDLKGIISRLDYIKKLGADIIWLNPIYRSPNV
DNGYDISDYRAIDPTFGSLTDFKELLTKAHELGLKIMMDLVVNHSSDENEFKQSRQGENP
YRDYYIWRDPVDGHEPTNWGSYFSGSAWQYDETSQYYLHLFAVKQPDNLWENEAVRHSVYD
IMNWWADLGVDGFRMDVINLISKPAVYKDVP TAPGMQYGDVEFPVANGHRMHEFLQEMHQAV
MAKHDLVTVGETPGATTDDAKKYANLEQTELMNVFEFEHVGLDGNPNPALGKWSDDKKVSLPE
LRDNLVKWQTQLNGKAWNSLYWNNHDQPRVVSFRFGNDDPKYRVVSAKMLATMLHCLQGTPYI
YAGEELGMTNTTFNSLSDYRDLESINAYHQLVDEEHLVDGKTMSRYLAIHSRDNARTPMQWD
DSKNAGFSDAEPWIAVNP NYSEINAKAALADPSSVFYHYQKLIQMRHDLPMTEGKFALVNG
NELDEQVFAYTRDDGETTLLVVANFTKETIKREYAAGQGKLLLSNYEDDMGETLRPYEAKVY
EFSSKR.

[0185] An exemplary isomaltase enzyme variant may comprise an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 47. In certain embodiments, the isomaltase enzyme variant comprises at least one amino acid substitution (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 amino acid substitutions) and an amino acid sequence that has at least 98%, 98.5%, 99% or 99.5% sequence identity to SEQ ID NO: 47. In certain embodiments, the isomaltase comprises substitutions at positions corresponding to the amino acids sequence set forth in SEQ ID NO: 47, including: the E93K, K115I, R132K, D226S, E310A, L366M, I421A, A444G, A531D, and F560L substitutions; the E93K, K115I, R132K, D226S, E310A, I421A, A444G, E524Q,

A531D, and F560L substitutions; the E93K, K115I, R132K, D226S, E310A, I421A, A444G,
 A531D, and F560V substitutions; the E93K, K115I, R132K, D226S, L366M, I421A, A444G,
 E524Q, A531D, and F560L substitutions; the E93K, K115I, R132K, D226S, L366M, I421A,
 A444G, A531D, and F560V substitutions; the E93K, K115I, R132K, D226S, I421A, A444G,
 5 E524Q, A531D, and F560V substitutions; the K115I, R132K, D226S, E310A, L366M,
 I421A, A444G, E524Q, A531D, and F560L substitutions; the K115I, R132K, D226S,
 E310A, L366M, I421A, A444G, A531D, and F560V substitutions; the K115I, R132K,
 D226S, E310A, I421A, A444G, E524Q, A531D, and F560V substitutions; the K115I,
 R132K, D226S, L366M, I421A, A444G, E524Q, A531D, and F560V substitutions; the
 10 E93K, K115I, R132K, A211E, D226S, I421A, A444G, E524Q, A531D, and F560V
 substitutions; the E93K, K115I, R132K, D226S, L366M, I421A, A444G, E524Q, A531D,
 and F560V substitutions; the E93K, K115I, R132K, D226S, E275D, I421A, A444G, E524Q,
 A531D, and F560V substitutions; the T89A, E93K, K115I, R132K, D226S, I421A, A444G,
 E524Q, A531D, and F560V substitutions; the E93K, K115I, R132K, H182R, D226S, I421A,
 15 A444G, E524Q, A531D, and F560V substitutions; the E93K, K115I, E122D, R132K,
 D226S, I421A, A444G, E524Q, A531D, and F560V substitutions; the E93K, K115I, D226S,
 I421A, A444G, E524Q, A531D, and F560V substitutions; the E93K, K115I, D226S, I421A,
 A444G, E524Q, A531D, and F560L substitutions; the K115I, A211E, D226S, I421A,
 A444G, A531D, and F560V substitutions; the K115I, D226S, L366M, I421A, A444G,
 20 E524Q, A531D, and F560L substitutions; the K115I, A211E, D226S, I421A, A444G,
 E524Q, A531D, and F560L substitutions; the K115I, R132K, D226S, E310A, L366M,
 I421A, A444G, A531D, and F560L substitutions; the E93K, K115I, D226S, E310A, I421A,
 A444G, A531D, and F560L substitutions; the K115I, D226S, E310A, I421A, A444G,
 A531D, and F560V substitutions; the E93K, K115I, D226S, L366M, I421A, A444G, A531D,
 25 and F560L substitutions; the K115I, D226S, I421A, A444G, E524Q, A531D, and F560V
 substitutions; the K115I, D226S, E310A, I421A, A444G, E524Q, A531D, and F560L
 substitutions; the K115I, A211E, D226S, L366M, I421A, A444G, A531D, and F560L
 substitutions; the K115I, A211E, D226S, E310A, I421A, A444G, A531D, and F560L
 substitutions; the E93K, K115I, D226S, I421A, A444G, A531D, and F560V substitutions;
 30 the E93K, K115I, A211E, D226S, I421A, A444G, A531D, and F560L substitutions; or the
 K115I, D226S, L366M, I421A, A444G, A531D, and F560V substitutions.

[0186] Additional exemplary isomaltase enzymes can be found on the world wide web at
brenda-enzymes.org/enzyme.php?ecno=3.2.1.10&onlyTable=Sequence.

[0187] A contemplated isomaltase enzyme may comprise the amino acid sequence of any one of SEQ ID NOs: 37-41, or an amino acid sequence that has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 42-46. Other isomaltase enzymes that may be used in combination with the recombinant invertase enzymes described herein are described in International Patent Application Publication No. WO 2024/015943, which claims priority to U.S. Provisional Patent Application No. 63/388,845, filed on July 13, 2022.

[0188] In certain embodiments, the isomaltase enzyme comprises one or more conservative substitutions relative to isomaltase enzyme disclosed herein. In other embodiments, the isomaltase enzyme comprises one or more non-conservative substitutions relative to isomaltase enzyme disclosed herein.

[0189] In certain embodiments, the composition comprises (i) an invertase comprising the amino acid sequence of SEQ ID NO: 14 (variant V78 with a FAKS signal sequence) or SEQ ID NO: 29 (variant 78 without a signal sequence) and (ii) one or more of the isomaltase enzymes described in this Section II.

[0190] In certain embodiments, the composition comprises (i) an invertase comprising the amino acid sequence of SEQ ID NO: 49 (*Sc_S288C* invertase with F172Y and N429A mutations relative to SEQ ID NO: 2, with wild-type signal sequence) or SEQ ID NO: 50 (*Sc_S288C* invertase with F172Y and N429A mutations relative to SEQ ID NO: 2 without a signal sequence) and (ii) one or more of the isomaltase enzymes described in this Section II.

III. Enzyme Production

[0191] Methods for producing invertase and/or isomaltase enzymes are known in the art. For example, DNA molecules encoding an invertase and/or isomaltase enzyme can be chemically synthesized using the sequence information provided herein. Synthetic DNA molecules can be ligated to other appropriate nucleotide sequences, including, *e.g.*, expression control sequences, to produce conventional gene expression constructs encoding the desired invertase and/or isomaltase enzyme.

[0192] Nucleic acids encoding desired invertase and/or isomaltase enzymes can be incorporated (ligated) into expression vectors, which can be introduced into host cells through conventional transfection or transformation techniques. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the invertase and/or isomaltase enzyme.

[0193] An exemplary DNA sequence encoding a wild-type *S. cerevisiae* invertase is provided as SEQ ID NO: 35.

[0194] SEQ ID NO: 35 (wild-type *S. cerevisiae* invertase DNA sequence):

ATGCTTTTGAAGCTTTCCTTTTCTTTTGGCTGGTTTTGCAGCCAAAATATCTGCATCAAT
 5 GACAAACGAAACTAGCGATAGACCTTTGGTCCACTTCACACCCAACAAGGGCTGGATGAATG
 ACCCAAATGGGTTGTGGTACGATGAAAAAGATGCCAAATGGCATCTGTACTTTCAATACAAC
 CCAAATGACACCGTATGGGGTACGCCATTGTTTTGGGGCCATGCTACTTCCGATGATTTGAC
 TCATTGGGAAGATGAACCCATTGCTATCGCTCCCAAGCGTAACGATTCAGGTGCTTTCTCTG
 GCTCCATGGTGGTTGATTACAACAACACGAGTGGGTTTTTCAATGATACTATTGATCCAAGA
 10 CAAAGATGCGTTGCGATTTGGACTTATAACACTCCTGAAAGTGAAGAGCAATACATTAGCTA
 TTCTCTTGATGGTGGTTACACTTTTACTGAATACCAAAAAGAACCCTGTTTTAGCTGCCAACT
 CCACTCAATTGAGAGATCCAAAGGTGTTCTGGTATGAACCTTCTCAAAAATGGATTATGACG
 GCTGCCAAATCACAAGACTACAAAATTGAAATTTACTCCTCTGATGACTTGAAGTCCTGGAA
 GCTAGAATCTGCATTTGCTAATGAAGGTTTCTTAGGCTACCAATATGAATGTCCAGGTTTGA
 15 TTGAAGTCCCAACTGAGCAAGATCCTTCCAAATCCTATTGGGTCATGTTTATTTCTATCAAT
 CCAGGTGCACCTGCTGGCGGTTCCCTTCAACCAATATTTTGTGGATCCTTCAATGGTACTCA
 TTTTGAAGCGTTTGACAATCAATCTAGAGTGGTAGATTTTGGTAAGGACTACTATGCCTTG
 CAACTTTCTTCAACACAGACCCAACGTACGGTTCAGCATTAGGTATTGCCTGGGCTTCAAAC
 TGGGAGTACAGTGCCTTTGTCCCAACTAACCCATGGAGATCATCCATGTCTTTGGTCCGCAA
 20 GTTTTCTTTGAACACTGAATATCAAGCTAATCCAGAGACTGAATTGATCAATTTGAAAGCCG
 AACCAATATTGAACATTAGTAATGCTGGTCCCTGGTCTCGTTTTGCTACTAACACAACCTCTA
 ACTAAGGCCAATTCTTACAATGTGATTTGAGCAACTCGACTGGTACCCTAGAGTTTGAGTT
 GGTTTACGCTGTTAACACCACACAAACCATATCCAAATCCGTCTTTCCCGACTTATCACTTT
 GGTTCAAGGGTTTAGAAGATCCTGAAGAATATTTAAGAATGGGTTTTGAAGCCAGTGCTTCT
 25 TCCTTCTTTTGGACCGTGGTAACTCTAAGGTCAAGTTTGTCAAGGAGAACCCATATTTAC
 AACAGAATGTCTGTCAACAACCAACCATTTCAAGTCTGAGAACGACCTAAGTTACTATAAAG
 TGTACGGCCTACTGGATCAAAACATCTTGGAATTGTACTTCAACGATGGAGATGTGGTTTCT
 ACAAATACCTACTTCATGACCACCGGTAACGCTCTAGGATCTGTGAACATGACCACTGGTGT
 CGATAATTTGTTCTACATTGACAAGTTCCAAGTAAGGGAAGTAAAATAG

30 [0195] An exemplary DNA sequence encoding a wild-type *L. fermentum* isomaltase is provided in SEQ ID NO: 48.

[0196] SEQ ID NO: 48 (wild-type *L. fermentum* isomaltase DNA sequence):

ATGATATATACACCAAAATGGTGGTGGCAAAATTCAGTTGTCTACCAAGTCTATCCACGGAG
 TTTTCAAGACAGCAATCATGATGGCATTGGTGATTTAAAAGGAATCATCAGTCGGCTTGACT
 ATATTAAAAACTAGGTGCTGATATTATCTGGCTCAATCCAATCTACCGTTTCGCCAAACGTT
 GATAATGGGTATGACATCAGTGATTATCGGGCAATTGATCCAACCTTTTGGCTCATTGACCGA
 5 CTTTAAGGAGCTGCTGACAAAGGCTCATGAACTTGGATTAAAAATAATGATGGATCTGGTGG
 TCAATCACTCATCAGATGAAAATGAATGGTTTAAGCAAAGCCGCCAAGGAAAAGAAAATCCA
 TACCGTGACTATTATATCTGGCGTGATCCAGTCGATGGCCATGAACCAACTAATTGGGGATC
 ATACTTCTCTGGTTCAGCTTGGCAGTACGATGAGACAAGCGGTCAATACTACCTTCACCTGT
 TCGCTGTAAAGCAGCCGGATCTAAACTGGGAAAATGAAGCCGTTTCGTCATTCCGTATACGAC
 10 ATCATGAATTGGTGGGCAGATCTCGGTGTCGATGGATTTTGAATGGATGTCATCAACTTAAT
 TTCAAACCGGCTGTCTACAAAGACGTTCCAACCGCGCCCGGAATGCAGTATGGCGATGTGC
 AGCCGGTTGTTGCCAATGGTCATCGGATGCACGAATTCCTTCAAGAAATGCATCAAGCTGTA
 ATGGCCAAGCATGATTTGGTAACAGTTGGCGAAACACCTGGTGCTACAACAGATGATGCCAA
 GAAGTACGCCAACCTTGAACAGACTGAGCTGAACATGGTTTTTGGAGTTTGGAGCATGTCGGCT
 15 TAGACGGTAACGACAACCCAGCTTTAGGAAAATGGAGCGACAAAAAGTCAGTCTGCCAGAA
 TTGCGAGATAACCTGGTGAAATGGCAGACTCAGCTGAATGGTAAGGCTTGGAACCTCGCTTTA
 CTGGAACAACCATGATCAGCCACGCGTCGTATCCCGTTTTGGCAATGATGATCCAAAATATC
 GTGTCGTTTTCGGCTAAAATGTTGGCAACAATGCTGCACTGTCTGCAAGGCACGCCTTATATC
 TACGCAGGTGAGGAACTGGGAATGACCAACACGACCTTTAACTCTTTGTCTGACTATCGTGA
 20 TTTGGAGAGCATTAATGCCTACCATCAGCTGGTTGACGAAGAGCATCTAGTTGATGGAAAGA
 CAATGAGCAGATATCTGGCAATTCATTCTCGGGACAATGCCAGAACGCCAATGCAATGGGAT
 GACAGCAAGAAATGCCGGATTTTCGGATGCTGAACCTTGGATCGCGGTCAATCCCAACTATTC
 AGAGATCAACGCAAAGGCGGCACTGGCGGATCCATCATCCGTCTTCTATCACTACCAGAAAC
 TCATTCAAATGCGCCATGACTTGCCAGTAATGACAGAAGGAAAGTTTGC GTTGGTCAATGGC
 25 AACGAATTGGATGAGCAGGTCTTTGCTTACACGCGTGATGATGGAGAAACAACGCTGCTTGT
 AGTAGCCAACTTTACTAAGGAAACAATCAAGCGAGAATACGCGGCTGGTCAAGGCAAGCTCT
 TATTAAGCAACTACGAGGATGACATGGGAGAACTCTGCGTCCATATGAAGCTAAAGTATAT
 GAATTCAGTTCAAAGAGGTAA

[0197] Nucleic acids encoding a recombinant mutant invertase and/or isomaltase may be
 30 generated by mutating a nucleotide sequence encoding a wild-type invertase, *e.g.*, SEQ ID
 NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3, or a wild-type isomaltase, *e.g.*, SEQ ID NOs: 37-
 41, using methods known in the art. SEQ ID Nos: 1 and 2 represent isomaltase protein
 sequences containing N-terminal signal sequences that can be removed post-translationally.

SEQ ID NO: 3 represents a corresponding mature isomaltase protein without an -N-terminal signal sequence. Furthermore, nucleic acids encoding such recombinant invertase and/or isomaltase enzymes may be codon optimized for expression in a heterologous cell, *e.g.*, an *Escherichia coli*, *Saccharomyces cerevisiae*, or *Pichia pastoris* cell, using methods known in the art.

[0198] In one embodiment, the disclosure relates to a nucleotide sequence encoding a recombinant mutant invertase that comprises the following substitutions: E120A, D122E, and P479A, relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V6 herein.

[0199] In one embodiment, the disclosure relates to a nucleotide sequence encoding a recombinant mutant invertase that comprises the following substitutions: T140L, E156Q, and L549I relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V9 herein.

[0200] In one embodiment, the disclosure relates to a nucleotide sequence encoding a recombinant mutant invertase that comprises the following substitutions: S476A, P479A, and Q597E relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V14 herein.

[0201] In one embodiment, the disclosure relates to a nucleotide sequence encoding a recombinant mutant invertase that comprises the following substitutions: K259L, S476N, and K514P relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V22 herein.

[0202] In one embodiment, the disclosure relates to a nucleotide sequence encoding a recombinant mutant invertase that comprises the following substitutions: K277T, E415S, and V581I relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V25 herein.

[0203] In one embodiment, the disclosure relates to a nucleotide sequence encoding a recombinant mutant invertase that comprises the following substitutions: A123G, T140L, and F143Y relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V32 herein.

[0204] In one embodiment, the disclosure relates to a nucleotide sequence encoding a recombinant mutant invertase that comprises the following substitutions: N390D, S476N, and K519R relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V38 herein.

[0205] In one embodiment, the disclosure relates to a nucleotide sequence encoding a recombinant mutant invertase that comprises the following substitutions: E382Q, T573S, and V581I relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V60 herein.

- 5 [0206] In one embodiment, the disclosure relates to a nucleotide sequence encoding a recombinant mutant invertase that comprises the following substitutions: D122E, T470S, and S476N relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V63 herein.

- [0207] In one embodiment, the disclosure relates to a nucleotide sequence encoding a
10 recombinant mutant invertase that comprises the following substitutions: T140Q, P479A, and K514P relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V70 herein.

- [0208] In one embodiment, the disclosure relates to a nucleotide sequence encoding a
recombinant mutant invertase that comprises the following substitutions: F172Y, N429A, and
15 P479A relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V78 herein.

- [0209] In one embodiment, the disclosure relates to a nucleotide sequence encoding a
recombinant mutant invertase that comprises the following substitutions: E120A, V178I, and
L549I relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V83
20 herein.

- [0210] In one embodiment, the disclosure relates to a nucleotide sequence encoding a
recombinant mutant invertase that comprises the following substitutions: E382Q, S537D, and
N589K relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V87
herein.

- 25 [0211] In one embodiment, the disclosure relates to a nucleotide sequence encoding a
recombinant mutant invertase that comprises the following substitutions: T140L, G586S, and
F596I relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V89
herein.

- [0212] In one embodiment, the disclosure relates to a nucleotide sequence encoding a
30 recombinant mutant invertase that comprises the following substitutions: E382Q, L459F, and
K519R relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as V90
herein.

[0213] In one embodiment, the disclosure relates to a nucleotide sequence encoding a recombinant mutant invertase that comprises the following substitutions: F172Y, N429A, P479A, T140L, E382Q, and A501N relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as VE36 herein.

- 5 [0214] In one embodiment, the disclosure relates to a nucleotide sequence encoding a recombinant mutant invertase that comprises the following substitutions: F172Y, N429A, P479A, T140L, D272N, and E285H relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as VE01 herein.

