

COMPOSITIONS AND METHODS FOR PRODUCING REBAUDIOSIDE M

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/374,461, filed on September 2, 2022, the content of which is herein incorporated by reference in its entirety.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The contents of the electronic sequence listing (ARZE_038_01WO_Se-
qList_ST26.xml; Size: 681,823 bytes; and Date of Creation: September 1, 2023) are herein incorporated by reference in its entirety.

FIELD OF THE DISCLOSURE

[0003] The present disclosure relates to enzymes and biocatalytic processes for producing steviol glycosides. The present disclosure particularly relates to the use of glycosyltransferases that can transfer a glucose moiety from an ADP-glucose sugar donor to steviol glycosides.

BACKGROUND

[0004] Excess sugar consumption has been linked to worldwide health epidemics including diabetes and heart disease. Healthcare systems incur exorbitant costs associated with treating these diseases. Replacing added sugar in food with a low calorie, high-intensity sweetener would have significant health and economic impact.

[0005] The species *Stevia rebaudiana* is commonly grown for its sweet leaves, which have traditionally been used as a sweetener. Stevia extract is 200-300 times sweeter than sugar and is used commercially as a high intensity sweetener. The main glycoside components of stevia leaf are steviosides and rebaudiosides. Over ten different steviol glycosides are present in appreciable quantities in the leaf. The principal sweetening compounds are stevioside and rebaudioside A. Rebaudioside A (Reb A) is considered higher value compared to stevioside, because of its increased sweetness and decreased bitterness.

[0006] Further, when compared to Reb A, the sweetness and bitterness profile of rebaudioside M (Reb M) is improved. Reb M, however, is present at very low quantities in the stevia leaf. Accordingly, improved methods for converting glucose sources into low-order steviol glycosides are required.

SUMMARY OF THE INVENTION

[0007] The present disclosure provides enzymes, particularly non-natural enzymes, and methods to use those enzymes to transfer a sugar moiety to a substrate steviol glycoside. Specifically, beta-1,3-glycosyltransferases (also referred to herein as “B13GTs”) are disclosed that can glycosylate the C3’ of the 13-O-glucose and/or the 19-O-glucose of a Steviol glycoside substrate. The present disclosure provides methods to use the disclosed beta-1,3-glycosyltransferases in combination with a sucrose synthase (SuSy) and optionally a beta-1,2-glycosyltransferase (B12GT) to make Reb M.

[0008] In contrast to native glycosyltransferases, the disclosure provides glycosyltransferase polypeptides that can utilize adenosine diphosphate (ADP)-glucose as the sugar donor to glycosylate the C3’ of the 13-O-glucose and/or 19-O-glucose of a steviol glycoside substrate. The disclosure provides glycosyltransferase polypeptides that comprise an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-517. The glycosyltransferase polypeptide may comprise, or consist of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-517. The polypeptides may comprise one or more peptide tags used for solubility, expression and/or purification; for example, a polyhistidine tag of between 4 and 10 histidine residues, preferably 6 histidine residues. Other suitable tags include, but are not limited to, glutathione *S*-transferase (GST), FLAG, maltose binding protein (MBP), calmodulin binding peptide (CBP), and Myc tag. Suitable linkers include, but are not limited to, polypeptides composed of glycine and serine, such as GSGS, polyglycine linkers, EAAAK repeats, and sequences containing cleavage sites for enzymes such as factor Xa, enterokinase, and thrombin.

[0009] The disclosure additionally provides a method to utilize the disclosed B13GTs to make steviol glycosides, specifically Reb M. Starting with a feed containing Reb A and/or stevioside, the enzymes B13GT, B12GT and SuSy can be combined with sucrose, ADP and the steviol glycoside feed to convert the Reb A and/or stevioside to Reb M. In some embodiments, the method comprises contacting a stevia leaf extract purified to contain greater than 50% Reb A (RA50), ADP, and sucrose with a beta-1,3-glycosyltransferase, beta-1,2 glycosyltransferase and sucrose synthase to make Reb M. In another embodiment, the method comprises contacting a stevia leaf extract purified to contain greater than 60% Reb A (RA60), ADP, and sucrose with a beta-1,3-glycosyltransferase, beta-1,2 glycosyltransferase, and sucrose synthase to make Reb M. In another embodiment, the method comprises contacting a stevia leaf extract purified

