AN IMPROVED GALACTOSE OXIDASE ENZYME PANEL FOR OXIDATION OF SECONDARY ALCOHOLS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore provisional application No. 10202250709V, filed 11 August 2022, the contents of it being hereby incorporated by reference in its entirety for all purposes.

TECHNICAL FIELD

[0002] The present disclosure generally relates to a modified galactose oxidase capable of oxidizing a wide range of secondary alcohols to ketones.

BACKGROUND

[0003] Alcohol oxidation is an important transformation in chemical synthesis due to the convenience of hydroxyl and carbonyl functionalities as synthetic intermediates. Present chemical alcohol oxidation methodologies typically involve elevated temperatures, stoichiometric oxidants, and toxic by-products.

[0004] In contrast, biocatalytic alcohol oxidation by enzymes such as galactose oxidase possess advantages such as high selectivity, mild reaction conditions, benign by-products, and sustainable operation in water instead of organic solvents. However, one inherent drawback of excellent biocatalytic selectivity is their limited substrate scope.

[0005] Galactose oxidase is a radical-copper enzyme. Since its discovery, various research groups have worked on expanding its substrate scope. The most well studied variant of galactose oxidase for oxidation of secondary alcohols is the M3-5 variant, which comprises eight mutations (S33P, M93V, G218E, W313F, R353M, Q429T, V517A, N558D) with respect to wild-type galactose oxidase from *Fusarium sp.* M3-5 encompasses a relatively wide substrate scope, including selected benzylic secondary alcohols. However, no enzyme variants so far have been able to accept bulky secondary alcohols.

[0006] There is therefore a need to develop modified galactose oxidase enzymes that can oxidize a wide range of secondary alcohols to ketones, to create a panel of biocatalysts for an important transformation in chemical synthesis.

SUMMARY

[0007] In one aspect, the present disclosure refers to a modified galactose oxidase comprising a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 6, wherein SEQ ID NO: 6 is a polypeptide sequence of galactose oxidase variant M3-5, wherein the galactose oxidase variant M3-5 comprises mutations of S33P, M93V, G218E, W313F, R353M, Q429T, V517A, and N558D relative to the wildtype galactose oxidase from *Fusarium graminearum* (SEQ ID NO: 4), and wherein the polypeptide sequence of the modified galactose oxidase further comprises at least one mutation at one or more positions selected from the group consisting of 24, 26, 28, 45, 49, 65, 69, 104, 114, 117, 153, 157, 172, 173, 175, 193, 194, 195, 197, 198, 202, 214, 217, 218, 220, 224, 226, 239, 240, 250, 259, 265, 268, 269, 284, 294, 313, 314, 325, 341, 346, 348, 352, 353, 356, 389, 401, 429, 447, 485, 487, 537, 538, 546, 559, 576, 599, and 662, wherein the position of the mutation is numbered with reference to SEQ ID NO: 6.

[0008] In one aspect, the present disclosure refers to a polynucleotide sequence encoding the modified galactose oxidase disclosed herein.

[0009] In one aspect, the present disclosure refers to an expression vector comprising the polynucleotide sequence disclosed herein.

[0010] In one aspect, the present disclosure refers to a host cell comprising the polynucleotide sequence disclosed herein or the expression vector disclosed herein.

[0011] In one aspect, the present disclosure refers to a method of producing a modified galactose oxidase, comprising culturing the host cell disclosed herein under suitable culture conditions such that the modified galactose oxidase is produced.

[0012] In one aspect, the present disclosure refers to a method of producing a ketone from a secondary alcohol, comprising contacting the secondary alcohol with the modified galactose oxidase disclosed herein under suitable conditions for an oxidation reaction.

[0013] In one aspect, the present disclosure refers to a kit for use in the method disclosed herein, wherein the kit comprises the modified galactose oxidase disclosed herein.

BRIEF DESCRIPTION OF DRAWINGS

[0014] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0015] Fig.1 shows an expanded substrate scope of the identified galactose oxidase variants. Up to 64 substrates were tested were test in the panel.

[0016] Fig. 2 highlights the performance of top performing galactose oxidase variant against five (5) selected examples of bulky benzylic secondary alcohol substrates with reference to the M3-5 variant as benchmark.

[0017] Fig. 3 shows the mutation details on five (5) selected galactose oxidase variants (incorporating three or more synergistic mutations on top of the M3-5 variant) and their high performance liquid chromatography (HPLC) yields of ortho-substituted bulky benzylic secondary alcohol substrates compared to mutant GOh1052 as the benchmark.

[0018] Fig. 4 shows the enzyme profile of 13 top performing galactose oxidase variants in terms of activity (high performance liquid chromatography yield from secondary alcohol substrate 152, 1-phenyl-1-butanol), residual activity (described in **Fig. 6**), protein expression, and ratio of soluble to insoluble protein compared to GOh1052 variant (F217A, N268W from M3-5) as the benchmark.

[0019] Fig. 5 shows the measured colorimetric activities of 12 selected galactose oxidase variants as well as M3-5 benchmark against the 64 secondary alcohol substrate panel described in **Fig. 1**. Mutations are given in the top row, variant identifier numbers are given in the second row, and substrate identifier numbers are given in the first column. Colour coded heatmap comparisons have been done horizontally per substrate.

[0020] Fig. 6 shows the procedure for measuring residual activity of a galactose oxidase variant.

DEFINITION OF TERMS

[0021] The following words and terms used herein shall have the meaning indicated:

[0022] As used in this application, the singular form "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a primer" includes a plurality of primers, including mixtures and combinations thereof.

[0023] As used herein, the terms "increase" and "decrease" refer to the relative alteration of a chosen trait or characteristic in a subset of a population in comparison to the same trait or characteristic as present in the whole population. An increase thus indicates a change on a positive scale, whereas a decrease indicates a change on a negative scale. The term "change", as used herein, also refers to the difference between a chosen trait or characteristic of an isolated

population subset in comparison to the same trait or characteristic in the population as a whole. However, this term is without valuation of the difference seen.

[0024] As used herein, the term "about" in the context of concentration of a substance, size of a substance, length of time, or other stated values means +/- 5% of the stated value, or +/- 4% of the stated value, or +/- 3% of the stated value, or +/- 2% of the stated value, or +/- 1% of the stated value, or +/- 0.5% of the stated value.

[0025] Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0026] The disclosure illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the disclosure claimed. Thus, it should be understood that although the present disclosure has been specifically disclosed by preferred embodiments and optional features, modification and variation of the disclosures embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this disclosure.

[0027] The disclosure has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of the disclosure with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0028] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0029] As used herein, the terms "modified galactose oxidase", "galactose oxidase mutant" and "galactose oxidase variant" are used interchangeably, when referring to altered forms of a galactose oxidase resulting from genetic changes or modifications.

[0030] The secondary alcohol may include, but is not limited, a bulky secondary alcohol or an unactivated secondary alcohol. In one example, the bulky secondary alcohol comprises one or more bulky substituents. In one example, an unactivated secondary alcohol is an aliphatic secondary alcohol without a benzene ring in the alpha position adjacent to the alcohol group. The term “bulky” when used in relation to the secondary alcohol refers to an alcohol that is sterically bulky (or large or complex). In one example, the sterically bulky alcohol is an alkyl alcohol (carbon-based, C-OH). Examples of sterically bulky alcohols are 1-phenyl-1-butanol, diphenylmethanol, alpha-tetralol, and the like (such as those disclosed herein), as well as derivatives thereof. Similarly, the term “bulky” when used in relation to “substituents” refers to a substituent that is sterically bulky (or large or complex). In one example, the bulky secondary alcohol is an alcohol comprising one or more bulky substituents. In one example, the bulky substituent is selected from the group consisting of cycloalkyl, aryl, heteroaryl, alkyl, alkylene, alkynyl, haloalkyl, carboxyl, and carbonyl, wherein the cycloalkyl, aryl, heteroaryl, alkyl, alkylene, alkynyl, haloalkyl, carboxyl, and carbonyl is optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, alkyl halide, alkylene, alkynyl, alkoxy, cyano, oxo, nitro, amino, thiol, carboxyl, ester and hydroxyl. In one example, the secondary alcohol comprises the formula R^1R^2CHOH, wherein R^1 and R^2 are independently selected from the group consisting of cycloalkyl, aryl, heteroaryl, alkyl, alkylene, alkynyl, haloalkyl, carboxyl, and carbonyl, wherein the cycloalkyl, aryl, heteroaryl, alkyl, alkylene, alkynyl, haloalkyl, carboxyl, and carbonyl is optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, alkyl halide, alkylene, alkynyl, alkoxy, cyano, oxo, nitro, amino, thiol, carboxyl, ester and hydroxyl.

[0031] The present disclosure includes within its scope all isomeric forms of the compounds disclosed herein, including all diastereomeric isomers, racemates and enantiomers. Thus,

formulae (I) and (II) should be understood to include, for example, *E*, *Z*, *cis*, *trans*, (R), (S), (L), (D), (+), and/or (-) forms of the compounds, as appropriate in each case.

[0032] The term "optionally substituted" as used herein means the group to which this term refers may be unsubstituted, or may be substituted with one or more groups independently selected from alkyl, alkenyl, alkynyl, thioalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, halo, carboxyl, haloalkyl, haloalkynyl, hydroxyl, alkoxy, thioalkoxy, alkenyloxy, haloalkoxy, haloalkenyloxy, nitro, amino, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroheterocyclyl, alkylamino, dialkylamino, alkenylamine, alkynylamino, acyl, alkenoyl, alkynoyl, acylamino, diacylamino, acyloxy, alkylsulfonyloxy, heterocycloxy, heterocycloamino, haloheterocycloalkyl, alkylsulfenyl, alkylcarbonyloxy, alkylthio, acylthio, phosphorus-containing groups such as phosphono and phosphinyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cyano, cyanate, isocyanate, -C(O)NH(alkyl), and -C(O)N(alkyl)$_2$.

[0033] The term "cycloalkyl" as used herein refers to cyclic saturated aliphatic groups and includes within its meaning monovalent ("cycloalkyl"), and divalent ("cycloalkylene"), saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals having from 3 to 10 carbon atoms, e.g., 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. Examples of cycloalkyl groups include but are not limited to cyclopropyl, 2-methylcyclopropyl, cyclobutyl, cyclopentyl, 2-methylcyclopentyl, 3-methylcyclopentyl, cyclohexyl, and the like. In one example, the term "fused polycyclic hydrocarbon radical" refers to a fused polycyclic hydrocarbon group, ring, or system, wherein the carbon atoms within the fused polycyclic hydrocarbon group, ring or system are covalently bonded.

[0034] The term "aromatic group", or variants such as "aryl" or "arylene" as used herein refers to monovalent ("aryl") and divalent ("arylene") single, polynuclear, conjugated and fused residues of aromatic hydrocarbons having from 6 to 10 carbon atoms. Examples of such groups include phenyl, biphenyl, naphthyl, phenanthrenyl, and the like.

[0035] The term "heteroaromatic group" and variants such as "heteroaryl" or "heteroarylene" as used herein, includes within its meaning monovalent ("heteroaryl") and divalent ("heteroarylene"), single, polynuclear, conjugated and fused aromatic radicals having 6 to 20 atoms wherein 1 to 6 atoms are heteroatoms selected from O, N, NH and S. Examples of such groups include pyridyl, 2,2'-bipyridyl, phenanthrolinyl, quinolinyl, thiophenyl, and the like. In one example, the term "fused aromatic radical" refers to a fused aromatic group, ring, or

system, wherein the atoms within the fused aromatic group, ring, or system are covalently bonded.

[0036] As used herein, the term "alkyl" includes within its meaning monovalent ("alkyl") and divalent ("alkylene") straight chain or branched chain saturated aliphatic groups having from 1 to 10 carbon atoms, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. For example, the term alkyl includes, but is not limited to, methyl, ethyl, 1-propyl, isopropyl, 1-butyl, 2-butyl, isobutyl, tert-butyl, amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, pentyl, isopentyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl, 2-ethylpentyl, 3-ethylpentyl, heptyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, 5-methylheptyl, 1-methylheptyl, octyl, nonyl, decyl, and the like.

[0037] The term "alkenyl" includes within its meaning monovalent ("alkenyl") and divalent ("alkenylene") straight or branched chain unsaturated aliphatic hydrocarbon groups having from 2 to 10 carbon atoms, e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms and having at least one double bond, of either *E, Z, cis* or *trans* stereochemistry where applicable, anywhere in the alkyl chain. Examples of alkenyl groups include but are not limited to ethenyl, vinyl, allyl, 1-methylvinyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl, 3-butentyl, 1,3-butadienyl, 1-pentenyl, 2-pententyl, 3-pentenyl, 4-pentenyl, 1,3-pentadienyl, 2,4-pentadienyl, 1,4-pentadienyl, 3-methyl-2-butenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 1,3-hexadienyl, 1,4-hexadienyl, 2-methylpentenyl, 1-heptenyl, 2-heptentyl, 3-heptenyl, 1-octenyl, 1-nonenyl, 1-decenyl, and the like.

[0038] The term "alkynyl" as used herein includes within its meaning monovalent ("alkynyl") and divalent ("alkynylene") straight or branched chain unsaturated aliphatic hydrocarbon groups having from 2 to 10 carbon atoms and having at least one triple bond anywhere in the carbon chain. Examples of alkynyl groups include but are not limited to ethynyl, 1-propynyl, 1-butynyl, 2-butynyl, 1-methyl-2-butynyl, 3-methyl-1-butynyl, 1-pentynyl, 1-hexynyl, methylpentynyl, 1-heptynyl, 2-heptynyl, 1-octynyl, 2-octynyl, 1-nonyl, 1-decynyl, and the like.

[0039] The term "haloalkyl" refers to a straight-or branched-chain alkenyl group having from 2-12 carbon atoms in the chain and where one or more hydrogens is substituted with a halogen.

Illustrative haloalkyl groups include trifluoromethyl, 2-bromopropyl, 3-chlorohexyl, 1-iodo-isobutyl, and the like.

[0040] The term "carboxyl" as used herein is intended to mean the radical-C (O) OH.

[0041] The term “carbonyl” as used herein refers to a functional group comprising a carbon atom with a double bond to an oxygen atom, which can be written as C(=O) or as C(O).

[0042] The term “halogen” or variants such as “halide” or “halo” as used herein refers to fluorine, chlorine, bromine and iodine.

[0043] The term “alkyl halide” as used herein includes within its meaning straight or branched chain alkyl groups having from 2-12 carbon atoms and in which one or more of its hydrogen atoms are substituted with the corresponding number of halogen atoms. An alkyl halide with C_n carbon atoms may have at least one up to 2_n+1 halogen atoms, and the halogen atoms may be the same or different. Examples of alkyl halide groups include but are not limited to CF_3, C_2F_5, CHF_2, $CHCl_2$, $CHBr_2$, C_2Cl_5 and the like.

[0044] The term "alkoxy" as used herein refers to straight chain or branched alkyloxy groups. Examples include methoxy, ethoxy, n-propoxy, isopropoxy, tert-butoxy, and the like.

[0045] The term “cyano”, used interchangeably with “nitrile”, refers to a group comprising a carbon atom with a triple bond to a nitrogen atom, which can be written as –C≡N or -CN.

[0046] The term "oxo" as used herein is intended to mean the radical =O.

[0047] The term “amino” as used herein refers to groups of the form $–NR_aR_b$ wherein R_a and R_b are individually selected from the group including but not limited to hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, and optionally substituted aryl groups.

[0048] The term “nitro” as used herein refers to a functional group comprising a nitrogen atom joined to two oxygen atoms, which can be written as –O–N=O or $-NO_2$.

[0049] The term “thiol” as used herein refers to a group comprising the formula –SH.

[0050] The term “ester” as used herein refers to a group represented by the general formula R_cCOOR_d. R_c and/or R_d may include, but is not limited to alkyl, aryl, haloalkyl.

[0051] The term “hydroxyl” as used herein refers to the group –OH.

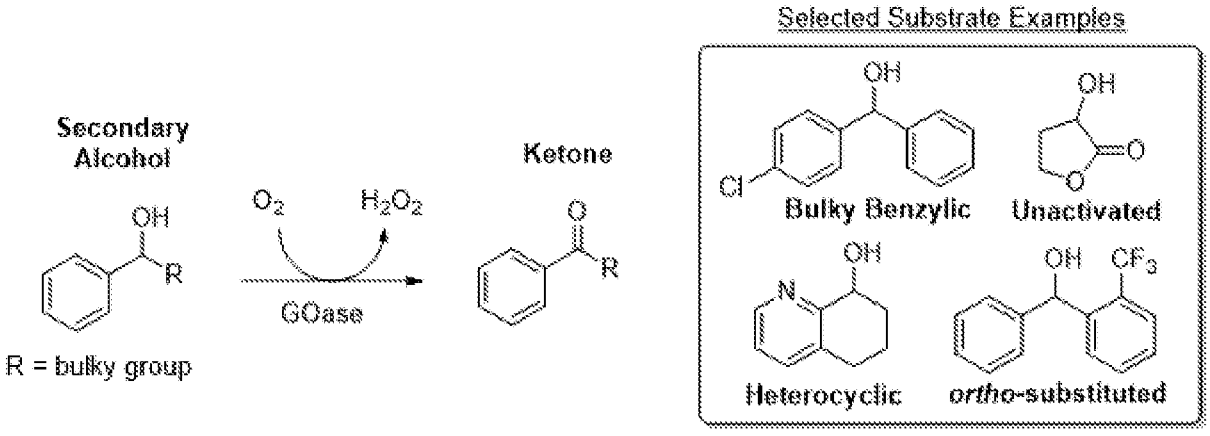
[0052] In the phrase "even numbered sequences", the term “even number” refers to any number that is a multiple of 2. In one example, even numbered sequences of SEQ ID NOS: 1-298 refer to SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and so on until SEQ ID NO: 298. In

another example, even numbered sequences of SEQ ID NOS: 77-298 refer to SEQ ID NOS: 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, and so on until SEQ ID NO: 298.

[0053] In the phrase "odd numbered sequences", the term “odd number” refers to any number that is not a multiple of 2. In one example, odd numbered sequences of SEQ ID NOS: 1-298 refer to SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and so on until SEQ ID NO: 297. In another example, odd numbered sequences of SEQ ID NOS: 77-298 refer to SEQ ID NOS: 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101 and so on until SEQ ID NO: 297.

DETAILED DESCRIPTION

[0054] The present disclosure is directed to a panel of modified galactose oxidase enzymes based on copper-dependent galactose oxidase from *Fusarium graminearum*. The present disclosure discloses 118 unique mutations (either singly or in combination) from the backbone M3-5 variant that convey improved enhancement in activity and/or stability, and/or solubility, and/or protein expression, and/or selectivity compared to M3-5 variant. The present disclosure describes galactose oxidase variants with enhanced protein expression, solubility, thermal stability and enzymatic activity, and reduced enantioselectivity, compared to galactose oxidase variants known in the art, and methods of producing the same. Galactose oxidases catalyse the oxidation of secondary alcohol substrates to ketones in accordance with the chemical equation shown below:



[0055] The inventors therefore screened and identified galactose oxidase variants that are capable of accepting a wide range of bulky secondary alcohols (e.g., biphenyl, C_8 alkyl sidechain, ortho-substitution) with good tolerance of halogen (e.g., Cl, Br), alkene, alkyne, and nitro functional groups. The galactose oxidase variants disclosed herein are also capable of accepting unactivated secondary alcohols as substrates.

[0056] In one aspect, the present disclosure refers to a modified galactose oxidase comprising a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 6, wherein SEQ ID NO: 6 is a polypeptide sequence of galactose oxidase variant M3-5, wherein the galactose oxidase variant M3-5 comprises mutations of S33P, M93V, G218E, W313F, R353M, Q429T, V517A, and N558D relative to the wildtype galactose oxidase from *Fusarium graminearum* (SEQ ID NO: 4), and wherein the polypeptide sequence of the modified galactose oxidase further comprises at least one mutation at one or more positions selected from the group consisting of 24, 26, 28, 45, 49, 65, 69, 104, 114, 117, 153, 157, 172, 173, 175, 193, 194, 195, 197, 198, 202, 214, 217, 218, 220, 224, 226, 239, 240, 250, 259, 265, 268, 269, 284, 294, 313, 314, 325, 341, 346, 348, 352, 353, 356, 389, 401, 429, 447, 485, 487, 537, 538, 546, 559, 576, 599, and 662, wherein the position of the mutation is numbered with reference to SEQ ID NO: 6.

[0057] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the mutation is selected from the group consisting of A24H, A24N, A26H, A26R, I28F, I28M, I28R, Q45D, Q45E, Q45N, E49K, F65P, N69D, N69E, N69F, N69H, N69L, N69M, N69T, N69W, N69Y, W104G, W104N, S114N, S114R, T117D, T153K, T153R, G157N, S172E, S173K, S173R, T175E, T175H, T175I, T175K, V193T, P194C, A195S, A195T, A195V, A197C, A197G, A198I, T202D, T202I, T202K, T202Q, N214R, N214S, F217A, F217C, F217D, F217E, F217G, F217H, F217I, F217K, F217L, F217M, F217N, F217P, F217Q, F217R, F217S, F217T, F217V, F217Y, E218F, E218K, E218P, E218Y, S220T, I224W, L226Q, L226V, D239E, D239P, D239Q, R240G, F250E, F250Y, N259T, T265S, N268W, D269N, I284S, I284Y, G294A, F313R, S314C, Y325F, N341W, A346L, K348S, Y352T, Y352W, M353F, N356K, N356R, N356W, K389I, A401D, T429W, T447A, I485A, I485K, I485R, F487R, L537G, C538K, C538R, F546M, S559P, S576D, Y599M, Q662D, and Q662P.

[0058] In one specific example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the mutation is selected from the group consisting of I28R, A24H, A195S, A195T, A195V, A198I, T202I, F217A, E218F, E218K, L226Q, D239E, N259T, N268W, S314C, Y352W, Y599M, and combinations thereof.