- [0215] In one embodiment, the disclosure relates to a nucleotide sequence encoding a
10 recombinant mutant invertase that comprises the following substitutions: F172Y, N429A, P479A, T140L, D272N, and L549I relative to SEQ ID NO: 2; *e.g.*, a recombinant mutant invertase referred to as VE03 herein.

- [0216] In one embodiment, the disclosure relates to a nucleotide sequence encoding a
15 recombinant mutant invertase that comprises the following substitutions: F172Y, N429A, P479A, T140L, D272N, and V581I substitutions relative to SEQ ID NO: 2; *e.g.*, a recombinant mutant invertase referred to as VE04 herein.

- [0217] In one embodiment, the disclosure relates to a nucleotide sequence encoding a
20 recombinant mutant invertase that comprises the following substitutions: F172Y, N429A, P479A, T140L, E285H, and V581I substitutions relative to SEQ ID NO: 2; *e.g.*, a recombinant mutant invertase referred to as VE08 herein.

- [0218] In one embodiment, the disclosure relates to a nucleotide sequence encoding a
recombinant mutant invertase that comprises the following substitutions: F172Y, N429A, P479A, T140L, E382Q, and A123G substitutions relative to SEQ ID NO: 2; *e.g.*, a recombinant mutant invertase referred to as VE38 herein.

- 25 [0219] In one embodiment, the disclosure relates to a nucleotide sequence encoding a recombinant mutant invertase that comprises the following substitutions: F172Y, N429A, P479A, T140L, E382Q, and N512G substitutions relative to SEQ ID NO: 2; *e.g.*, a recombinant mutant invertase referred to as VE39 herein.

- [0220] In one embodiment, the disclosure relates to a nucleotide sequence encoding a
30 recombinant mutant invertase that comprises the following substitutions: F172Y, N429A, P479A, T140L, E382Q, and V178I substitutions relative to SEQ ID NO: 2; *e.g.*, a recombinant mutant invertase referred to as VE40 herein.

[0221] In one embodiment, the disclosure relates to a nucleotide sequence encoding a recombinant mutant invertase that comprises the following substitutions: the F172Y, N429A, P479A, T140L, E382Q, and N390D substitutions relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as VE44 herein.

- 5 [0222] In one embodiment, the disclosure relates to a nucleotide sequence encoding a recombinant mutant invertase that comprises the following substitutions: F172Y, N429A, P479A, T140L, E382Q, A501N, and D272N substitutions relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as VE361 herein.

- [0223] In certain embodiments, the recombinant mutant invertase comprises the F172Y,
10 N429A, P479A, T140L, E382Q, A501N, D272N, and E285H substitutions relative to SEQ ID NO: 2, *e.g.*, a recombinant mutant invertase referred to as VE362 herein.

[0224] An exemplary DNA sequence encoding a recombinant mutant invertase referred to as VE362 herein is provided in SEQ ID NO: 63.

[0225] SEQ ID NO: 63 (DNA sequence encoding VE362 invertase):

15 ATGAGATTCCCATCTATTTTCACCGCTGTCTTGTTGCTGCCTCCTCTGCATTGGCTGCCCC
TGTTAACTACTACCACTGAAGACGAGACTGCTCAAATTCAGCTGAAGCAGTTATCGGTTACT
CTGACCTTGAGGGTGATTTTCGACGTCGCTGTTTTGCCTTTCTCTAACTCCACTAACAACGGT
TTGTTGTTTCATTAACACCACTATCGCTTCCATTGCTGCTAAGGAAGAGGGTGTCTCTCTCGA
GAAAAGAGAGGGCCGAAGCTTCTATGACCAATGAACTTCTGACAGACCATTGGTTCACTTCA
20 CACCCAACAAGGGTTGGATGAACGACCCAAACGGACTTTGGTATGATGAGAAAGATGCAAAA
TGGCATCTGTATTTCCAGTACAATCCTAATGACACGGTCTGGGGTTTGCCACTGTTTTGGGG
TCACGCAACTTCTGATGACTTAACTCATTGGGAAGATGAGCCAATCGCTATCGCTCCTAAGC
GTAACGATTTCAGGTGCCTACTCCGGATCCATGGTTGTGCGATTATAATAACTAGTGGTTTC
TTCAACGATACAATTGATCCCAGACAGCGATGTGTGGCAATTTGGACCTACAATACACCAGA
25 ATCAGAGGAACAATACATTTTCGTACTCCTTGGATGGTGGTTACACGTTCACTGAATATCAGA
AAAACCCTGTCTTGGCTGCGAATAGTACACAATTTAGAGATCCGAAAGTTTTCTGGTATGAG
CCTTCACAAAAATGGATCATGACTGCTGCAAAGTCCCAGGACTACAAGATTGAGATTTACTC
TTCAGACAATTTGAAGTCATGGAAATTGGAGAGCGCTTTTGCCAATCATGGATTCTCTGGGCT
ATCAATATGAGTGCCCTGGATTGATAGAAGTACCAACAGAACAAAGATCCATCCAAGTCTTAC
30 TGGGTGATGTTTATCAGCATTAATCCTGGTGCTCCCGCTGGCGGATCTTTTAACCAATACTT
TGTGGGTTTCCTTCAACGGTACACATTTTGAAGCATTTGATAACCAATCCAGAGTTGTTGACT
TTGGTAAAGACTATTATGCTTTACAACTTTCTTCAACACCGACCCCACTTACGGTTCTGCA
TTGGGTATAGCTTGGGCAAGCAATTGGCAATACTCCGCATTCGTCCCTACTAATCCATGGCG

TTCCTCTATGAGTCTGGTCCGTAAGTTTTCTCTCAATACTGAGTATCAGGCCAACCCAGAGA
 CAGAACTGATCAACTTAAAAGCTGAGCCTATTCTGAATATCTCTGCTGCCGGTCCATGGTCA
 AGATTCGCAACAAATACAACCCTGACTAAAGCTAATAGCTACAATGTGGATCTTAGCAATAG
 TACGGGTACCCTGGAATTGCAATTGGTTTATGCAGTAAACACAACCTCAGACCATCTCGAAAT
 5 CCGTATTCGCTGACTTATCCCTATGGTTTAAGGGTCTTGAGGATCCAGAGGAATACTTGAGA
 ATGGGTTTTGAGAATAGTGCCTCCTCGTTCTTTTTGGACAGGGGAAATTCGAAAGTGAAGTT
 CGTTAAAGAGAAATCCATATTTCACTAATAGAATGAGCGTGAATAATCAACCGTTTAAAGTCTG
 AAAATGACTTGTCTACTACAAAGTATATGGCTTGTTGGACCAAACATCCTTGAACATATAC
 TTTAATGATGGGGATGTTGTCTCAACTAATACTTACTTTATGACCACAGGAAACGCTTTGGG
 10 ATCAGTTAACATGACAACGGGTGTTGATAATCTTTTCTATATTGACAAGTTTCAAGTTAGGG
 AAGTTAAGTAATAG.

[0226] Specific expression and purification conditions will vary depending upon the expression system employed. For example, if a gene is to be expressed in *E. coli*, *S. cerevisiae*, or *P. pastoris*, it can be cloned into an expression vector by positioning the engineered gene downstream from a suitable bacterial or yeast promoter, *e.g.*, Trp or Tac, and a prokaryotic or eukaryotic signal sequence. In yeast, it may be advantageous to introduce the coding gene into the yeast genome (such as by homologous recombination) for higher expression. The expressed protein accumulates in refractile or inclusion bodies or is secreted, and can be harvested after disruption of the cells by French press or sonication, or by simply collecting the supernatant if secreted. The refractile bodies then are solubilized, and the proteins refolded and cleaved by methods known in the art. Secreted proteins can be further purified using methods known in the art such as ion exchange, affinity chromatography, or salt precipitation.

[0227] An invertase and/or isomaltase enzyme can be produced by growing (culturing) a host cell transfected with an expression vector encoding such invertase and/or isomaltase enzyme, under conditions that permit expression of the invertase and/or isomaltase enzyme. Following expression, the invertase and/or isomaltase enzyme can be harvested and purified or isolated using techniques known in the art, *e.g.*, affinity tags such as glutathione-S-transferase (GST) and histidine tags.

[0228] Exemplary methods for recombinant expression and purification of invertase enzymes are described in Mohandesi *et al.* (2016) BIOTECH 6(2):129. Exemplary methods for recombinant expression and purification of isomaltase enzymes are described in Deng *et al.* (2014) FEBS OPEN BIO 4:200–212.

[0229] In certain embodiments, an invertase and/or isomaltase enzyme is dried, *e.g.*, spray-dried or lyophilized. Pharmaceutical proteins may be dried in many ways, *e.g.*, by removal of water, organic solvent or liquid polymer by means including drying with N₂, air or inert gases, vacuum oven drying, lyophilization, washing with a volatile organic solvent followed by evaporation of the solvent, evaporation in a fume hood, tray drying, fluid bed drying, spray drying, vacuum drying, or roller drying.

[0230] Spray drying invertase and/or isomaltase enzymes allows water to be separated from the invertase and/or isomaltase enzyme preparation, allowing for continuous production of dry solids in powder, granulate, or agglomerate form from liquid feedstocks such as emulsions and pumpable suspensions. Spray drying involves the atomization of a liquid feedstock comprising invertase and/or isomaltase enzyme into a spray of droplets and contacting the droplets with hot air or gas in a drying chamber. The atomization process may be conducted using a two-fluid atomizer that mixes the liquid feedstock with a drying gas such as compressed air or nitrogen. Operating conditions and dryer design are selected according to the drying characteristics of the invertase and/or isomaltase enzyme and the desired powder qualities. Exemplary methods for spray drying enzymes are described in United States Patent Application Publication No. 2015/0353913. The invertase and isomaltase enzyme may be spray dried separately or combined and spray dried together.

[0231] It is contemplated that a disclosed invertase and/or isomaltase enzyme may be modified, engineered or chemically conjugated. For example, it is contemplated that a disclosed invertase and/or isomaltase enzyme can be conjugated to an effector agent using standard *in vitro* conjugation chemistries. If the effector agent is a polypeptide, the invertase and/or isomaltase enzyme can be chemically conjugated to the effector or joined to the effector as a fusion protein. Construction of fusion proteins is within ordinary skill in the art.

[0232] In certain embodiments, depending upon a particular mode of administration or site of activity, a disclosed invertase and/or isomaltase enzyme can be modified with a moiety that improves its stabilization and/or retention in circulation, *e.g.*, in blood, serum, or other tissues. For example, a disclosed invertase and/or isomaltase enzyme may be conjugated to a polymer, *e.g.*, a substantially non-antigenic polymer, such as a polyalkylene oxide or a polyethylene oxide. In certain embodiments, a disclosed invertase and/or isomaltase enzyme is conjugated to a water soluble polymer, *e.g.*, a hydrophilic polyvinyl polymer, *e.g.*, polyvinylalcohol or polyvinylpyrrolidone. Examples of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols,

polyoxyethylenated polyols, copolymers thereof and block copolymers thereof. Additional useful polymers include polyoxyalkylenes such as polyoxyethylene, polyoxypropylene, and block copolymers of polyoxyethylene and polyoxypropylene, polymethacrylates, carbomers, and branched or unbranched polysaccharides.

5 IV. Pharmaceutical Compositions

[0233] For therapeutic use, an invertase and/or isomaltase enzyme described herein preferably is combined with a pharmaceutically acceptable carrier and/or an excipient. The term “pharmaceutically acceptable” as used herein refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment,
10 suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0234] The term “pharmaceutically acceptable carrier” as used herein refers to buffers, carriers, and excipients suitable for use in contact with the tissues of human beings and
15 animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable carriers include any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and
20 preservatives. For examples of carriers, stabilizers and adjuvants, see, *e.g.*, *see e.g.*, Adeboye Adejare, REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY (23rd ed. 2020). Pharmaceutically acceptable carriers include buffers, solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is
25 known in the art.

[0235] In another aspect, the disclosure relates to a pharmaceutical composition comprising: an invertase enzyme, *e.g.*, a recombinant mutant invertase as described herein; a separate isomaltase enzyme; and a pharmaceutically acceptable carrier and/or an excipient. In certain embodiments, the invertase and/or the isomaltase is dried (*e.g.*, spray-dried or lyophilized).

30 [0236] In certain embodiments of the pharmaceutical compositions disclosed herein (i) the invertase is a microbial invertase (*e.g.*, derived from *Saccharomyces cerevisiae*), or a functional fragment or variant thereof, (ii) the invertase comprises a sequence of any one of SEQ ID NOs: 1-33 or 51-61, or a functional fragment or variant thereof, (iii) the invertase is

a recombinant mutant *S. cerevisiae* invertase as described herein, (iv) the isomaltase is a microbial isomaltase (e.g., derived from *S. cerevisiae* or *L. fermentum*, or a functional fragment or variant of either of the foregoing, (v) the isomaltase comprises any one of SEQ ID NOs: 37-41, or a functional fragment or variant thereof, (vi) the isomaltase comprises a

5 sequence of SEQ ID NO: 47, (vii) the isomaltase comprises substitutions at positions corresponding to the amino acids sequence set forth in SEQ ID NO: 47, including: the E93K, K115I, R132K, D226S, E310A, L366M, I421A, A444G, A531D, and F560L substitutions; the E93K, K115I, R132K, D226S, E310A, I421A, A444G, E524Q, A531D, and F560L substitutions; the E93K, K115I, R132K, D226S, E310A, I421A, A444G, A531D, and F560V

10 substitutions; the E93K, K115I, R132K, D226S, L366M, I421A, A444G, E524Q, A531D, and F560L substitutions; the E93K, K115I, R132K, D226S, L366M, I421A, A444G, A531D, and F560V substitutions; the E93K, K115I, R132K, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions; the K115I, R132K, D226S, E310A, L366M, I421A, A444G, E524Q, A531D, and F560L substitutions; the K115I, R132K, D226S, E310A, L366M, I421A, A444G, A531D, and F560V substitutions; the K115I, R132K, D226S, L366M, I421A, A444G, E524Q, A531D, and F560V substitutions; the E93K, K115I, R132K, A211E, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions; the E93K, K115I, R132K, D226S, L366M, I421A, A444G, E524Q, A531D, and F560V substitutions; the E93K, K115I, R132K, D226S, L366M, I421A, A444G, E524Q, A531D, and F560V substitutions; the E93K, K115I, R132K, D226S, E275D, I421A, A444G, E524Q, A531D, and F560V substitutions; the

20 T89A, E93K, K115I, R132K, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions; the E93K, K115I, R132K, H182R, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions; the E93K, K115I, E122D, R132K, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions; the E93K, K115I, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions; the E93K, K115I, D226S, I421A, A444G, E524Q, A531D, and F560L substitutions; the K115I, A211E, D226S, I421A, A444G, A531D, and F560V substitutions; the K115I, D226S, L366M, I421A, A444G, E524Q, A531D, and F560L substitutions; the K115I, A211E, D226S, I421A, A444G, E524Q, A531D, and F560L substitutions; the K115I, R132K, D226S, E310A, L366M, I421A, A444G, A531D, and

30 F560L substitutions; the E93K, K115I, D226S, E310A, I421A, A444G, A531D, and F560L substitutions; the K115I, D226S, E310A, I421A, A444G, A531D, and F560V substitutions; the E93K, K115I, D226S, L366M, I421A, A444G, A531D, and F560L substitutions; the K115I, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions; the K115I, D226S,

E310A, I421A, A444G, E524Q, A531D, and F560L substitutions; the K115I, A211E, D226S, L366M, I421A, A444G, A531D, and F560L substitutions; the K115I, A211E, D226S, E310A, I421A, A444G, A531D, and F560L substitutions; the E93K, K115I, D226S, I421A, A444G, A531D, and F560V substitutions; the E93K, K115I, A211E, D226S, I421A, A444G, A531D, and F560L substitutions; or the K115I, D226S, L366M, I421A, A444G, A531D, and F560V substitutions, (viii) the isomaltase is a recombinant mutant isomaltase set forth in International Patent Application No. PCT/US2023/070180, filed on July 13, 2023, or (ix) or a combination of any one of features (i) – (viii). In certain embodiments, the invertase comprises the amino acid sequence of SEQ ID NO: 51, or a functional fragment thereof. In certain embodiments, the invertase comprises the amino acid sequence of SEQ ID NO: 52, or a functional fragment thereof. In certain embodiments, the invertase comprises the amino acid sequence of SEQ ID NO: 53, or a functional fragment thereof.

[0237] In certain embodiments, the composition is formulated as an oral dosage form. In certain embodiments, the composition is formulated as a liquid, powder, sachet, granulate, pellet, micropellet, tablet, or minitab. In certain embodiments, the composition is formulated as a liquid, powder, sachet, or tablet. In certain embodiments, the composition has a shelf-life at room temperature of at least 3 months, 6 months, 9 months, 12 months, 15 months, 18 months, 21 months, 24 months, 36 months, 48 months, 60 months, 72 months, 84 months, 96 months, 108 months, or 120 months.

[0238] In certain embodiments, the invertase and/or isomaltase enzymes can be formulated, or co-administered (either at the same time or sequentially), for example, by an enteral route (*e.g.*, orally), with a pH increasing agent, for example, a protein pump inhibitor (PPI), to enhance the stability of the invertase and/or isomaltase enzyme, for example, in an acidic environment, for example, in the gastrointestinal tract.

[0239] Proton pump inhibitors are a group of drugs whose main action is pronounced and long-lasting reduction of gastric acid production. Proton pump inhibitors act by blocking the hydrogen/potassium adenosine triphosphatase enzyme system (the H⁺/K⁺ ATPase, or more commonly just gastric proton pump) of the gastric parietal cell. The proton pump is the terminal stage in gastric acid secretion, being directly responsible for secreting H⁺ ions into the gastric lumen, making it an ideal target for inhibiting acid secretion. Examples of proton pump inhibitors include: Omeprazole (brand names: LOSEC[®], PRILOSEC[®], ZEGERID[®]); Lansoprazole (brand names: PREVACID[®], ZOTON[®], INHIBITOL[®]); Esomeprazole (brand

names: NEXIUM®); and Pantoprazole (brand names: PROTONIX®, SOMAC®, PANTOLOC®).

[0240] Pharmaceutical compositions containing a recombinant invertase and/or isomaltase enzyme disclosed herein can be presented in a dosage unit form and can be prepared by any suitable method. A pharmaceutical composition should be formulated to be compatible with its intended route of administration. The pharmaceutical compositions may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions, dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form will depend upon the intended mode of administration and therapeutic application. In certain embodiments, the composition is formulated as an oral dosage form. The oral dosage form may, for example, be formulated as a liquid, powder, sachet, granulate, pellet, micropellet, tablet, or minitab. In some embodiments, the composition is formulated as a liquid, powder, sachet, or tablet.

[0241] Although the compositions preferably are formulated for administration enterally (for example, orally), such compositions can be administered by a parenteral mode (*e.g.*, intravenous, subcutaneous, intraperitoneal, or intramuscular injection). The phrases "parenteral administration" and "administered parenterally" as used herein mean modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and infrasternal injection and infusion.