to contain greater than 90% stevioside (RS90), ADP, and sucrose with a beta-1,3-glucosyltransferase, beta-1,2-glucosyltransferase, and sucrose synthase to make Reb M. In another embodiment, the method comprises contact a stevia leaf extract purified to contain greater than 99% stevioside (RS99), ADP, and sucrose with a beta-1,3-glucosyltransferase, beta-1,2-glucosyltransferase, and sucrose synthase to make Reb M.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] The accompanying drawings are included to provide a further understanding of the disclosure. The drawings illustrate embodiments of the disclosure and together with the description, serve to explain the principles of the embodiments of the disclosure.

[0011] **FIG. 1** Chemical structure of rebaudioside M.

[0012] **FIG. 2** Partial steviol glycoside network showing conversion of stevioside and Reb A to Reb M through additions of glucose monomers catalyzed by a beta-1,2-glucosyltransferase (B12GT) and beta-1,3-glucosyltransferase (B13GT). For each molecule, the central hexagon represents the steviol glycoside core, circles represent beta-1,2-linked glucose monomers and squares represent beta-1,3-linked glucose monomers.

[0013] **FIG. 3** SDS-PAGE gel of designed beta-1,3-glucosyltransferases expressed in *Pichia pastoris* and purified by immobilized metal affinity chromatography.

DETAILED DESCRIPTION

[0014] The present disclosure provides enzymes and biocatalytic processes for preparing a composition comprising a target steviol glycoside by contacting a starting composition comprising a substrate steviol glycoside, sucrose, and nucleotide diphosphate (NDP) with one or more NDP-glycosyltransferase polypeptides and a sucrose synthase, thereby producing a composition comprising a target steviol glycoside comprising one or more additional glucose units than the substrate steviol glycoside.

Definitions

[0015] The term “a” or “an” refers to one or more of that entity, i.e. can refer to plural referents. As such, the terms “a,” “an,” “one or more,” and “at least one” are used interchangeably herein. In addition, reference to “an element” by the indefinite article “a” or “an” does not exclude the possibility that more than one of the elements is present, unless the context clearly requires that there is one and only one of the elements.

[0016] Throughout this application, ADP, GDP, UDP, CDP, and TDP are used to refer, respectively, to adenosine diphosphate, guanosine-5'-diphosphate, uridine diphosphate, cytidine diphosphate, and thymidine diphosphate.

[0017] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device or the method being employed to determine the value, or the variation that exists among the samples being measured. Unless otherwise stated or otherwise evident from the context, the term "about" means within 10% above or below the reported numerical value (except where such number would exceed 100% of a possible value or go below 0%). When used in conjunction with a range or series of values, the term "about" applies to the endpoints of the range or each of the values enumerated in the series, unless otherwise indicated. As used in this application, the terms "about" and "approximately" are used as equivalents.

[0018] As used herein the term "sequence identity" refers to the extent to which two optimally aligned polynucleotides or polypeptide sequences are invariant throughout a window of alignment of residues, e.g. nucleotides or amino acids. An "identity fraction" for aligned segments of a test sequence and a reference sequence is the number of identical residues which are shared by the two aligned sequences divided by the total number of residues in the reference sequence segment, i.e. the entire reference sequence or a smaller defined part of the reference sequence. "Percent identity" is the identity fraction times 100. Comparison of sequences to determine percent identity can be accomplished by a number of well-known methods, including for example by using mathematical algorithms, such as, for example, those in the BLAST suite of sequence analysis programs. Unless noted otherwise, the term "sequence identity" in the claims refers to sequence identity as calculated by Clustal Omega® using default parameters.

[0019] As used herein, "biocatalysis" or "biocatalytic" refers to the use of natural catalysts, such as protein enzymes, to perform chemical transformations on organic compounds. Biocatalysis is alternatively known as biotransformation or biosynthesis. Both isolated and whole-cell biocatalysis methods are known in the art. Biocatalyst protein enzymes can be naturally occurring or recombinant proteins.