[0059] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the mutation is selected from the group consisting of:

- M353F;

- S314C;
- L537G;
- F217V;
- F217P;
- F217Q;
- F217N;
- F217S;
- N268W;
- E218P;
- F217I;
- F217T;
- F217L;
- F217E;
- F217Y;
- F217A;
- F217C;
- F217D;
- F217G;
- F217H;
- F217K;
- F217M;
- F217R;
- a combination of F217P and E218P;
- a combination of F217P and L537G;
- a combination of F217P, E218P and L537G;
- a combination of F217V and E218P;
- a combination of F217V and L537G;
- a combination of F217V, E218P and L537G;
- a combination of E218P and L537G;
- a combination of F217A and N268W;
- a combination of F217A, N268W and M353F;

- a combination of F217A, N268W and S314C;
- a combination of F217G and N268W;
- a combination of F217G, N268W and M353F;
- a combination of F217A, N268W and A195S;
- a combination of F217A, N268W and V193T;
- a combination of F217A, N268W and N259T;
- a combination of F217A, N268W and Y325F;
- a combination of F217A, N268W and G294A;
- a combination of F217A, N268W and A195T;
- a combination of F217A, N268W and Y352T;
- a combination of F217A, N268W and N341W;
- a combination of F217A, N268W and A197C;
- a combination of F217A, N268W and R240G;
- a combination of F217A, N268W and A198I;
- a combination of F217A, N268W and K348S;
- a combination of F217A, N268W and T202D;
- a combination of F217A, N268W and T202Q;
- a combination of F217A, N268W and T202I;
- a combination of F217A, N268W and T202K;
- a combination of F217A, N268W and P194C;
- a combination of F217A, N268W and A346L;
- a combination of F217A, N268W and K389I;
- a combination of F217A, N268W and Y352W;
- a combination of F217A, N268W and C538K;
- a combination of F217A, N268W and C538R;
- a combination of F217A, N268W and F250E;
- a combination of F217A, N268W and F250Y;
- a combination of F217A, N268W and N356K;
- a combination of F217A, N268W and N356R;
- a combination of F217A, N268W and N356W;
- a combination of F217A, N268W and F487R;

- a combination of F217A, N268W and T429W;
- a combination of F217A, N268W and E218K;
- a combination of F217A, N268W and F313R;
- a combination of F217A, N268W and I284Y;
- a combination of F217A, N268W and A24H;
- a combination of F217A, N268W and I28R;
- a combination of F217A, N268W and I28M;
- a combination of F217A, N268W and S576D;
- a combination of F217A, N268W and I284S;
- a combination of F217A, N268W and F65P;
- a combination of F217A, N268W and T153R;
- a combination of F217A, N268W and A197G;
- a combination of F217A, N268W and T265S;
- a combination of F217A, N268W and S220T;
- a combination of F217A, N268W and N214S;
- a combination of F217A, N268W and I485A;
- a combination of F217A, N268W and N214R;
- a combination of F217A, N268W and D269N;
- a combination of F217A, N268W and I485R;
- a combination of F217A, N268W and I485K;
- a combination of F217A, N268W and D239E;
- a combination of F217A, N268W and T117D;
- a combination of F217A, N268W and S114R;
- a combination of F217A, N268W and S559P;
- a combination of F217A, N268W and S114N;
- a combination of F217A, N268W and Q662P;
- a combination of F217A, N268W and S173R;
- a combination of F217A, N268W and D239P;
- a combination of F217A, N268W and S172E;
- a combination of F217A, N268W and Q662D;
- a combination of F217A, N268W and S173K;

- a combination of F217A, N268W and A195V;
- a combination of F217A, N268W and E218F;
- a combination of F217A, N268W and L226Q;
- a combination of F217A, N268W and I224W;
- a combination of F217A, N268W and A26R;
- a combination of F217A, N268W and I28F;
- a combination of F217A, N268W and E218Y;
- a combination of F217A, N268W and L226V;
- a combination of F217A, N268W and D239Q;
- a combination of F217A, N268W and F546M;
- a combination of F217A, N268W and A24N;
- a combination of F217A, N268W and A26H;
- a combination of F217A, N268W and Y599M;
- a combination of F217A, N268W and G157N;
- a combination of F217A, N268W and Q45D;
- a combination of F217A, N268W and Q45E;
- a combination of F217A, N268W and Q45N;
- a combination of F217A, N268W and E49K;
- a combination of F217A, N268W and N69D;
- a combination of F217A, N268W and N69E;
- a combination of F217A, N268W and N69F;
- a combination of F217A, N268W and N69H;
- a combination of F217A, N268W and N69L;
- a combination of F217A, N268W and N69M;
- a combination of F217A, N268W and N69T;
- a combination of F217A, N268W and N69W;
- a combination of F217A, N268W and N69Y;
- a combination of F217A, N268W and W104G;
- a combination of F217A, N268W and W104N;
- a combination of F217A, N268W and T175E;
- a combination of F217A, N268W and T175H;

- a combination of F217A, N268W and T175I;
- a combination of F217A, N268W and T175K;
- a combination of F217A, N268W and A401D;
- a combination of F217A, N268W, A195T and Y352W;
- a combination of F217A, N268W, N341W and Y352T;
- a combination of F217A, N268W, A198I and N259T;
- a combination of F217A, N268W, A195S and N341W;
- a combination of F217A, N268W, T153K and A195S;
- a combination of F217A, N268W, I28R and K389I;
- a combination of F217A, N268W, T153R and A195T;
- a combination of F217A, N268W, I28R, T153R, A198I and N341W;
- a combination of F217A, N268W, A195T, N259T and N341W;
- a combination of F217A, N268W, I28R, A195T, N259T and K389I;
- a combination of F217A, N268W, I28R, N259T and N341W;
- a combination of F217A, N268W, I28R, A195S and K389I;
- a combination of F217A, N268W, A195T and N341W;
- a combination of F217A, N268W, I28R, A195T, N341W and K389I;
- a combination of F217A, N268W, I28R, A195T, N259T and T447A;
- a combination of F217A, N268W, I28R, A195S, N259T and N341W;
- a combination of F217A, N268W, I28R, A195T, N259T, N341W and K389I; and
- a combination of F217A, N268W, T153R and N341W.

[0060] In one example, there is provided a galactose oxidase variant GOh1006, which has a M353F mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1008, which has a S314C mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1013, which has a L537G mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1015, which has a F217V mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1016, which has a F217P mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1017, which has a F217Q mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase

variant GOh1018, which has a F217N mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1020, which has a F217S mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1021, which has a N268W mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1025, which has a E218P mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1030, which has a F217I mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1031, which has a F217T mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1032, which has a F217L mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1034, which has a F217E mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1035, which has a F217Y mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1036, which has a F217A mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1037, which has a F217C mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1038, which has a F217D mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1039, which has a F217G mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1040, which has a F217H mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1041, which has a F217K mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1042, which has a F217M mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1043, which has a F217R mutation in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1045, which has F217P and E218P mutations in addition to those in the M3-5 variant. In one example, a modified galactose oxidase having the F217P and E218P mutations corresponds to galactose oxidase variant GOh1045. In one example, there is provided a galactose oxidase variant GOh1046, which has FF217P and L537G mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1047, which has F217P, E218P, and L537G mutations in addition to those in the

M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1048, which has F217V and E218P mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1049, which has F217V and L537G mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1050, which has F217V, E218P, and L537G mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1051, which has E218P and L537G mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1052, which has F217A and N268W mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1053, which has F217A, N268W, and M353F mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1054, which has F217A, N268W, and S314C mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1055, which has F217G and N268W mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh1056, which has F217G, N268W, and M353 mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2001, which has F217A, N268W, and A195S mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2002, which has F217A, N268W, and V193T mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2008, which has F217A, N268W, and N259T mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2010, which has F217A, N268W, and Y325F mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2011, which has F217A, N268W, and G294A mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2012, which has F217A, N268W, and A195T mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2013, which has F217A, N268W, and Y352T mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2014, which has F217A, N268W, and N341W mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2015, which has F217A, N268W, and A197C mutations in addition to those in the M3-5 variant. In

one example, there is provided a galactose oxidase variant GOh2016, which has F217A, N268W, and R240G mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2017, which has F217A, N268W, and A198I mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2018, which has F217A, N268W, and K348S mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2019, which has F217A, N268W, and T202D mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2020, which has F217A, N268W, and T202Q mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2021, which has F217A, N268W, and T202I mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2022, which has F217A, N268W, and T202K mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2023, which has F217A, N268W, and P194C mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2025, which has F217A, N268W, and A346L mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2026, which has F217A, N268W, and K389I mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2027, which has F217A, N268W, and Y352W mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2028, which has F217A, N268W, and C538K mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2029, which has F217A, N268W, and C538R mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2030, which has F217A, N268W, and F250E mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2031, which has F217A, N268W, and F250Y mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2032, which has F217A, N268W, and N356K mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2033, which has F217A, N268W, and N356R mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2034, which has F217A, N268W, and N356W mutations in addition to those in the M3-5 variant. In one example, there is

provided a galactose oxidase variant GOh2037, which has F217A, N268W, and F487R mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2038, which has F217A, N268W, and T429W mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2039, which has F217A, N268W, and E218K mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2041, which has F217A, N268W, and F313R mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2043, which has F217A, N268W, and I284Y mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2044, which has F217A, N268W, and A24H mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2045, which has F217A, N268W, and I28R mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2046, which has F217A, N268W, and I28M mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2047, which has F217A, N268W, and S576D mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2048, which has F217A, N268W, and I284S mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2050, which has F217A, N268W, and F65P mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2053, which has F217A, N268W, and T153R mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2055, which has F217A, N268W, and A197G mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2056, which has F217A, N268W, and T265S mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2057, which has F217A, N268W, and S220T mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2058, which has F217A, N268W, and N214S mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2061, which has F217A, N268W, and I485A mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2064, which has F217A, N268W, and N214R mutations in addition to those in the M3-5 variant. I In one example, there is

provided a galactose oxidase variant GOh2066, which has F217A, N268W, and D269N mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2067, which has F217A, N268W, and I485R mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2068, which has F217A, N268W, and I485K mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2070, which has F217A, N268W, and D239E mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2071, which has F217A, N268W, and T117D mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2072, which has F217A, N268W, and S114R mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2073, which has F217A, N268W, and S559P mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2074, which has F217A, N268W, and S114N mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2075, which has F217A, N268W, and Q662P mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2076, which has F217A, N268W, and S173R mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2077, which has F217A, N268W, and D239P mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2078, which has F217A, N268W, and S172E mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2079, which has F217A, N268W, and Q662D mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2080, which has F217A, N268W, and S173K mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2081, which has F217A, N268W, and A195V mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2082, which has F217A, N268W, and E218F mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2083, which has F217A, N268W, and L226Q mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2084, which has F217A, N268W, and I224W mutations in addition to those in the

M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2085, which has F217A, N268W, and I28F mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2086, which has F217A, N268W, and I28F mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2087, which has F217A, N268W, and E218Y mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2088, which has F217A, N268W, and L226V mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2089, which has F217A, N268W, and D239Q mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2090, which has F217A, N268W, and F546M mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2091, which has F217A, N268W, and A24N mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2092, which has F217A, N268W, and A26H mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2093, which has F217A, N268W, and Y599M mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2094, which has F217A, N268W, and G157N mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2097, which has F217A, N268W, and Q45D mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2098, which has F217A, N268W, and Q45E mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2099, which has F217A, N268W, and Q45N mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2100, which has F217A, N268W, and E49K mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2101, which has F217A, N268W, and N69D mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2102, which has F217A, N268W, and N69E mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2103, which has F217A, N268W, and N69F mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2104, which has F217A, N268W, and N69H mutations in addition to those in the M3-5 variant. In one

example, there is provided a galactose oxidase variant GOh2105, which has F217A, N268W, and N69L mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2106, which has F217A, N268W, and N69M mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2107, which has F217A, N268W, and N69T mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2108, which has F217A, N268W, and N69W mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2109, which has F217A, N268W, and N69Y mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2110, which has F217A, N268W, and W104G mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2111, which has F217A, N268W, and W104N mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2114, which has F217A, N268W, and T175E mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2115, which has F217A, N268W, and T175H mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2116, which has F217A, N268W, and T175I mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2117, which has F217A, N268W, and T175K mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh2119, which has F217A, N268W, and A401D mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3001, which has F217A, N268W, A195T, and Y352W mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3002, which has F217A, N268W, N341W, and Y352T mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3003, which has F217A, N268W, A198I, and N259T mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3004, which has F217A, N268W, A195S, and N341W mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3005, which has F217A, N268W, T153K, and A195S mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3006, which has F217A, N268W, I28R, and K389I mutations in

addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3007, which has F217A, N268W, T153R, and A195T mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3008, which has F217A, N268W, I28R, T153R, A198I, and N341W mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3009, which has F217A, N268W, A195T, N259T, and N341W mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3013, which has F217A, N268W, I28R, A195T, N259T, and K389I mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3015, which has F217A, N268W, I28R, N259T, and N341W mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3018, which has F217A, N268W, I28R, A195S, and K389I mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3019, which has F217A, N268W, A195T, and N341W mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3023, which has F217A, N268W, I28R, A195T, N341W mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3026, which has F217A, N268W, I28R, A195T, N259T, and T447A mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3032, which has F217A, N268W, I28R, A195S, N259T, and N341W mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3038, which has F217A, N268W, I28R, A195T, N259T, N341W, and K389I mutations in addition to those in the M3-5 variant. In one example, there is provided a galactose oxidase variant GOh3049, which has F217A, N268W, T153R, and N341W mutations in addition to those in the M3-5 variant. In one example, a modified galactose oxidase having the F217A, N268W, T153R, and N341W mutations corresponds to galactose oxidase variant GOh3049.

[0061] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence of at least one modified galactose oxidase variant set forth in the even numbered sequences of SEQ ID NOS: 7-298. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of

the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence of at least one modified galactose oxidase variant set forth in the following even numbered sequences: SEQ ID NOS: 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, or 298. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 8. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 10. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 12. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 14. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 16. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 18. In one example, the present disclosure refers to the modified galactose oxidase

disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 20. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 40. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 60. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 80. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 100. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 150. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 200. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 250. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 298.

[0062] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in the even numbered sequences of SEQ ID NOS: 7-298. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in the following even numbered sequences: SEQ ID NOS: 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, or 298. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 8. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 10. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 12. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 14. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 16. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 18. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 20. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide

sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 40. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 60. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 80. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 100. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 150. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 200. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 250. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 298.

[0063] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the mutation is selected from the group consisting of:

(a) S314C;

(b) N268W;

(c) F217A;

(d) a combination of F217A and N268W;

(e) a combination of F217A, N268W and A195S;

(f) a combination of F217A, N268W and N259T;

(g) a combination of F217A, N268W and A195T;

(h) a combination of F217A, N268W and A198I;

(i) a combination of F217A, N268W and T202I;

(j) a combination of F217A, N268W and Y352W;

(k) a combination of F217A, N268W and A24H;

(l) a combination of F217A, N268W and I28R;

(m) a combination of F217A, N268W and D239E;

(n) a combination of F217A, N268W and A24N;

(o) a combination of F217A, N268W and E218K;

(p) a combination of F217A, N268W and E218F;

(q) a combination of F217A, N268W and Y599M;

(r) a combination of F217A, N268W and L226Q;

(s) a combination of F217A, N268W and A195V;

(t) a combination of F217A, N268W, A195T and Y352W;

(u) a combination of F217A, N268W, I28R and K389I;

(v) a combination of F217A, N268W, I28R, A195T, N259T, and K389I; and

(w) a combination of F217A, N268W, I28R, A195T, N259T, N341W, and K389I.

[0064] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the modified galactose oxidase comprises the mutations of F217A and N268W.

[0065] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:68.

[0066] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the modified galactose oxidase further comprises at least one mutation selected from the group consisting of A24H, A24N, A26H, A26R, I28F, I28M, I28R, Q45D, Q45E, Q45N, E49K, F65P, N69D, N69E, N69F, N69H, N69L, N69M, N69T, N69W, N69Y, W104G, W104N, S114N, S114R, T117D, T153K, T153R, G157N, S172E, S173K, S173R, T175E, T175H, T175I, T175K, V193T, P194C, A195SX, A195T, A195V, A197C, A197G, A198I, T202D, T202I, T202K, T202Q, N214R, N214S, E218F, E218K, E218P, E218Y, S220T, I224W, L226Q, L226V, D239E, D239P, D239Q, R240G, F250E, F250Y, N259T, T265S, D269N, I284S, I284Y, G294A, F313R, S314C, Y325F, N341W, A346L, K348S, Y352T, Y352W, M353F, N356K, N356R, N356W, K389I, A401D, T429W, T447A, I485A, I485K, I485R, F487R, L537G, C538K, C538R, F546M, S559P, S576D, Y599M, Q662D, and Q662P, wherein the position of the mutation is numbered with reference to SEQ ID NO: 6.

[0067] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence of at least one modified galactose oxidase variant set forth in SEQ ID NOS: 70, 72 or the even numbered sequences of SEQ ID NOS: 77-298. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 70. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 72. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence of at least one modified galactose oxidase variant set forth in the following even numbered sequences: SEQ ID NOS: 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, or 298. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 78. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 80. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 90. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 90. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 100. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 120. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 140. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 160. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 180. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 200. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 250. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence set forth in SEQ ID NO: 298.

[0068] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NOS: 70, 72 or the even numbered sequences of SEQ ID NOS: 77-298. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 70. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 72. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in the following even numbered sequences: SEQ ID NOS: 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, or 298. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 78. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 80. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 90. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 100. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 120. In one example, the present disclosure refers to the

modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 140. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 160. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 180. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 200. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 250. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NO: 298.

[0069] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the modified galactose oxidase comprises one or more of the following properties:

(a) enhanced protein expression by at least 1.01-fold compared to the galactose oxidase variant M3-5 or by at least 1.01-fold compared to the modified galactose oxidase disclosed herein;

(b) enhanced solubility by at least 1.01-fold compared to the galactose oxidase variant M3-5 or by at least 1.01-fold compared to the modified galactose oxidase disclosed herein;

(c) enhanced thermal stability by at least 1.01-fold compared to the galactose oxidase variant M3-5 or by at least 1.01-fold compared to the modified galactose oxidase disclosed herein at a temperature of at least 50°C;

(d) increased enzymatic activity by at least 1.01-fold compared to the galactose oxidase variant M3-5 or by at least 1.01-fold compared to the modified galactose oxidase disclosed herein, wherein the enzymatic activity comprises converting a secondary alcohol to a ketone, wherein optionally the secondary alcohol is a bulky secondary alcohol or unactivated secondary alcohol; and

(e) reduced enantioselectivity by at least 1.01-fold compared to the galactose oxidase variant M3-5 or by at least 1.01-fold compared to the modified galactose oxidase disclosed herein.

[0070] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the modified galactose oxidase has enhanced protein expression by at least 1.01-fold compared to the galactose oxidase variant M3-5 or by at least 1.01-fold compared to the modified galactose oxidase variant GOh1052 that has F217A and N268W mutations. In one example, the modified galactose oxidase has enhanced protein expression by about 1.01 to 1.73-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced protein expression by at least about 1.06-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced protein expression by at least about 1.1-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced protein expression by at least about 1.2-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced protein expression by at least about 1.3-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced protein expression by at least about 1.4-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced protein expression by at least about 1.5-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced protein expression by at least about 1.6-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced protein expression by at least about 1.7-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced protein expression by about 1.01 to 10.09-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced protein expression by at least about 1.01-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced protein expression by at least about 2-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced protein expression by at least about 3-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced protein expression by at least about 4-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced protein expression by at least about 5-fold compared

to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced protein expression by at least about 6-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced protein expression by at least about 7-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced protein expression by at least about 8-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced protein expression by at least about 9-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced protein expression by at least about 10-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced protein expression by at least about 10-fold compared to the galactose oxidase variant GOh1052. In one specific example, the modified galactose oxidase has enhanced protein expression by at least 1.51-fold compared to the galactose oxidase variant M3-5. In one specific example, the modified galactose oxidase has enhanced protein expression by at least 2.58-fold compared to the galactose oxidase variant GOh1052. In one specific example, the modified galactose oxidase has enhanced protein expression by at least 4.29-fold compared to the galactose oxidase variant GOh1052.

[0071] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the modified galactose oxidase has enhanced solubility by at least 1.01-fold compared to the galactose oxidase variant M3-5 or by at least 1.01-fold compared to the modified galactose oxidase variant GOh1052 that has the F217A and N268W mutations. In one example, the modified galactose oxidase has enhanced solubility by about 1.01 to 1.67-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced solubility by at least about 1.1-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced solubility by at least about 1.2-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced solubility by at least about 1.3-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced solubility by at least about 1.4-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced solubility by at least about 1.5-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced solubility by at least about 1.6-fold compared to the galactose oxidase variant M3-5. In one specific example, the modified galactose oxidase has enhanced solubility

by at least about 1.13-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced solubility by about 1.01 to 4.57-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced solubility by at least about 1.01-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced solubility by at least about 1.02-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced solubility by at least about 2-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced solubility by at least about 3-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced solubility by at least about 4-fold compared to the galactose oxidase variant GOh1052. In one specific example, the modified galactose oxidase has enhanced solubility by at least about 1.01-fold compared to the galactose oxidase variant GOh1052. In one specific example, the modified galactose oxidase has enhanced solubility by at least about 2.82-fold compared to the galactose oxidase variant GOh1052. In one specific example, the modified galactose oxidase has enhanced solubility by at least about 4.57-fold compared to the galactose oxidase variant GOh1052. In one example, solubility of the galactose oxidase variant is determined using the ratio of protein expression of soluble proteins over insoluble proteins. The higher the value, the more soluble it is. Solubility is then compared against either M3-5 (SEQ ID NO.: 6) or GOh1052 (SEQ ID NO.: 68), where the fold improvement is determined.

[0072] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the modified galactose oxidase has enhanced thermal stability by at least 1.01-fold compared to the modified galactose oxidase variant GOh1052 that has F217A and N268W mutations at a temperature of at least 50°C or by at least 1.52-fold compared to the modified galactose oxidase variant M3-5 at a temperature of at least 55°C. In one example, the modified galactose oxidase has enhanced thermal stability by about 1.52 to 1.61-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced thermal stability by at least about 1.6-fold compared to the galactose oxidase variant M3-5. In one specific example, the modified galactose oxidase has enhanced thermal stability by at least about 1.52-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has enhanced thermal stability by about 1.01 to 1.61-fold compared to the galactose oxidase variant GOh1052. In one example, the modified

galactose oxidase has enhanced thermal stability by at least about 1.01-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced thermal stability by at least about 1.1-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced thermal stability by at least about 1.2-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced thermal stability by at least about 1.3-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced thermal stability by at least about 1.4-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced thermal stability by at least about 1.5-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase has enhanced thermal stability by at least about 1.6-fold compared to the galactose oxidase variant GOh1052.In one specific example, the modified galactose oxidase has enhanced thermal stability by at least about 1.50-fold compared to the galactose oxidase variant GOh1052. In one specific example, the modified galactose oxidase has enhanced thermal stability by at least about 1.61-fold compared to the galactose oxidase variant GOh1052. In one example, thermal stability of the galactose oxidase variants is tested using a two-phase approach. In one example, higher temperatures of 50°C and 55°C were tested in the two-phase approach by: (1) heating the enzyme at temperature for 30 minutes and then followed by (2) a contacting step of the heated enzyme and substrate combined at 25°C for a specified reaction time, typically for 24 hours. In one example, 50°C was the temperature at which the enzymes were incubated at for 30 minutes before being used in the standard 25°C activity assay, which was done to assess thermal stability of the enzyme by comparison of the thermally challenged activity against enzyme activity that was not thermally challenged. In one example, 55°C was the temperature at which the enzymes were incubated at for 30 minutes before being used in the standard 25°C activity assay, which was done to assess thermal stability of the enzyme by comparison of the thermally challenged activity against enzyme activity that was not thermally challenged. These are distinct from the 40°C activity assay, which is an activity assay conducted at higher temperatures to assess enzyme performance at higher temperatures. Although it can also be taken as a measure of thermal stability, the measure used to assess thermal stability was the former (50°C for 30 minutes thermal challenge assay).

[0073] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the modified galactose oxidase has increased enzymatic activity by at least 1.01-fold compared to the galactose oxidase variant M3-5 or by at least 1.01-fold compared to the modified galactose oxidase variant GOh1052 that has F217A and N268W mutations, wherein the enzymatic activity comprises converting a secondary alcohol to a ketone, wherein optionally the secondary alcohol is a bulky secondary alcohol or unactivated secondary alcohol. In one example, the modified galactose oxidase has increased enzymatic activity by at least 1.1-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has increased enzymatic activity by at least 2-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has increased enzymatic activity by at least 3-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has increased enzymatic activity by at least 4-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has increased enzymatic activity by at least 5-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has increased enzymatic activity by at least 6-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has increased enzymatic activity by at least 7-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has increased enzymatic activity by at least 8-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase has increased enzymatic activity by at least 9-fold compared to the galactose oxidase variant M3-5. In one specific example, the modified galactose oxidase has increased enzymatic activity by 1.85-fold compared to the galactose oxidase variant M3-5. In one specific example, the modified galactose oxidase has increased enzymatic activity by 2.18-fold compared to the galactose oxidase variant M3-5. In one specific example, the modified galactose oxidase has increased enzymatic activity by at least 1.5-fold better than M3-5 for Substrate 128 (alpha-tetralol). In one specific example, the modified galactose oxidase has increased enzymatic activity by at least 1.5-fold better than M3-5 for Substrate 128 (alpha-tetralol). In one specific example, the modified galactose oxidase has increased enzymatic activity by at least 1.04-fold better than GOh1052 for substrate 152 (1-phenyl-1-butanol). In one specific example, the modified galactose oxidase has increased enzymatic activity by at least 1.04-fold better than GOh1052 for substrate 152 (1-phenyl-1-butanol). In one specific example, the modified galactose oxidase has increased enzymatic activity by at least 1.01-fold

better than M3-5 for 1 or more substrates in the 64-substrate panel in **Fig. 1**. In one specific example, the modified galactose oxidase has increased enzymatic activity by at least 1.1-fold better than M3-5 for 1 or more substrates in the 64-substrate panel in **Fig. 1**. In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, wherein the modified galactose oxidase comprises increased enzymatic activity by at least 10-fold compared to the galactose oxidase variant M3-5 or by at least 1.2-fold compared to the modified galactose oxidase disclosed herein, wherein the enzymatic activity comprises converting a secondary alcohol to a ketone, wherein optionally the secondary alcohol is a bulky secondary alcohol or unactivated secondary alcohol.