[0242] The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable for stable storage at high concentration. Sterile injectable solutions can be prepared by incorporating an agent described herein in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating an agent described herein into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze drying that yield a powder of an agent described herein plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use

of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

- 5 [0243] Depending upon the mode of administration, for example, by parenteral administration, it may be desirable to produce a pharmaceutical formulation that is sterile. Sterilization can be accomplished by any suitable method, *e.g.*, filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.
- 10 [0244] In certain embodiments, a disclosed composition comprises a polyionic reagent which may, *e.g.*, coat the invertase and/or isomaltase enzyme (*e.g.*, the composition comprises a polyionic coating). Exemplary polyionic reagents include PSS (poly(Sodium 4-styrenesulfonate), PAA (poly Acrylic acid sodium salt), PMG (poly(methylene-co-guanidine) hydrochloride), DS (dextran sulfate), PMA (poly(methyl acrylate)), or PVS
- 15 (polyvinylsiloxane).

- [0245] In certain embodiments, a disclosed composition and/or dosage comprises: (i) about 750 to about 75,000, about 750 to about 60,000, about 750 to about 45,000, about 750 to about 30,000, about 750 to about 15,000, about 750 to about 10,000, about 750 to about 7500, about 750 to about 5,000, about 750 to about 2500, about 750 to about 1,000, about
- 20 1,000 to about 75,000, about 1,000 to about 60,000, about 1,000 to about 45,000, about 1,000 to about 30,000, about 1,000 to about 15,000, about 1,000 to about 10,000, about 1,000 to about 7,500, about 1,000 to about 5,000, about 1,000 to about 2,500, about 2,500 to about 75,000, about 2,500 to about 60,000, about 2,500 to about 45,000, about 2,500 to about 30,000, about 2,500 to about 15,000, about 2,500 to about 10,000, about 2,500 to about
- 25 7,500, about 2,500 to about 5,000, about 5,000 to about 75,000, about 5,000 to about 60,000, about 5,000 to about 45,000, about 5,000 to about 30,000, about 5,000 to about 15,000, about 5,000 to about 10,000, about 5,000 to about 7,500, about 7,500 to about 75,000, about 7,500 to about 60,000, about 7,500 to about 45,000, about 7,500 to about 30,000, about 7,500 to about 15,000, about 7,500 to about 10,000, about 10,000 to about 75,000, about 10,000 to
- 30 about 60,000, about 10,000 to about 45,000, about 10,000 to about 30,000, about 10,000 to about 15,000, about 15,000 to about 75,000, about 15,000 to about 60,000, about 15,000 to about 45,000, about 15,000 to about 30,000, about 30,000 to about 75,000, about 30,000 to about 60,000, about 30,000 to about 45,000, about 45,000 to about 75,000, about 45,000 to

about 60,000, or about 60,000 to about 75,000 international units (I.U.) of invertase enzyme;
and/or (ii) about 750 to about 75,000, about 750 to about 60,000, about 750 to about 45,000,
about 750 to about 30,000, about 750 to about 15,000, about 750 to about 10,000, about 750
to about 7,500, about 750 to about 5,000, about 750 to about 2,500, about 750 to about 1,000,
5 about 1,000 to about 75,000, about 1,000 to about 60,000, about 1,000 to about 45,000, about
1,000 to about 30,000, about 1,000 to about 15,000, about 1,000 to about 10,000, about 1,000
to about 7,500, about 1,000 to about 5,000, about 1,000 to about 2,500, about 2,500 to about
75,000, about 2,500 to about 60,000, about 2,500 to about 45,000, about 2,500 to about
30,000, about 2,500 to about 15,000, about 2,500 to about 10,000, about 2,500 to about
10 7,500, about 2,500 to about 5,000, about 5,000 to about 75,000, about 5,000 to about 60,000,
about 5,000 to about 45,000, about 5,000 to about 30,000, about 5,000 to about 15,000, about
5,000 to about 10,000, about 5,000 to about 7,500, about 7,500 to about 75,000, about 7,500
to about 60,000, about 7,500 to about 45,000, about 7,500 to about 30,000, about 7,500 to
about 15,000, about 7,500 to about 10,000, about 10,000 to about 75,000, about 10,000 to
15 about 60,000, about 10,000 to about 45,000, about 10,000 to about 30,000, about 10,000 to
about 15,000, about 15,000 to about 75,000, about 15,000 to about 60,000, about 15,000 to
about 45,000, about 15,000 to about 30,000, about 30,000 to about 75,000, about 30,000 to
about 60,000, about 30,000 to about 45,000, about 45,000 to about 75,000, about 45,000 to
about 60,000, or about 60,000 to about 75,000 international units (I.U.) of isomaltase
20 enzyme.

[0246] In certain embodiments, a disclosed composition and/or dosage comprises: (i) about
5,000 to about 12,000, about 5,000 to about 11,000, about 5,000 to about 10,000, about 5,000
to about 9,000, about 5,000 to about 8,000, about 5,000 to about 7,000, about 5,000 to about
6,000, about 6,000 to about 12,000, about 6,000 to about 11,000, about 6,000 to about
25 10,000, about 6,000 to about 9,000, about 6,000 to about 8,000, about 6,000 to about 7,000,
about 7,000 to about 12,000, about 7,000 to about 11,000, about 7,000 to about 10,000, about
7,000 to about 9,000, about 7,000 to about 8,000, about 8,000 to about 12,000, about 8,000 to
about 11,000, about 8,000 to about 10,000, about 8,000 to about 9,000, about 9,000 to about
12,000, about 9,000 to about 11,000, about 9,000 to about 10,000, about 10,000 to about
30 12,000, about 10,000 to about 11,000, about 11,000 to about 12,000, about 5,000, about
6,000, about 7,000, about 8,000, about 8,500, about 9,000, about 10,000, about 11,000, or
about 12,000 international units (I.U.) of invertase enzyme; and/or (ii) about 5,000 to about
12,000, about 5,000 to about 11,000, about 5,000 to about 10,000, about 5,000 to about

9,000, about 5,000 to about 8,000, about 5,000 to about 7,000, about 5,000 to about 6,000, about 6,000 to about 12,000, about 6,000 to about 11,000, about 6,000 to about 10,000, about 6,000 to about 9,000, about 6,000 to about 8,000, about 6,000 to about 7,000, about 7,000 to about 12,000, about 7,000 to about 11,000, about 7,000 to about 10,000, about 7,000 to about 9,000, about 7,000 to about 8,000, about 8,000 to about 12,000, about 8,000 to about 11,000, about 8,000 to about 10,000, about 8,000 to about 9,000, about 9,000 to about 12,000, about 9,000 to about 11,000, about 9,000 to about 10,000, about 10,000 to about 12,000, about 10,000 to about 11,000, about 11,000 to about 12,000, about 5,000, about 6,000, about 7,000, about 8,000, about 8,500, about 9,000, about 10,000, about 11,000, or about 12,000

10 international units (I.U.) of isomaltase enzyme.

[0247] In certain embodiments, a disclosed invertase and/or isomaltase enzyme or composition is administered to a subject together with a meal or snack. In certain embodiments, a disclosed invertase and/or isomaltase enzyme or composition is administered to a subject together with each meal or snack that the subject eats. In certain embodiments, a disclosed invertase and/or isomaltase enzyme or composition is administered to a subject once every 7 days, once every 6 days, once every 5 days, once every 4 days, once every 3 days, once every 2 days, once every day, 2 times every day, 3 times every day, 4 times every day, 5 times every day, 6 times every day, or more than 6 times every day.

[0248] Depending upon the circumstances, the composition can be formulated as a powder, granulate, pellet, micropellet, or a minitab. The composition can be encapsulated in a capsule, *e.g.*, a hydroxypropyl methylcellulose (HPMC) capsule, soft gelatin capsule, or a hard gelatin capsule. Alternatively, the composition can be formulated as a tablet dosage form. The dosage form, *e.g.*, granules, tablets, minitab. pellets, micropellets, capsules can be enterically coated using, *e.g.*, one or more methacrylic polymers, such as methacrylic acid methylmethacrylate copolymers (*e.g.*, Eudragit L and S), and methacrylic acid ethyl acrylate copolymer (Eudragit L30D), hydroxyl propyl methyl cellulose acetate, hydroxy propyl methyl cellulose phthalate succinate and others.

[0249] In certain embodiments, the composition comprises less than 50%, less than 40%, less than 30%, less than 20%, less than 10% or less than 5% glycerol. In certain embodiments, the composition does not comprise glycerol.

[0250] In certain embodiments, a disclosed pharmaceutical composition has a shelf-life (*e.g.*, retains at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or

100% of its biological activity) at room temperature of at least 3 months, 6 months, 9 months, 12 months, 15 months, 18 months, 21 months, 24 months, 36 months, 48 months, 60 months, 72 months, 84 months, 96 months, 108 months, or 120 months. In certain embodiments, a disclosed pharmaceutical composition has a shelf-life at room temperature of from about 3 to about 120 months, from about 3 to about 96 months, from about 3 to about 72 months, from about 3 to about 48 months, from about 3 to about 24 months, from about 3 to about 21 months, from about 3 to about 18 months, from about 3 to about 15 months, from about 3 to about 12 months, from about 3 to about 9 months, from about 3 to about 6 months, from about 6 to about 120 months, from about 6 to about 96 months, from about 6 to about 72 months, from about 6 to about 48 months, from about 6 to about 24 months, from about 6 to about 21 months, from about 6 to about 18 months, from about 6 to about 15 months, from about 6 to about 12 months, from about 6 to about 9 months, from about 9 to about 120 months, from about 9 to about 96 months, from about 9 to about 72 months, from about 9 to about 48 months, from about 9 to about 24 months, from about 9 to about 21 months, from about 9 to about 18 months, from about 9 to about 15 months, from about 9 to about 12 months, from about 12 to about 120 months, from about 12 to about 96 months, from about 12 to about 72 months, from about 12 to about 48 months, from about 12 to about 24 months, from about 12 to about 21 months, from about 12 to about 18 months, from about 12 to about 15 months, from about 15 to about 120 months, from about 15 to about 96 months, from about 15 to about 72 months, from about 15 to about 48 months, from about 15 to about 24 months, from about 15 to about 21 months, from about 15 to about 18 months, from about 18 to about 120 months, from about 18 to about 96 months, from about 18 to about 72 months, from about 18 to about 48 months, from about 18 to about 24 months, from about 18 to about 21 months, from about 21 to about 120 months, from about 21 to about 96 months, from about 21 to about 72 months, from about 21 to about 48 months, from about 21 to about 24 months, from about 24 to about 120 months, from about 24 to about 96 months, from about 24 to about 72 months, from about 24 to about 48 months, from about 48 to about 120 months, from about 48 to about 96 months, from about 48 to about 72 months, from about 72 to about 120 months, from about 72 to about 96 months, or from about 96 to about 120 months. In certain embodiments, a disclosed pharmaceutical composition retains at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of its

biological activity after 12 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks at 40 °C and 75% relative humidity (RH).

V. Therapeutic Uses

[0251] The methods and compositions disclosed herein can be used to treat diseases or disorders. For example, the disclosure provides a method of treating sucrase-isomaltase deficiency (*e.g.*, congenital sucrase isomaltase deficiency (CSID) or acquired sucrase-isomaltase deficiency (ASID)) in a subject. The method comprises administering (*e.g.*, orally administering) to the subject an effective amount of (i) an invertase enzyme (*e.g.*, a recombinant mutant invertase enzyme described herein), and/or (ii) an isomaltase enzyme. For example, the method may comprise administering a disclosed pharmaceutical composition comprising (i) a dried (*e.g.*, spray-dried or lyophilized) invertase enzyme (*e.g.*, a recombinant mutant invertase enzyme described herein), and/or (ii) a dried (*e.g.*, spray-dried or lyophilized) isomaltase enzyme. In certain embodiments, the pharmaceutical composition is administered to the subject together with a meal or snack.

[0252] Under certain conditions, the method reduces abdominal pain, bloating, and/or nausea in the subject, and as a result, the method reduces a total symptom score (for example, measured by abdominal pain, bloating and nausea) for the subject. Furthermore, the invention permits greater diet liberalization in the subject (*e.g.*, allows the subject to better tolerate certain foods, *e.g.*, grains (*e.g.*, wheat, potatoes, corn, rice, and bread), fruits (*e.g.*, apples, bananas, apricots, and oranges) and/or vegetables (*e.g.*, beets, carrots, and black beans)).

[0253] The term “effective amount” as used herein refers to the amount of an active agent (*e.g.*, a disclosed invertase and/or isomaltase enzyme) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not intended to be limited to a particular formulation or administration route.

[0254] As used herein, “treat”, “treating” and “treatment” mean the treatment of a disease in a subject, *e.g.*, in a human. This includes: (a) inhibiting the disease, *i.e.*, arresting its development; and (b) relieving the disease, *i.e.*, causing regression of the disease state. As used herein, the terms “subject” and “patient” refer to an organism to be treated by the methods and compositions described herein. Such organisms preferably include, but are not limited to, mammals (*e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like), and more preferably includes humans.

[0255] The disclosure also provides a method of reducing an amount of sucrose and branched (1–6 linked) α -limit dextrin in a subject, for example, a subject with sucrase-isomaltase deficiency (*e.g.*, congenital sucrase-isomaltase deficiency (CSID) or acquired sucrase-isomaltase deficiency (ASID)). The method comprises administering (*e.g.*, orally administering) to the subject an effective amount of (i) an invertase enzyme (*e.g.*, a recombinant mutant invertase enzyme described herein), and/or (ii) an isomaltase. For example, the method may comprise administering a disclosed pharmaceutical composition comprising (i) a dried (*e.g.*, spray-dried or lyophilized) invertase (*e.g.*, a recombinant mutant invertase described herein), and (ii) a dried (*e.g.*, spray-dried or lyophilized) isomaltase. An amount of sucrose and branched (1–6 linked) α -limit dextrin in a subject may refer to an amount of sucrose and branched (1–6 linked) α -limit dextrin measured by a hydrogen breath test, *e.g.*, a sucrose methane hydrogen breath test or a ^{13}C -sucrose breath test. An amount of sucrose and branched (1–6 linked) α -limit dextrin in a subject may refer to an amount of sucrose and branched (1–6 linked) α -limit dextrin in a body fluid (*e.g.*, blood, plasma, serum, or urine), tissue and/or cell in the subject.

[0256] The methods and compositions described herein can be used alone or in combination with other therapeutic agents and/or modalities. The term administered “in combination,” as used herein, is understood to mean that two (or more) different treatments are delivered to the subject during the course of the subject’s affliction with the disorder, such that the effects of the treatments on the patient overlap at a point in time. In certain embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as “simultaneous” or “concurrent delivery”. In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In certain embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, *e.g.*, an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In certain embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

[0257] In certain embodiments, a method or composition described herein, is administered in combination with one or more fructose degrading enzymes. Exemplary fructose degrading enzymes include glucose (xylose) isomerases.

5 [0258] Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions of the present disclosure that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present disclosure that consist essentially of, or consist of, the recited processing steps. Similarly, throughout
10 the description, where compositions are described as consisting essentially of specific components, or where processes and methods are described as consisting essentially of specific steps, it is contemplated that, additionally, there are compositions of the present disclosure that consist of the recited components, and that there are processes and methods according to the present disclosure that consist of the recited processing steps.

15 [0259] In the application, where an element or component is said to be included in and/or selected from a list of recited elements or components, it should be understood that the element or component can be any one of the recited elements or components, or the element or component can be selected from a group consisting of two or more of the recited elements or components.

20 [0260] Further, it should be understood that elements and/or features of a composition or a method described herein can be combined in a variety of ways without departing from the spirit and scope of the present invention, whether explicit or implicit herein. For example, where reference is made to a particular compound, that compound can be used in various embodiments of compositions of the present invention and/or in methods of the present
25 invention, unless otherwise understood from the context. In other words, within this application, embodiments have been described and depicted in a way that enables a clear and concise application to be written and drawn, but it is intended and will be appreciated that embodiments may be variously combined or separated without parting from the present teachings and invention(s). For example, it will be appreciated that all features described and
30 depicted herein can be applicable to all aspects of the invention(s) described and depicted herein.

[0261] It should be understood that the expression “at least one of” includes individually each of the recited objects after the expression and the various combinations of two or more

of the recited objects unless otherwise understood from the context and use. The expression “and/or” in connection with three or more recited objects should be understood to have the same meaning unless otherwise understood from the context.

[0262] The use of the term “include,” “includes,” “including,” “have,” “has,” “having,” “contain,” “contains,” or “containing,” including grammatical equivalents thereof, should be understood generally as open-ended and non-limiting, for example, not excluding additional unrecited elements or steps, unless otherwise specifically stated or understood from the context.

[0263] Where the use of the term “about” is before a quantitative value, the present invention also includes the specific quantitative value itself, unless specifically stated otherwise. As used herein, the term “about” refers to a $\pm 10\%$ variation from the nominal value unless otherwise indicated or inferred.

[0264] It should be understood that the order of steps or order for performing certain actions is immaterial so long as the present invention remain operable. Moreover, two or more steps or actions may be conducted simultaneously.

[0265] The use of any and all examples, or exemplary language herein, for example, “such as” or “including,” is intended merely to illustrate better the present invention and does not pose a limitation on the scope of the invention unless claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the present invention.

EXAMPLES

[0266] The following Examples are merely illustrative and are not intended to limit the scope or content of the invention in any way.

Example 1: Invertase Selection and Engineering

[0267] This Example describes the design of recombinant mutant *S. cerevisiae* invertases with improved tolerance of low pH, resistance to digestion by pepsin and pancreatin, improved thermostability, and high catalytic activity.

Invertase Expression

[0268] Expression constructs encoding recombinant *S. cerevisiae* invertase (GenBank: AFN08663.1, SEQ ID NO: 1) were constructed for expression in the yeast expression strains *Pichia pastoris* and *S. cerevisiae*. Expression constructs were designed with or without a C-terminal or N-terminal 6x-His affinity tag (SEQ ID NO: 62) and were tested for expression and tolerance of the added His tag. Further expression constructs were designed with either

the natural invertase secretion leader intact (SEQ ID NO: 1) or with a substituted alpha-mating factor (FAKS) leader (SEQ ID NO: 2). The nucleic acid sequences of each expression construct were optimized for high expression in the corresponding host. The synthesized nucleic acid sequences encoding for the invertases were cloned into pD1204 (*S. cerevisiae*) or pD902 (*P. pastoris*).

[0269] For expression in *S. cerevisiae*, the strain CEN.PK2-1C was transformed chemically using the lithium acetate method (Ito *et al.* (1983) J. BACTERIOL. 153(1):163-168). Cells harboring plasmid were selected by plating on CM-URA media (MP Biomedicals, Santa Ana, CA) over 3 days at 30 °C. Three individual colonies for each construct were isolated and used to inoculate 500 µL of CM-URA media. The inoculated culture was incubated at 30 °C with shaking at 1,000 rpm for 16 hours. 40 µL of overnight culture was then used to inoculate 500 µL of YP induction media (1% yeast extract, 2% peptone, 0.1 M potassium phosphate, 2% raffinose, and 2% galactose). The induction culture was then incubated at 30 °C with shaking at 1,000 rpm for 72 hours. The induction culture was supplemented with 50 µL 20% galactose every 24 hours after induction. The supernatant was clarified by centrifugation and secreted invertase expression was analyzed by PAGE (4-12% Bis-tris, MOPS-buffered; Invitrogen).