[0020] As used herein, the term "steviol glycoside(s)" refers to a glycoside of steviol, including, but not limited to, naturally occurring steviol glycosides, synthetic steviol glycosides, e.g. enzymatically glycosylated steviol glycosides and combinations thereof. For example, the term steviol glycosides includes the known steviol glycosides steviol-13-O-glucoside, steviol-19-O-glucoside, rubusoside, steviol-1,2-bioside, steviol-1,3-bioside, rubusoside, dulcoside B,

dulcoside A, rebaudioside B, rebaudioside G, stevioside, rebaudioside C, rebaudioside F, rebaudioside A, rebaudioside I, rebaudioside E, rebaudioside E2, rebaudioside E3, rebaudioside H, rebaudioside L, rebaudioside K, rebaudioside J, rebaudioside M, rebaudioside D, rebaudioside N, rebaudioside O, rebaudioside Q, and rebaudioside AM.

5 [0021] As used herein, "starting composition" refers to any composition (generally an aqueous solution) containing one or more steviol glycosides, where the one or more steviol glycosides serve as the substrate for the biotransformation.

[0022] As used herein, the terms "polynucleotide" or "nucleic acid" are used interchangeably, unless indicated by context, and is used to refer to a polymeric form of nucleotides of any
10 length, either ribonucleotides or deoxyribonucleotides, typically DNA.

[0023] As used herein, "expression" refers to either or both steps, depending on context, of the two-step process by which polynucleotides are transcribed into mRNA and the transcribed mRNA is subsequently translated into a polypeptides.

[0024] "Under transcriptional control" means that transcription of a polynucleotide, usually a
15 DNA sequence, depends on its being operatively linked to an element that promotes transcription.

[0025] "Operatively linked" means that the polynucleotide elements are arranged in a manner that allows them to function in a cell; typically to produce polypeptides in the cell; for example the disclosure provides promoters operatively linked to the downstream sequences encoding
20 polypeptides.

[0026] The term "encode" refers to the ability of a polynucleotide to produce an mRNA or a polypeptide if it can be transcribed to produce the mRNA and then translated to produce the polypeptide or a fragment thereof. In each case, the polynucleotide is referred to as encoding the mRNA and encoding the polypeptide. The antisense strand is the complement of such a
25 nucleic acid, and the encoding sequence can be deduced therefrom. Similarly, a "coding sequence" refers to a region of a nucleic acid that encodes an mRNA or a polypeptide.

[0027] The term "promoter" as used herein refers to a control sequence that is a portion of a polynucleotide sequence that controls the initiation and rate of transcription of a coding sequence. An "enhancer" is a regulatory element that increases the expression of a target
30 sequence. A "promoter/enhancer" is a polynucleotide with sequences that provide both promoter and enhancer functions.

[0028] The regulatory elements, e.g. enhancers and promoters, may be "homologous" or "heterologous." A "homologous" regulatory element is one which is naturally linked with a given

polynucleotide in the genome; for example, it may be the promoter found natively in the organism upstream of the encoded polypeptide. A "heterologous" regulatory element is one which is placed in juxtaposition to a polynucleotide by means of recombinant molecular biological techniques but is not a combination found in nature. Often, promoters, enhancers and other regulatory elements are heterologous so as to facilitate expression of a polypeptide in a host cell other than one in which a polypeptide naturally occurs. Thus, "heterologous expression", as used herein, refers to producing an mRNA and/or a polypeptide in a host cell, such as a microorganism, where the polynucleotide is not found naturally or one or more regulatory elements are not naturally found operably linked to the polynucleotide in the host cell.

[0029] The term "polypeptide" is used here to refer to a molecule of two or more subunits of amino acids linked by peptide bonds. Typically, though not always, the polypeptides contain several hundred amino acids; for example, about 400 to about 900 amino acids.

[0030] A "plasmid" is a DNA molecule that is typically separate from and capable of replicating independently of the chromosomal DNA. In many cases, it is circular and double-stranded.