[0074] In one example, the present disclosure refers to the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 1.04-fold compared to the galactose oxidase variant M3-5 or by at least 1.15-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by about 1.04 to 12.9-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 1.1-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 2-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 3-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 4-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 5-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 6-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 7-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 8-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 9-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 10-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase disclosed

herein, reduced enantioselectivity by at least 11-fold compared to the galactose oxidase variant M3-5. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 12-fold compared to the galactose oxidase variant M3-5. In one specific example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by 12.9-fold compared to the galactose oxidase variant M3-5. In one specific example, the GOh1XXX modified galactose oxidase disclosed herein, has minimum reduced enantioselectivity of 0.8 R/S ratio from M3-5 experimental value of 20.6 R/S ratio (for substrate 128 – alpha-tetralol). In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by about 1.15 to 1.59-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 1.2-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 1.3-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 1.4-fold compared to the galactose oxidase variant GOh1052. In one example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by at least 1.5-fold compared to the galactose oxidase variant GOh1052. In one specific example, the modified galactose oxidase disclosed herein, reduced enantioselectivity by 1.59-fold compared to the galactose oxidase variant GOh1052. In one specific example, the GOh2XXX and GOh3XXX modified galactose oxidase disclosed herein, has minimum reduced enantioselectivity of 1.04 R/S ratio from M3-5 (for substrate 128 – alpha-tetralol) or from GOh1052 (for substrate 152 – 1-phenyl-1-butanol).

[0075] In one aspect, the present disclosure refers to a polynucleotide sequence encoding the modified galactose oxidase disclosed herein.

[0076] In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide sequence comprises at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more sequence identity to SEQ ID NO: 5, wherein SEQ ID NO: 5 is a polynucleotide sequence of the galactose oxidase variant M3-5, wherein the polynucleotide sequence disclosed herein comprises at least one mutation at one or more positions which leads to at least one mutation in the polypeptide sequence of the modified galactose oxidase disclosed herein.

[0077] In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide is codon optimized, wherein optionally the polynucleotide is codon optimized for protein expression in *Escherichia coli*. In one example, the polynucleotide is codon optimized for protein expression in other types of cells known in the art, such as yeast cells, mammalian cells or other suitable cells known in the art. In one example, the polynucleotide is codon optimized for protein expression in yeast cells such as *Saccharomyces cerevisiae* and *Pichia pastoris*. In one example, the polynucleotide is codon optimized for protein expression in mammalian cells such as Chinese Hamster Ovary (CHO) cells. The term "codon optimization" as used herein, refers to methods to improve codon composition of a polynucleotide without altering the amino acid sequence, since it is known in the art that most amino acids can be encoded by more than one codon.

[0078] In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide sequence is further linked to a control sequence. In one example, the control sequence is a promoter sequence or a termination sequence. In one example, the promoter sequence is a lac promoter sequence, CMV promoter sequence, SV40 promoter sequence, PBAD promoter sequence, GAL promoter sequence, AOX1 promoter sequence, or other promoter sequences that are well-known in the art. In one example, the PBAD promoter can be used for controlling gene expression in *Escherichia coli* (E. coli). In one example, the GAL promoter can be used for controlling gene expression in *Saccharomyces cerevisiae* (*S. cerevisiae*). In one example, the AOX1 promoter can be used for controlling gene expression in *Pichia pastoris* (*P. pastoris*). In one specific example, the promoter sequence is a lac promoter sequence. The term "control sequence" as used herein refers to a regulatory sequence or regulatory element within a polynucleotide sequence that regulates gene expression. Examples of "control sequences" that are well-known in the art are promoters, enhancers, repressors, and terminators.

[0079] In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises an odd numbered sequence of SEQ ID NOS: 7-298. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises an odd numbered sequence of SEQ ID NOS: 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143,

145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, or 297. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 7. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 9. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 11. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 13. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 15. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 17. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 19. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 21. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 31. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 41. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 51. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 61. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 71. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 81. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 91. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein,

wherein the polynucleotide comprises the sequence of SEQ ID NO: 101. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 151. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 201. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 251. In one example, the present disclosure refers to the polynucleotide sequence disclosed herein, wherein the polynucleotide comprises the sequence of SEQ ID NO: 297.

[0080] In one aspect, the present disclosure refers an expression vector comprising the polynucleotide sequence disclosed herein. In one example, the expression vector is selected from the group consisting of a plasmid and a viral vector. Suitable expression vectors for production of proteins (such as enzymes, such as the modified galactose oxidase of the present disclosure) are well known to those skilled in the art. Examples of plasmid vectors include pET28a, pcDNA3.1, pGEX, pCMV, pYES, and pGAPZ. In one example, the pYES plasmid vector is designed for protein expression in *Saccharomyces cerevisiae* (*S. cerevisiae*). In one example, the pGAPZ plasmid vector is designed for protein expression in *Pichia pastoris* (*P. pastoris*). In one specific example, the expression vector is a pET28a plasmid vector. Examples of viral vectors include Adeno-Associated Viral Vector (AAV) and lentiviral vector. In one example, the expression vector comprising the polynucleotide sequence disclosed herein may be vectors generally used for the production of proteins/enzymes which are known to those skilled in the art.

[0081] In one aspect, the present disclosure refers to a host cell comprising the polynucleotide sequence disclosed herein or the expression vector disclosed herein. In one example, the present disclosure refers to a host cell comprising the polynucleotide sequence disclosed herein. In one example, the present disclosure refers to a host cell comprising the expression vector disclosed herein. In one example, the host cell disclosed herein is a prokaryotic cell or eukaryotic cell. In one example, the host cell is a prokaryotic cell. In one specific example, the prokaryotic cell is *Escherichia coli*. In one example, the host cell comprising the polynucleotide sequence disclosed herein or the expression vector disclosed herein is *E. coli* BL21(DE3). In one example, the host cell comprising the polynucleotide sequence disclosed herein or the

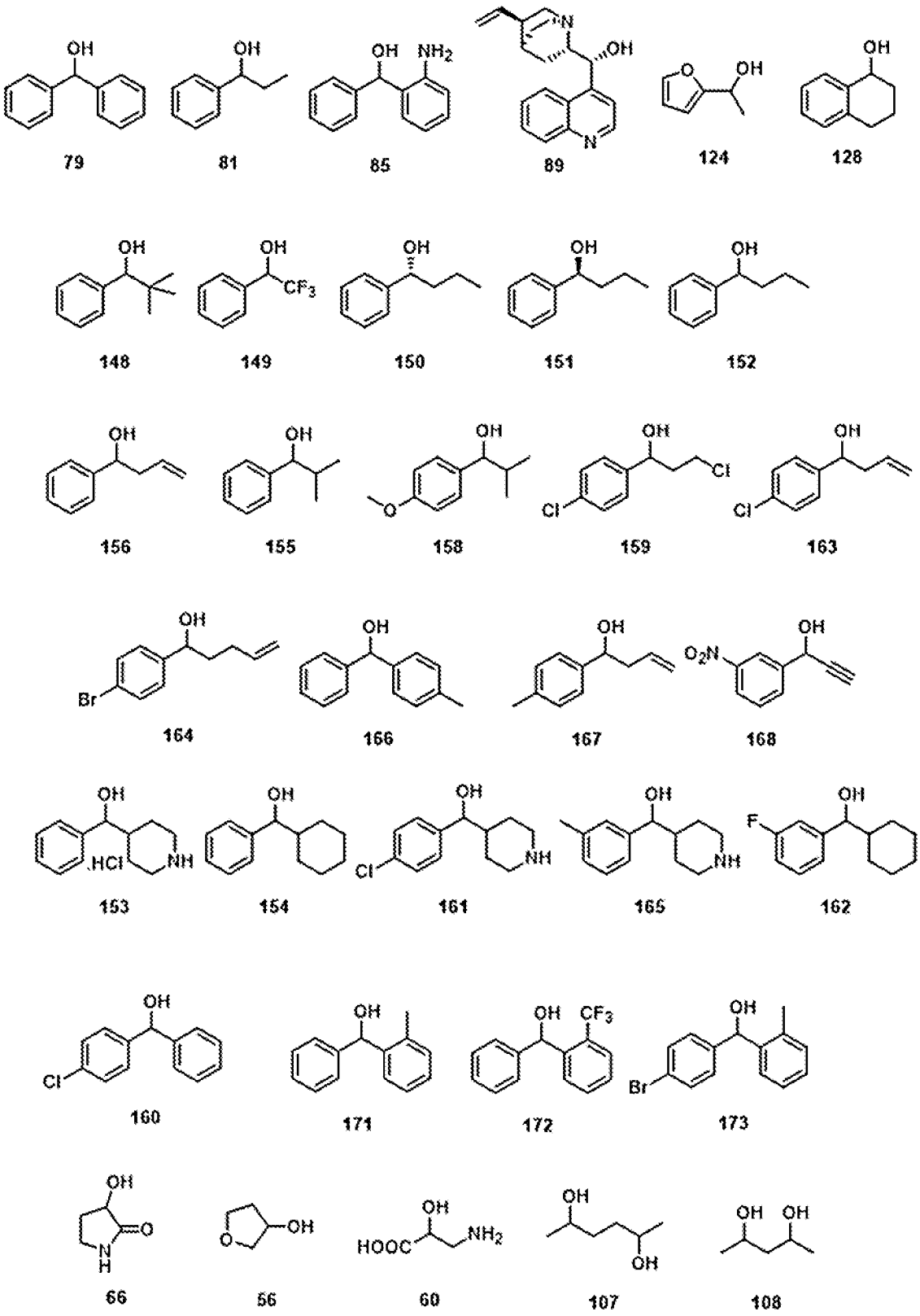
expression vector disclosed herein may be cells generally used for the production of proteins/enzymes which are known to those skilled in the art.

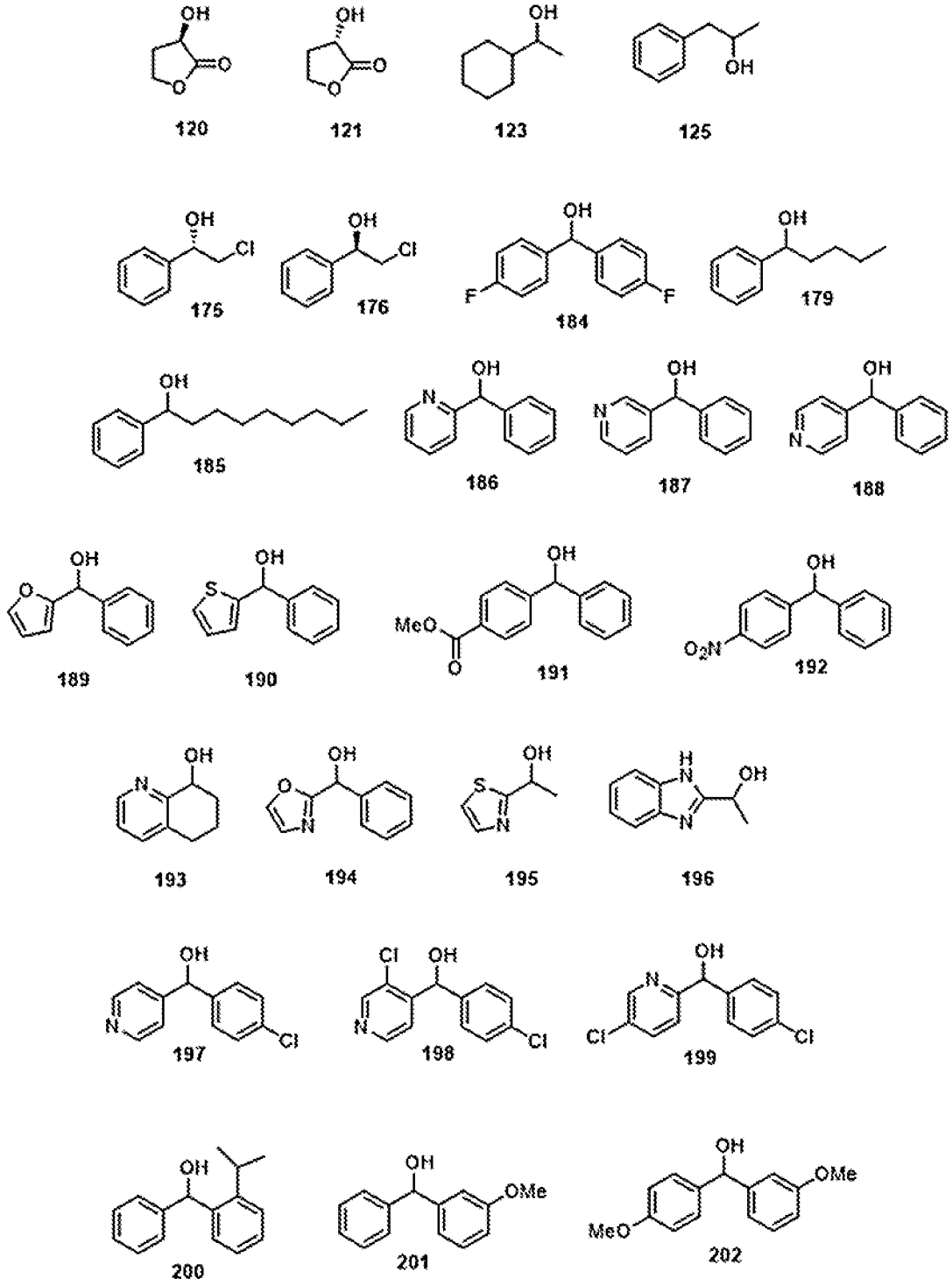
[0082] In one aspect, the present disclosure refers to a method of producing a modified galactose oxidase, comprising culturing the host cell disclosed herein under suitable culture conditions such that the modified galactose oxidase is produced. In one example, the method comprises culturing the host cell disclosed herein in a culture medium. In one example, the culture medium is lysogeny broth (LB) medium supplemented with kanamycin, or any other suitable culture medium known in the art. In one example, the method of producing the modified galactose oxidase disclosed herein, further comprises recovering the modified galactose oxidase from the culture and/or the host cell. In one example, the method of producing the modified galactose oxidase disclosed herein, further comprises recovering the modified galactose oxidase from the culture. In one example, the method of producing the modified galactose oxidase disclosed herein, further comprises recovering the modified galactose oxidase from the host cell. In one example, the method of producing the modified galactose oxidase disclosed herein, further comprises recovering the modified galactose oxidase from the culture and host cell. In one example, the method of recovering the modified galactose oxidase disclosed herein from the culture and host cell are commonly used methods of protein recovery from culture and host cells which are well-known to those skilled in the art. In one example, the modified galactose oxidase disclosed herein is recovered from the culture and host cell through cell harvesting by centrifugation, where the proteins from the culture medium are separated based on density differences. In one example, the modified galactose oxidase disclosed herein is recovered from the culture and host cell through cell harvesting by filtration, whereby culture media are filtered to separate host cells from the culture media using membranes filters. In one example, the modified galactose oxidase disclosed herein is recovered from the culture and host cell through cell lysis/disruption by mechanical or chemical disruption of host cells to disrupt cell walls/membranes and release intracellular proteins. In one example, the method of producing the modified galactose oxidase disclosed herein, further comprises purifying the modified galactose oxidase. In one example, the method of purifying the modified galactose oxidase disclosed herein is by affinity chromatography, using specific affinity tags such as His-tag and GST-tag. In one example, the method of purifying the modified galactose oxidase disclosed herein is by ion exchange chromatography, whereby proteins are separated based on charge differences. In one example, the method of purifying

the modified galactose oxidase disclosed herein is by size exclusion chromatography, whereby proteins are separated based on their size and molecular weight. In one specific example, the method of purifying the modified galactose oxidase disclosed herein is by affinity chromatography, using His-tag. In one example, the method of producing and purifying the modified galactose oxidase disclosed herein may be methods generally used for the production and purification of proteins, such as enzymes, which are known to those skilled in the art.

[0083] In one aspect, the present disclosure refers to a method of producing a ketone from a secondary alcohol, comprising contacting the secondary alcohol with the modified galactose oxidase disclosed herein under suitable conditions for an oxidation reaction. In one example, the secondary alcohol is a bulky secondary alcohol or unactivated secondary alcohol. In one example, the secondary alcohol is a bulky secondary alcohol which comprises one or more bulky substituents. In one example, the unactivated secondary alcohol is an aliphatic secondary alcohol without a benzene ring in the alpha position adjacent to the alcohol group.

[0084] In one example, the secondary alcohol comprises the formula R^1R^2CHOH, wherein R^1 and R^2 are independently selected from the group consisting of cycloalkyl, aryl, heteroaryl, alkyl, alkylene, alkynyl, haloalkyl, carboxyl, and carbonyl, wherein the cycloalkyl, aryl, heteroaryl, alkyl, alkylene, alkynyl, haloalkyl, carboxyl, and carbonyl is optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, alkyl halide, alkylene, alkynyl, alkoxy, cyano, oxo, nitro, amino, thiol, carboxyl, ester and hydroxyl. In one example, the secondary alcohol is selected from the group consisting of:


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[0085] In one example, the secondary alcohol is chiral or non-chiral. In one example, the secondary alcohol is chiral. In one example, the secondary alcohol is non-chiral. In one example, the chiral secondary alcohol is present as (S)-enantiomer, (R)-enantiomer, or a

mixture of (S)- and (R)-enantiomers. In one example, the chiral secondary alcohol is present as a racemic mixture.

[0086] In one example, the present disclosure refers to a method of producing a ketone from a secondary alcohol, comprising contacting the secondary alcohol with the modified galactose oxidase disclosed herein, wherein the contacting step is performed at a temperature of 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C or more. In one example, the contacting step is performed at a temperature of 25°C. In one example, the contacting step is performed at a temperature of 30°C. In one example, the contacting step is performed at a temperature of 35°C. In one example, the contacting step is performed at a temperature of 40°C. In one example, the contacting step is performed at a temperature of 45°C. In one example, the contacting step is performed at a temperature of 50°C. In one example, the contacting step is performed at a temperature of 55°C. In one example, the contacting step is performed at a temperature higher than 55°C. In one example, the contacting step refers to a step where the substrate and the enzyme are first mixed, before the oxidation reaction occurs. In one example, the contacting step is performed at a temperature of 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C or more, wherein optionally the temperature is 35°C. In one example, the oxidation reaction can be performed at lower temperatures than 40°C and can be conducted at 25°C. In some examples, certain galactose oxidase mutants have shown enzymatic activity even after a thermal challenge at 55°C. Optimal temperature for the reaction depends on, for example, enzyme variant, substrate as well as which output variable. Taking GOh1052 variant with substrate 152 (1-phenyl-1-butanol) as an example, although 40°C is the optimal temperature for fastest initial rate of reaction, 35°C is preferred over a 24-hour reaction period as it results in higher overall yields (72% at 35°C vs 55% at 40°C).

[0087] In one example, the method of producing a ketone from a secondary alcohol disclosed herein, further comprises converting the secondary alcohol into an active pharmaceutical ingredient (API). The term "active pharmaceutical ingredient" as used herein refers to a biologically active component of a pharmaceutical drug responsible for the therapeutic effect of the drug.

[0088] In one aspect, the present disclosure refers to a kit for use in the method disclosed herein, wherein the kit comprises the modified galactose oxidase disclosed herein. In one example, the kit disclosed herein comprises one or more of the following: (a) one or more reaction buffers; (b) one or more reaction vessels, wherein optionally the reaction vessel is a

HPLC vial; (c) one or more reaction solvents, wherein optionally the reaction solvent is 5% v/v dimethyl sulfoxide in 100 mM sodium phosphate buffer with a pH of 7; (d) one or more secondary alcohol substrates; (e) one or more co-enzymes to break down the by-product of the oxidation reaction, wherein optionally the co-enzyme is a horseradish peroxidase that breaks down the H_2O_2; and (f) instructions for performing the method disclosed herein. In one example, the one or more reaction buffer is Jones Reagent. In one example, the one or more reaction buffer is PCC (Pyridinium chlorochromate). In one example, the one or more reaction buffer contains an enzyme capable of catalysing the conversion of secondary alcohols to ketones. In one example, the enzyme is alcohol dehydrogenase. In one example, the one or more reaction buffer contains an enzyme and cofactors. In one example, the enzyme is alcohol dehydrogenase and the cofactor is NAD^+ (nicotinamide adenine dinucleotide) or $NADP^+$ (nicotinamide adenine dinucleotide phosphate). In some examples, the reagents in the kit as described herein may be provided in separate containers comprising the components independently distributed in one or more containers.

EXAMPLES

[0089] Methods

[0090] ***Galactose oxidase expression in E. coli and purification***

[0091] The wildtype galactose oxidase gene from *Fusarium graminearum* was synthesized (Twist Bioscience) and cloned into the expression vector pET28a (Novagen) using the *Nde*I and *Xho*I restriction sites. Eight additional mutations (S33P, M93V, G218E, W313F, R353M, Q429T, V517A, N558D) were incorporated into the wildtype galactose oxidase gene via splicing by overlap extension (SOE) method to produce GOh1001b variant. The GOh1001b plasmid was then transformed into *E. coli* BL21(DE3) strain. The *E. coli* containing the plasmid was grown in 250 mL lysogeny broth (LB) medium with 50 µg/mL kanamycin at 37°C until an absorbance of 0.5 at 600 nm was reached. Overexpression was induced by adding 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and incubation was continued at 16°C for 20 hours. Cells were harvested by centrifugation (2,450 x *g* for 15 minutes). The cell pellet was resuspended in 12 mL of 100 mM sodium phosphate buffer, pH 7.0 and the cells were lysed using a cell disruptor (Constant Systems) at 40 kPSI. The lysate was then centrifuged, and the clarified supernatant was added to TALON metal affinity resin (Clontech). The supernatant-resin mixture was incubated at 4°C with rotation for 2 hours. The His-tagged protein bound

resin was washed with binding buffer (100 mM sodium phosphate buffer, pH 7.0, 300 mM NaCl, 5 mM imidazole) and eluted with elution buffer (100 mM sodium phosphate buffer, pH 7.0, 300 mM NaCl, 250 mM imidazole). The elution was desalted using PD-10 desalting column (GE Healthcare) in 100 mM sodium phosphate buffer, pH 7.0. The protein concentration was measured using Pierce™ BCA Protein Assay kit (Thermo Fisher Scientific) against diluted albumin (BSA) standards as provided inside the kit. The purified protein was then diluted to 400 µg/mL and activated with 1 mM copper sulphate at 25°C for 15 minutes.

[0092] ***Codon optimization for GO01b variant***

[0093] Synthesized gene was ordered from Twist Bioscience. Utilizing their website tool, amino acid sequence was entered, and desired codon usage table selected (*E. coli*). Twist Bioscience's codon optimization algorithm subsequently provided the DNA nucleotide sequence. Algorithm results are not reproducible as identical amino acid sequences keyed in twice will return different DNA nucleotide sequences.