[0270] For expression in *P. pastoris*, 20 µg of expression plasmid was linearized by digestion with PmeI. The DNA was isolated by precipitation with ethanol and reconstituted in Qiagen EB buffer. For expression studies, the strain BG10 was transformed by electroporation. For protein engineering, the strain BG10 was transformed chemically using the lithium acetate method (Ito *et al.*, *supra*). Cells harboring plasmid were selected by plating on YPD media with 250 µg/mL zeocin (Teknova) over 3 days at 30 °C. Three individual colonies for each construct were isolated and used to inoculate 350 µL of BYPG media with 250 µg/mL zeocin in a deep-well microplate. The inoculated culture was then incubated at 30 °C with shaking at 1,000 rpm for 60 hours. 250 µL of the overnight culture was then added to 250 µL BYPM induction media (BYP media supplemented with 20% methanol). The induction culture was then incubated at 30 °C with shaking at 1,000 rpm for 72 hours. Cultures were supplemented with 50 µL BYP with 10% methanol every 12 hours after induction. Supernatants were clarified from cells by centrifugation and secreted invertase expression was analyzed by PAGE.

[0271] Expression yields were determined by PAGE densitometry (after deglycosylation with PNGaseF, if deglycosylated). For experiments using deglycosylated enzyme, culture

supernatant samples were treated with PNGaseF after denaturation according to manufacturer's protocol (NEB) and the samples were analyzed by PAGE. Full-length glycosylated or deglycosylated invertase band intensities were determined and intensity relative to BSA standards was used to estimate protein concentration.

- 5 [0272] Expression and yield of *S. cerevisiae* invertase in *P. pastoris* was typically better than in *S. cerevisiae*.

Invertase Activity and Stability Assays

- [0273] In the initial stages, when the resulting proteins tested without purification, specific activities were approximated by assaying units per amount of deglycosylated or glycosylated protein (1 unit of enzyme consumes 1 μ mole sucrose per minute at 37 °C).

- [0274] Activity assays were performed using the dinitro salicylate (DNS) colorimetric method to monitor reducing sugar formation upon hydrolysis of sucrose. Briefly, the DNS method detects the reducing sugars liberated by the action of hydrolase enzymes on carbohydrates, under specific pH and temperatures (Bailey (1988) APPLIED MICROBIOLOGY AND BIOTECHNOLOGY 29: 494–496). Upon reaction with a reducing sugar DNS is reduced to 3-amino-5-nitro salicylic acid and the reaction product can be monitored at 540 nm.

- [0275] Culture supernatants were diluted 1/20 into reaction buffer containing 237 mM NaCl and either: A) 50 mM glycine, pH 3.5 (buffer A); B) 50 mM sodium acetate, pH 4.7 (buffer B); C) 50 mM MES, pH 5.8 (buffer C); or D) 50 mM sodium phosphate, pH 6.8 (buffer D).
- 20 Diluted enzymes were then further diluted 10-fold into 10 mM sucrose in reaction buffer pre-equilibrated at 37 °C. Reaction progress was then estimated by the DNS method. To stop the reaction, 20 μ L of reaction was removed to 30 μ L DNS reagent (10 mg/mL dinitrosalicylic acid, 300 mg/mL tartrate, 0.4 N NaOH) and the mixture was immediately heated to 99 °C for 5 minutes. 40 μ L of the DNS reaction was diluted with 160 μ L water and the absorbance
- 25 was read at 540 nm. Reducing sugars present were determined relative to 1:1 glucose:fructose standards.

- [0276] Thermostability was assayed by treatment of invertase samples at 56.5 °C for 30 minutes prior to assaying residual activity at pH 4.7 (in reaction buffer B, described above) using the DNS method as described above.
- 30 [0277] Low pH tolerance assays were performed as follows. Culture supernatants including the invertase enzymes were diluted 1/20 into 237 mM NaCl, 50 mM glycine pH 2.8 and preincubated at 37 °C for 30 minutes. Preincubations were then diluted into 10 mM sucrose

in reaction buffer at a final pH 4.7 and activity was assayed using the DNS method as described above.

[0278] Resistance to pepsin was assayed by treating the invertase enzymes with 10 mg/mL soluble pepsin from porcine gastric mucosa (Sigma-Aldrich, St. Louis, MO) in 237 mM NaCl, 50 mM NaOAc pH 4 at 37 °C. Culture supernatants including the invertase enzymes were diluted 20-fold into 10 mg/mL pepsin and incubated for 2 hours at 37 °C. Residual invertase activity was then assayed by diluting the pepsin treatment reaction 20-fold into 10 mM sucrose in reaction buffer at a final pH of 4.7 and activity was assayed using the DNS method as described above. The overexpressed *S. cerevisiae* invertase and most invertase variants showed insignificant degradation under these conditions.

[0279] Resistance to pancreatin was assayed by treating the invertases with 3.4 mg/mL soluble porcine pancreatin (Sigma-Aldrich, St. Louis, MO) in 237 mM NaCl, 50 mM MES pH 6 at 37 °C. Culture supernatants were diluted 20-fold into 3.4 mg/mL pancreatin and incubated for 2 hours at 37 °C. Residual invertase activity was then assayed by diluting the pancreatin treatment reaction 20-fold into 10 mM sucrose in reaction buffer at a final pH of 4.7 and activity was assayed using the DNS method as described above.

Invertase Engineering

[0280] 95 recombinant *S. cerevisiae* invertase variants (denoted as V1-V95) were created using protein engineering principles, each containing three substitutions relative to the wild-type natural protein *S. cerevisiae* invertase (denoted as V0). **TABLE 2** shows certain exemplary amino acid mutations in *S. cerevisiae* invertase (SEQ ID NO: 2) that were analyzed in the invertase engineering program. The variants along with the wild-type (wt) *S. cerevisiae* invertase were expressed in *P. pastoris* and tested as culture supernatants for activity and stability. Each variant was tested for seven properties using the assays described above, including: 1) expression level, 2) activity at pH 3.5, 3) activity at pH 7, 4) thermostability, 5) stability at pH 2.8, 6) resistance to pepsin, and 7) resistance to pancreatin.

TABLE 2

E120A	K277T	N429A	K519R
D122E	K259L	L459F	S537D
A123G	L359F	T470S	L549I
T140L	T365S	T573S	V581I
T140Q	A378T	S476N	G586S

F143Y	E382Q	S476A	N589K
E156Q	N390D	P479A	F596I
F172Y	E415S	N512G	Q597E
V178I	L416Q	K514P	

[0281] Overall, 94 invertase variants were expressed and tested (one variant did not express) and compared against wild-type enzyme. In general, 13 invertase variants showed an improvement pH 2.8 stability, thermostability, pH 3.5 stability, and pH 7.0 activity over the parent invertase, 35 invertase displayed mixed properties, and 46 invertase variants displayed worse properties than the parent invertase. Exemplary results for the engineered invertase variants compared to parental, wild-type invertase (*S. cerevisiae* invertase) are shown in **TABLE 3** and the substitutions present in the various invertase variants (relative to *S. cerevisiae* invertase (SEQ ID NO: 2) listed in **TABLE 3** are shown in **TABLE 4**. **TABLE 3** includes the properties of the 13 invertase variants (V6, V9, V14, V25, V32, V38, V60, V63, V70, V78, V83, V87, V89, and V90), that showed one or more improved properties relative to parental invertase, and three exemplary invertase variants (V3, V75, and V82) that performed worse than the parental invertase.

TABLE 3

Invertase	Stability at pH2.8 ^{a)}	Thermostability ^{a)}	Activity at pH3.5 ^{a)}	Activity at pH7.0 ^{a)}
Parental invertase (V0)	~	~	~	~
V6	+	++	~	+
V9	++	+	~	~
V14	+	++	~	+
V22	+	-	-	-
V25	~	~	~	+
V32	+	+	+	~
V38	~	+	~	+
V60	+	+	~	~

Invertase	Stability at pH2.8^{a)}	Thermostability^{a)}	Activity at pH3.5^{a)}	Activity at pH7.0^{a)}
V63	~	+	~	~
V70	~	++	~	+
V78	+	++	+	++
V83	+	~	~	~
V87	+	+	~	~
V89	+	~	~	+
V90	+	+	~	~
V3	-	-	-	+
V75	-	-	-	-
V82	-	-	-	-

^a Relative impacts of the substitutions are indicated as strongly positive (++), moderately positive (+), moderately negative (-) or neutral (~) relative to the parent invertase.

TABLE 4

Name	Substitution 1	Substitution 2	Substitution 3	SEQ ID NO:
V6	E120A	D122E	P479A	SEQ ID NO: 4
V9	T140L	E156Q	L549I	SEQ ID NO: 5
V14	S476A	P479A	Q597E	SEQ ID NO: 6
V22	K259L	S476N	K514P	SEQ ID NO: 7
V25	K277T	E415S	V581I	SEQ ID NO: 8
V32	A123G	T140L	F143Y	SEQ ID NO: 9
V38	N390D	S476N	K519R	SEQ ID NO: 10
V60	E382Q	T573S	V581I	SEQ ID NO: 11
V63	D122E	T470S	S476N	SEQ ID NO: 12
V70	T140Q	P479A	K514P	SEQ ID NO: 13
V78	F172Y	N429A	P479A	SEQ ID NO: 14
V83	E120A	V178I	L549I	SEQ ID NO: 15

Name	Substitution 1	Substitution 2	Substitution 3	SEQ ID NO:
V87	E382Q	S537D	N589K	SEQ ID NO: 16
V89	T140L	G586S	F596I	SEQ ID NO: 17
V90	E382Q	L459F	K519R	SEQ ID NO: 18
V3	K277T	T365S	N512G	
V75	D122E	L359F	A378T	
V82	E120A	L416Q	S537D	

[0282] The relative impact of each substitution on each target property was estimated and is set forth in **TABLE 5** (amino acid numbering is relative to *S. cerevisiae* invertase (SEQ ID NO: 2). In particular, the impact of certain amino acid substitutions relative to wild-type *S. cerevisiae* invertase (SEQ ID NO: 2) that showed significant contribution to at least one of three key invertase properties: pH 7.0 activity, thermostability, or low pH survival, are summarized in **TABLE 5**.

TABLE 5

Amino Acid Substitution	Activity at pH 7.0 ^{a)}	Low pH Stability ^{a)}	Thermostability ^{a)}
A378T	+	--	-
A501N	~	~	+
D272N	+	~	~
E156Q	~	+	
E285H	~	~	++
E382Q	~	+	+
F172Y	+	+	~
G431R	~	+	-
G586S	+	~	~
K259L	-	++	~
K277T	+	~	-
L549I	+	~	+
N589K	~	+	+
P479A	++	+	++
T140L	~	+	++
V178I	~	+	-

Amino Acid Substitution	Activity at pH 7.0 ^{a)}	Low pH Stability ^{a)}	Thermostability ^{a)}
V399A	+	-	+
V477D	+	~	~
V581I	~	+	+
D122E	-	+	+

^a Relative impacts of substitutions are indicated as strongly positive (++), moderately positive (+), moderately negative (-) or neutral (~).

Further Characterization of Certain Invertase Variants

[0283] A number of invertase variants showing improved properties relative to the parental invertase in terms of activity and/or stability were further characterized. In particular, two studies were performed on independent preparations of the selected variants and the results are summarized in **TABLES 6** and **7**. **TABLE 6** summarizes the characteristics of three variants compared against wild-type parental invertase.

TABLE 6

Invertase Variant	Activity (U/mg protein) ^a				Stability			
	pH 3.5	pH 5.0	pH 6.2	pH 7.1	pH 2.5 ^b	Thermo-stability ^c (T _m , °C)	Pepsin ^d	Pancreatin ^d
V0 (Parent)	768	956	660	171	16	55	>80	>80
V78	1288	1520	1099	311	63	62	>80	>80
V22	770	1029	637	96	76	54	>80	>80
V6	1037	1188	830	247	60	61	>80	>80

^a Specific activity was estimated as μ mole sucrose consumed per minute per mg of deglycosylated invertase in reactions (single point measurements from single variant preparations for each reaction pH).

^b Stability at pH 2.5 was measured as the percent residual activity remaining after pretreatment at 37 °C for 30 minutes.

^c Thermostability was estimated as the observed temperature of 50% loss of activity after 30 minutes of pretreatment.

^d Percent residual activity after protease pretreatment. Protease stability was assessed at 37 °C at 10 mg/mL pepsin in SGF, pH 4 or 3.4 mg/mL pancreatin in SIF, pH 6. All variants showed <20% loss of activity under the assay conditions after 2 hours pretreatment.

[0284] Strong improvements in activity, low pH stability and thermostability were seen for variants V78 (F172Y, N429A, P479A; SEQ ID NO: 14) and V6 (E120A, D122E, P479A; SEQ ID NO: 4). Both variants V6 and V78 contained the broadly beneficial substitution P479A and at least one other substitution positive for one or more critical properties. V22 (K259L, S476N, K514P; SEQ ID NO: 7) showed an improvement in low pH stability. V22 contained the K259L substitution which was found to be beneficial for low pH stability but not high pH activity.

[0285] To further compare enzymes that were expressed under similar conditions, *Sc_S288C* (SEQ ID NO: 36) invertase was expressed in *P. pastoris* and assayed under identical conditions as certain of the better variants. Further variants of *Sc_S288C* invertase were tested that included the two additional substitutions found in V78 (F172Y and N429A) to assess their impact in the context of the *Sc_S288C* invertase sequence. The results of the comparison are summarized in **TABLE 7**.

TABLE 7

Variant	Activity (U/mg protein) ^a				Stability			
	pH 3.5	pH 5	pH 6	pH 7	pH 2.7 ^b	Thermo- stability ^c	Pepsin ^d	Pancreatin ^d
V0 (Parent)	960	1020	800	300	28	55	>80	>80
V78	1540	1670	1470	820	82	62	>80	>80
<i>ScS288C</i>	1230	1330	1107	580	89	64	nd	nd

	Activity (U/mg protein) ^a				Stability			
Variant	pH 3.5	pH 5	pH 6	pH 7	pH 2.7 ^b	Thermo- stability ^c	Pepsin ^d	Pancreatin ^d
<i>ScS288C</i> + F172Y + 429A	1240	1370	1190	690	97	65	nd	nd

^a Specific activity was estimated as μ mole sucrose consumed per minute per mg of deglycosylated invertase in reactions. Expression and reactions were performed in triplicate.

^b Stability at pH 2.7 was measured as the percent residual activity remaining after pretreatment at 37 °C for 30 minutes.

5 ^c Thermostability was estimated as the observed temperature of 50% loss of activity after 30 minutes of pretreatment.

^d Percent residual activity after protease pretreatment. Protease stability was assessed at 37 °C at 10 mg/mL pepsin in SGF, pH 4 or 3.4 mg/mL pancreatin in SIF, pH 6. All variants showed <20% loss of activity under the assay conditions after 2 hours pretreatment.

10 **[0286]** The activity profile for the *Sc_S288C* invertase was more comparable to that of the parent *S. cerevisiae* invertase, but similar differences were observed in low pH and thermostability. This was consistent with the expected impact of the P479A substitution on invertase activity. The variant V78 showed improvement in activity relative to *Sc_S288C* invertase but slightly poorer thermostability.

15 **[0287]** Together, these studies identified several substitutions that can be used alone or in combination to positively impact activity, low pH stability and thermostability of *S. cerevisiae* invertase. Variant V78 shows all-around significant improvement relative to the parental invertase.

Example 2: Engineering of Enhanced Invertase Variants

20 **[0288]** This Example describes the enhancement of a recombinant mutant *S. cerevisiae* invertase identified in Example 1 with improved tolerance of low pH, improved thermostability, and high catalytic activity.

Invertase Engineering

25 **[0289]** Recombinant *S. cerevisiae* invertase variants were created based on variant V78, each containing the three substitutions of V78 and three additional mutations relative to the wild-

type *S. cerevisiae* invertase. **TABLE 8** shows amino acid substitutions in each of the variants, where the locations of the substitutions are made with reference to SEQ ID NO: 2. During expression, the signal sequences are removed.

- [0290] The variants and the wild-type (wt) *S. cerevisiae* invertase were each expressed in *P. pastoris* and culture supernatants were tested for activity and stability. Each variant was tested for various properties using the assays described in Example 1 above, including: (i) activity at pH 3.6, 4.8, 6, and 7.1, (ii) thermostability, (iii) stability at pH 2.8, (iv) resistance to pepsin, and (v) resistance to pancreatin.

TABLE 8

Variant	Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	SEQ ID NO
V78	F172Y	N429A	P479A	-	-	-	29
VE01	F172Y	N429A	P479A	T140L	D272N	E285H	54
VE03	F172Y	N429A	P479A	T140L	D272N	L549I	55
VE04	F172Y	N429A	P479A	T140L	D272N	V581I	56
VE08	F172Y	N429A	P479A	T140L	E285H	V581I	57
VE36	F172Y	N429A	P479A	T140L	E382Q	A501N	51
VE38	F172Y	N429A	P479A	T140L	E382Q	A123G	58
VE39	F172Y	N429A	P479A	T140L	E382Q	N512G	59
VE40	F172Y	N429A	P479A	T140L	E382Q	V178I	60
VE44	F172Y	N429A	P479A	T140L	E382Q	N390D	61

10

- [0291] The new variants were expressed, tested, and compared against the parental V78 enzyme. In general, all nine invertase variants showed an improvement in pH 2.8 stability, thermostability, pH 3.6 activity, pH 4.8 activity, and pH 6 activity, with four of the variants further showing improved pH 7.1 activity over the parent invertase. Exemplary activity results for the engineered invertase variants compared to the parental invertase (V78) are shown in **TABLE 9** and stability results are shown in **TABLE 10**.

15

TABLE 9

	Specific Activity (μmole/min/mg @ 10 mM sucrose, 37°C)			
	SGF, pH 3.6 ^a	SGF, pH 4.8 ^a	SIF, pH 6 ^a	SIF, pH 7.1 ^a
V78	~	~	~	~
VE01	+	+	+	+

	Specific Activity (μmole/min/mg @ 10 mM sucrose, 37°C)			
	SGF, pH 3.6 ^a	SGF, pH 4.8 ^a	SIF, pH 6 ^a	SIF, pH 7.1 ^a
VE03	+	+	+	+
VE04	+	+	+	+
VE08	+	+	+	+
VE36	+	+	+	~
VE38	+	+	+	~
VE39	+	+	+	~
VE40	+	+	+	~
VE44	+	+	+	~

^a Relative impacts of substitutions are indicated as positive (+) or neutral (~) relative to the parental invertase V78.

TABLE 10

	Pepsin Stability (t _{1/2} , min) ^a	Pancreatin Stability (t _{1/2} , min) ^a	Thermostability (Residual @ 60 min) ^a	pH Stability (t _{1/2} , min) ^a
	32 mg/ml, pH 3.5	5 mg/ml, pH 6.0	62°C, SIF pH 5.0	pH 2.8
V78	~	~	~	~
VE01	~	~	+++	+++
VE03	~	~	++	+++
VE04	~	~	++	+++
VE08	~	~	+++	+++
VE36	~	~	+++	+++
VE38	~	~	++	+++
VE39	~	~	+	+++
VE40	~	~	++	+++
VE44	~	~	++	+++

^a Relative impacts of substitutions are indicated as very strongly positive (+++), strongly positive (++) , moderately positive (+), or neutral (~) relative to the parental invertase V78.