It is known in the art that while plasmid vectors often exist as extrachromosomal circular DNA molecules, plasmid vectors may also be designed to be stably integrated into a host chromosome either randomly or in a targeted manner. Many plasmids are commercially available for varied uses. The gene to be replicated is inserted into copies of a plasmid containing genes that make cells resistant to particular antibiotics, and a multiple cloning site (MCS, or polylinker), which is a short region containing several commonly used restriction sites allowing the easy insertion of DNA fragments at this location. Typically, the polypeptides disclosed herein are expressed from plasmids.

[0031] Reb M, the chemical structure of which is shown in **FIG. 1**, can be made by adding a beta-1,3-linked glucose monomer to the lower-order steviol glycosides Rebaudioside I (Reb I), or Reb AM or adding a beta-1,2-linked glucose monomer to Rebaudioside D (Reb D), as shown in **FIG. 2**. Native glycosyltransferases that perform these conversions use uridine diphosphate (UDP)-glucose as the glucose source for transferring to the lower-order steviol glycosides.

[0032] The present disclosure provides non-natural, engineered beta-1,3-adenosine diphosphate (ADP) glycosyltransferases (B13GTs) that can use an ADP-glucose sugar donor to add a beta-linked glucose monomer to the C3' of the 13-O-glucose and/or 19-O-glucose of a steviol glycoside substrate. For example, the disclosed B13GTs can perform the following conversions: stevioside to Reb A, stevioside to Rebaudioside E2 (Reb E2), Reb A to Reb I, Rebaudioside E (Reb E) to Reb D, Reb E to Rebaudioside AM (Reb AM), Reb E2 to Reb I, Reb D to

Reb M and Reb AM to Reb M. In a particular embodiment, the glycosyltransferase polypeptide is one of SEQ ID NOs: 2-517. In another embodiment, the glycosyltransferase polypeptide is a polypeptide sequence that is at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to one of SEQ ID NOs: 2-517.

[0033] In bioinformatics, several methods have been developed to find and determine related polypeptide sequences. For example, percent sequence identity, position-specific scoring matrices (PSSMs), and hidden Markov models (HMMs) are all commonly employed to find sequences that are similar to a given query sequence. Percent sequence identity calculates the number of amino acids that are shared between two sequences. Percent sequence identity is calculated in the context of a given alignment between two sequences. Percentage identity may be calculated using the alignment program Clustal Omega (available at www.ebi.ac.uk/Tools/msa/clustalo/) with default settings. The default transition matrix is Gonnet, gap opening penalty is 6 bits, and gap extension is 1 bit. Clustal Omega uses the HHalign algorithm and its default settings as its core alignment engine. The algorithm is described in Söding, J. (2005) 'Protein homology detection by HMM–HMM comparison'. *Bioinformatics* 21, 951-960.

[0034] Position-specific scoring matrices (PSSMs) are a concise way to represent many related sequences. PSSMs are often generated using multiple sequence alignments. The sequence search tool PSI-BLAST generates PSSMs and uses them to search for related polypeptide sequences. A PSSM used to score polypeptide sequences is a matrix (i.e. table) composed of 21 columns by N rows, where N is the length of the related sequences. Each row corresponds to a position within the polypeptide sequence and each column represents a different amino acid (or gap) that the residue position can take on. Each entry in the PSSM represents a score for the specific amino acid at the specific position within the polypeptide sequence. A sequence can be scored with a PSSM by first aligning the sequence to a reference sequence, and then calculating the following sum: $S_{PSSM} = \sum_{i=1}^N PSSM(i, aa_i)$, where i is the sequence position and aa_i is the amino acid at position i . Related polypeptide sequences will all have high PSSM scores, while unrelated sequences will yield low scores.

[0035] In some embodiments, the beta-1,3-glycosyltransferase polypeptide is prepared by expression in a host microorganism. Suitable host microorganisms include, but are not limited

to, *E. coli*, *Saccharomyces* sp., *Aspergillus* sp., *Pichia* sp., *Bacillus* sp. In a particular embodiment, the glycosyltransferase is expressed in *E. coli*. In a particular embodiment, the glycosyltransferase is expressed in *Pichia pastoris*.