[0094] ***Library construction and new variant construction***

[0095] Round 1: Saturation mutagenesis (NNK) at the actives sites was performed on GOh1001b variant via SOE method to generate libraries for high throughput (HTP) screening. Beneficial mutations from HTP screening were combined via SOE method.

[0096] Round 2: Site saturation mutagenesis libraries on GOh1052 variant were ordered from either Twist Bioscience or GenScript with codon usage for *E. coli* expression at each mutated position only.

[0097] Round 3: Beneficial mutations from Round 2 were combined via SOE method on GOh1052 variant. For combinatorial library on GOh1052 variant, oligos with wildtype DNA codon and mutation DNA codon at each position were ordered and pooled together with equal ratio to give a theoretical 50% rate of mutation incorporation. Combinatorial library was constructed via SOE method.

[0098] ***High throughput growth and lysis***

[0099] For each NNK library in round 1, 84 colonies for variants (32 codons, ~93% coverage), six (6) colonies for GOh1001b variant, and six (6) colonies for negative control (pET28a) were inoculated into a 96-well plate. For round 2 libraries, 19 colonies per position (i.e., 19 other amino acid residues from wildtype residue), six (6) colonies for GOh1052 variant, and six (6) colonies for negative control (pET28a) were inoculated into a 96-well plate. For round 3 combinatorial library, 84 colonies for variants, six (6) colonies for GOh1052 variant, and six

(6) colonies for negative control (pET28a) were inoculated into a 96-well plate (for five (5) plates, ~95% coverage) Individual colony was inoculated in 180 µL LB medium containing kanamycin (50 µg/mL) and cultured overnight at 37°C. 20 µL of cultures were transferred to 380 µL LB medium containing 50 µg/mL kanamycin and grown until an absorbance of 0.5 at 600 nm was reached. Overexpression was induced by adding 0.1 mM IPTG and incubation was continued at 16°C for 20 hours. Cells were harvested by centrifugation (2,182 x *g* for 10 min at 4°C). The cell pellets were lysed with 250 µL lysis buffer (100 mM sodium phosphate buffer, pH 7.0, 0.1% Triton-X, 1 mM copper sulphate) at room temperature for 2 hours at speed 6.5 on titer plate shaker (Barnstead). The lysates were obtained by centrifugation (2,182 x *g* for 20 min at 4°C).

[0100] ***ABTS-HRP* colorimetric assay (Fig. 5)***

[0101] HTP colorimetric assay conditions: Substrate (1 mM in DMSO), ABTS (2.5 mM in water), HRP (Toyobo, 25 µg/mL), and galactose oxidase lysates (25 µL) in a final volume of 200 µL with final concentration of dimethylsulfoxide (DMSO) at 5% v/v in 100mM sodium phosphate buffer (pH 7.0). HTP colorimetric assay was carried out using 96-well clear shallow well plates at room temperature and read at 405 nm using a microplate reader (Tecan Infinite M200 Pro) for the following timepoints: every 2 minutes for 1 hour, every 10 minutes for next 2 hours, every 30 minutes for next 5 hours, every 2 hours for next 16 hours for a total of 24 hours.

[0102] Focused secondary alcohol substrate panel colorimetric assay: Identical conditions to HTP colorimetric assay except with galactose oxidase purified protein (20 µg/mL) instead of galactose oxidase lysates. Better performing variants against secondary alcohol substrates were selected based on the following formula (maximum absorbance x initial velocity). *ABTS refers to 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), HRP refers to Horseradish peroxidase.

[0103] ***F217 Fitness Landscape (Enantiomeric Bias)***

[0104] HTP enzymatic reaction conditions: Substrate 128 (1 mM), HRP (25 µg/mL), and galactose oxidase purified protein (100 µg/mL) in a final volume of 200 µL with final concentration of DMSO at 5% v/v in 100mM sodium phosphate buffer (pH 7.0). HTP enzymatic reaction was carried out using 96-well deepwell plates with breathable seal at 25°C, 220 rpm. Reaction was stopped at 24 hours via addition of methanol (200 µL). The precipitated

protein was then removed by centrifugation (2,182 x *g* for 20 minutes). 10 µL of the reaction mixture was sampled for HPLC-UV analysis.

[0105] ***Thermostability Assay***

[0106] Enzyme heat treatment conditions: Purified enzyme (60 µL, 400 µg/mL) was incubated at 55°C for 30 minutes in PCR strip tubes using Bio-Rad PTC-200 Thermal Cycler. The heat-treated enzymatic solution was transferred to 1.5 mL tubes and the precipitated protein was removed by centrifugation (20,238 x *g* for 5 min at 4°C). 50 µL of the heat-treated enzymatic solution was transferred to a 96 deep well plate for activity assay. Activity assay: Substrate 128 (1 mM), HRP (25 µg/mL), and heat-treated galactose oxidase purified protein (100 µg/mL) in a final volume of 200 µL with final concentration of DMSO at 5% v/v in 100 mM sodium phosphate buffer (pH 7.0). Reactions were carried out in 96 deepwell plates with breathable seal at 25°C, 220 rpm. Reactions were stopped at 24 h via addition of methanol (200 µL). The precipitated protein was then removed by centrifugation (2,182 x *g* for 20 minutes at 4°C). 10 µL of the reaction mixture was sampled for HPLC-UV analysis. The full experimental materials include:

- Suitable reaction vessel (i.e. 2 mL HPLC vials were used)
- 5% v/v Dimethyl sulfoxide in 100 mM sodium phosphate buffer (pH = 7) as reaction solvent (200 µL)
- Secondary alcohol substrate (1 mM)
- Horseradish peroxidase (25 µg / mL) as a co-enzyme to breakdown H_2O_2 that is produced as a by-product of the oxidation reaction
- Galactose oxidase protein (40 µg / mL)
- Sufficient atmospheric oxygen content.

[0107] ***Enzyme Kinetics***

[0108] Kinetics colorimetric assay conditions for substrate 128: Substrate 128 (varying concentrations from 2.5 to 30 mM), ABTS (2.5 mM), HRP (25 µg/mL) and galactose oxidase purified protein (amount is variant dependent) in a final volume of 200 µL with final concentration of DMSO at 5% v/v in 100 mM sodium phosphate buffer (pH 7.0). Kinetic parameters were obtained by the best-fit model of initial velocity against substrate concentrations based on Michaelis-Menten equation using GraphPad Prism 8 (GraphPad Software).

[0109] Amount of galactose oxidase purified protein used: GOh1001b (100 µg/mL), GOh1021 (10 µg/mL), GOh1036 (10 µg/mL), GOh1052 (5 µg/mL)

[0110] ***High-Performance Liquid Chromatography (HPLC)***

[0111] ***HPLC validation***

[0112] Purified protein reaction conditions: Substrate (1 mM), HRP (25 µg/mL), and galactose oxidase purified protein (100 µg/mL) in a final volume of 200 µL with final concentration of DMSO at 5% v/v in 100mM sodium phosphate buffer (pH 7.0). Purified protein reaction was carried out in sealed 2 mL HPLC vials at 25°C, 220 rpm. Reaction was stopped at 24 hours via addition of methanol (200 µL). The precipitated protein was then removed by centrifugation (20,238 x *g* for 1 minute). 10 µL of the reaction mixture was sampled for HPLC-UV analysis.

[0113] 40°C heated activity assay conditions: The conditions are identical to the purified protein reaction conditions described above except that the reactions are conducted at 40°C temperature for a reaction period of 6 hours.

[0114] ***HPLC analysis***

[0115] HPLC analysis was carried out using Shimadzu Prominence system UFLC or Agilent HPLC 1200 Infinity series paired with CTC Analytics HTS PAL LC Autosampler.

[0116] Mobile phase A: Water; B: Acetonitrile. Column: Phenomenex, Luna® 5 µm C18(2) 100 Å, LC Column 150 x 4.6 mm, 1 mL/minute flow rate

[0117] Standards (200 µL) of 1 mM substrate and 1 mM product for each compound were prepared with 5% DMSO v/v in 100 mM sodium phosphate buffer (pH 7.0) then diluted with methanol (200 µL) before being run with each sample for quantification according to the following formula:

$$HPLC\ Yield\% = \frac{Peak\ Integral\ (Sample)}{Peak\ Integral\ (Standard)}$$

[0118] ***HRP-ABTS Colorimetric Assay***

[0119] ***HRP-ABTS Colorimetric Assay Screening Data***

[0120] Colorimetric activities were assessed calculated from two parameters measured via 405 nm absorbance data gathered by microplate reader (Tecan) according to the following formula:

$$Colorimetric\ Activity = Rate \times Maximum\ Absorbance \times 100$$

where,

Colorimetric Activity = measure of enzymatic activity

Rate = initial rate of increase in absorbance in the first 8 min

Maximum Absorbance = maximum absorbance recorded over the 24 h reaction period

[0121] Table 1 - Table of galactose oxidase mutations investigated in the present disclosure

Mutation	Position of mutation, numbered based on the protein sequence of the M3-5 variant of galactose oxidase
A24H	24
A24N	24
A26H	26
A26R	26
I28F	28
I28M	28
I28R	28
Q45D	45
Q45E	45
Q45N	45
E49K	49
F65P	65
N69D	69
N69E	69
N69F	69
N69H	69
N69L	69
N69M	69
N69T	69
N69W	69
N69Y	69
W104G	104
W104N	104
S114N	114
S114R	114
T117D	117
T153K	153
T153R	153
G157N	157
S172E	172
S173K	173
S173R	173
T175E	175
T175H	175
T175I	175
T175K	175

V193T	193
P194C	194
A195S	195
A195T	195
A195V	195
A197C	197
A197G	197
A198I	198
T202D	202
T202I	202
T202K	202
T202Q	202
N214R	214
N214S	214
F217A	217
F217C	217
F217D	217
F217E	217
F217G	217
F217H	217
F217I	217
F217K	217
F217L	217
F217M	217
F217N	217
F217P	217
F217Q	217
F217R	217
F217S	217
F217T	217
F217V	217
F217Y	217
E218F	218
E218K	218
E218P	218
E218Y	218
S220T	220
I224W	224
L226Q	226
L226V	226
D239E	239
D239P	239
D239Q	239

R240G	240
F250E	250
F250Y	250
N259T	259
T265S	265
N268W	268
D269N	269
I284S	284
I284Y	284
G294A	294
F313R	313
S314C	314
Y325F	325
N341W	341
A346L	346
K348S	348
Y352T	352
Y352W	352
M353F	353
N356K	356
N356R	356
N356W	356
K389I	389
A401D	401
T429W	429
T447A	447
I485A	485
I485K	485
I485R	485
F487R	487
L537G	537
C538K	538
C538R	538
F546M	546
S559P	559
S576D	576
Y599M	599
Q662D	662
Q662P	662

[0122] ***Exemplary minimum fold changes***

[0123] ***The following exemplary minimum fold changes are to be considered:***

- For GOh1XXX variant activity (Table 2) – 1.5-fold more than M3-5 for Substrate 128 (alpha-tetralol)
- For GOh2XXX and GOh3XXX variant activity (Tables 3 and 4 – 1.04-fold more than GOh1052 for substrate 152 (1-phenyl-1-butanol) indicated by "Y" in "Activity (HPLC)" column in Table 6.
- For GOhXXXX variant activity (Table 2 – 1.1-fold better than M3-5 for 1 or more substrates in the 64-substrate panel in **Fig. 1**.
- For GOh1XXX protein expression (Table 14) - 1.06-fold better than M3-5; For GOh1XXX solubility (Table 16) - 1.13-fold better than M3-5; and thermal stability (Table 12) - 1.52-fold better than M3-5
- For GOh2XXX and GOh3XXX protein expression (Table 13) - 1.01-fold better than GOh1052; solubility (Table 15) - 1.02-fold better than GOh1052; and thermal stability (Table 11) – 1.01-fold better than GOh1052
- For GOh1XXX enantioselectivity (Table 18 – minimum reduction of 0.8 R/S ratio from M3-5 experimental value of 20.6 R/S ratio (for substrate 128 – alpha-tetralol)
- For GOh2XXX and GOh3XXX enantioselectivity (Tables 17 and 18 – minimum 1.04-fold change reduction in R/S ratio from M3-5 (for substrate 128 – alpha-tetralol) or from GOh1052 (for substrate 152 – 1-phenyl-1-butanol)
- For ease of comparison, interpretation of reduced enantioselectivity is conveyed as absolute units as a ratio in conversion of (R)- and (S)-enantiomers of the substrate. Higher R/S ratios indicate higher enantioselectivity for (R)-enantiomers while R/S ratios closer to 1 indicate reduced enantioselectivity, with 1 R/S ratio being no enantioselectivity.

[0124] Table 2 - The colorimetric activity of purified galactose oxidase enzymes activity profile benchmarked against M3-5 backbone.

Mutant No.	Substrate	Mutations (on M3-5)	Activity Fold Change
M3-5	66, 120, 121, 128, 166	backbone	1.0
GOh1008	66	S314C	19.9
GOh1017	66	F217Q	8.4
GOh1018	66	F217N	3.1

GOh1042	66	F217M	3.0
GOh1039	66	F217G	2.2
GOh1020	66	F217S	2.0
GOh1036	66	F217A	1.3
GOh1006	66	M353F	1.1
GOh1021	120	N268W	3.3
GOh1015	121	F217V	2.1
GOh1017	128	F217Q	132.2
GOh1036	128	F217A	101.9
GOh1039	128	F217G	87.9
GOh1042	128	F217M	73.0
GOh1015	128	F217V	68.7
GOh1018	128	F217N	67.7
GOh1043	128	F217R	40.8
GOh1021	128	N268W	39.8
GOh1030	128	F217I	22.9
GOh1016	128	F217P	20.1
GOh1031	128	F217T	15.0
GOh1020	128	F217S	13.6
GOh1034	128	F217E	5.6
GOh1008	128	S314C	4.9
GOh1009	128	S314K	4.3
GOh1010	128	S314L	3.5
GOh1006	128	M353F	3.1
GOh1023	128	N268F	3.1
GOh1025	128	E218P	3.1
GOh1013	128	L537G	2.5
GOh1024	128	N268K	1.9
GOh1012	128	S314T	1.5
GOh1052	166	F217A, N268W	3643.3
GOh1048	166	F217V, E218P	452.9
GOh1051	166	E218P, L537G	192.2
GOh1045	166	F217P, E218P	188.9
GOh1046	166	F217P, L537G	85.4
GOh1049	166	F217V, L537G	58.3
GOh1055	166	F217G, N268W	47.4

[0125] 39 purified enzymes show improved activity profile benchmarked against M3-5 backbone (Table 2). The experimental conditions used were: Purified protein (10 µL, 400 µg/mL), 5 µL HRP (1mg/mL), 2.5 mM ABTS, 5% DMSO v/v, 1 mM substrate, 25°C, topped up to 200 µL with 100mM sodium phosphate (pH 7.0) buffer, 24 hours.

[0126] Table 3 - The colorimetric activity of purified galactose oxidase enzymes activity profile benchmarked against GOh1052 (F217A, N268W from M3-5) backbone for substrate 152.

Mutant No.	Mutations (on GOh1052)	Activity Fold Change
GOh1052	backbone	1.0
GOh2015	A197C	22.2
GOh2010	Y352F	18.2
GOh2072	S114R	13.3
GOh2013	Y352T	9.5
GOh2026	K389I	8.2
GOh2011	G294A	8.1
GOh2016	R240G	6.2
GOh2021	T202I	5.9
GOh2025	A346L	4.1
GOh2002	V193T	1.8
GOh2019	T202D	1.8
GOh2020	T202Q	1.8
GOh2018	K348S	1.5
GOh2022	T202K	1.4

[0127] 14 purified enzymes show improved activity profile benchmarked against GOh1052 (F217A, N268W from M3-5) backbone for substrate 152 (Table 3). The experimental conditions used were: Purified protein (10 µL, 400 µg/mL), 5 µL HRP (1 mg/mL), 2.5 mM ABTS, 5% DMSO v/v, 1 mM substrate 152, 25°C, topped up to 200 µL with 100 mM sodium phosphate (pH 7.0) buffer, 24 hours.

[0128] Table 4 - The High Performance Liquid Chromatography (HPLC) Yield Activity of purified galactose oxidase enzymes activity profile benchmarked against GOh1052 (F217A, N268W from M3-5) backbone for substrate 152.

Mutant No.	Mutations (on GOh1052)	Activity Fold Change
GOh1052	backbone	1.00
GOh2082	E218F	2.68
GOh2081	A195V	2.04
GOh2044	A24H	2.00
GOh2086	I28F	1.84
GOh2012	A195T	1.80

GOh2027	Y352W	1.76
GOh2045	I28R	1.76
GOh2085	A26R	1.76
GOh2070	D239E	1.68
GOh2084	I224W	1.64
GOh2087	E218Y	1.60
GOh2083	L226Q	1.56
GOh2091	A24N	1.52
GOh2023	P194C	1.36
GOh2092	A26H	1.36
GOh2039	E218K	1.28
GOh2090	F546M	1.28
GOh2001	A195S	1.24
GOh2080	S173K	1.20
GOh2014	N341W	1.16
GOh2076	S173R	1.16
GOh2079	Q662D	1.16
GOh2008	N259T	1.12
GOh2043	I284Y	1.08
GOh2046	I28M	1.08
GOh2047	S576D	1.08
GOh2017	A198I	1.04
GOh2048	I284S	1.04
GOh2055	A197G	1.04
GOh2071	T117D	1.04
GOh2075	Q662P	1.04
GOh2078	S172E	1.04
GOh2089	D239Q	1.04

[0129] 33 purified enzymes show improved activity profile benchmarked against GOh1052 (F217A, N268W from M3-5) backbone for substrate 152 (Table 4). The experimental conditions used were: purified protein (20 µL, 400 µg/mL), 5 µL HRP (1 mg/mL), 5% DMSO v/v, 1 mM substrate 152, 25°C, 220 rpm, topped up to 200 µL with 100 mM sodium phosphate (pH 7.0) buffer (in 2 mL HPLC vials), 6 hours.

[0130] Table 5 - The 40°C Activity of purified galactose oxidase enzymes activity profile benchmarked against GOh1052 (F217A, N268W from M3-5) backbone for substrate 152.

Mutant No.	**Mutations (on GOh1052)**	**HPLC Yield of Ketone**
GOh1052	backbone	33%

GOh3006	I28R, K389I	72%
GOh3008	I28R, T153R, A198I, N341W	64%
GOh3038	I28R, A195T, N259T, N341W, K389I	64%
GOh3013	I28R, A195T, N259T, K389I	63%
GOh3026	I28R, A195T, N259T, T447A	63%
GOh3023	I28R, A195T, N341W, K389I	61%
GOh2045	I28R	61%
GOh2044	A24H	59%
GOh2070	D239E	57%
GOh3032	I28R, A195S, N259T, N341W	56%
GOh3015	I28R, N259T, N341W	52%
GOh3018	I28R, A195S, K389I	52%
GOh2082	E218F	52%
GOh2083	L226Q	52%
GOh3003	A198I, N259T	51%
GOh2014	N341W	50%
GOh2017	A198I	50%
GOh2091	A24N	49%
GOh2086	I28F	47%
GOh2087	E218Y	47%
GOh2084	I224W	46%
GOh2085	A26R	44%
GOh2023	P194C	43%
GOh2119	A401D	43%
GOh2027	Y352W	41%
GOh2055	A197G	41%
GOh2089	D239Q	41%
GOh2092	A26H	41%
GOh2117	T175K	41%
GOh2081	A195V	40%
GOh2039	E218K	39%
GOh2114	T175E	39%
GOh2115	T175H	39%
GOh2008	N259T	38%
GOh2043	I284Y	36%
GOh2046	I28M	36%
GOh2098	Q45E	36%
GOh2116	T175I	36%
GOh2071	T117D	35%
GOh2090	F546M	35%
GOh2111	W104N	35%
GOh3002	N341W, Y352T	34%
GOh2048	I284S	34%

GOh2056	T265S	34%
GOh2076	S173R	34%

[0131] 45 purified enzymes show improved heated activity profile benchmarked against GOh1052 (F217A, N268W from M3-5) backbone for substrate 152 (Table 5). The experimental conditions used were: purified protein (20 µL, 400 µg/mL), 5 µL HRP (1 mg/mL), 5% DMSO v/v, 1 mM substrate 152, 40°C, 220 rpm, topped up to 200 µL with 100 mM sodium phosphate (pH 7.0) buffer (in 2 mL HPLC vials), 6 hours.