5 [0292] The relative impact of each substitution on each target property was estimated and is set forth in **TABLE 11** (amino acid numbering is relative to *S. cerevisiae* invertase (SEQ ID

NO: 2). In particular, the impact of certain amino acid substitutions relative to wild-type *S. cerevisiae* invertase (SEQ ID NO: 2) that showed significant contribution to at least one of three key invertase properties: pH 7.0 activity, or low pH stability, or thermostability, are summarized in **TABLE 11**.

5

TABLE 11

Amino Acid Substitution	Activity at pH 7.0 ^a	Low pH Stability ^a	Thermostability ^a
F172Y	+	+	~
P479A	++	+	++
N429A	~	~	+
L549I	+	~	~
T470S	~	+	
E120A	~	~	++
N512G	~	+	+
N390D	+	~	+
A123G	~	+	+
T140L	~	+	++
E382Q	~	+	-
V581I	~	+	+
D272N	~	++	~
E285H	~	~	+++

^a Relative impacts of substitutions are indicated as very strongly positive (+++), strongly positive (++), moderately positive (+), moderately negative (-) or neutral (~).

10 **[0293]** Together, these studies identified further substitutions and combinations of substitutions that can be used to positively impact activity, low pH stability and thermostability of *S. cerevisiae* invertase. Variant VE36 showed all-around significant improvement relative to the parental invertase.

Example 3: Engineering of Enhanced Invertase Variants for Enhanced Thermostability and Stability at Low pH

[0294] This Example describes the enhancement of a recombinant mutant *S. cerevisiae* invertase identified in Example 2 with improved tolerance of low pH and improved thermostability.

Invertase Engineering

[0295] Additional recombinant *S. cerevisiae* invertase variants were created based on variant VE36 identified in Example 2, each containing the six substitutions of VE36 and one or two further mutations relative to the wild-type *S. cerevisiae* invertase. **TABLE 12** shows amino acid substitutions in each of the two variants VE361 and VE362 as well as variant VE36, where the locations of the substitutions are made with reference to SEQ ID NO: 2. During expression the signal sequences are removed.

[0296] The variants VE361 and VE362 along with the wild-type (wt) *S. cerevisiae* invertase, V78 (variant from Example 1), and VE36 (variant from Example 2) were each expressed in *P. pastoris* and tested as culture supernatants for activity and stability. Each variant was tested for various properties using the assays described in Example 1 above, including: 1) activity at pH 3.6, 4.9, 6, and 7, 2) thermostability, 3) stability at pH 2.8, 4) resistance to pepsin, and 5) resistance to pancreatin.

TABLE 12

Variant	Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6	Sub 7	Sub 8	SEQ ID NO
VE36	F172Y	N429A	P479A	T140L	E382Q	A501N			51
VE361	F172Y	N429A	P479A	T140L	E382Q	A501N	D272N		52
VE362	F172Y	N429A	P479A	T140L	E382Q	A501N	D272N	E285H	53

[0297] Two invertase variants were expressed, tested, and compared against the wild-type, *sc_S288C*, V78, and VE36 enzymes. Exemplary activity results for the engineered invertase variants compared to the parental invertase (V78) are shown in **TABLE 9** and **FIG. 1**, and stability results are shown in **TABLE 10**. Graphs detailing the resistance of variants to pepsin and pancreatin are shown in **FIGs. 2A** and **2B**, respectively. The stability of the variants at pH 2.8 is shown in **FIG. 3**, and the thermostability of variants at 62°C is shown in **FIG. 4**. In general, the variants VE361 and VE362 had improved stability at pH 2.8 and increased thermostability compared to variants VE36 and V78, and variant VE36 also

showed improved stability at pH 2.8 and increased stability relative to variant V78 from Example 1.

TABLE 13

	Specific Activity (μmole/min/mg @ 10 mM sucrose, 37°C)			
	SGF, pH 3.6	SGF, pH 4.9	SIF, pH 6	SIF, pH 7
Wild-type	D	E	D	A
<i>Sc_S288C</i>	G	H	F	B
V78	G	H	G	C
VE36	I	I	G	B
VE361	H	H	F	B
VE362	I	I	F	B

- A=Specific Activity below 300; B= Specific Activity between 300-500; C=Specific Activity between 500-700; D=Specific Activity between 800-1000; E=Specific Activity between 1001-1200; F=Specific Activity between 1200-1400; G=Specific Activity between 1401-1600; H=Specific Activity between 1601-1800; I=Specific Activity above 1800

TABLE 14

	Thermostability (t1/2, min)	pH Stability (t1/2, min)	Pepsin Stability (t1/2, min)	Pancreatin Stability (t1/2, min)
	62°C, SIF pH 5.0	pH 2.8	32 mg/ml, pH 3.5	5 mg/ml, pH 6.0
Wild-type	A	C	>120	>120
<i>Sc_S288C</i>	F	D	>120	>120
V78	B	D	>120	>120
VE36	H	H	>120	>120
VE361	H	H	>120	>120
VE362	H	H	>120	>120

- A= t1/2 below 10; B= t1/2 between 10-50; C= t1/2 between 51-100; D= t1/2 between 101-200; E= t1/2 between 201-300; F= t1/2 between 301-400; G= t1/2 between 401-500; H= t1/2 above 500.

INCORPORATION BY REFERENCE

[0298] The entire disclosure of each of the patent and scientific documents referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[0299] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein.

- 5 Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

SEQUENCE LISTING

SEQ ID NO: 1 (wild-type *S. cerevisiae* invertase, AFN08663.1, with signal sequence in bold):

5 **MLLQAFLLAGFAAKIS**ASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYN
 PNDTVWGTPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGFFNDTIDPR
 QRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMT
 AAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISIN
 PGAPAGGSFNQYFVGFSFNGTHFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASN
 WEYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTL
 10 TKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASAS
 SFFLDRGNSKVVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVS
 TNYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK

15 SEQ ID NO: 2 (wild-type *S. cerevisiae* invertase, with FAKS signal sequence, with signal sequence in bold):

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNDTVWGTPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 20 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGFSFNGTHFEAFDNQSRVVDGKDYYALQTFNTDPTYGSA
 LGIAWASNWEYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 25 FNDGDVVSTNYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK

SEQ ID NO: 3 (wild-type *S. cerevisiae* invertase without signal sequence):

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGTPLFWGHATSDD
 LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 30 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGFSFNG
 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNYFMTTGNALGSVNMTT
 35 GVDNLFYIDKFQVREVK

SEQ ID NO: 4 (V6 invertase with FAKS signal sequence):

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDAKEAK
 WHLYFQYNPNDTVWGTPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 40 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGFSFNGTHFEAFDNQSRVVDGKDYYALQTFNTDPTYGSA
 LGIAWASNWEYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS

RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK

SEQ ID NO: 5 (V9 invertase with FAKS signal sequence):

5 MRFPSIFTAVLFAASSALAAPVNTTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNDTVWGLPLFWGHATSDDLTHWQDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 10 WVMFISINPGAPAGGSFNQYFVGFSFNTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWEYSFAFPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK

15 SEQ ID NO: 6 (V14 invertase with FAKS signal sequence):

MRFPSIFTAVLFAASSALAAPVNTTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNDTVWGTPPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 20 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGFSFNTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWEYSFAFPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKAVFADLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 25 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFEVREVK

SEQ ID NO: 7 (V22 invertase with FAKS signal sequence):

MRFPSIFTAVLFAASSALAAPVNTTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNDTVWGTPPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 30 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAALSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGFSFNTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWEYSFAFPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKNVFPDLSLWFKGLEDPEEYLR
 35 MGFEASASSFFLDRGNSPVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK

SEQ ID NO: 8 (V25 invertase with FAKS signal sequence):

MRFPSIFTAVLFAASSALAAPVNTTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 40 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNDTVWGTPPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWTLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGFSFNTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA

LGIAWASNWEYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETS LINLKAEPILNISNAGPWS
 RFATNTTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMTTGNALGSINMTTGVDNLFYIDKFQVREVK

5

SEQ ID NO: 9 (V32 invertase with FAKS signal sequence):

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDGK
 WHLYFQYNPNDTVWGLPLYWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 10 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGFSFNTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWEYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR
 15 MGFEASASSFFLDRGNSKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK

SEQ ID NO: 10 (V38 invertase with FAKS signal sequence):

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 20 WHLYFQYNPNDTVWGTPFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGFSFNTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWEYSAFVPTDPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 25 RFATNTTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKNVFPDLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVRENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK

SEQ ID NO: 11 (V60 invertase with FAKS signal sequence):

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 30 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNDTVWGTPFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGFSFNTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 35 LGIAWASNWQYS AFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMSTGNALGSINMTTGVDNLFYIDKFQVREVK

SEQ ID NO: 12 (V63 invertase with FAKS signal sequence):

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 40 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKEAK
 WHLYFQYNPNDTVWGTPFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY

WVMFISINPGAPAGGSFNQYFVGSFNGTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWEYSFAFPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTSQTISKNVFPDLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 5 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK

SEQ ID NO: 13 (V70 invertase with FAKS signal sequence):

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNDTVWGPPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 10 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSFNGTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWEYSFAFPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLR
 15 MGFEASASSFFLDRGNSPVKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK

SEQ ID NO: 14 (V78 invertase with FAKS signal sequence):

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 20 WHLYFQYNPNDTVWGTPPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAYSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSFNGTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWEYSFAFPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISAAGPWS
 25 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK

SEQ ID NO: 15 (V83 invertase with FAKS signal sequence):

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 30 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDAKDAK
 WHLYFQYNPNDTVWGTPPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVIDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSFNGTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 35 LGIAWASNWEYSFAFPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGILDQNILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK

SEQ ID NO: 16 (V87 invertase with FAKS signal sequence):

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 40 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNDTVWGTPPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY

WVMFISINPGAPAGGSFNQYFVGSFNGTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWQYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVKENPYFTNRMSVNNQPFKDENDLSYYKVYGLLDQNILELY
 5 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDKLFYIDKFQVREVK

SEQ ID NO: 17 (V89 invertase with FAKS signal sequence):

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 WHLYFQYNPNDTVWGLPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 10 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSFNGTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWEYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 RFATNTTLTKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR
 15 MGFEASASSFFLDRGNSKVKFVKENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTSVDNLFYIDKIQVREVK

SEQ ID NO: 18 (V90 invertase with FAKS signal sequence):

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPPFSNSTNNG
 LLFINTTIIASIAAKEEGVSLEKREAEASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAK
 20 WHLYFQYNPNDTVWGTPPLFWGHATSDDLTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGF
 FNDTIDPRQRCVAIWYNTPESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEY
 PSQKWIMTAAKSQDYKIEIYSSDDLKSWKLESAFANEGFLGYQYECPLIEVPTEQDPSKSY
 WVMFISINPGAPAGGSFNQYFVGSFNGTHFEAFDNQSRVVDGKDYALQTFNTDPTYGSA
 LGIAWASNWQYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISNAGPWS
 25 RFATNTTLTKANSYNVDLSNSTGTTEFELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLR
 MGFEASASSFFLDRGNSKVKFVRENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELY
 FNDGDVVSTNTYFMTTGNALGSVNMTTGVDNLFYIDKFQVREVK

SEQ ID NO: 19 (V6 invertase without signal sequence):

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDAKEAKWHLYFQYNPNDTVWGTPPLFWGHATSDD
 30 LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEYEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 35 ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK

SEQ ID NO: 20 (V9 invertase without signal sequence):

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
 40 LTHWQDEPIAIAPKRNDSGAFSGSMVVDYNNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEYEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF

ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK

SEQ ID NO: 21 (V14 invertase without signal sequence):

5 SMTNETSDRPLVHFTPNKGWMNDP NGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
LTHWEDEPIAIAIPKRND SGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEYEPSQKWIMTAAKSQDYKIEIYSSDDLKS
WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
10 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKAVFADLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK

SEQ ID NO: 22 (V22 invertase without signal sequence):

15 SMTNETSDRPLVHFTPNKGWMNDP NGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
LTHWEDEPIAIAIPKRND SGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEYEPSQKWIMTAALSQDYKIEIYSSDDLKS
WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
20 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKNVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSPVKFKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK

SEQ ID NO: 23 (V25 invertase without signal sequence):

25 SMTNETSDRPLVHFTPNKGWMNDP NGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
LTHWEDEPIAIAIPKRND SGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEYEPSQKWIMTAAKSQDYKIEIYSSDDLKS
WTLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
30 RKFSLNTEYQANPETSILNKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSINMTT
GVDNLFYIDKFQVREVK

35 SEQ ID NO: 24 (V32 invertase without signal sequence):

SMTNETSDRPLVHFTPNKGWMNDP NGLWYDEKDGKWHLYFQYNPNDTVWGLPLYWGHATSDD
LTHWEDEPIAIAIPKRND SGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEYEPSQKWIMTAAKSQDYKIEIYSSDDLKS
WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
40 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK

SEQ ID NO: 25 (V38 invertase without signal sequence):

SMTNETSDRPLVHFTPKNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
5 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTDPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKNVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVRENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
10 GVDNLFYIDKFQVREVK

SEQ ID NO: 26 (V60 invertase without signal sequence):

SMTNETSDRPLVHFTPKNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
15 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMSTGNALGSINMTT
20 GVDNLFYIDKFQVREVK

SEQ ID NO: 27 (V63 invertase without signal sequence):

SMTNETSDRPLVHFTPKNKGWMNDPNGLWYDEKEAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
25 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTSQTISKNVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
30 GVDNLFYIDKFQVREVK

SEQ ID NO: 28 (V70 invertase without signal sequence):

SMTNETSDRPLVHFTPKNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGPPLFWGHATSDD
LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
35 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSPVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
40 GVDNLFYIDKFQVREVK

SEQ ID NO: 29 (V78 invertase without signal sequence):

SMTNETSDRPLVHFTPKNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
LTHWEDEPIAIAPKRNDSGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI

SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 5 ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK

SEQ ID NO: 30 (V83 invertase without signal sequence):

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDAKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
 10 LTHWEDEPIAIAPKRNDSGAFSGSMVIDYNNTSGFFNDTIDPRQRCVAIWTYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 15 ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK

SEQ ID NO: 31 (V87 invertase without signal sequence):

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
 20 LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWTYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 25 ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
 FTNRMSVNNQPFKDENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDKLFYIDKFQVREVK

SEQ ID NO: 32 (V89 invertase without signal sequence):

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
 30 LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWTYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 35 ELVYAVNTTQTISKSVFPDLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
 SVDNLFYIDKIQVREVK

SEQ ID NO: 33 (V90 invertase without signal sequence):

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGTPLEFWGHATSDD
 40 LTHWEDEPIAIAPKRNDSGAFSGSMVVDYNNTSGFFNDTIDPRQRCVAIWTYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTLTKANSYNVDLSNSTGTFFEF

ELVYAVNTTQTISKSVFPDL SLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKV K FVREN PY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNI LELYFNDGDV VSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK

SEQ ID NO 34 (FAKS signal sequence):

- 5 MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVL PFSNSTNNG
LLFINTTIIASIAAKEEGVSLEKREAEA

SEQ ID NO: 35 (wild-type *S. cerevisiae* invertase DNA sequence JQ836661.1):

- ATGCTTTTGC AAGCTTTCCTTTTCTTTTGGCTGGTTTTGCAGCCAAAATATCTGCATCAAT
GACAAACGAACTAGCGATAGACCTTTGGTCCACTTCACACCCAACAAGGGCTGGATGAATG
10 ACCCAAATGGGTTGTGGTACGATGAAAAAGATGCCAAATGGCATCTGTACTTTCAATACAAC
CCAAATGACACCGTATGGGGTACGCCATTGTTTTGGGGCCATGCTACTTCCGATGATTGAC
TCATTGGGAAGATGAACCCATTGCTATCGCTCCCAAGCGTAACGATT CAGGTGCTTTCTCTG
GCTCCATGGTGGTTGATTACAACAACACGAGTGGGTTTTTCAATGATACTATTGATCCAAGA
CAAAGATGCGTTGCGATTG GACTTATAACACTCCTGAAAGTGAAGAGCAATACATTAGCTA
15 TTCTCTTGATGGTGGTTACACTTTTACTGAATACCAAAGAACCCTGTTTTAGCTGCCAACT
CCACTCAATTCAGAGATCCAAAGGTGTTCTGGTATGAACCTTCTCAAAAATGGATTATGACG
GCTGCCAAATCACAAGACTACAAAATTGAAATTTACTCCTCTGATGACTTGAAGTCCTGGAA
GCTAGAATCTGCATTTGCTAATGAAGGTTTCTTAGGCTACCAATATGAATGTCCAGGTTTGA
TTGAAGTCCCAACTGAGCAAGATCCTTCAAATCCTATTGGGTCATGTTTATTTCTATCAAT
20 CCAGGTGCACCTGCTGGCGGTTCTTCAACCAATATTTTGTGGATCCTTCAATGGTACTCA
TTTTGAAGCGTTTGACAATCAATCTAGAGTGGTAGATTTTGGTAAGGACTACTATGCCTTGC
AACTTTCTTCAACACAGACCCAACGTACGGTTCAGCATTAGGTATTGCCTGGGCTTCAAAC
TGGGAGTACAGTGCCTTTGTCCCAACTAACCCATGGAGATCATCCATGTCTTTGGTCCGCAA
GTTTTCTTTGAACACTGAATATCAAGCTAATCCAGAGACTGAATTGATCAATTTGAAAGCCG
25 AACCAATATTGAACATTAGTAATGCTGGTCCCTGGTCTCGTTTTGCTACTAACACAACCTCTA
ACTAAGGCCAATTCTTACAATGTGATTTGAGCAACTCGACTGGTACCCTAGAGTTTGAGTT
GGTTTACGCTGTTAACACCACACAAACCATATCCAAATCCGTCTTTCCCGACTTATCACTTT
GGTTCAAGGGTTTAGAAGATCCTGAAGAATATTTAAGAATGGGTTTTGAAGCCAGTGCTTCT
TCCTTCTTTTTTGGACCGTGGTAACCTAAGGTCAAGTTTGTCAAGGAGAACCCATATTTTAC
30 AAACAGAATGTCTGTCAACAACCAACCATTCAGTCTGAGAACGACCTAAGTTACTATAAAG
TGTACGGCCTACTGGATCAAAACATCTTGGAATTGTACTTCAACGATGGAGATGTGGTTTCT
ACAAATACCTACTTCATGACCACCGGTAACGCTCTAGGATCTGTGAACATGACCACTGGTGT
CGATAATTTGTTCTACATTGACAAGTTCCAAGTAAGGGAAGTAAAATAG

SEQ ID NO: 36 (*Sc*_S288C invertase):

- 35 MLLQAFLELLAGFAAKISASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYN
PNDTVWGTPLFWGHATSDDL TNWEDQPIAIAPKRND SGAFSGSMVVDYNN TSGFFNDTIDPR
QRCVAIWTYNTP ESEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWYEPSQKWIMT
AAKSQDYKIEIYSSDDLKSWKLESAFANEGLGYQYECPLIEVPTEQDPSKSYWVMFISIN
PGAPAGGSFNQYFVG SFNGTHFEAFDNQSRVVD FGKDYYALQTF FNTDPTYGSALGIAWASN
40 WEYSAFVPTNPWRSSMSLVRKFS LNTEYQANPETELINLKAEPILNISNAGPWSRFATNTTL
TKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEVSAS
SFFLDRGNSKV K FVREN PYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNI LELYFNDGDV V S
TNTYFMTTGNALGSVNMTT GVDNLFYIDKFQVREVK

- 45 SEQ ID NO: 37 (Isomaltase):

MTISSAHPETEPKWWKEATFYQIYPASFKDSNDDGWGDMKGIASKLEYIKELGADAIWISPF
 YDSPQDDMGYDIANYEKVWPTYGTNEDCFALIEKTHKLGMKFITDLVINHCSSEHEWFKESR
 SSKTNPKRDWFFWRPPKGYDAEGKPIPPNNWKSIFYGGSAAWTFDEKTQEFYLRFLCSTQPDNLN
 WENEDCRKAIYESAVGYWLDHGVDGFRIDVGSLSYKVVGLPDAPVVDKNSTWQSSDPYTLNG
 5 PRIHEFHQEMNQFIRNRVKDGREIMTVGEMQHASEDETKRLYTSASRHELSELFNFSTHDVGT
 SPLFRYNLVPFELKDWKIALAELFRYINGTDCWSTIYLENHDQPRISITRFGDDSPKNRVISG
 KLLSVLLSALTGTLYVYQGQELGQINFKNWPVEKYEDVEIRNNYNAIKEEHGENSEEMKKFL
 EAIALISRDHARTPMQWSREEPNAGFSGPSAKPWFYLNDSFREGINVEDEIKDPNSVLNFWK
 EALKFRKAHKDITVYGYDFEFIDLDNKKLFSFTKKYNNKTLFAALNFSSDATDFKIPNDSS
 10 FKLEFGNYPKKEVDASSRTLKPWEGRYIYSE

SEQ ID NO: 38 (Isomaltase):

MTISSAHPETEPKWWKEATFYQIYPASFKDSNDDGWGDMKGIASKLEYIKELGADAIWISPF
 YDSPQDDMGYDIANYEKVWPTYGTNEDCFALIEKTHKLGMKFITDLVINHCSSEHEWFKESR
 15 SSKTNPKRDWFFWRPPKGYDAEGKPIPPNNWRSIFYGGSAAWTFDEKTQEFYLRFLCSTQPDNLN
 WENEDCRKAIYESAVGYWLDHGVDGFRIDVGSLSYKVVGLPDAPVIDENSKWQSPDFTMNG
 PRIHEFHQEMNQFIRNRVKDGREIMTVGEMQHATDETKRLYTSASRHELSELFNFSTHDVGT
 SPKFRQNLIPYELKDWKVALAELFRYVNGTDCWSTIYLENHDQPRISITRFGDDSPKNRVISG
 KLLSVLLVLSLGTLYVYQGQELGEINFKNWPIEKYEDVEVRNNYDAIKEEHGENSKEMKRFL
 20 EAIALISRDHARTPMQWSREEPNAGFSGPNAKPWFYLNDSFREGINAEDESKDPNSVLNFWK
 EALRFRKAHKDITVYGYDFEFIDLDNKKLFSFTKKYDNKTLFAALNFSSDSIDFTIPNNSSS
 FKLEFGNYPRSEVDASSRTLKPWEGRYIYSE

SEQ ID NO: 39 (Isomaltase):

MTISSAHPETEPKWWKEATFYQIYPASFKDSNDDGWGDMKGIASKLEYIKELGTDAIWISPF
 YDSPQDDMGYDIANYEKVWPTYGTNEDCFALIEKTHKLGMKFITDLVINHCSSEHEWFKESR
 SSKTNPKRDWFFWRPPKGYDAEGKPIPPNNWRSIFYGGSAAWTFDEKTQEFYLRFLCSTQPDNLN
 WENEDCRKAIYESAVGYWLDHGVDGFRIDVGSLSYKVVGLPDAPVIDENSKWQLSDPFTMNG
 PRIHEFHQEMNQFIRNRVKDGREIMTVGEMRHATDETKRLYTSASRHELSELFNFSTHDVGT
 30 SPKFRQNLIPYELKDWKVALAELFRYVNGTDCWSTIYLENHDQPRISITRFGDDSPKNRVISG
 KLLSVLLVLSLGTLYVYQGQELGEINFKNWPIEKYEDVEVRNNYDAIKEEHGENSKEMKRFL
 EAIALISRDHARTPMQWSREEPNAGFSGPNAKPWFYLNDSFREGINAEDESKDPNSVLNFWK
 EALRFRKAHKDITVYGYDFEFIDLDNKKLFSFTKKYDNKTLFAALNFSSDSIDFTIPNNSSS
 FKLEFGNYPRSEVDASSRTLKPWEGRYIYSE

35

SEQ ID NO: 40 (Isomaltase):

MTISSAHPETEPKWWKEATFYQIYPASFKDSNDDGWGDMKGIASKLEYIKELGTDAIWISPF
 YDSPQDDMGYDIANYEKVWPTYGTNEDCFALIEKTHKLGMKFITDLVINHCSSEHEWFKESR
 SSKTNPKRDWFFWRPPKGYDAEGKPIPPNNWRSIFYGGSAAWTFDEKTQEFYLRFLCSTQPDNLN
 40 WENEDCRKAIYESAVGYWLDHGVDGFRIDVGSLSYKVVGLPDAPVIDENSKWQLSDPFTMNG
 PRIHEFHQEMNQFIRNRVKDGREIMTVGEMRHATDETKRLYTSASRHELSELFNFSTHDVGT
 SPKFRQNLIPYELKDWKVALAELFRYVNGTDCWSTIYLENHDQPRISITRFGDDSPKNRVISG
 KLLSVLLVLSLGTLYVYQGQELGEINFKNWPIEKYEDVEVRNNYDAIKEEHGENSKEMKRFL
 EAIALISRDHARTPMQWSREEPNAGFSGPNAKPWFYLNDSFREGINAEDESKDPNSVLNFWK
 45 EALRFRKAHKDITVYGYDFEFIDLDNKKLFSFTKKYDNKTLFAALNFSSDSIDFTIPNNSSS
 FKLEFGNYPRSEVDASSRTLKPWEGRYIYSE

SEQ ID NO: 41 (Isomaltase):

MTIIHNPKWKEATVYQIYPASFKDSNNDGWGDLAGITSKLDYVKELGVDAIWVCPFYDSPQ
EDMGYDIANYEKVWPRYGTNEDCFQMIIEEAHKRGIKVIVDLVINHCSEEHWFKESRSSKAN
PKRDWFFWRPPKGYDEKGNPIPPNNWRSFFGGSAWRYDEKTGEFFLHVFBALGQPDFNWEENE
5 CRKAIYDSSVGYWLRHNVDFRIDVGSMSKVEGLPDAPITDPTVPYQKGTEFFINGPRIHE
YHKEMHNYMLSQVPEGKEIMTVGEVGIGNEDDFRVYTSAGEGELNMMFNFKHTSVGENPKCK
YELIPFTLKDFKLALAESFLFIENTDCWSTIYLENHDQPRSVSRFGSDSPKWREISSKMLAT
LIISLTGTVFIYQGQELGMPNFKNRKIEQIKVEGTGTAAIKRDYGEDSEKMKKFFEALAL
ISRDHGRTPFPWSADEPSAGFSKDAKPWIDMNESFRDGINAEAEKDKNSVFFFWKKALQVR
10 KEHKDILVYGHNFQFIDLDNDKLFMTKDTDNKKMFAVFNFSSDNTDFSVPDNEASYTMFFG
NYANSNGDSRTLQPWEGRLYLLK

SEQ ID NO: 42 (Isomaltase):

ATGACTATTTCTTCTGCACATCCAGAGACAGAACCAAAGTGGTGGAAAGAGGCCACGTTCTA
15 TCAAATTTACCCAGCAAGTTTCAAAGACTCTAATGACGATGGCTGGGGTGACATGAAAGGGA
TTGCCTCCAAGCTGGAGTACATCAAAGAGCTTGGTGCCGATGCCATTTGGATCTCACCATTC
TACGACTCGCCACAAGATGATATGGGTACGATATTGCCAACTACGAAAAGGTCTGGCCAAC
ATATGGTACGAATGAAGACTGCTTTGCCCTTGATCGAAAAGACACATAAGCTTGGTATGAAAT
TTATCACCGACTTGGTCATCAATCACTGTTCCAGCGAACATGAATGGTTCAAAGAGAGCAGA
20 TCCTCGAAGACTAATCCAAAGCGTGAAGTGTCTTCTGGAGACCTCCTAAAGGTTATGACGC
CGAAGGCAAGCCAATTCCTCCAAACAATTGGAAATCCTATTTTGGTGGTTCCGCATGGACCT
TCGATGAAAAGACACAAGAATTCTACTTGCCTTTGTTTTGCTCCACTCAACCTGATTGAAAT
TGGGAGAATGAAGACTGTAGAAAGGCAATCTACGAAAGTGCCGTTGGATACTGGTTAGACCA
TGGTGTAGACGGCTTTAGAATTGATGTCGGAAGTTTGTACTCCAAAGTTGTAGGTTTACCAG
25 ATGCTCCTGTTGTTGACAAAACTCGACTTGGCAATCCAGTGATCCATACACATTGAATGGA
CCACGTATTCACGAGTTCCATCAAGAAATGAATCAATTCATCAGAAACAGAGTGAAGGATGG
CAGGGAGATTATGACAGTTGGTGAATGCAACATGCCTCCGACGAAACTAAGAGACTTTATA
CGAGTGCTTCAAGACACGAACTTAGTGAGTTATTTAACTTTTCCCACTGATGTGGGGACT
TCACCTTTGTTCCGTTACAACCTTGGTCCCATTGAACTGAAGGATTGGAAGATTGCCCTTGC
30 TGAGCTGTTCAAGTACATTAATGGTACAGATTGTTGGTCAACAATCTATCTGGAAAATCACG
ACCAACCTCGTTCAATTACGAGATTTGGTGACGATTCTCCCAAGAACCGTGTTATTTCTGGT
AAGTTACTCTCTGTGTGCTAAGTGCTTGAACCGTACTCTATATGTGTATCAGGGACAAGA
GCTTGGCCAAATCAATTTCAAGAACTGGCCTGTTGAAAAGTACGAGGATGTGAAATCAGAA
ACAACATCAATGCCATTAAAGAAGAGCATGGGGGAAACTCAGAGGAGATGAAAAAGTTTTTA
35 GAAGCCATTGCCCTTATCTCCAGGGACCATGCTAGAACACCTATGCAATGGTCTCGTGAGGA
GCCAAATGCTGGTTTTTCTGGTCTAGTGCTAAACCATGGTTTTACTTGAACGACTCTTTCA
GAGAAGGCATTAACGTCGAAGATGAAATCAAGGATCCCAACTCGGTTTTGAACTTCTGGAAG
GAGGCCTTGAAGTTTAGAAAGGCGCATAAAGACATTACTGTGTACGGATACGATTTGAGTT
TATTGATTTAGACAATAAGAAGTTGTTTAGCTTCACAAAGAAGTACAACAATAAAACATTGT
40 TTGCGGCTTTGAACTTTAGCTCTGATGCGACAGATTTCAAGATTCCAAATGATGATTCATCG
TTCAAGTTAGAGTTTGGAAACTATCCAAAGAAGGAGGTAGATGCCTCTTCCAGAACATTGAA
GCCATGGGAAGGAAGAATATATATCAGCGAATGA

SEQ ID NO: 43 (Isomaltase):

45 ATGACTATTTCTTCTGCACATCCAGAAACAGAACCCAAGTGGTGGAAAGAGGCCACAATCTA
TCAAATTTATCCCGCAAGTTTCAAAGACTCCAACAACGATGGGTGGGGTGATATGAAGGGTA
TTGCCTCCAAGTTGGAGTATATCAAAGAGCTTGGTGCCGATGCCATTTGGATCTCACCATTC

TACGACTCGCCACAAGATGATATGGGTTACGATATTGCCAACTACGAAAAGGTCTGGCCAAC
CTACGGTACGAACGAGGACTGCTTTGCCTTGATCGAAAAGACACATAAGCTTGGTATGAAAT
TTATCACCGACTTAGTCATCAACCATTGCTCGAGCGAACATGAATGGTTCAAAGAGAGCAGA
TCCTCAAAAACCAATCCAAAACGTGACTGGTTCTTCTGGAGACCTCCTAAGGGTTATGACGC
5 CGAAGGCAAGCCAATTCTCCAAACAATTGGAGGTCTTACTTCGGTGGTTCTGCATGGACGT
TCGATGAAAAGACACAAGAGTTTTACTTGCGTTTGTTTTGCTCCACCCAACCTGATTTGAAT
TGGGAGAATGAGGACTGTAGAAAGGCAATCTACGAAAGTGCCGTTGGATACTGGTTAGACCA
TGGTGTAGACGGCTTTAGAATTGATGTGGGAAGCTTGTAAGTTGCTGGTCTACCAG
ACGCTCCTGTGATTGACGAAAACCTCAAAGTGGCAACCCAGTGATCCTTTCACAATGAACGGA
10 CCACGTATCCATGAGTTTCATCAAGAAATGAACAAGTTCATCAGAAACAGAGTGAAGGATGG
CAGAGAAATTATGACAGTTGGTGAAATGCAACATGCTACTGATGAAACCAAGAGGTTGTATA
CAAGTGCATCAAGACACGAACCTTAGTGAGTTATTCAACTTTTCCCACACTGATGTCGGGACT
TCGCCCCAAGTTCCGTCAAAATTTGATCCCATATGAACTAAAGGATTGGAAGGTTGCCCTTGC
TGAACCTTTTCAGATACGTTAACGGAACCTGATTGTTGGTCAACAATTTATCTGGAAAAATCAGC
15 ACCAACCTCGTTCAATTACCAGATTTGGTGACGATTCTCCTAAGAACCGTGTTATTTCTGGT
AAGTTGTTGTCTGTGTTGTTGGTGTCACTGAGCGGTACTCTATATGTGTACCAAGGACAGGA
ACTGGGTGAAATCAATTTCAAGAACTGGCCTATCGAAAAATACGAGGATGTGCAAGTTAGAA
ATAACTATGATGCGATCAAGGAAGAGCATGGAGAAAACCTCGAAGGAGATGAAGAGGTTTTTG
GAAGCAATTGCCCTTATTTCCAGAGACCATGCTAGAACACCTATGCAATGGTCTCGTGAGGA
20 GCCAATGCTGGTTTTTCTGGTCCTAATGCTAAACCATGGTTTTACTTGAACGAGTCTTTCA
GGGAAGGAATTAACGCTGAAGACGAATCCAAGGATCCCAACTCGGTTTTGAACTTCTGGAAG
GAGGCCTTGAGATTTAGAAAGGCACACAAGGATATTACTGTATATGGATATGATTTTGAGTT
TATTGATTTGGACAATAAGAACTGTTTCAGCTTCACAAAGAAAATACGACAACAAAACATTGT
TTGCTGCTTTGAACTTCAGCTCTGATTTCGATTGACTTCACGATTCCAAACAATAGCTCGTCG
25 TTTAAGTTGGAATTCGGAAACTACCCAAGAAGCGAAGTTGATGCGTCTTCCAGAACATTGAA
GCCATGGGAGGGCAGAATTTACATATCTGAATGA

SEQ ID NO: 44 (Isomaltase):

ATGACTATTTCTTCTGCACATCCAGAAACAGAACCCAAGTGGTGGAAAGAGGCCACAATTTA
30 TCAAATTTATCCCGCAAGTTTCAAAGACTCCAACAACGATGGTTGGGGTGATATGAAGGGTA
TTGCCCTCCAAGTTGGAGTACATCAAAGAGCTTGGTACCGATGCCATTTGGATCTCGCCATTT
TACGACTCGCCACAAGATGATATGGGTTACGATATTGCCAACTACGAAAAGGTCTGGCCAAC
CTACGGTACGAACGAGGACTGCTTTGCCTTGATCGAAAAGACACATAAGCTTGGTATGAAAT
TTATCACCGACTTAGTCATCAACCATTGCTCCAGCGAACATGAATGGTTCAAAGAGAGCAGA
35 TCCTCAAAAACCAATCCAAAACGTGACTGGTTCTTCTGGAGACCTCCTAAGGGTTATGACGC
CGAAGGCAAGCCAATTCTCCAAACAATTGGAGGTCTTACTTCGGTGGTTCTGCATGGACGT
TCGATGAAAAGACACAAGAGTTTTACTTGCGTTTGTTTTGCTCCACCCAACCTGATCTAAAC
TGGGAGAACGAAGACTGCAGAAAGGCAATCTACGAAAGTGCCGTTGGATACTGGTTAGACCA
TGGTGTAGACGGCTTTAGAATTGATGTGGGAAGCTTGTAAGTTGCTGGTCTACCAG
40 ACGCTCCTGTGATTGACGAAAACCTCAAAGTGGCAACTCAGTGATCCTTTCACAATGAACGGA
CCACGTATCCATGAGTTTCATCAAGAAATGAACAAGTTCATCAGAAACAGAGTGAAGGATGG
CAGAGAAATTATGACAGTTGGTGAAATGCGACATGCTACTGATGAGACCAAGAGGTTGTATA
CAAGTGCCTCAAGACACGAACCTTAGTGAGTTATTCAACTTTTCCCACACTGATGTCGGGACT
TCGCCCCAAGTTCCGTCAAAATTTGATACCATATGAACTAAAGGATTGGAAGGTTGCCCTTGC
45 TGAACCTTTTCAGATACGTTAACGGAACCTGATTGTTGGTCAACAATTTATCTGGAAAAATCAGC
ACCAACCTCGTTCAATTACCAGATTTGGTGACGATTCTCCAAAAACCGTGTTATTTCTGGT
AAGTTGTTGTCTGTGTTGTTGGTGTCACTGAGCGGTACTCTATATGTGTACCAAGGACAGGA
ACTGGGTGAAATCAATTTCAAGAACTGGCCTATCGAAAAATACGAGGATGTGCAAGTTAGAA

ATAACTATGATGCGATCAAGGAAGAGCATGGAGAAAACCTCGAAGGAGATGAAGAGGTTTTTG
GAAGCAATTGCCCTTATTTCCAGAGACCATGCTAGAACACCTATGCAATGGTCTCGTGAGGA
GCCAAATGCTGGTTTTTCTGGTCCTAATGCTAAACCATGGTTTTACTTGAACGAGTCTTTCA
GGGAAGGAATTAACGCTGAAGACGAATCCAAGGACCCCAACTCGGTTTTGAACTTCTGGAAG
5 GAGGCCTTGAGATTTAGAAAGGCACACAAGGATATTACTGTGTATGGATATGATTTTGAGTT
TATTGATTTGGACAATAAGAACTGTTTCAGCTTCACAAAGAAAATACGACAACAAAACATTGT
TTGCTGCTTTGAACTTCAGCTCTGATTTCGATCGACTTCACGATTCCAAACAATAGCTCATCG
TTTAAGTTGGAATTCGGAAACTACCCAAGAAGTGAAGTTGATGCGTCTTCCAGAACATTGAA
GCCATGGGAGGGCAGAATTTACATATCTGAATGA