[0036] The B13GT polypeptide can be provided in any suitable form, including free, immobilized or as a whole cell system. The degree of purity of the glycosyltransferase polypeptide may vary, e.g., it may be provided as a crude, semi-purified or purified enzyme preparation(s). In one embodiment, the glycosyltransferase polypeptide is free. In another embodiment, the glycosyltransferase polypeptide is immobilized to a solid support, for example on an inorganic or organic support. In some embodiments, the solid support is derivatized cellulose, glass, ceramic, methacrylate, styrene, acrylic, a metal oxide, or a membrane. In some embodiments, the glycosyltransferase polypeptide is immobilized to the solid support by covalent attachment, adsorption, cross-linking, entrapment, or encapsulation.

[0037] In yet another embodiment, the B13GT polypeptide is provided in the form of a whole cell system, for example as a living fermentative microbial cell, or as dead and stabilized microbial cell, or in the form of a cell lysate.

[0038] The present disclosure provides a biocatalytic process for the preparation of a composition comprising one or more target steviol glycosides from a starting composition comprising one or more substrate steviol glycosides, wherein the target steviol glycoside comprises steviol glycosides with one or more additional glucose units than the substrate steviol glycoside. The biocatalytic process comprises contacting an engineered B13GT, a sucrose synthase (SuSy) (unless ADP-glucose is directly supplied), and optionally a B12GT with a starting composition containing one or more steviol glycosides, a non-UDP nucleotide diphosphate, and sucrose. In another embodiment, the biocatalytic process comprises contacting an engineered B13GT, a SuSy, and optionally a B12GT with a starting composition containing one or more steviol glycosides, a non-UDP nucleotide diphosphate, and sucrose. In some embodiments, the method comprises contacting RA50, ADP, and sucrose with an engineered B1,3 glycosyltransferase, a B12GT, and a SuSy to make Reb M. In another embodiment, the method comprises contacting RA60, ADP, and sucrose with an engineered B13GT, a B12GT, and a SuSy to make Reb M. In another embodiment, the method comprises contacting stevioside, ADP, and sucrose with an engineered B1,3 glycosyltransferase, a B12GT, and a SuSy to make Reb M. In another embodiment, the method comprises contacting Reb A, ADP, and sucrose with an engineered B13GT, a B12GT, and a SuSy to make Reb M. In another embodiment, the method comprises contacting RS90, ADP, and sucrose with an engineered B13GT, a B12GT, and a SuSy to make

Reb M. In another embodiment, the method comprises contacting RS99, ADP, and sucrose with an engineered B13GT, B12GT, and a SuSy to make Reb M.

[0039] In one embodiment, the B13GT polypeptide is one of SEQ ID NOs: 2-517. In another embodiment, the glycosyltransferase polypeptide is a polypeptide sequence that is at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to one of SEQ ID NOs: 2-517.

[0040] In one embodiment the sucrose synthase is any polypeptide with sucrose synthase activity. In another embodiment, the sucrose synthase is derived from an organism from the Bacteria domain. In another embodiment, the sucrose synthase is derived from an organism from the Plantae kingdom. In another embodiment, the sucrose synthase is derived from an organism from the proteobacteria, deferribacteres, or cyanobacteria phylum. In another embodiment, the sucrose synthase is derived from the species *Acidithiobacillus caldus*, *Nitrosomonas europaea*, *Denitrovibrio acetiphilus*, *Thermosynechococcus elongatus*, *Oryza sativa*, *Arabidopsis thaliana*, or *Coffea arabica*. In another embodiment, the sucrose synthase is an engineered sucrose synthase with a polypeptide sequence that is at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a sucrose synthase derived from the species *Acidithiobacillus caldus*, *Nitrosomonas europaea*, *Denitrovibrio acetiphilus*, *Thermosynechococcus elongatus*, *Oryza sativa*, *Arabidopsis thaliana*, or *Coffea arabica*.