[0132] Table 6 - The activity, stability, solubility, expression, and enantioselectivity of modified galactose oxidases

Name	Mutations on top of M3-5	Activity (HPLC)	Activity (Colorimetric)	Thermal Stability	Solubility	Expression	Relaxed Enantioselectivity
GOh1006	M353F		Y+	Y	Y	Y	
GOh1008	S314C		Y+		Y	Y	
GOh1013	L537G	Y	Y+		Y	Y	
GOh1015	F217V	Y	Y+				Y
GOh1016	F217P	Y	Y+				Y
GOh1017	F217Q	Y	Y+			Y	
GOh1018	F217N	Y	Y+			Y	
GOh1020	F217S	Y	Y+	Y		Y	Y
GOh1021	N268W	Y	Y+		Y		
GOh1025	E218P	Y	Y+			Y	
GOh1030	F217I	Y					Y
GOh1031	F217T	Y			Y		Y
GOh1032	F217L				Y		Y
GOh1034	F217E		Y+		Y		Y
GOh1035	F217Y				Y	Y	
GOh1036	F217A		Y+	Y	Y		Y
GOh1037	F217C						Y
GOh1038	F217D				Y		Y
GOh1039	F217G		Y+			Y	Y
GOh1040	F217H					Y	Y
GOh1041	F217K				Y		Y
GOh1042	F217M		Y+				Y
GOh1043	F217R		Y+				Y
GOh1045	F217P, E218P		Y+				
GOh1046	F217P, L537G		Y+				

GOh1047	F217P, E218P, L537G		Y+				
GOh1048	F217V, E218P		Y+				
GOh1049	F217V, L537G		Y+				
GOh1050	F217V, E218P, L537G		Y+				
GOh1051	E218P, L537G		Y+				
GOh1052	F217A, N268W		Y+				
GOh1053	F217A, N268W, M353F		Y+				
GOh1054	F217A, N268W, S314C		Y+				
GOh1055	F217G, N268W		Y+				
GOh1056	F217G, N268W, M353F		Y+				
GOh2001	F217A, N268W, A195S	Y	Y+		Y		Y
GOh2002	F217A, N268W, V193T		Y+				
GOh2008	F217A, N268W, N259T		Y+	Y			
GOh2010	F217A, N268W, Y325F		Y+				
GOh2011	F217A, N268W, G294A		Y+				
GOh2012	F217A, N268W, A195T	Y	Y+		Y		
GOh2013	F217A, N268W, Y352T		Y+				
GOh2014	F217A, N268W, N341W		Y+	Y		Y	Y
GOh2015	F217A, N268W, A197C		Y+				

GOh2016	F217A, N268W, R240G		Y+				
GOh2017	F217A, N268W, A198I		Y+	Y			
GOh2018	F217A, N268W, K348S		Y+				
GOh2019	F217A, N268W, T202D		Y+				Y
GOh2020	F217A, N268W, T202Q		Y+			Y	
GOh2021	F217A, N268W, T202I		Y+	Y		Y	
GOh2022	F217A, N268W, T202K		Y+			Y	
GOh2023	F217A, N268W, P194C	Y	Y+	Y		Y	
GOh2025	F217A, N268W, A346L		Y+			Y	Y
GOh2026	F217A, N268W, K389I		Y+		Y	Y	
GOh2027	F217A, N268W, Y352W	Y	Y+			Y	Y
GOh2028	F217A, N268W, C538K		Y+			Y	
GOh2029	F217A, N268W, C538R		Y+		Y	Y	
GOh2030	F217A, N268W, F250E				Y		
GOh2031	F217A, N268W, F250Y				Y		
GOh2032	F217A, N268W, N356K		Y+		Y		
GOh2033	F217A, N268W, N356R		Y+		Y	Y	

GOh2034	F217A, N268W, N356W		Y+		Y	Y	
GOh2037	F217A, N268W, F487R		Y+				
GOh2038	F217A, N268W, T429W			Y	Y	Y	
GOh2039	F217A, N268W, E218K	Y	Y+				
GOh2041	F217A, N268W, F313R			Y	Y	Y	
GOh2043	F217A, N268W, I284Y		Y+	Y			
GOh2044	F217A, N268W, A24H	Y	Y+	Y			
GOh2045	F217A, N268W, I28R	Y	Y+	Y	Y		
GOh2046	F217A, N268W, I28M		Y+	Y	Y		
GOh2047	F217A, N268W, S576D				Y		
GOh2048	F217A, N268W, I284S		Y+	Y	Y		
GOh2050	F217A, N268W, F65P			Y	Y		
GOh2053	F217A, N268W, T153R			Y			
GOh2055	F217A, N268W, A197G			Y	Y		
GOh2056	F217A, N268W, T265S			Y	Y		
GOh2057	F217A, N268W, S220T				Y		
GOh2058	F217A, N268W, N214S				Y		

GOh2061	F217A, N268W, I485A				Y		
GOh2064	F217A, N268W, N214R			Y	Y		
GOh2066	F217A, N268W, D269N				Y	Y	
GOh2067	F217A, N268W, I485R				Y	Y	
GOh2068	F217A, N268W, I485K				Y		
GOh2070	F217A, N268W, D239E	Y	Y+	Y		Y	
GOh2071	F217A, N268W, T117D		Y+				
GOh2072	F217A, N268W, S114R		Y+		Y	Y	
GOh2073	F217A, N268W, S559P				Y	Y	
GOh2074	F217A, N268W, S114N				Y		
GOh2075	F217A, N268W, Q662P		Y+		Y		
GOh2076	F217A, N268W, S173R		Y+				
GOh2077	F217A, N268W, D239P				Y		
GOh2078	F217A, N268W, S172E				Y	Y	
GOh2079	F217A, N268W, Q662D		Y+		Y	Y	
GOh2080	F217A, N268W, S173K	Y	Y+		Y		
GOh2081	F217A, N268W, A195V	Y	Y+		Y	Y	

GOh2082	F217A, N268W, E218F	Y	Y+	Y	Y	Y	
GOh2083	F217A, N268W, L226Q	Y		Y	Y	Y	
GOh2084	F217A, N268W, I224W	Y		Y	Y		
GOh2085	F217A, N268W, A26R	Y		Y			
GOh2086	F217A, N268W, I28F	Y	Y+	Y			
GOh2087	F217A, N268W, E218Y	Y		Y	Y		
GOh2088	F217A, N268W, L226V				Y		
GOh2089	F217A, N268W, D239Q			Y	Y		
GOh2090	F217A, N268W, F546M	Y			Y		
GOh2091	F217A, N268W, A24N	Y	Y+	Y	Y		
GOh2092	F217A, N268W, A26H	Y		Y			
GOh2093	F217A, N268W, Y599M			Y		Y	
GOh2094	F217A, N268W, G157N			Y	Y	Y	
GOh2097	F217A, N268W, Q45D			Y	Y		
GOh2098	F217A, N268W, Q45E			Y	Y	Y	
GOh2099	F217A, N268W, Q45N					Y	
GOh2100	F217A, N268W, E49K			Y			

GOh2101	F217A, N268W, N69D				Y	Y	
GOh2102	F217A, N268W, N69E				Y	Y	
GOh2103	F217A, N268W, N69F			Y			
GOh2104	F217A, N268W, N69H			Y			
GOh2105	F217A, N268W, N69L			Y		Y	
GOh2106	F217A, N268W, N69M			Y			
GOh2107	F217A, N268W, N69T			Y			
GOh2108	F217A, N268W, N69W			Y			
GOh2109	F217A, N268W, N69Y			Y		Y	
GOh2110	F217A, N268W, W104G	Y				Y	
GOh2111	F217A, N268W, W104N	Y		Y		Y	
GOh2114	F217A, N268W, T175E	Y		Y		Y	
GOh2115	F217A, N268W, T175H	Y		Y		Y	
GOh2116	F217A, N268W, T175I			Y		Y	
GOh2117	F217A, N268W, T175K	Y		Y	Y	Y	
GOh2119	F217A, N268W, A401D	Y			Y	Y	
GOh3001	F217A, N268W, A195T, Y352W	Y	Y+		Y		

GOh3002	F217A, N268W, N341W, Y352T	Y		Y		Y	
GOh3003	F217A, N268W, A198I, N259T			Y			
GOh3004	F217A, N268W, A195S, N341W					Y	
GOh3005	F217A, N268W, T153K, A195S	Y			Y	Y	
GOh3006	F217A, N268W, I28R, K389I	Y	Y+	Y			
GOh3007	F217A, N268W, T153R, A195T	Y					
GOh3008	F217A, N268W, I28R, T153R, A198I, N341W	Y		Y			
GOh3009	F217A, N268W, A195T, N259T, N341W	Y				Y	
GOh3013	F217A, N268W, I28R, A195T, N259T, K389I	Y	Y+	Y	Y		
GOh3015	F217A, N268W, I28R, N259T, N341W	Y		Y	Y	Y	
GOh3018	F217A, N268W, I28R, A195S, K389I	Y		Y	Y		

GOh3019	F217A, N268W, A195T, N341W	Y			Y	Y	
GOh3023	F217A, N268W, I28R, A195T, N341W, K389I	Y			Y		
GOh3026	F217A, N268W, I28R, A195T, N259T, T447A	Y		Y	Y		
GOh3032	F217A, N268W, I28R, A195S, N259T, N341W	Y		Y	Y	Y	
GOh3038	F217A, N268W, I28R, A195T, N259T, N341W, K389I	Y	Y+	Y			
GOh3049	F217A, N268W, T153R, N341W			Y			

[0133] For "Activity (HPLC)":

[0134] Interpretation is as follows:

(a) if a "Y" is obtained for a GOh1XXX modified galactose oxidase tested, then the activity of said modified galactose oxidase is at least 10-fold better than the M3-5 variant for substrate 128 (alpha-tetralol); and

(b) if a "Y" is obtained for a GOh2XXX or GOh3XXX modified galactose oxidase tested, then the activity of said modified galactose oxidase is at least 1.2-fold better than GOh1052 for substrate 152 (1-phenyl-1-butanol).

[0135] For GOhXXXX variant activity – the minimum fold change for said variant to be considered as having better activity is 1.01-fold better than M3-5 for 1 or more substrates in the 64-substrate panel (indicated in **Fig. 1** of the present disclosure).

[0136] For "Activity (Colorimetric)":

[0137] Interpretation is as follows:

(a) if a "Y+" is obtained for any of modified galactose oxidase tested, then said modified galactose oxidase is better than the M3-5 variant for some substrate in the colorimetric substrate panel.

[0138] For "Stability" (thermal stability), "Solubility" and "Expression" (protein expression):

[0139] Interpretation is as follows:

(a) if a "Y" is obtained for a GOh1XXX modified galactose oxidase tested, then the properties of said modified galactose oxidase is better than the M3-5 variant by 1.01-fold; and (b) if a "Y" is obtained for a GOh2XXX or GOh3XXX modified galactose oxidase tested, then the properties of said modified galactose oxidase is better than Goh1052 by 1.01-fold.

[0140] For the measure of "Relaxed Enantioselectivity"

[0141] Interpretation is as follows:

[0142] (a) if a "Y" is obtained for any of the modified galactose oxidase tested, then the enantioselectivity of said modified galactose oxidase is reduced by 1.01-fold compared to that of the M3-5 variant baseline.

[0143] For ease of comparison, interpretation of reduced enantioselectivity is conveyed as absolute units as a ratio in conversion of (R)- and (S)-enantiomers of the substrate. Higher R/S ratios indicate higher enantioselectivity for (R)-enantiomers while R/S ratios closer to 1 indicate reduced enantioselectivity, with 1 R/S ratio being no enantioselectivity.

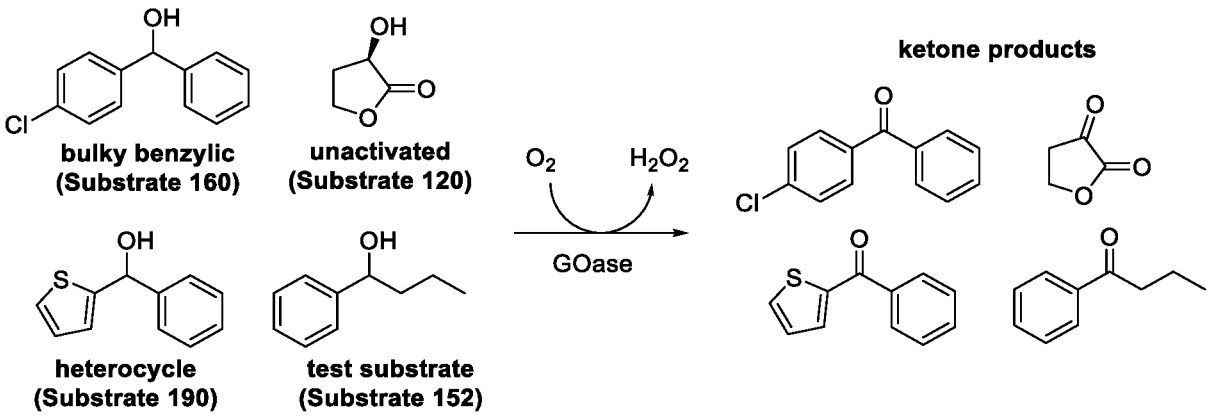
[0144] For GOh1XXX enantioselectivity – minimum reduction of 0.8 R/S ratio from M3-5 experimental value of 20.6 R/S ratio (for substrate 128 – alpha-tetralol)

[0145] For GOh2XXX and GOh3XXX enantioselectivity – minimum reduction of 0.22 R/S ratio from GOh1052 experimental value of 1.67 R/S ratio (for substrate 152 – 1-phenyl-1-butanol).

[0146] ***Results***

[0147] ***Identification of galactose oxidase mutants***

[0148] High throughput screening of 6,692 galactose oxidase variants from 352 amino acid positions against substrate 152 have revealed that 31 galactose oxidase variants (0.46%) show improved enzymatic activity (≥1.2x baseline, Table 7). The oxidation reaction of selected examples of bulky benzylic, unactivated, and heterocyclic aliphatic secondary alcohol substrates to ketones by galactose oxidase mutants are shown in the chemical equation below:



[0149] Table 7 - Table showing the Activity Hits from High Throughput Screening of 6,692 galactose oxidase variants

Variant	Activity vs GOh1052
E218F	2.13
Y599M	1.84
L226Q	1.66
A24H	1.51
A195V	1.49
A26R	1.35
I28A	1.33
L226R	1.33
I224W	1.32
I28M	1.31
D239Q	1.31
K508P	1.31
A401D	1.30
D239E	1.30
I28P	1.29
E218Y	1.29
A26F	1.28
A24N	1.28
I28D	1.28
T542G	1.28
L226V	1.27
I28R	1.26
I28E	1.26
G157N	1.26
A26H	1.25

E218W	1.25
T616V	1.23
I28F	1.23
A24G	1.22
I28H	1.21
A26D	1.20

[0150] 31 Activity Hits were obtained from High Throughput Screening of 6,692 galactose oxidase variants. For the screening, GOh1052 was used as the backbone with 10 mutations (S33P, M93V, F217A, G218E, N268W, W313F, R353M, Q429T, V517A, N558D) from wild-type galactose oxidase from *Fusarium graminearum*. Screening conditions are described in paragraph [0109] and the Activity calculations are described in paragraph [0128] above.

[0151] Table 8 - Selected first generation galactose oxidase variant examples based on M3-5 backbone with improved activity for chosen bulky benzylic and unactivated alcohol substrates.

Name	**Mutations**	**Activity (bulky benzylic – substrate 160)**	**Activity (unactivated – substrate 120)**	**Comment**
M3-5	backbone	1.54	135	Baseline
GOh1008	S314C	2.04	264	unactivated activity
GOh1021	N268W	5.47	446	unactivated activity
GOh1036	F217A	6921	4.50	bulky benzylic activity
GOh1052	F217A, N268W	5884	2.87	generalist activity

As described in paragraph [0110] above, *Activity = initial rate * max absorbance * 100. Max absorbance = indirect measure of ketone yield based on 405nm absorbance of a colorimetric probe (ABTS) measuring* H_2O_2.

[0152] Table 9 - Selected second generation variant examples based on GOh1052 (F217A, N268W from M3-5) backbone with improved properties. Up to 10^4-fold activity improvement from baseline M3-5 variant.

Name	**Mutations**	**Activity (bulky benzylic – substrate 160)**	**Activity (hetero-cycle – substrate 190)**	**R/S Ratio[a] – substrate 152**	**Residual Activity[b] – substrate 152**	**Comment**
GOh1052	backbone	5884	4862	1.67	56%	baseline

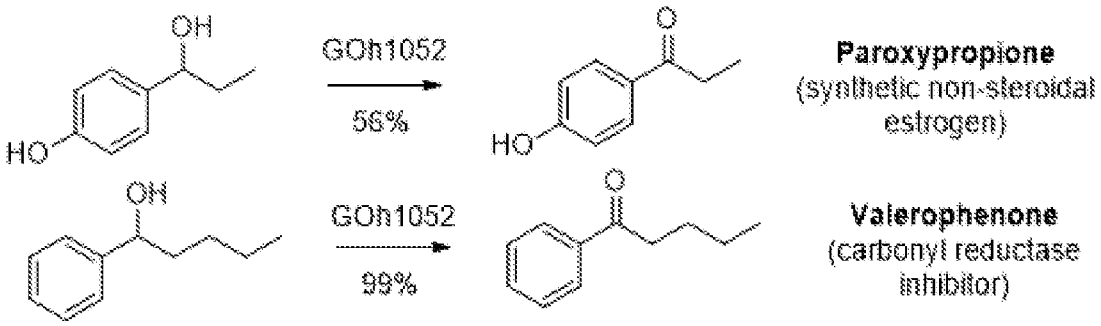
GOh2001	A195S	5996	2125	1.05	39%	activity, relaxed enantioselectivity
GOh2008	N259T	6529	5394	2.00	84%	activity
GOh2012	A195T	6393	2874	1.73	3%	activity
GOh2017	A198I	5662	1034	-	79%	thermostable
GOh2021	T202I	2376	296	-	74%	thermostable
GOh2027	Y352W	2116	1885	1.45	38%	*ortho*-substrate activity
GOh2044	A24H	8582	36573	-	74%	activity
GOh2045	I28R	6469	9346	-	79%	activity & thermostable
GOh2070	D239E	11412	8105	-	78%	activity & thermostable
GOh2091	A24N	-	-	-	82%	activity & thermostable

As described in paragraph [0110] above, *Activity = initial rate * max absorbance * 100. Max absorbance = indirect measure of ketone yield based on 405nm absorbance of a colorimetric probe (ABTS) measuring H_2O_2.* [a]*R/S ratio = [(R-isomer substrate 152 converted) / (S-isomer substrate 152 converted)] starting from a 50/50 mixture of each.* [b]*Residual activity = (substrate 152 ketone yield using enzyme heated at 50°C for 30 min) / (substrate 152 ketone yield using fresh enzyme).*

[0153] Selected examples of additional combinatorial third generation variants (incorporating 4 or more synergistic mutations to the M3-5 variant) including galactose oxidase variant GOh3001 (A195T, F217A, N268W, Y352W) showed improved generalist activity and improved acceptance of ortho-substituted substrates (**Fig. 3**), and galactose oxidase variant GOh3006 (I28R, F217A, N268W, K389I) showed synergistic mutations that combine desirable traits of high activity and thermostability (**Fig. 4**).

[0154] ***Synthesis of active pharmaceutical ingredient (API) molecules using galactose oxidase variants***

[0155] Identified beneficial enzyme mutations were applied to synthesize API molecules. The chemical equation below shows two examples of the biocatalytic synthesis of APIs by galactose oxidase variant GOh1052.



[0156] ***Analysis of the enzyme generalist activity of 12 galactose oxidase mutants***

[0157] To determine the enzyme generalist activity of galactose oxidase mutants, purified galactose oxidase enzyme mutants were screened against a panel of 60 secondary alcohols. The screening conditions were:

- 10 µL purified enzyme (400 µg/mL)
- 5 µL horseradish peroxidase (1 mg/mL)
- 1 mM secondary alcohol substrate
- 2.5 mM ABTS (colorimetric indicator)
- 5% dimethylsulfoxide v/v in 100 mM sodium phosphate buffer (pH = 7)
- 200 µL total reaction volume

[0158] Table 10 below lists 12 galactose oxidase mutants with good enzyme generalist activity compared to the M3-5 variant across the 60-substrate panel. The 60 secondary alcohol substrate panel is shown in **Fig. 1**. The activity fold improvements have been rounded to the nearest 100.

[0159] Table 10 - Table of 12 galactose oxidase mutants with good generalist activity across the 60-substrate panel.

Mutant	**Mutations**	**Averaged Activity Fold vs M3-5 (across 60 Substrates)**
GOh1001b (M3-5)	Backbone	1
GOh1036	F217A	900
GOh1052	F217A, N268W	600
GOh2001	F217A, N268W, A195S	600
GOh2008	F217A, N268W, N259T	800

GOh2012	F217A, N268W, A195T	900
GOh2044	F217A, N268W, A24H	1500
GOh2045	F217A, N268W, I28R	1200
GOh2081	F217A, N268W, A195V	1100
GOh2082	F217A, N268W, E218F	2000
GOh3001	F217A, N268W, A195T, Y352W	1000
GOh3013	F217A, N268W, I28R, A195T, N259T, K389I	2000
GOh3038	F217A, N268W, I28R, A195T, N259T, N341W, K389I	2100

[0160] The activity values for 13 galactose oxidase mutants against 60 substrates are shown in **Fig. 5** (colour comparisons are done horizontally per substrate). **Fig. 5** shows that the 13 galactose oxidase mutants had generally higher activity values compared to the M3-5 variant. The "Averaged Activity Fold" was obtained by sequentially: (1) taking the colorimetric activity fold change of each substrate by the selected galactose oxidase variant (e.g., GOh1052) over benchmark M3-5; and (2) taking the average of all the fold changes of a given galactose oxidase variant (e.g. GOh1052) for all 60 substrates in **Fig. 5**).

[0161] ***Analysis of the thermostability of galactose oxidase mutants***

[0162] The thermostability profile of 55 purified galactose oxidase mutants were compared against the thermostability profile of GOh1052 (F217A, N268W from M3-5) backbone. As shown in **Fig. 6**, the purified galactose oxidase enzymes were heated at 50°C or at 55°C for 30 minutes, and the enzymatic activities of the resulting heat-treated enzymes were measured and compared with the GOh1052 control. Table 11 shows higher residual activity of the heat-treated galactose oxidase mutants compared to GOh1052 control.

[0163] Table 11 - Table of 55 thermostable mutants compared to GOh1052 (F217A, N268W from M3-5) backbone.

Mutant No.	**Mutations (on GOh1052)**	**Residual Activity (50°C 30 min)**
GOh1052	backbone	56%

GOh3006	I28R, K389I	90%
GOh2008	N259T	84%
GOh2091	A24N	82%
GOh2104	N69H	82%
GOh2045	I28R	79%
GOh2105	N69L	79%
GOh2017	A198I	79%
GOh3015	I28R, N259T, N341W	78%
GOh2082	E218F	78%
GOh2070	D239E	78%
GOh2089	D239Q	76%
GOh3003	A198I, N259T	75%
GOh2041	F313R	75%
GOh2084	I224W	75%
GOh2021	T202I	74%
GOh2044	A24H	74%
GOh2106	N69M	74%
GOh3008	I28R, T153R, A198I, N341W	74%
GOh2086	I28F	73%
GOh2038	T429W	72%
GOh2093	Y599M	72%
GOh2097	Q45D	72%
GOh2100	E49K	72%
GOh2046	I28M	70%
GOh2087	E218Y	70%
GOh2109	N69Y	70%
GOh2114	T175E	70%
GOh3032	I28R, A195S, N259T, N341W	69%
GOh3049	T153R, N431W	69%
GOh2083	L226Q	68%

GOh2014	N341W	68%
GOh2092	A26H	67%
GOh2085	A26R	67%
GOh3002	N341W, Y352T	66%
GOh2055	A197G	66%
GOh2115	T175H	66%
GOh2116	T175I	66%
GOh2043	I284Y	65%
GOh2103	N69F	65%
GOh2108	N69W	65%
GOh3018	I28R, A195S, K389I	65%
GOh2064	N214R	63%
GOh2048	I284S	62%
GOh2107	N69T	62%
GOh2117	T175K	62%
GOh3038	I28R, A195T, N259T, N341W, K389I	62%
GOh2050	F65P	61%
GOh2098	Q45E	61%
GOh2023	P194C	60%
GOh2094	G157N	60%
GOh3026	I28R, A195T, N259T, T447A	58%
GOh2053	T153R	58%
GOh2056	T265S	57%
GOh2111	W104N	57%
GOh3013	I28R, A195T, N259T, K389I	57%

[0164] Table 12 thermostable mutants compared to M3-5 control backbone for substrate 128

Mutant No.	**Mutations (on M3-5)**	**Residual Activity (55°C 30 min)**
M3-5	Backbone	36%

GOh1036	F217A	58%
GOh1020	F217S	55%
GOh1006	M353F	55%

[0165] ***Analysis of the protein expression profile of galactose oxidase mutants***

[0166] The protein expression profiles of purified galactose oxidase mutants were compared against the protein expression profile of GOh1052 (F217A, N268W from M3-5) backbone. Tables 13 and 14 show higher protein expression level of purified galactose oxidase mutants compared to the protein expression level of GOh1052 control and M3-5 control, respectively.

[0167] Table 13 - Table showing improved protein expression profile of 46 purified galactose oxidase mutants versus GOh1052 (F217A, N268W from M3-5) backbone.

Mutant No.	**Mutations (on GOh1052)**	**Relative Expression**
GOh1052	backbone	1.00
GOh3002	N341W, Y352T	10.09
GOh2119	A401D	6.34
GOh2117	T175K	4.29
GOh2115	T175H	3.46
GOh3004	A195S, N341W	3.36
GOh2102	N69E	2.96
GOh2116	T175I	2.58
GOh2114	T175E	2.47
GOh2101	N69D	2.06
GOh2110	W104G	2.04
GOh2041	F313R	2.00
GOh2105	N69L	1.67
GOh2021	T202I	1.64
GOh2029	C538R	1.62
GOh2109	N69Y	1.58
GOh2022	T202K	1.52
GOh2098	Q45E	1.47

GOh2094	G157N	1.38
GOh2099	Q45N	1.35
GOh2038	T429W	1.34
GOh2028	C538K	1.34
GOh2020	T202Q	1.33
GOh2066	D269N	1.32
GOh3019	A195T, N341W	1.32
GOh2025	A346L	1.31
GOh2034	N356W	1.25
GOh3005	T153K, A195S	1.22
GOh3032	I28R, A195S, N259T, N341W	1.21
GOh2111	W104N	1.19
GOh2067	I485R	1.17
GOh3015	I28R, N259T, N341W	1.17
GOh2033	N356R	1.17
GOh2081	A195V	1.15
GOh2078	S172E	1.14
GOh2026	K389I	1.14
GOh2070	D239E	1.11
GOh2072	S114R	1.11
GOh2093	Y599M	1.08
GOh2079	Q662D	1.05
GOh2023	P194C	1.05
GOh2073	S559P	1.03
GOh2083	L226Q	1.03
GOh2082	E218F	1.03
GOh2027	Y352W	1.02
GOh2014	N341W	1.01
GOh3009	A195T, N259T, N341W	1.01

[0168] Table 14 - Table showing improved protein expression profile of 10 purified galactose oxidase mutants versus M3-5 control backbone.