10

SEQ ID NO: 45 (Isomaltase):

ATGACTATTTCTTCTGCACATCCAGAAACAGAACCCCAAGTGGTGGAAAGAGGCCACAATTTA
TCAAATTTATCCCGCAAGTTTCAAAGACTCCAACAACGATGGTTGGGGTGATATGAAGGGTA
TTGCCTCCAAGTTGGAGTACATCAAAGAGCTTGGTACCGATGCCATTTGGATCTCGCCATTT
15 TACGACTCGCCACAAGATGATATGGGTACGATATTGCCAACTACGAAAAGGTCTGGCCAAC
CTACGGTACGAACGAGGACTGCTTTGCCCTTGATCGAAAAGACACATAAGCTTGGTATGAAAT
TTATCACCGACTTAGTCATCAACCATTGCTCCAGCGAACATGAATGGTTCAAAGAGAGCAGA
TCCTCAAAAACCAATCCAAAACGTGACTGGTTCTTCTGGAGACCTCCTAAGGGTTATGACGC
CGAAGGCAAGCCAATTCCTCCAAACAATTGGAGGTCTTACTTCGGTGGTTCTGCATGGACGT
20 TCGATGAAAAGACACAAGAGTTTTACTTGCGTTTGTGTTTGTCTCCACCCAACCTGATCTAAAC
TGGGAGAACGAAGACTGCAGAAAGGCAATCTACGAAAGTGCCGTTGGATACTGGTTAGACCA
TGGTGTAGACGGCTTTAGAATTGATGTGGGAAGCTTGTACTCCAAGGTTGCTGGTCTACCAG
ACGCTCCTGTGATTGACGAAAACCTCAAAGTGGCAACTCAGTGATCCTTTCACAATGAACGGA
CCACGTATCCATGAGTTTCATCAAGAAATGAACAAGTTCATCAGAAACAGAGTGAAGGATGG
25 CAGAGAAATTATGACAGTTGGTGAAATGCGACATGCTACTGATGAGACCAAGAGGTTGTATA
CAAGTGCGTCAAGACACGAACCTTAGTGAGTTATTCAACTTTTCCCACACTGATGTCGGGACT
TCGCCCAAGTTCCGTCAAAATTTGATACCATATGAACTAAAGGATTGGAAGGTTGCCCTTGC
TGAATTTTTCAGATACGTTAACGGAACCTGATTGTTGGTCAACAATTTATCTGGAAAATCACG
ACCAACCTCGTTCAATTACCAGATTTGGTGACGATTCTCCCAAAAACCGTGTTATTTCTGGT
30 AAGTTGTTGTCTGTGTTGTTGGTGTCACTGAGCGGTACTCTATATGTGTACCAAGGACAGGA
ACTGGGTGAAATCAATTTCAAGAACTGGCCTATCGAAAAATACGAGGATGTGCAAGTTAGAA
ATAACTATGATGCGATCAAGGAAGAGCATGGAGAAAACCTCGAAGGAGATGAAGAGGTTTTTG
GAAGCAATTGCCCTTATTTCCAGAGACCATGCTAGAACACCTATGCAATGGTCTCGTGAGGA
GCCAAATGCTGGTTTTTCTGGTCCTAATGCTAAACCATGGTTTTACTTGAACGAGTCTTTCA
35 GGGAAGGAATTAACGCTGAAGACGAATCCAAGGACCCCAACTCGGTTTTGAACTTCTGGAAG
GAGGCCTTGAGATTTAGAAAGGCACACAAGGATATTACTGTGTATGGATATGATTTTGAGTT
TATTGATTTGGACAATAAGAACTGTTTCAGCTTCACAAAGAAAATACGACAACAAAACATTGT
TTGCTGCTTTGAACTTCAGCTCTGATTTCGATCGACTTCACGATTCCAAACAATAGCTCATCG
TTTAAGTTGGAATTCGGAAACTACCCAAGAAGTGAAGTTGATGCGTCTTCCAGAACATTGAA
40 GCCATGGGAGGGCAGAATTTACATATCTGAATGA

SEQ ID NO: 46 (Isomaltase):

ATGACGATCATCCATAATCCTAAATGGTGGAAAGAAGCCACCGTCTACCAAATTTATCCTGC
TAGTTTCAAGGACTCCAACAATGACGGATGGGGTGATTTAGCTGGGATTACCTCCAAACTTG
45 ACTATGTCAAAGAATTAGGTGTCGATGCGATATGGGTGTGCCATTTTATGACTCTCCTCAA
GAAGATATGGGTACGATATTGCCAACTATGAGAAGGTTTGGCCACGTTACGGTACCAATGA
GGATTGTTTCCAAATGATAGAAGAAGCTCATAAGAGAGGTATCAAGGTTATTGTTGACCTGG
TTATCAACCACTGTTCTGAAGAGCATGAATGGTTCAAGGAGAGTAGATCTTCTAAGGCTAAT

CCAAAGCGTGACTGGTTTTTCTGGAGACCTCCAAAGGGTTACGATGAGAAAGGTAATCCAAT
 TCCTCCCAACAATTGGAGATCTTTCTTTGGTGGATCTGCTTGGAGATATGACGAAAAAACTG
 GTGAGTTCTTTCTGCGATGTGTTTGGCTCTTGGCCAACCTGATTTCATTGGGAAAAATGAAGAG
 TGCCGTAAAGCCATTTATGACTCCTCAGTGGGCTATTGGCTACGTCACAACGTTGATGGCTT
 5 TCGTATTGACGTAGGCAGCATGTATTCTAAGGTTGAGGGCTACCCGATGCTCCTATCACTG
 ATCCTACAGTTCCTTACCAAAAAGGAACTGAATTTTTTCATAAATGGGCCACGTATCCATGAG
 TATCATAAAGAAAATGCATAACTACATGCTCTCGCAAGTTCCAGAGGGTAAAGAAAATCATGAC
 TGTTGGAGAAGTTGGTATCGGGAATGAAGACGACTTCAGAGTTTATACCAGTGCCAAAAGAAG
 GAGAACTAAATATGATGTTCAACTTCAAGCACACCTCAGTAGGTGAAAACCCAAAAATGCAAG
 10 TACGAACCTTATTCCCTTTACTTTAAAAAGACTTTAAGCTGGCTCTTGCTGAAAGCTTTCTGTT
 TATCGAAAAACAGATTGCTGGTGCACCATCTATTTGGAGAATCATGATCAACCTCGTAGCG
 TTTTCGCGTTTTGGCTCAGATTCTCCTAAATGGCGTGAGATTTTCGTCCAAGATGTTGGCTACT
 CTCATCATTTTCATTGACTGGTACTGTTTTTATCTACCAAGGACAAGAATTGGGTATGCCCAA
 CTTCAAAAACAGAAAAATTGAGCAAAATCAAATGCGTTGAAGGTACTGGTACATATGCCGCCA
 15 TTAACCGTGATTATGGTGAAGATTCTGAAAAGATGAAAAAGTTTTTTGAAGCATTAGCTTTG
 ATCTCTAGAGATCATGGGAGAACACCATTTCCCATGGAGTGCTGATGAGCCTTCTGCAGGTTT
 CTCGAAGGATGCCAAACCATGGATTGACATGAATGAATCATTTAGAGACGGAATCAACGCAG
 AAGCTGAGTTAAAAGATAAAAACTCAGTCTTCTTTTTCTGGAAAAAGGCCTTACAAGTTAGA
 AAGGAGCACAAAGATATCCTTGTTTATGGACATAACTTTTCAAGTTCATTGATCTTGATAATGA
 20 CAAGCTATTTCATGTTTACAAAGGACACAGATAACAAAAAAATGTTTGCTGTTTTTCAATTTCA
 GTAGCGATAATACGGACTTTTTCAGTTCCTGATAATGAGGCCTCCTACACCATGTTTTTTGGC
 AACTATGCAAACTCAAATGGTGATTCCCGCACCTTGCAGCCATGGGAAGGAAGACTTTACTT
 GTTGAAGTAA

25 SEQ ID NO: 47 (wild-type *L. fermentum* isomaltase)

MIYTPKWWWQNSVYQVYPRSFQDSNHDGIGDLKGIISRLDYIKKLGADIIWLNPIYRSPNV
 DNGYDISDYRAIDPTFGSLTDFKELLTKAHELGLKIMMDLVVNHSSDENEFKQSRQKGNP
 YRDYYIWRDPVDGHEPTNWGSYFSGSAWQYDETSQYYLHLFAVKQPDNLWENEAVRHSVYD
 IMNWWADLGVDGFRMDVINLISKPAVYKDVPTAPGMQYGDVEPVVANGHRMHEFLQEMHQAV
 30 MAKHDLVTVGETPGATTDDAKKYANLEQTELMNVFEFEHVGLDGNNDNPALGKWSDDKKVSLPE
 LRDNLVKWQTQLNGKAWNSLYWNNHDQPRVVSFRGNDDPKYRVVSAKMLATMLHCLQGTPYI
 YAGEELGMTNTTFNSLSYDRDLESINAYHQLVDEEHLVDGKTMSRYLAIHSRDNARTPMQWD
 DSKNAGFSDAEPWIAVNPYSEINAKAALADPSSVFYHYQKLIQMRHDLPMTEGKFALVNG
 NELDEQVFAYTRDDGETTLLVVANFTKETIKREYAAGQGKLLLSNYEDDMGETLRPYEAKVY
 35 EFSSKR

SEQ ID NO: 48 (wild-type *L. fermentum* isomaltase DNA sequence):

ATGATATATACACCAAAATGGTGGTGGCAAAATTCAGTTGTCTACCAAGTCTATCCACGGAG
 TTTTCAAGACAGCAATCATGATGGCATTGGTGATTTAAAAGGAATCATCAGTCGGCTTGACT
 ATATTAAAAAACTAGGTGCTGATATTATCTGGCTCAATCCAATCTACCGTTCCGCAAAACGTT
 40 GATAATGGGTATGACATCAGTGATTATCGGGCAATTGATCCAACCTTTTGGCTCATTGACCGA
 CTTTAAGGAGCTGCTGACAAAGGCTCATGAACTTGGATTAAAAATAATGATGGATCTGGTGG
 TCAATCACTCATCAGATGAAAATGAATGGTTTAAAGCAAAGCCGCCAAGGAAAAGAAAAATCCA
 TACCGTGACTATTATATCTGGCGTGATCCAGTCGATGGCCATGAACCAACTAATTGGGGATC
 ATACTTCTCTGGTTCAGCTTGGCAGTACGATGAGACAAGCGGTCAATACTACCTTCACCTGT
 45 TCGCTGTAAAGCAGCCGGATCTAAACTGGGAAAATGAAGCCGTTTCGTCATTCCGTATACGAC
 ATCATGAATTGGTGGGCAGATCTCGGTGTCGATGGATTTCGAATGGATGTCATCAACTTAAT
 TTCAAAACCGGCTGTCTACAAAGACGTTCCAACCGCGCCCGGAATGCAGTATGGCGATGTCG

AGCCGGTTGTTGCCAATGGTCATCGGATGCACGAATTCTTCAAGAAATGCATCAAGCTGTA
 ATGGCCAAGCATGATTTGGTAACAGTTGGCGAAACACCTGGTGCTACAACAGATGATGCCAA
 GAAGTACGCCAACCTTGAACAGACTGAGCTGAACATGGTTTTTGGAGTTTGGAGCATGTCGGCT
 TAGACGGTAACGACAACCCAGCTTTAGGAAAATGGAGCGACAAAAAAGTCAGTCTGCCAGAA
 5 TTGCGAGATAACCTGGTGAAATGGCAGACTCAGCTGAATGGTAAGGCTTGGAACCTCGCTTTA
 CTGGAACAACCATGATCAGCCACGCGTCGTATCCCGTTTTGGCAATGATGATCCAAAAATATC
 GTGTCGTTTTCGGCTAAAATGTTGGCAACAATGCTGCACTGTCTGCAAGGCACGCCTTATATC
 TACGCAGGTGAGGAACTGGGAATGACCAACACGACCTTTAACTCTTTGTCTGACTATCGTGA
 TTTGGAGAGCATTAATGCCTACCATCAGCTGGTTGACGAAGAGCATCTAGTTGATGGAAAGA
 10 CAATGAGCAGATATCTGGCAATTCATTCTCGGGACAATGCCAGAACGCCAATGCAATGGGAT
 GACAGCAAGAATGCCGGATTTTCGGATGCTGAACCTTGGATCGCGGTCAATCCCAACTATTC
 AGAGATCAACGCAAAGGCGGCACTGGCGGATCCATCATCCGTCTTCTATCACTACCAGAAAC
 TCATTCAAATGCGCCATGACTTGCCAGTAATGACAGAAGGAAAAGTTTGC GTTGGTCAATGGC
 AACGAATTGGATGAGCAGGTCTTTGCTTACACGCGTGATGATGGAGAAACAACGCTGCTTGT
 15 AGTAGCCAACTTTACTAAGGAAACAATCAAGCGAGAATACGCGGCTGGTCAAGGCAAGCTCT
 TATTAAGCAACTACGAGGATGACATGGGAGAACTCTGCGTCCATATGAAGCTAAAGTATAT
 GAATTCAGTTCAAAGAGGTAA

SEQ ID NO: 49 (*Sc*_S288C invertase with F172Y and N429A mutations relative to SEQ ID

20 NO: 2, the native signal sequence is in bold):

MLLQAFLLFLLAGFAAKISASMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYN
 PNDTVWGTPFLFWGHATSDDL TNWEDQPIAIAPKRND SGAYSGSMVVDYNNTSGFFNDTIDPR
 QRCVAIWTYNTPESSEEQYISYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWYEPSQKWIMT
 AAKSQDYKIEIYSSDDLKSWKLESFAFANEGFLGYQYECPLIEVPTEQDP SKSYWVMFISIN
 25 PGAPAGGSFNQYFVGSFNGTHFEAFDNQSRVDFGKDYYALQTFNTDPTYGSALGIAWASN
 WEYSAFVPTNPWRSSMSLVRKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTL
 TKANSYNVDLSNSTGTLEFELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEVSAS
 SFFLDRGNSKV KVFVENPYFTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVS
 TNTYFMTTGNALG SVNMTTGVDNLFYIDKFQVREVK

30 SEQ ID NO: 50 (*Sc*_S288C invertase with F172Y and N429A mutations relative to SEQ ID

NO: 2, without signal sequence):

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGTPFLFWGHATSDD
 LTNWEDQPIAIAPKRND SGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWTYNTPESSEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWYEPSQKWIMTAAKSQDYKIEIYSSDDLK
 35 WKLESFAFANEGFLGYQYECPLIEVPTEQDP SKSYWVMFISINPGAPAGGSFNQYFVGSFNG
 THFEAFDNQSRVDFGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEVSASSFFLDRGNSKV KVFVENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVS TNTYFMTTGNALG SVNMTT
 40 GVDNLFYIDKFQVREVK

SEQ ID NO: 51 (VE36 invertase without signal sequence)

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
 LTHWEDEPIAIAPKRND SGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWTYNTPESSEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWYEPSQKWIMTAAKSQDYKIEIYSSDDLK

WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFN
 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFENSASSFFLDRGNSKVKFVKENPY
 5 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK

SEQ ID NO: 52 (VE361 invertase without signal sequence)

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
 LTHWEDEPIAIAIPKRND SGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 10 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDNLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFN
 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFENSASSFFLDRGNSKVKFVKENPY
 15 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK

SEQ ID NO: 53 (VE362 invertase without signal sequence)

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
 LTHWEDEPIAIAIPKRND SGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 20 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDNLKS
 WKLESAFANHGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFN
 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFENSASSFFLDRGNSKVKFVKENPY
 25 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK

SEQ ID NO: 51 (VE36 invertase without signal sequence)

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
 30 LTHWEDEPIAIAIPKRND SGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFN
 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
 35 ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFENSASSFFLDRGNSKVKFVKENPY
 FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
 GVDNLFYIDKFQVREVK

SEQ ID NO: 52 (VE361 invertase without signal sequence)

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
 40 LTHWEDEPIAIAIPKRND SGAYSGSMVVDYNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDNLKS
 WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFN
 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
 RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF

ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFENSASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK

SEQ ID NO: 53 (VE362 invertase without signal sequence)

5 SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
LTHWEDEPIAIAPKRND SGAYSGSMVVDYNN TSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDNLKS
WKLESAFANHGF LGYQYECPLIEVPTEQDP SKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVD FGKDYYALQTF FNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
10 RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFENSASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK

15 SEQ ID NO: 54 (VE01 invertase without signal sequence)

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
LTHWEDEPIAIAPKRND SGAYSGSMVVDYNN TSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDNLKS
WKLESAFANHGF LGYQYECPLIEVPTEQDP SKSYWVMFISINPGAPAGGSFNQYFVGSFNG
20 THFEAFDNQSRVVD FGKDYYALQTF FNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK

25

SEQ ID NO: 55 (VE03 invertase without signal sequence)

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
LTHWEDEPIAIAPKRND SGAYSGSMVVDYNN TSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDNLKS
30 WKLESAFANEGLGYQYECPLIEVPTEQDP SKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVD FGKDYYALQTF FNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLDQNIILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
35 GVDNLFYIDKFQVREVK

SEQ ID NO: 56 (VE04 invertase without signal sequence)

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
LTHWEDEPIAIAPKRND SGAYSGSMVVDYNN TSGFFNDTIDPRQRCVAIWYNTPESEEQYI
40 SYSLDGGYTTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDNLKS
WKLESAFANEGLGYQYECPLIEVPTEQDP SKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVD FGKDYYALQTF FNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY

FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSINMTT
GVDNLFYIDKFQVREVK

SEQ ID NO: 57 (VE08 invertase without signal sequence)

5 SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
LTHWEDEPIAIAPKRND SGAYSGSMVVDYNN TSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
WKLESAFANHGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWEYSAFVPTNPWRSSMSLV
10 RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSINMTT
GVDNLFYIDKFQVREVK

15 SEQ ID NO: 58 (VE38 invertase without signal sequence)

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDGKWHLYFQYNPNDTVWGLPLFWGHATSDD
LTHWEDEPIAIAPKRND SGAYSGSMVVDYNN TSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
WKLESAFANEGLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
20 THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK
25

SEQ ID NO: 59 (VE39 invertase without signal sequence)

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
LTHWEDEPIAIAPKRND SGAYSGSMVIDYNN TSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
30 WKLESAFANEGLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
35 GVDNLFYIDKFQVREVK

SEQ ID NO: 60 (VE40 invertase without signal sequence)

SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
LTHWEDEPIAIAPKRND SGAYSGSMVIDYNN TSGFFNDTIDPRQRCVAIWYNTPESEEQYI
40 SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
WKLESAFANEGLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVDGKDYYALQTFNTDPTYGSALGIAWASNWQYSAFVPTNPWRSSMSLV
RKFSLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFADLSLWFKGLEDPEEYLRMGFEASASSFFLDRGNSKVKFVKENPY

FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK

SEQ ID NO: 61 (VE44 invertase without signal sequence)

5 SMTNETSDRPLVHFTPNKGWMNDPNGLWYDEKDAKWHLYFQYNPNDTVWGLPLFWGHATSDD
LTHWEDEPIAIAPKRND SGAYSGSMVVDYNNNTSGFFNDTIDPRQRCVAIWYNTPESEEQYI
SYSLDGGYTFTEYQKNPVLAANSTQFRDPKVFWEPSQKWIMTAAKSQDYKIEIYSSDDLKS
WKLESAFANEGFLGYQYECPLIEVPTEQDPSKSYWVMFISINPGAPAGGSFNQYFVGSFNG
THFEAFDNQSRVVDGKDYALQTFNTDPTYGSALGIAWASNWQYSAFVPTDPWRSSMSLV
10 RKFSNLNTEYQANPETELINLKAEPILNISAAGPWSRFATNTTLTKANSYNVDLSNSTGTLEF
ELVYAVNTTQTISKSVFADLSLWFKGLEDP EEYL RMGF EASASSFFLDRGNSKV K FVKENPY
FTNRMSVNNQPFKSENDLSYYKVYGLLDQNILELYFNDGDVVSTNTYFMTTGNALGSVNMTT
GVDNLFYIDKFQVREVK

15 SEQ ID NO: 63 (DNA sequence encoding VE362 invertase)

ATGAGATTCCCATCTATTTTCACCGCTGTCTTGTTTCGCTGCCTCCTCTGCATTGGC
TGCCCCTGTAACTACTACCACTGAAGACGAGACTGCTCAAATCCAGCTGAAGCA
GTTATCGGTTACTCTGACCTTGAGGGTGATTTTCGACGTCGCTGTTTTGCCTTTCTC
TAACCTCCACTAACACGGTTTGTTGTTTCAATTAACACCACTATCGCTTCCATTGCTG
20 CTAAGGAAGAGGGTGTCTCTCTCGAGAAAAGAGAGGCCGAAGCTTCTATGACCA
ATGAAACTTCTGACAGACCATTGGTTCACTTCACACCCAACAAGGGTTGGATGAA
CGACCCAAACGGACTTTGGTATGATGAGAAAGATGCAAAATGGCATCTGTATTT
CCAGTACAATCCTAATGACACGGTCTGGGGTTTGCCACTGTTTTGGGGTCACGCA
ACTTCTGATGACTTAATCATTGGGAAGATGAGCCAATCGCTATCGCTCCTAAGC
25 GTAACGATT CAGGTGCCTACTCCGGATCCATGGTTGTCGATTATAATAACACTAG
TGGTTTCTTCAACGATACAATTGATCCCAGACAGCGATGTGTGGCAATTTGGACC
TACAATACACCAGAATCAGAGGAACAATACATTTTCGTACTCCTTGGATGGTGGTT
ACACGTTCACTGAATATCAGAAAAACCCTGTCTTGGCTGCGAATAGTACACAATT
TAGAGATCCGAAAGTTTTCTGGTATGAGCCTTACAAAAATGGATCATGACTGCT
30 GCAAAGTCCCAGGACTACAAGATTGAGATTTACTCTTCAGACAATTTGAAGTCAT
GGAAATTGGAGAGCGCTTTTGCCAATCATGGATTCCTGGGCTATCAATATGAGTG
CCCTGGATTGATAGAAGTACCAACAGAACAGATCCATCCAAGTCTTACTGGGT
GATGTTTATCAGCATTAATCCTGGTGCTCCCGCTGGCGGATCTTTTAACCAATACT
TTGTGGGTTTCTTCAACGGTACACATTTTGAAGCATTTGATAACCAATCCAGAGT
35 TGTTGACTTTGGTAAAGACTATTATGCTTTACAACTTTCTTCAACACCGACCCCA
CTTACGGTTCTGCATTGGGTATAGCTTGGGCAAGCAATTGGCAATACTCCGCATT
CGTCCCTACTAATCCATGGCGTTCTCTATGAGTCTGGTCCGTAAGTTTTCTCTCA
ATACTGAGTATCAGGCCAACCCAGAGACAGA ACTGATCAACTTAAAAGCTGAGC
CTATTCTGAATATCTCTGCTGCCGGTCCATGGTCAAGATTTCGCAACAAATACAAC
40 CCTGACTAAAGCTAATAGCTACAATGTGGATCTTAGCAATAGTACGGGTACCCTG
GAATTCGAATTGGTTTATGCAGTAAACACA ACTCAGACCATCTCGAAATCCGTAT
TCGCTGACTTATCCCTATGGTTTAAGGGTCTTGAGGATCCAGAGGAATACTTGAG
AATGGGTTTTGAGAATAGTGCCTCCTCGTTCTTTTTGGACAGGGGAAATTCGAAA
GTGAAGTTCGTTAAAGAGAATCCATATTTCACTAATAGAATGAGCGTGAATAATC
45 AACCGTTTAAAGTCTGAAAATGACTTGTCTACTACAAAGTATATGGCTTGTGGA
CCAAAACATCCTTGA ACTATACTTTAATGATGGGGATGTTGTCTCACTAATACT

TACTTTATGACCACAGGAAACGCTTTGGGATCAGTTAACATGACAACGGGTGTTG
ATAATCTTTTCTATATTGACAAGTTTCAAGTTAGGGAAGTTAAGTAATAG

CLAIMS

1. A recombinant mutant *S. cerevisiae* invertase enzyme, wherein the invertase comprises one or more amino acid substitutions selected from the group consisting of:
 - (a) a substitution of a T residue at a position corresponding to position 51 of SEQ ID NO: 3;
 - (b) a substitution of an F residue at a position corresponding to position 83 of SEQ ID NO: 3;
 - (c) a substitution of an E residue at a position corresponding to position 293 of SEQ ID NO: 3;
 - (d) a substitution of an N residue at a position corresponding to position 340 of SEQ ID NO: 3;
 - (e) a substitution of a P residue at a position corresponding to position 390 of SEQ ID NO: 3; and
 - (f) a substitution of an A residue at a position corresponding to position 412 of SEQ ID NO: 3; ora combination of any of the foregoing substitutions, and is catalytically active to digest sucrose into glucose and fructose under physiological conditions (*e.g.*, of the stomach and/or small intestine).
2. The invertase of claim 1, wherein, in the invertase:
 - (a) the T residue at a position corresponding to position 51 of SEQ ID NO: 3 is substituted by L (T51L);
 - (b) the F residue at a position corresponding to position 83 of SEQ ID NO: 3 is substituted by Y (F83Y);
 - (c) the E residue at a position corresponding to position 293 of SEQ ID NO: 3 is substituted by Q (E293Q);
 - (d) the N residue at a position corresponding to position 340 of SEQ ID NO: 3 is substituted by A (N340A);
 - (e) the P residue at a position corresponding to position 390 of SEQ ID NO: 3 is substituted by A (P390A);
 - (f) the A residue at a position corresponding to position 412 of SEQ ID NO: 3 is substituted by N (A412N); orthe invertase comprises a combination of any of the foregoing substitutions.

3. The invertase of claim 1 or 2, wherein the invertase further comprises a substitution at a D residue at a position corresponding to position 183 of SEQ ID NO: 3.
4. The invertase of claim 3, wherein the D residue at the position corresponding to position 183 of SEQ ID NO: 3 is substituted by an N (D183N).
5. The invertase of any one of claims 1-4, wherein the invertase further comprises a substitution at an E residue at a position corresponding to position 196 of SEQ ID NO: 3.
6. The invertase of claim 5, wherein the E residue at the position corresponding to position 196 of SEQ ID NO: 3 is substituted by an H (E196H).
7. The invertase of any one of claims 1-6, wherein the invertase comprises four, five, six, seven, or eight mutations relative to the corresponding wild-type invertase.
8. The invertase of any one of claims 1-7, wherein the invertase comprises F83Y, N340A, P390A, T51L, E293Q, and A412N substitutions.
9. The invertase of any one of claims 1-7, wherein the invertase comprises F83Y, N340A, P390A, T51L, E293Q, A412N, and D183N substitutions.
10. The invertase of any one of claims 1-7, wherein the invertase comprises F83Y, N340A, P390A, T51L, E293Q, A412N, D183N, and E196H substitutions.
11. The invertase of any one of claims 1-10, wherein the invertase comprises the amino acid sequence of any one of SEQ ID NOs: 53, 51-52, or 54-61 or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 53, 51-52, or 54-61.
12. A recombinant mutant *S. cerevisiae* invertase enzyme comprising a substitution or combination of substitutions listed in **TABLE 8** or **TABLE 12**, wherein the invertase enzyme is catalytically active to digest sucrose into glucose and fructose under physiological conditions (*e.g.*, of the stomach and/or small intestine).
13. The invertase of any one of claims 1-12, wherein the invertase has a specific activity at about pH 3.5 (*e.g.*, pH 3.6) of at least 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, or 2,000 μmol sucrose consumed per minute per milligram of invertase.

14. The invertase of any one of claims 1-13, wherein the invertase has at least 1-fold, 1.5-fold, 2-fold, 2.5-fold, or 3-fold higher activity at about pH 3.5 (*e.g.*, pH 3.6), compared to a corresponding wild-type invertase.
15. The invertase of any one of claims 1-14, wherein the invertase has a specific activity at about pH 5.0 (*e.g.*, pH 4.9) of at least 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,100, or 2,200 μmol sucrose consumed per minute per milligram of invertase.
16. The invertase of any one of claims 1-15, wherein the invertase has at least 1-fold, 1.5-fold, 2-fold, 2.5-fold, or 3-fold higher activity at about pH 5.0 (*e.g.*, pH 4.9), compared to a corresponding wild-type invertase.
17. The invertase of any one of claims 1-16, wherein the invertase has a specific activity at about pH 6.0 or 6.2 of at least 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, or 1,800 μmol sucrose consumed per minute per milligram of invertase.
18. The invertase of any one of claims 1-17, wherein the invertase has at least 1-fold, 1.5-fold, 2-fold, 2.5-fold, or 3-fold higher activity at about pH 6.0 or 6.2, compared to a corresponding wild-type invertase.
19. The invertase of any one of claims 1-18, wherein the invertase has a specific activity at about pH 7.0 or 7.1 of at least 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, or 1,300 μmol sucrose consumed per minute per milligram of invertase.
20. The invertase of any one of claims 1-19, wherein the invertase has at least 1-fold, 1.5-fold, 2-fold, 2.5-fold, or 3-fold higher activity at about pH 7.0 or 7.1, compared to a corresponding wild-type invertase.
21. The invertase of any one of claims 1-20, wherein the invertase retains at least 80%, 90%, or 95% of activity following incubation at about pH 2.8 for about 2 hours.
22. The invertase of any one of claims 1-21, wherein the invertase retains at least 80%, 90%, or 95% of activity following incubation at about 62 °C for about 1 hour.
23. The invertase of any one of claims 1-22, wherein the invertase retains at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% of activity following incubation at about pH 2.5 for about 30 minutes.

24. The invertase of any one of claims 1-23, wherein the invertase has at least 1-fold, 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, or 5-fold higher stability at about pH 2.5 compared to a corresponding wild-type invertase.
25. The invertase of any one of claims 1-24, wherein the invertase has a melting temperature (T_m) of at least 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, or 68 °C.
26. The invertase of any one of claims 1-25, wherein the invertase has a T_m that is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 °C higher than a corresponding wild-type invertase.
27. The invertase of any one of claims 1-26, wherein the invertase has higher stability in the presence of pancreatin or pepsin compared to a corresponding wild-type invertase.
28. A nucleic acid encoding the invertase of any one of claims 1-27.
29. An expression vector comprising the nucleic acid of claim 28.
30. A cell comprising the expression vector of claim 29.
31. The cell of claim 30, wherein the cell is a *P. pastoris* or *S. cerevisiae* cell.
32. A method of producing a recombinant mutant *S. cerevisiae* invertase enzyme, the method comprising:
 - a) growing the cell of claim 30 or 31 under conditions so that the cell expresses the invertase, and
 - b) purifying the invertase.
33. A pharmaceutical composition comprising the invertase of any one of claims 1-27 and a pharmaceutically acceptable carrier and/or an excipient.
34. A pharmaceutical composition comprising the invertase enzyme of any one of claims 1-27, optionally an isomaltase enzyme, and a pharmaceutically acceptable carrier and/or an excipient.
35. The pharmaceutical composition of claim 34, wherein the invertase is dried.
36. The pharmaceutical composition of claim 34 or 35, wherein the composition is formulated for delivery as a powder or compressed into a tablet.
37. The pharmaceutical composition of claim 34 or 35, wherein the isomaltase is dried.
38. The pharmaceutical composition of claim 37, wherein the composition is formulated for delivery as a powder or compressed into a tablet.

39. The pharmaceutical composition of any one of claims 33-38, wherein the isomaltase is a microbial isomaltase, or a functional fragment or variant thereof.
40. The pharmaceutical composition of any one of claims 33-39, wherein the isomaltase is derived from *Saccharomyces cerevisiae*.
41. The pharmaceutical composition of claim 40, wherein the isomaltase comprises the amino acid sequence of any one of SEQ ID NOs: 37-41 or a functional fragment or variant thereof.
42. The pharmaceutical composition of any one of claims 33-41, wherein the isomaltase comprises a recombinant mutant *Lactobacillus fermentum* isomaltase (SEQ ID NO: 47) or a functional fragment or variant thereof.
43. The pharmaceutical composition of claim 42, wherein the isomaltase further comprises:
- (a) K115I, R132K, D226S, E310A, L366M, I421A, A444G, A531D, and F560V substitutions;
 - (b) E93K, K115I, R132K, D226S, E310A, L366M, I421A, A444G, A531D, and F560L substitutions;
 - (c) E93K, K115I, R132K, D226S, E310A, I421A, A444G, E524Q, A531D, and F560L substitutions;
 - (d) E93K, K115I, R132K, D226S, E310A, I421A, A444G, A531D, and F560V substitutions;
 - (e) E93K, K115I, R132K, D226S, L366M, I421A, A444G, E524Q, A531D, and F560L substitutions;
 - (f) E93K, K115I, R132K, D226S, L366M, I421A, A444G, A531D, and F560V substitutions;
 - (g) E93K, K115I, R132K, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions;
 - (h) K115I, R132K, D226S, E310A, L366M, I421A, A444G, E524Q, A531D, and F560L substitutions;
 - (i) K115I, R132K, D226S, E310A, I421A, A444G, E524Q, A531D, and F560V substitutions;
 - (j) K115I, R132K, D226S, L366M, I421A, A444G, E524Q, A531D, and F560V substitutions;
 - (k) E93K, K115I, R132K, A211E, D226S, I421A, A444G, E524Q, A531D, and F560V

substitutions;

(l) E93K, K115I, R132K, D226S, L366M, I421A, A444G, E524Q, A531D, and F560V

substitutions;

(m) E93K, K115I, R132K, D226S, E275D, I421A, A444G, E524Q, A531D, and F560V

substitutions;

(n) T89A, E93K, K115I, R132K, D226S, I421A, A444G, E524Q, A531D, and F560V

substitutions;

(o) E93K, K115I, R132K, H182R, D226S, I421A, A444G, E524Q, A531D, and F560V

substitutions;

(p) E93K, K115I, E122D, R132K, D226S, I421A, A444G, E524Q, A531D, and F560V

substitutions;

(q) E93K, K115I, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions;

(r) E93K, K115I, D226S, I421A, A444G, E524Q, A531D, and F560L substitutions;

(s) K115I, A211E, D226S, I421A, A444G, A531D, and F560V substitutions;

(t) K115I, D226S, L366M, I421A, A444G, E524Q, A531D, and F560L substitutions;

(u) K115I, A211E, D226S, I421A, A444G, E524Q, A531D, and F560L substitutions;

(v) K115I, R132K, D226S, E310A, L366M, I421A, A444G, A531D, and F560L

substitutions;

(w) E93K, K115I, D226S, E310A, I421A, A444G, A531D, and F560L substitutions;

(x) K115I, D226S, E310A, I421A, A444G, A531D, and F560V substitutions;

(y) E93K, K115I, D226S, L366M, I421A, A444G, A531D, and F560L substitutions;

(z) K115I, D226S, I421A, A444G, E524Q, A531D, and F560V substitutions;

(aa) K115I, D226S, E310A, I421A, A444G, E524Q, A531D, and F560L substitutions;

(bb) K115I, A211E, D226S, L366M, I421A, A444G, A531D, and F560L

substitutions;

(cc) K115I, A211E, D226S, E310A, I421A, A444G, A531D, and F560L substitutions;

(dd) E93K, K115I, D226S, I421A, A444G, A531D, and F560V substitutions;

(ee) E93K, K115I, A211E, D226S, I421A, A444G, A531D, and F560L substitutions;

or

(ff) K115I, D226S, L366M, I421A, A444G, A531D, and F560V substitutions.

44. The pharmaceutical composition of claim 42 or 43, wherein the invertase comprises the amino acid sequence of any one of SEQ ID NOs: 51-61 or a functional fragment thereof.

45. The pharmaceutical composition of claim 42 or 43, wherein the invertase comprises the amino acid sequence of any one of SEQ ID NOs: 51-53 or a functional fragment thereof.
46. The pharmaceutical composition of any one of claims 33-45, wherein the composition is formulated as an oral dosage form.
47. The pharmaceutical composition of claim 46, wherein the composition is formulated as a liquid, powder, sachet, granulate, pellet, micropellet, tablet, or minitabket.
48. The pharmaceutical composition of claim 47, wherein the composition is formulated as a liquid, powder, sachet, or tablet.
49. The pharmaceutical composition of any one of claims 33-48, wherein the composition has a shelf-life at room temperature of at least 3 months, 6 months, 9 months, 12 months, 15 months, 18 months, 21 months, 24 months, 36 months, 48 months, 60 months, 72 months, 84 months, 96 months, 108 months, or 120 months.
50. A method of treating sucrase-isomaltase deficiency (*e.g.*, congenital sucrase-isomaltase deficiency (CSID) or acquired sucrase-isomaltase deficiency (ASID)) in a subject in need thereof, the method comprising orally administering to the subject an effective amount of the pharmaceutical composition of any one of claims 33-49.
51. The method of claim 50, wherein the pharmaceutical composition is administered to the subject together with a meal or snack.
52. A method of reducing sucrose and branched (1–6 linked) α -limit dextrin concentration in a subject, the method comprising orally administering to the subject an effective amount of the pharmaceutical composition of any one of claims 33-49.
53. The method of claim 52, wherein the method reduces sucrose and branched (1–6 linked) α -limit dextrin concentration in the subject as measured by a hydrogen breath test.
54. A method of treating sucrase-isomaltase deficiency (*e.g.*, congenital sucrase-isomaltase deficiency (CSID) or acquired sucrase-isomaltase deficiency (ASID)) in a subject in need thereof, the method comprising orally administering to the subject an invertase enzyme of any one of claims 1-27 and optionally an isomaltase enzyme.

55. The method of claim 54, wherein the isomaltase is the recombinant mutant isomaltase of any one of claims 39-43.
56. The method of any one of claims 50-55, wherein the subject is a mammal.
57. The method of any one of claims 50-56, wherein the subject is a human.