[0041] In one embodiment the B12GT is any polypeptide that can glycosylate the C2' of the 13-O-glucose and/or 19-O-glucose of a steviol glycoside substrate. In another embodiment, the B12GT is derived from an organism from the Plantae kingdom. In another embodiment, the B12GT is derived from an organism from the Angiosperm clade. In another embodiment, the B12GT is derived from the species *Hordeum vulgare*, *Oryza sativa*, *Solanum lycopersicum*, *Lycium barbarum*, *Solanum tuberosum*, or *Stevia rebaudiana*. In another embodiment, the B12GT is an engineered B12GT with a polypeptide sequence that is at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a B12GT derived from the species *Hordeum vulgare*, *Oryza sativa*, *Solanum lycopersicum*, *Lycium barbarum*, *Solanum tuberosum*, or *Stevia rebaudiana*.

[0042] In some embodiments, the B13GT, B12GT and/or sucrose synthase polypeptides are prepared by expression in a host microorganism. Suitable host microorganisms include, but are not limited to, *E. coli*, *Saccharomyces* sp., *Aspergillus* sp., *Pichia* sp., *Bacillus* sp. In a particular embodiment, the glycosyltransferase and sucrose synthase are expressed in *E. coli*. In an-

other embodiment, the glycosyltransferase and sucrose synthase are expressed in *Pichia pastoris*. In another embodiment, the B13GT, B12GT and/or sucrose synthase polypeptides are prepared by cell-free expression.

[0043] The B13GT, B12GT and sucrose synthase polypeptides can be provided in any suitable form, including free, immobilized or as a whole cell system. The degree of purity of the polypeptides may vary, e.g., they may be provided as a crude, semi-purified or purified enzyme preparation(s). In one embodiment, the B13GT, B12GT and/or SuSy polypeptide is free. In another embodiment, the B13GT, B12GT and/or SuSy polypeptide is immobilized to a solid support, for example on an inorganic or organic support. In some embodiments, the solid support is derivatized cellulose, glass, ceramic, methacrylate, styrene, acrylic, a metal oxide, or a membrane. In some embodiments, the B13GT, B12GT and/or SuSy polypeptide is immobilized to the solid support by covalent attachment, adsorption, cross-linking, entrapment, or encapsulation.

[0044] In yet another embodiment, the B13GT, B12GT and/or SuSy polypeptide is provided in the form of a whole cell system, for example as a living fermentative microbial cell, or as dead and stabilized microbial cell, or in the form of a cell lysate.

[0045] The steviol glycoside component(s) of the starting composition serve as a substrate(s) for the production of the target steviol glycoside(s), as described herein. The target steviol glycosides differ chemically from their corresponding substrate steviol glycoside(s) by the addition of one or more glucose units.

[0046] The starting steviol glycoside composition can contain at least one substrate steviol glycoside. In one embodiment, the substrate steviol glycoside is selected from the group consisting of steviol, steviol-13-O-glucoside, steviol-19-O-glucoside, rubusoside, steviol-1,2-bioside, steviol-1,3-bioside, rubusoside, dulcoside B, dulcoside A, rebaudioside B, rebaudioside G, stevioside, rebaudioside C, rebaudioside F, rebaudioside A, rebaudioside I, rebaudioside E, rebaudioside E2, rebaudioside H, rebaudioside L, rebaudioside K, rebaudioside J, rebaudioside M, rebaudioside AM, rebaudioside D, rebaudioside N, rebaudioside O, rebaudioside Q, an isomer thereof, a synthetic steviol glycoside or combinations thereof. In another embodiment the starting steviol glycoside composition comprises stevioside and Reb A. In another embodiment the starting steviol glycoside composition comprises stevioside. In yet another embodiment the starting steviol glycoside composition comprises Reb A.

[0047] The starting steviol glycoside composition may be synthetic or purified (partially or entirely), commercially available or prepared. One example of a starting composition useful in

the method of the present disclosure is an extract obtained from purification of *Stevia rebaudiana* plant material (e.g. leaves). Another example of a starting composition is a commercially available *stevia* extract brought into solution with a solvent. Yet another example of a starting composition is a commercially available mixture of steviol glycosides brought into solution with a solvent. Other suitable starting compositions include by-products of processes to isolate and purify steviol glycosides.