Mutant No.	Mutations (on M3-5)	Relative Expression
M3-5	Backbone	1.00
GOh1006	M353F	1.73
GOh1008	S314C	1.51
GOh1013	L537G	1.64
GOh1017	F217Q	1.06
GOh1018	F217N	1.16
GOh1020	F217S	1.41
GOh1025	E218P	1.36
GOh1035	F217Y	1.10
GOh1039	F217G	1.25
GOh1040	F217H	1.19

[0169] ***Analysis of the protein solubility of galactose oxidase mutants***

[0170] The protein solubilities of purified galactose oxidase mutants were compared against the protein solubility of GOh1052 (F217A, N268W from M3-5) backbone. Tables 15 and 16 show increased protein solubility of purified galactose oxidase mutants compared to the protein solubility of GOh1052 control, and M3-5 control, respectively.

[0171] Table 15 - Table showing increased protein solubility of 58 purified galactose oxidase mutants versus GOh1052 (F217A, N268W from M3-5) backbone.

Mutant No.	Mutations (on GOh1052)	Soluble/Insoluble Ratio
GOh1052	backbone	0.44
GOh3023	I28R, A195T, N341W, K389I	2.01
GOh2082	E218F	1.24
GOh2056	T265S	1.12
GOh2079	Q662D	1.03
GOh3018	I28R, A195S, K389I	0.99

GOh3013	I28R, A195T, N259T, K389I	0.99
GOh2041	F313R	0.95
GOh2084	I224W	0.84
GOh2081	A195V	0.80
GOh2068	I485K	0.79
GOh2033	N356R	0.76
GOh2119	A401D	0.74
GOh2080	S173K	0.74
GOh2047	S576D	0.73
GOh2038	T429W	0.72
GOh2075	Q662P	0.71
GOh2067	I485R	0.71
GOh3032	I28R, A195S, N259T, N341W	0.71
GOh2083	L226Q	0.70
GOh2073	S559P	0.68
GOh2074	S114N	0.67
GOh2048	I284S	0.66
GOh2089	D239Q	0.65
GOh2030	F250E	0.64
GOh3019	A195T, N341W	0.64
GOh2046	I28M	0.63
GOh3001	A195T, Y352W	0.62
GOh3015	I28R, N259T, N341W	0.61
GOh2088	L226V	0.61
GOh3026	I28R, A195T, N259T, T447A	0.61
GOh3005	T153K, A195S	0.60
GOh2050	F65P	0.60
GOh2045	I28R	0.59
GOh2091	A24N	0.58
GOh2098	Q45E	0.58
GOh2101	N69D	0.57

GOh2064	N214R	0.57
GOh2094	G157N	0.55
GOh2032	N356K	0.53
GOh2058	N214S	0.53
GOh2087	E218Y	0.52
GOh2061	I485A	0.52
GOh2102	N69E	0.51
GOh2078	S172E	0.51
GOh2031	F250Y	0.50
GOh2012	A195T	0.50
GOh2117	T175K	0.49
GOh2097	Q45D	0.48
GOh2072	S114R	0.47
GOh2055	A197G	0.47
GOh2034	N356W	0.46
GOh2029	C538R	0.46
GOh2090	F546M	0.46
GOh2077	D239P	0.46
GOh2066	D269N	0.45
GOh2026	K389I	0.45
GOh2057	S220T	0.45
GOh2001	A195S	0.45

[0172] Table 16 - Table showing increased protein solubility of 11 purified galactose oxidase mutants versus M3-5 control backbone.

Mutant No.	**Mutations (on M3-5)**	**Soluble/Insoluble Ratio**
M3-5	Backbone	0.58
GOh1038	F217D	0.97
GOh1013	L537G	0.94
GOh1008	S314C	0.78
GOh1032	F217L	0.78

GOh1031	F217T	0.78
GOh1006	M353F	0.77
GOh1036	F217A	0.77
GOh1021	N268W	0.76
GOh1041	F217K	0.73
GOh1035	F217Y	0.69
GOh1034	F217E	0.66

[0173] ***Analysis of relaxed enantioselectivity profile of galactose oxidase mutants***

[0174] Table 17 - Table showing relaxed enantioselectivity profile of five purified enzymes (for substrate 152) versus GOh1052 (F217A, N268W from M3-5) backbone.

Mutant No.	**Mutations (on GOh1052)**	**R/S Ratio**
GOh1052	backbone	1.67
GOh2001	A195S	1.05
GOh2025	A346L	1.25
GOh2019	T202D	1.27
GOh2014	N341W	1.35
GOh2027	Y352W	1.45

[0175] Table 18 - Table showing relaxed enantioselectivity profile of 15 purified enzymes at F217 position (for substrate 128) versus M3-5 backbone.

Mutant No.	**Mutations (on M3-5)**	**R/S Ratio**
M3-5	Backbone	20.6
GOh1036	F217A	1.6
GOh1039	F217G	3.4
GOh1016	F217P	4.1
GOh1041	F217K	4.1
GOh1042	F217M	4.7
GOh1015	F217V	4.9
GOh1038	F217D	6.0
GOh1032	F217L	6.4
GOh1030	F217I	6.7
GOh1043	F217R	6.9
GOh1020	F217S	7.0
GOh1031	F217T	7.1
GOh1037	F217C	8.6

GOh1034	F217E	13.5
GOh1040	F217H	19.8

[0176] The fold change ratios can be calculated as the benchmark R/S ratio divided by the mutant R/S ratio, which gives the fold decrease in enantioselectivity. For example, for GOh1036 benchmarked to M3-5, calculation is: 20.6 (M3-5 R/S ratio) / 1.6 (GOh1036 R/S ratio) = 12.875 (round up to 12.9).

[0177] ***Construction of the combinatorial library targeting generalist activity and stability of galactose oxidase mutants***.

[0178] Combinatorial library targeting generalist activity and stability was constructed from the following eight mutations (six amino acid mutation positions)

- Generalist Activity: A195S, A195T, N259T, K389I
- Stability: I28R, T153R, T153K, N341W

[0179] Purified galactose oxidase proteins from the combinatorial library have been found to surpass single mutants in the characteristics shown in Table 19.

[0180] Table 19

Characteristic	**Single Mutant (mutations on GOh1052)**	**Fold vs GOh1052 backbone**	**Combinatorial (mutations on GOh1052)**	**Fold vs GOh1052 backbone**
40°C Activity	GOh2045 (I28R)	1.85	GOh3006 (I28R, K389I)	2.18
Thermal Stability	GOh2008 (N259T)	1.50	GOh3006 (I28R, K389I)	1.61
Protein Expression	GOh2041 (F313R)	2.00	GOh3002 (N341W, Y352T)	10.09
Solubility	GOh2082 (E218F)	2.82	GOh3023 (I28R, A195T, N341W, K389I)	4.57

[0181] Synergistic mutations I28R and K389I (GOh3006) have been found to combine the desirable traits of both generalist activity and thermostability.

[0182] The present disclosure describes for the first time, galactose oxidase variants that convey superior enhancement in enzymatic activity towards bulky secondary alcohols and unactivated secondary alcohols.

[0183] The galactose oxidase variants of the present disclosure have the following advantages:

1. The galactose oxidase variants disclosed herein are capable of oxidising secondary alcohols and oxidising unactivated secondary alcohols.
2. The galactose oxidase variants disclosed herein has reduced enantioselectivity compared to modified galactose oxidase known in the art.
3. The galactose oxidase variants disclosed herein has improved enzyme solubility.
4. The galactose oxidase variants disclosed herein has improved protein expression.
5. The galactose oxidase variants disclosed herein has improved enzyme thermal stability.
6. The galactose oxidase variants disclosed herein possess overall improvement in enzyme properties such as activity, selectivity, expression, thermal stability and/or solubility.
7. The galactose oxidase variants disclosed herein can be used as a part of/all of the process for the manufacturing of APIs.
8. The galactose oxidase variants disclosed herein are useful in applications requiring the use of biocatalysts, e.g., bioremediation, cleaning, food production.

SEQUENCE LISTING

SEQ ID NO	Sequence Name	Sequence
1	GO01 = Wildtype galactose oxidase from *Fusarium graminearum*; has additional His-tag at the C-terminal	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcttctgctccaattggttctgctatttccagaaacaattgggctgtta cttgtgattctgctcaatctggtaatgaatgtaacaaggctattgatggtaacaaggatac tttttggcatactttctacggtgctaacggagatccaaaaccacctcacacttacactatc gatatgaagactactcaaaacgttaatggtttgtctatgttgcctagacaagatggtaacc aaaatggttggattggtagacatgaagtttacttgtcttctgatggtactaattggggttct cctgttgcttccggttcttggtttgctgattctactactaaatactctaacttcgagactaga ccagctagatatgttagattggttgctgttactgaagctaatggtcaaccttggacttctat tgctgagattaacgtttttcaggcttcttcttacactgctccacaacctggtttgggtagat ggggtccaactattgatttgccaattgttcctgctgctgctgctattgagcctacttctggt agagttttgatgtggtcttcttatagaaatgatgcttttggtggttctccaggtggtattactt tgacttcttcttgggacccatctactggtattgtttctgatagaactgttactgttactaagc atgatatgttctgtccaggtatttctatggatggtaatggtcaaattgttgttactggtggta acgatgctaagaaaacttctttgtacgattcttcttctgattcttggattccaggtcctgatat gcaagttgctagaggttatcaatcttctgctactatgtctgatggtagagtttttactattgg tggttcttggtctggtggtgttttcgaaaagaacggagaggtttactctccatcttctaaga cttggacttctttgccaaatgctaaagttaaccctatgttgactgctgataagcaaggtttg tatagatctgataatcacgcttggttgtttggttggaagaaaggttctgttttccaagctgg tccttctactgctatgaactggtactatacttctggttctggagatgttaagtctgctggtaa aagacaatctaatagaggtgttgctccagatgctatgtgtggtaacgctgttatgtacgat gctgttaagggtaaaattttgactttcggtggttctcctgattatcaagattctgatgctact actaacgctcatatcatcactttgggtgaaccaggtacttctcctaatactgtttttgcttct aacggtttgtactttgctagaactttccacacttctgttgttttgccagatggttctactttcat tactggtggtcaaagaagaggtattccttttgaggattctactccagttttcactcctgaaa tctacgttccagagcaagatactttctacaagcaaaaccctaattctatcgttagagtttac cattctatttctttgttgttgccagatggtagagttttcaacggtggtggtggtttgtgtgga gattgtactactaaccacttcgatgctcaaattttcactccaaactacttgtacaactctaa cggtaacttggctactagacctaagatcactagaacttctactcaatctgttaaggttggt ggtagaatcactatttctactgattcttctatcactaaggcttctttgatcagatatggtactg ctactcacactgttaatactgatcaaagaagaatcccattgactttgactaacaatggtgg taactcttactcttttcaagttccatctgattctggtgttgctttgcctggttattggatgttgtt cgttatgaactctgctggtgttccttctgttgcttctactattagagttactcaagcggccg cactcgagcaccaccaccaccaccactga
2	GO01 = Wildtype galactose oxidase from *Fusarium graminearum*; same protein sequence as GO01b but has additional His-tag at the C-terminal	MGSSHHHHHHSSGLVPRGSHMGTASAPIGSAISRNNW AVTCDSAQSGNECNKAIDGNKDTFWHTFYGANGDPK PPHTYTIDMKTTQNVNGLSMLPRQDGNQNGWIGRHE VYLSSDGTNWGSPVASGSWFADSTTKYSNFETRPARY VRLVAVTEANGQPWTSIAEINVFQASSYTAPQPGLGR WGPTIDLPIVPAAAAIEPTSGRVLMWSSYRNDAFGGSP GGITLTSSWDPSTGIVSDRTVTVTKHDMFCPGISMDGN GQIVVTGGNDAKKTSLYDSSSDSWIPGPDMQVARGYQ SSATMSDGRVFTIGGSWSGGVFEKNGEVYSPSSKTWT SLPNAKVNPMLTADKQGLYRSDNHAWLFGWKKGSVF QAGPSTAMNWYYTSGSGDVKSAGKRQSNRGVAPDA

		MCGNAVMYDAVKGKILTFGGSPDYQDSDATTNAHIIT LGEPGTSPNTVFASNGLYFARTFHTSVVLPDGSTFITGG QRRGIPFEDSTPVFTPEIYVPEQDTFYKQNPNSIVRVYH SISLLLPDGRVFNGGGGLCGDCTTNHFDAQIFTPNYLY NSNGNLATRPKITRTSTQSVKVGGRITISTDSSITKASLI RYGTATHTVNTDQRRIPLTLTNNGGNSYSFQVPSDSGV ALPGYWMLFVMNSAGVPSVASTIRVTQAAALEHHHH HH
3	GO01b = *E. coli* optimized version of GO01; no C-terminal His-tag	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatctcacgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccatgctgccgc gccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctccg acggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcacc accaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgaca gaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccagc tcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcct atcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggagc agctaccgtaacgacgcgttcggcggcagcccgggcggcatcactctgacgtcgagt tgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacgac atgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcggc aatgacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccggg gccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacggc cgtgtctttacaatcggcggctcatggagtggcggagtgtttgagaagaatggcgaag tctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatcccatg ttaaccgccgacaaacagggcctgtaccgcagcgacaaccatgcgtggctgttcggc tggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtattaca cgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcggcgt ggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggcaaga tcctgacatttggcgggagtccggactaccaggacagtgacgcgacaaccaatgccc acattattaccttggggagccggggacgagcccgaacacggtcttcgcgtccaatg gcctctatttcgcgcgtaccttcatacgtcggtggtcctgccggacggatcaaccttat caccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttacacc ggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattgtac gcgtgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggagga ggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaatta tctgtataattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaaccca gtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaaagc gtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccgtatt ccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcggactc gggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtccc gagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa
4	GO01b = *E. coli* optimized version of GO01; no C-terminal His-tag	MGSSHHHHHHSSGLVPRGSHMGTASAPIGSAISRNNW AVTCDSAQSGNECNKAIDGNKDTFWHTFYGANGDPK PPHTYTIDMKTTQNVNGLSMLPRQDGNQNGWIGRHE VYLSSDGTNWGSPVASGSWFADSTTKYSNFETRPARY VRLVAVTEANGQPWTSIAEINVFQASSYTAPQPGLGR

		WGPTIDLPIVPAAAAIEPTSGRVLMWSSYRNDAFGGSP GGITLTSSWDPSTGIVSDRTVTVTKHDMFCPGISMDGN GQIVVTGGNDAKKTSLYDSSSDSWIPGPDMQVARGYQ SSATMSDGRVFTIGGSWSGGVFEKNGEVYSPSSKTWT SLPNAKVNPMLTADKQGLYRSDNHAWLFGWKKGSVF QAGPSTAMNWYYTSGSGDVKSAGKRQSNRGVAPDA MCGNAVMYDAVKGKILTFGGSPDYQDSDATTNAHIIT LGEPGTSPNTVFASNGLYFARTFHTSVVLPDGSTFITGG QRRGIPFEDSTPVFTPEIYVPEQDTFYKQNPNSIVRVYH SISLLLPDGRVFNGGGGLCGDCTTNHFDAQIFTPNYLY NSNGNLATRPKITRTSTQSVKVGGRITISTDSSITKASLI RYGTATHTVNTDQRRIPLTLTNNGGNSYSFQVPSDSGV ALPGYWMLFVMNSAGVPSVASTIRVTQAAA
5	GOh1001b = GO01b + 8 mutations from Turner's ChemBiochem 2008 paper to change substrate specificity more towards secondary alcohols; backbone for Round 1; termed as M3-5 variant for comparison to Turner's paper	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcgttcgaaggcagcccgggcggcatcactctgacgtcga gttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacg acatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcg gcaatgacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa gtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatcccat gttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcgg ctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtattac acgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcggc gtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggcaa gatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatgc ccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtccaat ggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaacctttt atcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttacac cggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattgta cgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggagg aggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaatt atctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaaccc agtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaaag cgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccgtat tccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcggactc gggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtccc gagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa

6	GOh1001b = GO01b + 8 mutations from Turner's ChemBiochem 2008 paper to change substrate specificity more towards secondary alcohols; backbone for Round 1; termed as M3-5 variant for comparison to Turner's paper	MGSSHHHHHHSSGLVPRGSHMGTASAPIGSAIPRNNW AVTCDSAQSGNECNKAIDGNKDTFWHTFYGANGDPK PPHTYTIDMKTTQNVNGLSVLPRQDGNQNGWIGRHEV YLSSDGTNWGSPVASGSWFADSTTKYSNFETRPARYV RLVAVTEANGQPWTSIAEINVFQASSYTAPQPGLGRW GPTIDLPIVPAAAAIEPTSGRVLMWSSYRNDAFEGSPG GITLTSSWDPSTGIVSDRTVTVTKHDMFCPGISMDGNG QIVVTGGNDAKKTSLYDSSSDSWIPGPDMQVARGYQS SATMSDGRVFTIGGSFSGGVFEKNGEVYSPSSKTWTSL PNAKVNPMLTADKQGLYMSDNHAWLFGWKKGSVFQ AGPSTAMNWYYTSGSGDVKSAGKRQSNRGVAPDAM CGNAVMYDAVKGKILTFGGSPDYTDSDATTNAHIITL GEPGTSPNTVFASNGLYFARTFHTSVVLPDGSTFITGG QRRGIPFEDSTPVFTPEIYVPEQDTFYKQNPNSIVRAYH SISLLLPDGRVFNGGGGLCGDCTTNHFDAQIFTPNYLY DSNGNLATRPKITRTSTQSVKVGGRITISTDSSITKASLI RYGTATHTVNTDQRRIPLTLTNNGGNSYSFQVPSDSGV ALPGYWMLFVMNSAGVPSVASTIRVTQAAA
7	GOh1006	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcgttcgaaggcagcccgggcggcatcactctgacgtcga gttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacg acatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcg gcaatgacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa gtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatcccat gttaaccgccgacaaacagggcctgtactttagcgacaaccatgcgtggctgttcggc tggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtattaca cgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcggcgt ggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggcaaga tcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatgccc acattattacccttggggagccggggacgagcccgaacacggtcttcgcgtccaatg gcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaaccttttat caccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttacacc ggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattgtac gcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggagga ggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaatta tctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaaccca

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8	GOh1006	MGSSHHHHHHSSGLVPRGSHMGTASAPIGSAIPRNNW AVTCDSAQSGNECNKAIDGNKDTFWHTFYGANGDPK PPHTYTIDMKTTQNVNGLSVLPRQDGNQNGWIGRHEV YLSSDGTNWGSPVASGSWFADSTTKYSNFETRPARYV RLVAVTEANGQPWTSIAEINVFQASSYTAPQPGLGRW GPTIDLPIVPAAAAIEPTSGRVLMWSSYRNDAFEGSPG GITLTSSWDPSTGIVSDRTVTVTKHDMFCPGISMDGNG QIVVTGGNDAKKTSLYDSSSDSWIPGPDMQVARGYQS SATMSDGRVFTIGGSFSGGVFEKNGEVYSPSSKTWTSL PNAKVNPMLTADKQGLYFSDNHAWLFGWKKGSVFQ AGPSTAMNWYYTSGSGDVKSAGKRQSNRGVAPDAM CGNAVMYDAVKGKILTFGGSPDYTDSDATTNAHIITL GEPGTSPNTVFASNGLYFARTFHTSVVLPDGSTFITGG QRRGIPFEDSTPVFTPEIYVPEQDTFYKQNPNSIVRAYH SISLLLPDGRVFNGGGGLCGDCTTNHFDAQIFTPNYLY DSNGNLATRPKITRTSTQSVKVGGRITISTDSSITKASLI RYGTATHTVNTDQRRIPLTLTNNGGNSYSFQVPSDSGV ALPGYWMLFVMNSAGVPSVASTIRVTQAAA
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10	GOh1008	MGSSHHHHHHSSGLVPRGSHMGTASAPIGSAIPRNNW AVTCDSAQSGNECNKAIDGNKDTFWHTFYGANGDPK PPHTYTIDMKTTQNVNGLSVLPRQDGNQNGWIGRHEV YLSSDGTNWGSPVASGSWFADSTTKYSNFETRPARYV RLVAVTEANGQPWTSIAEINVFQASSYTAPQPGLGRW GPTIDLPIVPAAAAIEPTSGRVLMWSSYRNDAFEGSPG GITLTSSWDPSTGIVSDRTVTVTKHDMFCPGISMDGNG QIVVTGGNDAKKTSLYDSSSDSWIPGPDMQVARGYQS SATMSDGRVFTIGGSFCGGVFEKNGEVYSPSSKTWTSL PNAKVNPMLTADKQGLYMSDNHAWLFGWKKGSVFQ AGPSTAMNWYYTSGSGDVKSAGKRQSNRGVAPDAM CGNAVMYDAVKGKILTFGGSPDYTDSDATTNAHIITL GEPGTSPNTVFASNGLYFARTFHTSVVLPDGSTFITGG QRRGIPFEDSTPVFTPEIYVPEQDTFYKQNPNSIVRAYH SISLLLPDGRVFNGGGGLCGDCTTNHFDAQIFTPNYLY DSNGNLATRPKITRTSTQSVKVGGRITISTDSSITKASLI RYGTATHTVNTDQRRIPLTLTNNGGNSYSFQVPSDSGV ALPGYWMLFVMNSAGVPSVASTIRVTQAAA

11	GOh1013	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcgttcgaaggcagcccgggcggcatcactctgacgtcga gttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacg acatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcg gcaatgacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa gtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatcccat gttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcgg ctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtattac acgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcggc gtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggcaa gatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatgc ccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtccaat ggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaaccttt atcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttacac cggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattgta cgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggagg aggaggttgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaatt atctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaaccc agtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaaag cgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccgtat tccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcggactc gggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtccc gagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa
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25	GOh1025	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcgttccctggcagcccgggcggcatcactctgacgtcgag ttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacga catgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcgg caatgacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccgg ggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacgg ccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaag tctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatcccatg ttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcggct ggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtattaca cgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcggcgt ggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggcaaga tcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatgccc acattattacccttggggagccggggacgagcccgaacacggtcttcgcgtccaatg gcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaaccttttat caccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttacacc ggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattgtac gcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggagga ggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaatta tctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaaccca gtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaaagc gtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccgtatt ccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcggactc gggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtccc gagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa

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27	GOh1030	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcgattgaaggcagcccgggcggcatcactctgacgtcga gttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacg acatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcg gcaatgacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa gtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatcccat gttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcgg ctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtattac acgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcggc gtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggcaa gatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatgc ccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtccaat ggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaaccttt atcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttacac cggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattgta cgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggagg aggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaatt atctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaaccc

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29	GOh1031	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcgacggaaggcagcccgggcggcatcactctgacgtcga gttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacg acatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcg gcaatgacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa

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35	GOh1035	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcgtatgaaggcagcccgggcggcatcactctgacgtcga gttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacg acatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcg gcaatgacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa gtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatcccat gttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcgg ctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtattac acgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcggc gtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggcaa gatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatgc ccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtccaat ggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaaccttt atcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttacac cggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattgta cgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggagg aggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaatt atctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaaccc agtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaaag cgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccgtat tccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcggactc gggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtccc gagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa

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37	GOh1036	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggc ggcaatgacgccaagaagacgagcctctatgacagtagcagcgacagttggatccc ggggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagac ggccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcga agtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatccc atgttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcg gctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtatt acacgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcgg cgtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggca agatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatg cccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtcca atggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaacct ttatcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttac accggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattg tacgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggag gaggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaa ttatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaac

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45	GOh1040	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcgcatgaaggcagcccgggcggcatcactctgacgtcga gttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacg acatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcg gcaatgacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa gtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatcccat gttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcgg ctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtattac acgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcggc gtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggcaa gatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatgc ccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtccaat ggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaaccttt atcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttacac cggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattgta cgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggagg aggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaatt atctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaaccc agtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaaag cgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccgtat tccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcggactc gggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtccc gagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa

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47	GOh1041	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcgaaagaaggcagcccgggcggcatcactctgacgtcga gttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacg acatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcg gcaatgacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa gtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatcccat gttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcgg ctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtattac acgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcggc gtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggcaa gatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatgc ccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtccaat ggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaaccttt atcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttacac cggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattgta cgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggagg aggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaatt atctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaaccc

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49	GOh1042	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcgatggaaggcagcccgggcggcatcactctgacgtcga gttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacg acatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcg gcaatgacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa

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65	GOh1051	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcgttcccaggcagcccgggcggcatcactctgacgtcga gttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacg acatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcg gcaatgacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa gtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatcccat gttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcgg ctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtattac acgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcggc gtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggcaa gatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatgc ccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtccaat ggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaaccttt atcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttacac cggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattgta cgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggagg aggagggtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaat tatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaacc cagtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaaa gcgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccgt attccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcggac tcgggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtcc cgagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa

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85	GOh2011	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggc ggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatccc ggggccggacatgcaggtggcacgcgcgtaccagtccagcgcgaccatgtcagac ggccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcga agtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatccc atgttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcg gctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtatt acacgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcgg cgtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggca agatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatg cccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtcca atggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaacct ttatcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttac accggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattg tacgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggag gaggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaa ttatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaac ccagtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaa agcgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccg tattccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcgga ctcgggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtc ccgagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa

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87	GOh2012	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccaccgccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggc ggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatccc ggggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagac ggccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcga agtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatccc atgttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcg gctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtatt acacgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcgg cgtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggca agatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatg cccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtcca atggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaacct ttatcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttac accggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattg tacgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggag gaggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaa ttatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaac

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95	GOh2016	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgacggtaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggc ggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatccc ggggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagac ggccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcga agtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatccc atgttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcg gctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtatt acacgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcgg cgtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggca agatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatg cccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtcca atggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaacct ttatcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttac accggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattg tacgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggag gaggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaa ttatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaac ccagtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaa agcgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccg tattccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcgga ctcgggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtc ccgagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa

96	GOh2016	MGSSHHHHHHSSGLVPRGSHMGTASAPIGSAIPRNNW AVTCDSAQSGNECNKAIDGNKDTFWHTFYGANGDPK PPHTYTIDMKTTQNVNGLSVLPRQDGNQNGWIGRHEV YLSSDGTNWGSPVASGSWFADSTTKYSNFETRPARYV RLVAVTEANGQPWTSIAEINVFQASSYTAPQPGLGRW GPTIDLPIVPAAAAIEPTSGRVLMWSSYRNDAAEGSPG GITLTSSWDPSTGIVSDGTVTVTKHDMFCPGISMDGNG QIVVTGGWDAKKTSLYDSSSDSWIPGPDMQVARGYQS SATMSDGRVFTIGGSFSGGVFEKNGEVYSPSSKTWTSL PNAKVNPMLTADKQGLYMSDNHAWLFGWKKGSVFQ AGPSTAMNWYYTSGSGDVKSAGKRQSNRGVAPDAM CGNAVMYDAVKGKILTFGGSPDYTDSDATTNAHIITL GEPGTSPNTVFASNGLYFARTFHTSVVLPDGSTFITGG QRRGIPFEDSTPVFTPEIYVPEQDTFYKQNPNSIVRAYH SISLLLPDGRVFNGGGGLCGDCTTNHFDAQIFTPNYLY DSNGNLATRPKITRTSTQSVKVGGRITISTDSSITKASLI RYGTATHTVNTDQRRIPLTLTNNGGNSYSFQVPSDSGV ALPGYWMLFVMNSAGVPSVASTIRVTQAAA
97	GOh2017	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcgattattgaaccaacgtcaggccgcgtactgatgtggagc agctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcga gttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacg acatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcg gctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa gtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatcccat gttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcgg ctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtattac acgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcggc gtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggcaa gatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatgc ccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtccaat ggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaaccttt atcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttacac cggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattgta cgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggagg aggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaatt atctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaaccc

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99	GOh2018	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggc ggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatccc ggggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagac ggccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcga

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101	GOh2019	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccagattcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggc ggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatccc ggggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagac ggccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcga agtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatccc atgttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcg gctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtatt acacgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcgg cgtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggca agatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatg cccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtcca atggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaacct ttatcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttac accggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattg tacgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggag gaggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaa ttatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaac ccagtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaa agcgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccg tattccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcgga ctcgggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtc ccgagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa
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105	GOh2021	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaatttcaggccgcgtactgatgtggagc agctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcga gttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacg acatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcg gctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa gtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatcccat gttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcgg ctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtattac acgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcggc gtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggcaa gatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatgc ccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtccaat ggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaaccttt atcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttacac cggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattgta cgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggagg aggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaatt atctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaaccc agtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaaag cgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccgtat tccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcggactc gggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtccc gagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa

106	GOh2021	MGSSHHHHHHSSGLVPRGSHMGTASAPIGSAIPRNNW AVTCDSAQSGNECNKAIDGNKDTFWHTFYGANGDPK PPHTYTIDMKTTQNVNGLSVLPRQDGNQNGWIGRHEV YLSSDGTNWGSPVASGSWFADSTTKYSNFETRPARYV RLVAVTEANGQPWTSIAEINVFQASSYTAPQPGLGRW GPTIDLPIVPAAAAIEPISGRVLMWSSYRNDAAEGSPGG ITLTSSWDPSTGIVSDRTVTVTKHDMFCPGISMDGNGQ IVVTGGWDAKKTSLYDSSSDSWIPGPDMQVARGYQSS ATMSDGRVFTIGGSFSGGVFEKNGEVYSPSSKTWTSLP NAKVNPMLTADKQGLYMSDNHAWLFGWKKGSVFQA GPSTAMNWYYTSGSGDVKSAGKRQSNRGVAPDAMC GNAVMYDAVKGKILTFGGSPDYTDSDATTNAHIITLG EPGTSPNTVFASNGLYFARTFHTSVVLPDGSTFITGGQR RGIPFEDSTPVFTPEIYVPEQDTFYKQNPNSIVRAYHSIS LLLPDGRVFNGGGGLCGDCTTNHFDAQIFTPNYLYDS NGNLATRPKITRTSTQSVKVGGRITISTDSSITKASLIRY GTATHTVNTDQRRIPLTLTNNGGNSYSFQVPSDSGVAL PGYWMLFVMNSAGVPSVASTIRVTQAAA
107	GOh2022	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaaaatcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggc ggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatccc ggggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagac ggccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcga agtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatccc atgttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcg gctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtatt acacgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcgg cgtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggca agatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatg cccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtcca atggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaacct ttatcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttac accggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattg tacgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggag gaggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaa ttatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaac

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108	GOh2022	MGSSHHHHHHSSGLVPRGSHMGTASAPIGSAIPRNNW AVTCDSAQSGNECNKAIDGNKDTFWHTFYGANGDPK PPHTYTIDMKTTQNVNGLSVLPRQDGNQNGWIGRHEV YLSSDGTNWGSPVASGSWFADSTTKYSNFETRPARYV RLVAVTEANGQPWTSIAEINVFQASSYTAPQPGLGRW GPTIDLPIVPAAAAIEPKSGRVLMWSSYRNDAAEGSPG GITLTSSWDPSTGIVSDRTVTVTKHDMFCPGISMDGNG QIVVTGGWDAKKTSLYDSSSDSWIPGPDMQVARGYQS SATMSDGRVFTIGGSFSGGVFEKNGEVYSPSSKTWTSL PNAKVNPMLTADKQGLYMSDNHAWLFGWKKGSVFQ AGPSTAMNWYYTSGSGDVKSAGKRQSNRGVAPDAM CGNAVMYDAVKGKILTFGGSPDYTDSDATTNAHIITL GEPGTSPNTVFASNGLYFARTFHTSVVLPDGSTFITGG QRRGIPFEDSTPVFTPEIYVPEQDTFYKQNPNSIVRAYH SISLLLPDGRVFNGGGGLCGDCTTNHFDAQIFTPNYLY DSNGNLATRPKITRTSTQSVKVGGRITISTDSSITKASLI RYGTATHTVNTDQRRIPLTLTNNGGNSYSFQVPSDSGV ALPGYWMLFVMNSAGVPSVASTIRVTQAAA
109	GOh2023	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtatgtgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggagc agctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcga gttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacg acatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcg gctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa

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111	GOh2025	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggc ggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatccc ggggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagac ggccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcga agtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatccc atgttaaccctggacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcg gctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtatt acacgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcgg cgtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggca agatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatg cccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtcca atggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaacct ttatcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttac accggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattg tacgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggag gaggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaa ttatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaac ccagtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaa agcgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccg tattccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcgga ctcgggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtc ccgagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa
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113	GOh2026	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggc ggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatccc ggggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagac ggccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcga agtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatccc atgttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcg gctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtatt acacgtcaggaagcggggacgtgatctcagccggcaagcgccagtcgaaccgcgg cgtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggca agatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatg cccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtcca atggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaacct ttatcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttac accggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattg tacgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggag gaggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaa ttatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaac ccagtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaa agcgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccg tattccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcgga ctcgggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtc ccgagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa
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125	GOh2032	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggc ggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatccc ggggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagac ggccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcga agtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatccc atgttaaccgccgacaaacagggcctgtacatgagcgacaagcatgcgtggctgttcg gctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtatt acacgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcgg cgtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggca agatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatg cccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtcca atggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaacct ttatcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttac accggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattg tacgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggag gaggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaa ttatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaac ccagtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaa agcgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccg tattccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcgga ctcgggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtc ccgagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa

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135	GOh2039	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcgaaaggcagcccgggcggcatcactctgacgtcga gttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacacg acatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggcg gctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatcccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa gtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatcccat gttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcgg ctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtattac acgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcggc gtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggcaa gatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatgc ccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtccaat ggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaaccttt atcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttacac cggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattgta cgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggagg aggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaatt atctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaaccc agtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaaag cgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccgtat tccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcggactc gggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtccc gagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa

136	GOh2039	MGSSHHHHHHSSGLVPRGSHMGTASAPIGSAIPRNNW AVTCDSAQSGNECNKAIDGNKDTFWHTFYGANGDPK PPHTYTIDMKTTQNVNGLSVLPRQDGNQNGWIGRHEV YLSSDGTNWGSPVASGSWFADSTTKYSNFETRPARYV RLVAVTEANGQPWTSIAEINVFQASSYTAPQPGLGRW GPTIDLPIVPAAAAIEPTSGRVLMWSSYRNDAAKGSPG GITLTSSWDPSTGIVSDRTVTVTKHDMFCPGISMDGNG QIVVTGGWDAKKTSLYDSSSDSWIPGPDMQVARGYQS SATMSDGRVFTIGGSFSGGVFEKNGEVYSPSSKTWTSL PNAKVNPMLTADKQGLYMSDNHAWLFGWKKGSVFQ AGPSTAMNWYYTSGSGDVKSAGKRQSNRGVAPDAM CGNAVMYDAVKGKILTFGGSPDYTDSDATTNAHIITL GEPGTSPNTVFASNGLYFARTFHTSVVLPDGSTFITGG QRRGIPFEDSTPVFTPEIYVPEQDTFYKQNPNSIVRAYH SISLLLPDGRVFNGGGGLCGDCTTNHFDAQIFTPNYLY DSNGNLATRPKITRTSTQSVKVGGRITISTDSSITKASLI RYGTATHTVNTDQRRIPLTLTNNGGNSYSFQVPSDSGV ALPGYWMLFVMNSAGVPSVASTIRVTQAAA
137	GOh2041	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggc ggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatccc ggggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagac ggccgtgtctttacaatcggcggctcacgtagtggcggagtgtttgagaagaatggcg aagtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatcc catgttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttc ggctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtat tacacgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcg gcgtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggc aagatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaat gcccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtcc aatggcctctatttcgcgcgtaccttcatacgtcggtggtcctgccggacggatcaacc tttatcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttac accggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattg tacgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggag gaggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaa ttatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaac

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139	GOh2043	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggc ggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggtatccg gggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagacg gccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcgaa

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141	GOh2044	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtacccattctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggc ggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatccc ggggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagac ggccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcga agtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatccc atgttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcg gctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtatt acacgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcgg cgtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggca agatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatg cccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtcca atggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaacct ttatcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttac accggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattg tacgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggag gaggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaa ttatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaac ccagtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaa agcgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccg tattccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcgga ctcgggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtc ccgagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa
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145	GOh2046	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatgggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacgggc ggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatccc ggggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagac ggccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcga agtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgaatccc atgttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcg gctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtatt acacgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcgg cgtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggca agatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatg cccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtcca atggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaacct ttatcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttac accggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattg tacgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggag gaggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaa ttatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaac ccagtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaa agcgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccg tattccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcgga ctcgggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtc ccgagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa

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294	GOh3032	MGSSHHHHHHSSGLVPRGSHMGTASAPRGSAIPRNNW AVTCDSAQSGNECNKAIDGNKDTFWHTFYGANGDPK PPHTYTIDMKTTQNVNGLSVLPRQDGNQNGWIGRHEV YLSSDGTNWGSPVASGSWFADSTTKYSNFETRPARYV RLVAVTEANGQPWTSIAEINVFQASSYTAPQPGLGRW GPTIDLPIVPSAAAIEPTSGRVLMWSSYRNDAAEGSPG GITLTSSWDPSTGIVSDRTVTVTKHDMFCPGISMDGTG QIVVTGGWDAKKTSLYDSSSDSWIPGPDMQVARGYQS

		SATMSDGRVFTIGGSFSGGVFEKNGEVYSPSSKTWTSL PNAKVWPMLTADKQGLYMSDNHAWLFGWKKGSVFQ AGPSTAMNWYYTSGSGDVKSAGKRQSNRGVAPDAM CGNAVMYDAVKGKILTFGGSPDYTDSDATTNAHIITL GEPGTSPNTVFASNGLYFARTFHTSVVLPDGSTFITGG QRRGIPFEDSTPVFTPEIYVPEQDTFYKQNPNSIVRAYH SISLLLPDGRVFNGGGGLCGDCTTNHFDAQIFTPNYLY DSNGNLATRPKITRTSTQSVKVGGRITISTDSSITKASLI RYGTATHTVNTDQRRIPLTLTNNGGNSYSFQVPSDSGV ALPGYWMLFVMNSAGVPSVASTIRVTQAAA
295	GOh3038	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctcgtggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgac agaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagccag ctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgcc tatcgtacccaccgccgcggcgattgaaccaacgtcaggccgcgtactgatgtggag cagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtcg agttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaacac gacatgttttgcccggggatcagcatggacggcaccggccagatcgtcgtcacgggc ggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatccc ggggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcagac ggccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcga agtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgtggccc atgttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttcg gctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtatt acacgtcaggaagcggggacgtgatctcagccggcaagcgccagtcgaaccgcgg cgtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggca agatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaatg cccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtcca atggcctctatttcgcgcgtacctttcatacgtcggtggtcctgccggacggatcaacct ttatcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttac accggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattg tacgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggag gaggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaa ttatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaac ccagtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaa agcgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccg tattccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcgga ctcgggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtc ccgagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa

296	GOh3038	MGSSHHHHHHSSGLVPRGSHMGTASAPRGSAIPRNNW AVTCDSAQSGNECNKAIDGNKDTFWHTFYGANGDPK PPHTYTIDMKTTQNVNGLSVLPRQDGNQNGWIGRHEV YLSSDGTNWGSPVASGSWFADSTTKYSNFETRPARYV RLVAVTEANGQPWTSIAEINVFQASSYTAPQPGLGRW GPTIDLPIVPTAAAIEPTSGRVLMWSSYRNDAAEGSPG GITLTSSWDPSTGIVSDRTVTVTKHDMFCPGISMDGTG QIVVTGGWDAKKTSLYDSSSDSWIPGPDMQVARGYQS SATMSDGRVFTIGGSFSGGVFEKNGEVYSPSSKTWTSL PNAKVWPMLTADKQGLYMSDNHAWLFGWKKGSVFQ AGPSTAMNWYYTSGSGDVISAGKRQSNRGVAPDAMC GNAVMYDAVKGKILTFGGSPDYTDSDATTNAHIITLG EPGTSPNTVFASNGLYFARTFHTSVVLPDGSTFITGGQR RGIPFEDSTPVFTPEIYVPEQDTFYKQNPNSIVRAYHSIS LLLPDGRVFNGGGGLCGDCTTNHFDAQIFTPNYLYDS NGNLATRPKITRTSTQSVKVGGRITISTDSSITKASLIRY GTATHTVNTDQRRIPLTLTNNGGNSYSFQVPSDSGVAL PGYWMLFVMNSAGVPSVASTIRVTQAAA
297	GOh3049	atgggcagcagccatcatcatcatcacagcagcggcctggtgccgcgcggcag ccatatgggtaccgcctctgcccctatcggctcagccatcccgcgcaataactgggcc gtaacgtgcgacagcgcgcagagcggcaacgagtgcaataaagccatcgacggca ataaagacaccttctggcacacgttttatggcgcaaatggcgacccgaagccaccgca tacctataccattgacatgaaaaccacccagaatgtgaacgggctgtccgtgctgccg cgccaggacggcaatcagaacggatggatcggccgtcacgaggtatatttaagctcc gacggcaccaactggggatccccggtcgcgtcgggcagctggttcgccgacagcac caccaagtattccaattttgaaacccgtcctgcacgctacgtgcgcttagtggccgtgc gtgaggcgaacggccagccatggacctcaatcgccgaaatcaatgtattccaagcca gctcctataccgcgccgcagccaggacttgggcgttggggcccgaccatcgacctgc ctatcgtacccgcagccgcggcgattgaaccaacgtcaggccgcgtactgatgtgga gcagctaccgtaacgacgcggcggaaggcagcccgggcggcatcactctgacgtc gagttgggatccatccacgggcatcgtctccgaccgcaccgtgaccgtcacgaaaca cgacatgttttgcccggggatcagcatggacggcaacggccagatcgtcgtcacggg cggctgggacgccaagaagacgagcctctatgacagtagcagcgacagttggatcc cggggccggacatgcaggtggcacgcgggtaccagtccagcgcgaccatgtcaga cggccgtgtctttacaatcggcggctcatttagtggcggagtgtttgagaagaatggcg aagtctatagcccctcatccaaaacgtggacgtcacttccgaacgcgaaggtgtggcc catgttaaccgccgacaaacagggcctgtacatgagcgacaaccatgcgtggctgttc ggctggaagaaggggagcgtctttcaggcgggcccgtcgaccgcaatgaattggtat tacacgtcaggaagcggggacgtgaaatcagccggcaagcgccagtcgaaccgcg gcgtggcgccggacgcaatgtgcggcaatgccgtgatgtatgacgcggtcaaaggc aagatcctgacatttggcgggagtccggactacaccgacagtgacgcgacaaccaat gcccacattattacccttggggagccggggacgagcccgaacacggtcttcgcgtcc aatggcctctatttcgcgcgtaccttcatacgtcggtggtcctgccggacggatcaacc tttatcaccggcggacagcgccgtggcatccccttcgaagactcgacccccgtctttac accggagatttatgtgccggaacaggacacgttttataaacagaatccgaactcgattg tacgcgcgtatcactccatcagccttcttctcccggacggacgtgtctttaatggcggag gaggactgtgcggggactgcacgaccaatcattttgacgcgcagatctttaccccgaa ttatctgtatgattcaaatgggaatctcgcgacccgtccgaaaattacccgtacatcaac

		ccagtccgtcaaagtgggcggccgcattacgatcagtaccgacagcagcattaccaa agcgtcactgattcgttacggcaccgcaacccatacagtcaacacggaccagcgccg tattccgttaaccctgaccaataacggtggcaattcgtatagcttccaggtaccgtcgga ctcgggcgtggcgctgccaggatactggatgctgtttgtgatgaatagcgcgggcgtc ccgagcgtagcgagcacgatccgtgtgacccaggccgcagcttgataa
298	GOh3049	MGSSHHHHHHSSGLVPRGSHMGTASAPIGSAIPRNNW AVTCDSAQSGNECNKAIDGNKDTFWHTFYGANGDPK PPHTYTIDMKTTQNVNGLSVLPRQDGNQNGWIGRHEV YLSSDGTNWGSPVASGSWFADSTTKYSNFETRPARYV RLVAVREANGQPWTSIAEINVFQASSYTAPQPGLGRW GPTIDLPIVPAAAAIEPTSGRVLMWSSYRNDAAEGSPG GITLTSSWDPSTGIVSDRTVTVTKHDMFCPGISMDGNG QIVVTGGWDAKKTSLYDSSSDSWIPGPDMQVARGYQS SATMSDGRVFTIGGSFSGGVFEKNGEVYSPSSKTWTSL PNAKVWPMLTADKQGLYMSDNHAWLFGWKKGSVFQ AGPSTAMNWYYTSGSGDVKSAGKRQSNRGVAPDAM CGNAVMYDAVKGKILTFGGSPDYTDSDATTNAHIITL GEPGTSPNTVFASNGLYFARTFHTSVVLPDGSTFITGG QRRGIPFEDSTPVFTPEIYVPEQDTFYKQNPNSIVRAYH SISLLLPDGRVFNGGGGLCGDCTTNHFDAQIFTPNYLY DSNGNLATRPKITRTSTQSVKVGGRITISTDSSITKASLI RYGTATHTVNTDQRRIPLTLTNNGGNSYSFQVPSDSGV ALPGYWMLFVMNSAGVPSVASTIRVTQAAA

CLAIMS

What is claimed is:

1. A modified galactose oxidase comprising a polypeptide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 6,

wherein SEQ ID NO: 6 is a polypeptide sequence of galactose oxidase variant M3-5, wherein the galactose oxidase variant M3-5 comprises mutations of S33P, M93V, G218E, W313F, R353M, Q429T, V517A, and N558D relative to the wildtype galactose oxidase from *Fusarium graminearum* (SEQ ID NO: 4),

and wherein the polypeptide sequence of the modified galactose oxidase further comprises at least one mutation at one or more positions selected from the group consisting of 24, 26, 28, 45, 49, 65, 69, 104, 114, 117, 153, 157, 172, 173, 175, 193, 194, 195, 197, 198, 202, 214, 217, 218, 220, 224, 226, 239, 240, 250, 259, 265, 268, 269, 284, 294, 313, 314, 325, 341, 346, 348, 352, 353, 356, 389, 401, 429, 447, 485, 487, 537, 538, 546, 559, 576, 599, and 662, wherein the position of the mutation is numbered with reference to SEQ ID NO: 6.

2. The modified galactose oxidase of claim 1, wherein the mutation is selected from the group consisting of A24H, A24N, A26H, A26R, I28F, I28M, I28R, Q45D, Q45E, Q45N, E49K, F65P, N69D, N69E, N69F, N69H, N69L, N69M, N69T, N69W, N69Y, W104G, W104N, S114N, S114R, T117D, T153K, T153R, G157N, S172E, S173K, S173R, T175E, T175H, T175I, T175K, V193T, P194C, A195S, A195T, A195V, A197C, A197G, A198I, T202D, T202I, T202K, T202Q, N214R, N214S, F217A, F217C, F217D, F217E, F217G, F217H, F217I, F217K, F217L, F217M, F217N, F217P, F217Q, F217R, F217S, F217T, F217V, F217Y, E218F, E218K, E218P, E218Y, S220T, I224W, L226Q, L226V, D239E, D239P, D239Q, R240G, F250E, F250Y, N259T, T265S, N268W, D269N, I284S, I284Y, G294A, F313R, S314C, Y325F, N341W, A346L, K348S, Y352T, Y352W, M353F, N356K, N356R, N356W, K389I, A401D, T429W, T447A, I485A, I485K, I485R, F487R, L537G, C538K, C538R, F546M, S559P, S576D, Y599M, Q662D, and Q662P.

3. The modified galactose oxidase of claim 2, wherein the mutation is selected from the group consisting of I28R, A24H, A195S, A195T, A195V, A198I, T202I, F217A, E218F, E218K, L226Q, D239E, N259T, N268W, S314C, Y352W, Y599M, and combinations thereof.

4. The modified galactose oxidase of claim 2, wherein the mutation is selected from the group consisting of:

- M353F;
- S314C;
- L537G;
- F217V;
- F217P;
- F217Q;
- F217N;
- F217S;
- N268W;
- E218P;
- F217I;
- F217T;
- F217L;
- F217E;
- F217Y;
- F217A;
- F217C;
- F217D;
- F217G;
- F217H;
- F217K;
- F217M;
- F217R;
- a combination of F217P and E218P;
- a combination of F217P and L537G;

- a combination of F217P, E218P and L537G;
- a combination of F217V and E218P;
- a combination of F217V and L537G;
- a combination of F217V, E218P and L537G;
- a combination of E218P and L537G;
- a combination of F217A and N268W;
- a combination of F217A, N268W and M353F;
- a combination of F217A, N268W and S314C;
- a combination of F217G and N268W;
- a combination of F217G, N268W and M353F;
- a combination of F217A, N268W and A195S;
- a combination of F217A, N268W and V193T;
- a combination of F217A, N268W and N259T;
- a combination of F217A, N268W and Y325F;
- a combination of F217A, N268W and G294A;
- a combination of F217A, N268W and A195T;
- a combination of F217A, N268W and Y352T;
- a combination of F217A, N268W and N341W;
- a combination of F217A, N268W and A197C;
- a combination of F217A, N268W and R240G;
- a combination of F217A, N268W and A198I;
- a combination of F217A, N268W and K348S;
- a combination of F217A, N268W and T202D;
- a combination of F217A, N268W and T202Q;
- a combination of F217A, N268W and T202I;
- a combination of F217A, N268W and T202K;
- a combination of F217A, N268W and P194C;
- a combination of F217A, N268W and A346L;
- a combination of F217A, N268W and K389I;
- a combination of F217A, N268W and Y352W;
- a combination of F217A, N268W and C538K;

- a combination of F217A, N268W and C538R;
- a combination of F217A, N268W and F250E;
- a combination of F217A, N268W and F250Y;
- a combination of F217A, N268W and N356K;
- a combination of F217A, N268W and N356R;
- a combination of F217A, N268W and N356W;
- a combination of F217A, N268W and F487R;
- a combination of F217A, N268W and T429W;
- a combination of F217A, N268W and E218K;
- a combination of F217A, N268W and F313R;
- a combination of F217A, N268W and I284Y;
- a combination of F217A, N268W and A24H;
- a combination of F217A, N268W and I28R;
- a combination of F217A, N268W and I28M;
- a combination of F217A, N268W and S576D;
- a combination of F217A, N268W and I284S;
- a combination of F217A, N268W and F65P;
- a combination of F217A, N268W and T153R;
- a combination of F217A, N268W and A197G;
- a combination of F217A, N268W and T265S;
- a combination of F217A, N268W and S220T;
- a combination of F217A, N268W and N214S;
- a combination of F217A, N268W and I485A;
- a combination of F217A, N268W and N214R;
- a combination of F217A, N268W and D269N;
- a combination of F217A, N268W and I485R;
- a combination of F217A, N268W and I485K;
- a combination of F217A, N268W and D239E;
- a combination of F217A, N268W and T117D;
- a combination of F217A, N268W and S114R;
- a combination of F217A, N268W and S559P;

- a combination of F217A, N268W and S114N;
- a combination of F217A, N268W and Q662P;
- a combination of F217A, N268W and S173R;
- a combination of F217A, N268W and D239P;
- a combination of F217A, N268W and S172E;
- a combination of F217A, N268W and Q662D;
- a combination of F217A, N268W and S173K;
- a combination of F217A, N268W and A195V;
- a combination of F217A, N268W and E218F;
- a combination of F217A, N268W and L226Q;
- a combination of F217A, N268W and I224W;
- a combination of F217A, N268W and A26R;
- a combination of F217A, N268W and I28F;
- a combination of F217A, N268W and E218Y;
- a combination of F217A, N268W and L226V;
- a combination of F217A, N268W and D239Q;
- a combination of F217A, N268W and F546M;
- a combination of F217A, N268W and A24N;
- a combination of F217A, N268W and A26H;
- a combination of F217A, N268W and Y599M;
- a combination of F217A, N268W and G157N;
- a combination of F217A, N268W and Q45D;
- a combination of F217A, N268W and Q45E;
- a combination of F217A, N268W and Q45N;
- a combination of F217A, N268W and E49K;
- a combination of F217A, N268W and N69D;
- a combination of F217A, N268W and N69E;
- a combination of F217A, N268W and N69F;
- a combination of F217A, N268W and N69H;
- a combination of F217A, N268W and N69L;
- a combination of F217A, N268W and N69M;

- a combination of F217A, N268W and N69T;
- a combination of F217A, N268W and N69W;
- a combination of F217A, N268W and N69Y;
- a combination of F217A, N268W and W104G;
- a combination of F217A, N268W and W104N;
- a combination of F217A, N268W and T175E;
- a combination of F217A, N268W and T175H;
- a combination of F217A, N268W and T175I;
- a combination of F217A, N268W and T175K;
- a combination of F217A, N268W and A401D;
- a combination of F217A, N268W, A195T and Y352W;
- a combination of F217A, N268W, N341W and Y352T;
- a combination of F217A, N268W, A198I and N259T;
- a combination of F217A, N268W, A195S and N341W;
- a combination of F217A, N268W, T153K and A195S;
- a combination of F217A, N268W, I28R and K389I;
- a combination of F217A, N268W, T153R and A195T;
- a combination of F217A, N268W, I28R, T153R, A198I and N341W;
- a combination of F217A, N268W, A195T, N259T and N341W;
- a combination of F217A, N268W, I28R, A195T, N259T and K389I;
- a combination of F217A, N268W, I28R, N259T and N341W;
- a combination of F217A, N268W, I28R, A195S and K389I;
- a combination of F217A, N268W, A195T and N341W;
- a combination of F217A, N268W, I28R, A195T, N341W and K389I;
- a combination of F217A, N268W, I28R, A195T, N259T and T447A;
- a combination of F217A, N268W, I28R, A195S, N259T and N341W;
- a combination of F217A, N268W, I28R, A195T, N259T, N341W and K389I; and
- a combination of F217A, N268W, T153R and N341W.

5. The modified galactose oxidase of claim 4, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence of at least one modified galactose oxidase variant set forth in the even numbered sequences of SEQ ID NOS: 7-298.

6. The modified galactose oxidase of claim 5, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in the even numbered sequences of SEQ ID NOS: 7-298.

7. The modified galactose oxidase of claim 4, wherein the mutation is selected from the group consisting of:
(a) S314C;
(b) N268W;
(c) F217A;
(d) a combination of F217A and N268W;
(e) a combination of F217A, N268W and A195S;
(f) a combination of F217A, N268W and N259T;
(g) a combination of F217A, N268W and A195T;
(h) a combination of F217A, N268W and A198I;
(i) a combination of F217A, N268W and T202I;
(j) a combination of F217A, N268W and Y352W;
(k) a combination of F217A, N268W and A24H;
(l) a combination of F217A, N268W and I28R;
(m) a combination of F217A, N268W and D239E;
(n) a combination of F217A, N268W and A24N;
(o) a combination of F217A, N268W and E218K;
(p) a combination of F217A, N268W and E218F;
(q) a combination of F217A, N268W and Y599M;
(r) a combination of F217A, N268W and L226Q;
(s) a combination of F217A, N268W and A195V;
(t) a combination of F217A, N268W, A195T and Y352W;

(u) a combination of F217A, N268W, I28R and K389I;

(v) a combination of F217A, N268W, I28R, A195T, N259T, and K389I; and

(w) a combination of F217A, N268W, I28R, A195T, N259T, N341W, and K389I.

8. The modified galactose oxidase of claim 4 or 7, wherein the modified galactose oxidase comprises the mutations of F217A and N268W.

9. The modified galactose oxidase of claim 8, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:68.

10. The modified galactose oxidase of claim 8 or 9, wherein the modified galactose oxidase further comprises at least one mutation selected from the group consisting of A24H, A24N, A26H, A26R, I28F, I28M, I28R, Q45D, Q45E, Q45N, E49K, F65P, N69D, N69E, N69F, N69H, N69L, N69M, N69T, N69W, N69Y, W104G, W104N, S114N, S114R, T117D, T153K, T153R, G157N, S172E, S173K, S173R, T175E, T175H, T175I, T175K, V193T, P194C, A195SX, A195T, A195V, A197C, A197G, A198I, T202D, T202I, T202K, T202Q, N214R, N214S, E218F, E218K, E218P, E218Y, S220T, I224W, L226Q, L226V, D239E, D239P, D239Q, R240G, F250E, F250Y, N259T, T265S, D269N, I284S, I284Y, G294A, F313R, S314C, Y325F, N341W, A346L, K348S, Y352T, Y352W, M353F, N356K, N356R, N356W, K389I, A401D, T429W, T447A, I485A, I485K, I485R, F487R, L537G, C538K, C538R, F546M, S559P, S576D, Y599M, Q662D, and Q662P, wherein the position of the mutation is numbered with reference to SEQ ID NO: 6.

11. The modified galactose oxidase of claim 10, wherein the polypeptide sequence of the modified galactose oxidase has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequence of at least one modified galactose oxidase variant set forth in SEQ ID NOS: 70, 72 or the even numbered sequences of SEQ ID NOS: 77-298.

12. The modified galactose oxidase of claim 11, wherein the polypeptide sequence of the modified galactose oxidase comprises a polypeptide sequence set forth in SEQ ID NOS: 70, 72 or the even numbered sequences of SEQ ID NOS: 77-298.

13. The modified galactose oxidase of any one of claims 1-12, wherein the modified galactose oxidase comprises one or more of the following properties:

(a) enhanced protein expression by at least 1.01-fold compared to the galactose oxidase variant M3-5 or by at least 1.01-fold compared to the modified galactose oxidase of claim 8;

(b) enhanced solubility by at least 1.01-fold compared to the galactose oxidase variant M3-5 or by at least 1.01-fold compared to the modified galactose oxidase of claim 8;

(c) enhanced thermal stability by at least 1.01-fold compared to the galactose oxidase variant M3-5 or by at least 1.01-fold compared to the modified galactose oxidase of claim 8 at a temperature of at least 50°C;

(d) increased enzymatic activity by at least 1.01-fold compared to the galactose oxidase variant M3-5 or by at least 1.01-fold compared to the modified galactose oxidase of claim 8, wherein the enzymatic activity comprises converting a secondary alcohol to a ketone, wherein optionally the secondary alcohol is a bulky secondary alcohol or unactivated secondary alcohol; and

(e) reduced enantioselectivity by at least 1.01-fold compared to the galactose oxidase variant M3-5 or by at least 1.01-fold compared to the modified galactose oxidase of claim 8.

14. A polynucleotide sequence encoding the modified galactose oxidase of any one of claims 1-13.

15. The polynucleotide sequence of claim 14, wherein the polynucleotide sequence comprises at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more sequence identity to SEQ ID NO: 5, wherein SEQ ID NO: 5 is a polynucleotide sequence of the galactose oxidase variant M3-5, wherein the polynucleotide sequence of claim 14 comprises at least one mutation at one or more positions which leads

to at least one mutation in the polypeptide sequence of the modified galactose oxidase in any one of claims 1-13.

16. The polynucleotide sequence of claim 14 or 15, wherein the polynucleotide is codon optimized, wherein optionally the polynucleotide is codon optimized for protein expression in *Escherichia coli*.

17. The polynucleotide sequence of any one of claims 14-16, wherein the polynucleotide sequence is further linked to a control sequence, wherein optionally the control sequence is a promoter sequence or a termination sequence, wherein optionally the promoter sequence is a lac promoter sequence.

18. The polynucleotide sequence of any one of claims 14-17, wherein the polynucleotide comprises an odd numbered sequence of SEQ ID NOS: 7-298.

19. An expression vector comprising the polynucleotide sequence of any one of claims 14-18.

20. The expression vector of claim 19, wherein the expression vector is selected from the group consisting of a plasmid and a viral vector, wherein optionally the expression vector is a pET28a plasmid vector.

21. A host cell comprising the polynucleotide sequence of any one of claims 14-18 or the expression vector of claim 19 or 20.

22. The host cell of claim 21, wherein the host cell is a prokaryotic cell or eukaryotic cell, wherein optionally the host cell is a prokaryotic cell, and wherein optionally the prokaryotic cell is *Escherichia coli*.

23. A method of producing a modified galactose oxidase, comprising culturing the host cell of claim 21 or 22 under suitable culture conditions such that the modified galactose oxidase is produced.

24. The method of claim 23, further comprises recovering the modified galactose oxidase from the culture and/or the host cell.

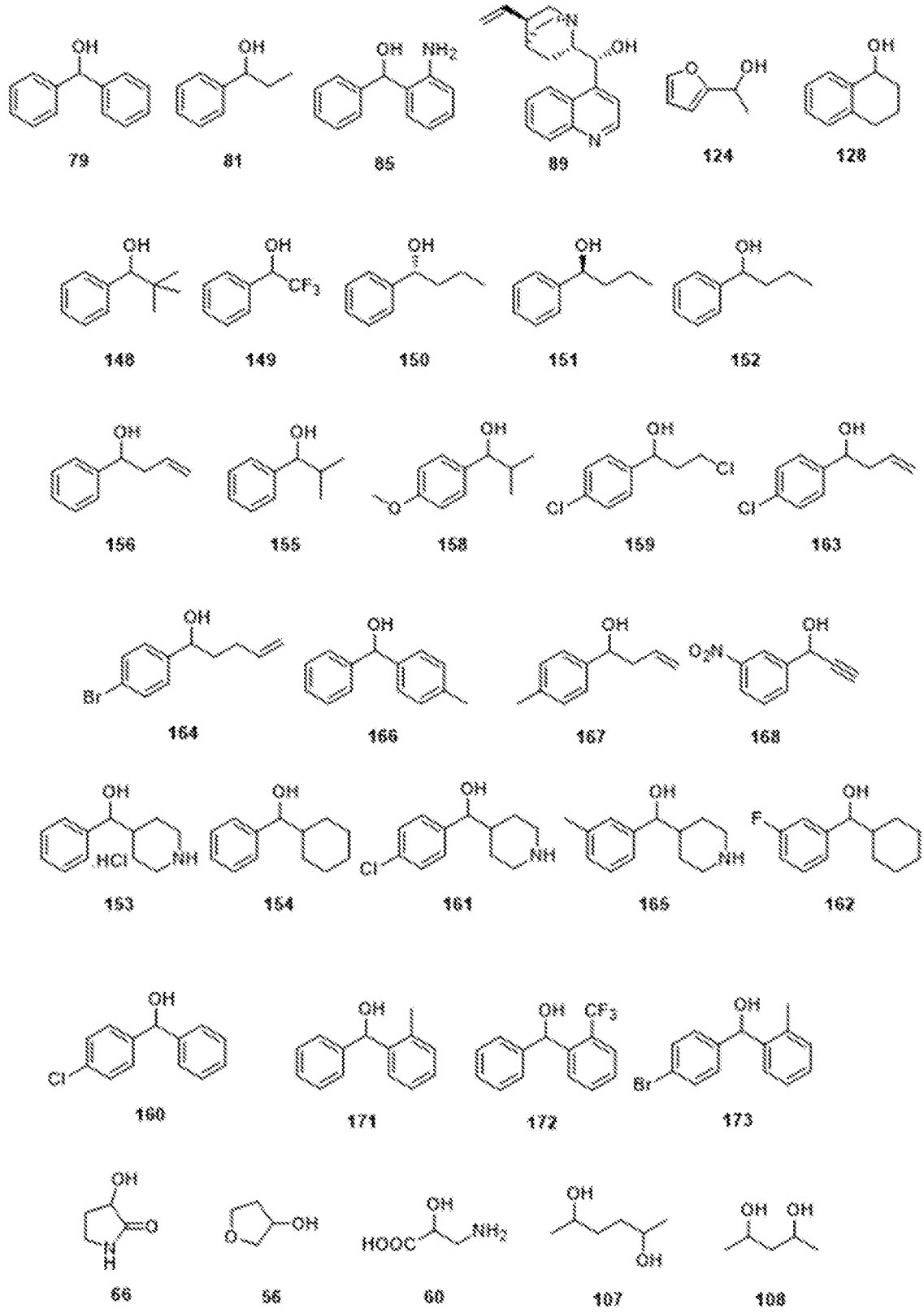
25. The method of claim 23 or 24, further comprises purifying the modified galactose oxidase.

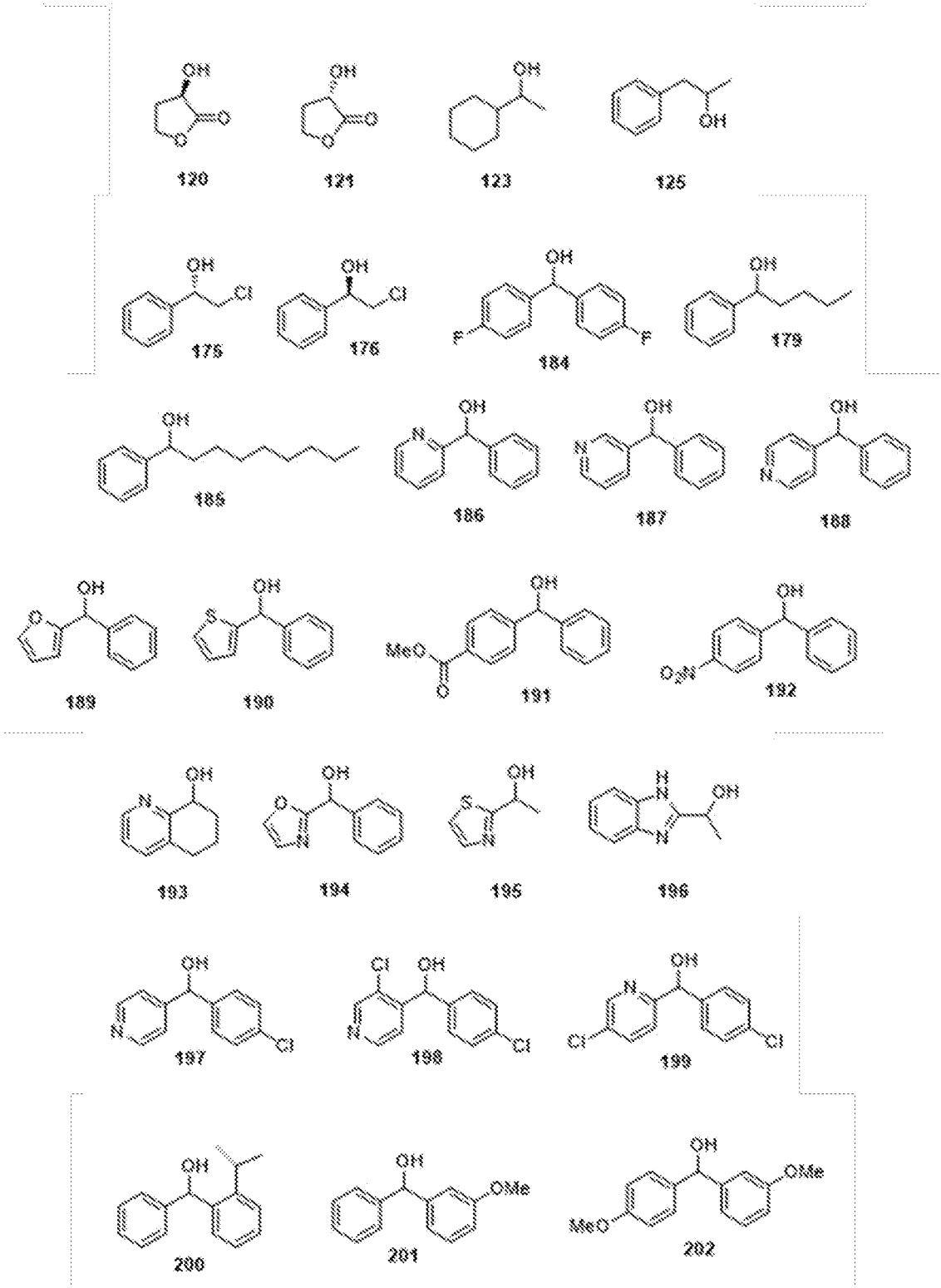
26. A method of producing a ketone from a secondary alcohol, comprising contacting the secondary alcohol with the modified galactose oxidase of any one of claims 1-13 under suitable conditions for an oxidation reaction.

27. The method of claim 26, wherein the secondary alcohol is a bulky secondary alcohol or unactivated secondary alcohol, wherein the bulky secondary alcohol comprises one or more bulky substituents, and wherein the unactivated secondary alcohol is an aliphatic secondary alcohol without a benzene ring in the alpha position adjacent to the alcohol group.

28. The method of claim 26 or 27, wherein the secondary alcohol comprises the formula R^1R^2CHOH, wherein R^1 and R^2 are independently selected from the group consisting of cycloalkyl, aryl, heteroaryl, alkyl, alkylene, alkynyl, haloalkyl, carboxyl, and carbonyl, wherein the cycloalkyl, aryl, heteroaryl, alkyl, alkylene, alkynyl, haloalkyl, carboxyl, and carbonyl is optionally substituted with one or more substituents selected from the group consisting of alkyl, halogen, alkyl halide, alkylene, alkynyl, alkoxy, cyano, oxo, nitro, amino, thiol, carboxyl, ester and hydroxyl.

29. The method of any one of claims 26-28, wherein the secondary alcohol is selected from the group consisting of:


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30. The method of any one of claims 26-29, wherein the secondary alcohol is chiral or non-chiral, wherein optionally the chiral secondary alcohol is present as (S)-enantiomer, (R)-

enantiomer, or a mixture of (S)- and (R)-enantiomers, wherein optionally the chiral secondary alcohol is present as a racemic mixture.

31. The method of any one of claims 26-30, wherein the contacting step is performed at a temperature of 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C or more, wherein optionally the temperature is 35°C.

32. The method of any one of claims 26-31, further comprising converting the secondary alcohol into an active pharmaceutical ingredient.

33. A kit for use in the method of any one of claims 26-32, wherein the kit comprises the modified galactose oxidase of any one of claims 1-13.

34. The kit of claim 33, wherein the kit further comprises one or more of the following:
(a) one or more reaction buffers;
(b) one or more reaction vessels, wherein optionally the reaction vessel is a HPLC vial;
(c) one or more reaction solvents, wherein optionally the reaction solvent is 5% v/v dimethyl sulfoxide in 100 mM sodium phosphate buffer with a pH of 7;
(d) one or more secondary alcohol substrates;
(e) one or more co-enzymes to break down the by-product of the oxidation reaction, wherein optionally the co-enzyme is a horseradish peroxidase that breaks down the H_2O_2; and
(f) instructions for performing the method of any one of claims 23-30.