[0048] In one embodiment, the starting composition comprises a purified substrate steviol glycoside. For example, the starting composition may comprise greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 85%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, greater than 99%, or greater than 99.6% of one or more substrate steviol glycosides by weight on an anhydrous basis.

[0049] In another embodiment, the starting composition comprises a partially purified substrate steviol glycoside composition. For example, the starting composition contains greater than 0.5%, greater than 1%, greater than 2%, greater than 3%, greater than 4%, greater than 5%, greater than 10%, greater than 20%, greater than 30%, greater than 40%, or greater than 50%, of one or more substrate steviol glycosides by weight on an anhydrous basis.

[0050] In another embodiment, the substrate steviol glycoside is purified rebaudioside A, or isomers thereof. In a particular embodiment, the substrate steviol glycoside contains greater than 99% rebaudioside A, or isomers thereof, by weight on an anhydrous basis. In another embodiment, the substrate steviol glycoside comprises partially purified rebaudioside A. In a particular embodiment, the substrate steviol glycoside contains greater than 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% rebaudioside A by weight on an anhydrous basis.

[0051] In yet another embodiment, the substrate steviol glycoside comprises purified stevioside, or isomers thereof. In a particular embodiment, the substrate steviol glycoside contains greater than 99% stevioside, or isomers thereof, by weight on an anhydrous basis. In another embodiment, the substrate steviol glycoside comprises partially purified stevioside. In a particular embodiment, the substrate steviol glycoside contains greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% stevioside by weight on an anhydrous basis.

[0052] In yet another embodiment, the substrate steviol glycoside is a combination of stevioside and rebaudioside A. In a particular embodiment, the substrate steviol glycoside contains greater than 5% stevioside and greater than 5% Reb A, greater than 10% stevioside and greater than 10% Reb A, greater than 20% stevioside and greater than 20% Reb A, greater than 30%

stevioside and greater than 30% Reb A, greater than 40% stevioside and greater than 40% Reb A, greater than 45% stevioside and greater than 45% Reb A, greater than 40% stevioside and greater than 50% Reb A, greater than 30% stevioside and greater than 60% Reb A, greater than 20% stevioside and greater than 70% Reb A, greater than 10% stevioside and greater than 80% Reb A, greater than 5% stevioside and greater than 90% Reb A, greater than 50% stevioside and greater than 40% Reb A, greater than 60% stevioside and greater than 30% Reb A, greater than 70% stevioside and greater than 20% Reb A, greater than 80% stevioside and greater than 10% Reb A, or greater than 90% stevioside and greater than 5% Reb A by weight on an anhydrous basis.

10 [0053] In still another embodiment, the substrate steviol glycoside is derived from stevia leaf extract. In one embodiment, RA50, stevia leaf extract purified to contain greater than 50% Reb A, is used as the steviol glycoside substrate. In one embodiment, RA50 is used at a concentration between 1 mg/ml and 800 mg/ml. In another embodiment, RA50 is used at a concentration of 100 mg/ml. In another embodiment, RA60, stevia leaf extract purified to contain greater than 60% Reb A, is used as the steviol glycoside substrate. In another embodiment, RA60 is used at a concentration between 1 mg/ml and 800 mg/ml. In another embodiment, RA60 is used at a concentration of 100 mg/ml. In another embodiment, RS90, stevia leaf extract purified to contain greater than 90% stevioside, is used as the steviol glycoside substrate. In another embodiment, RS99, stevia leaf extract purified to contain greater than 99% stevioside, is used as the steviol glycoside substrate. In another embodiment, RS90 is used at a concentration between 1 mg/ml and 800 mg/ml. In another embodiment, RS90 is used at a concentration of 100 mg/ml.

[0054] The reaction can be carried out with a nucleotide cofactor that can be converted to an NDP-glucose by sucrose synthase. In some embodiments, the nucleotide can be ADP, GDP, UDP, CDP, or TDP. In some embodiments, the nucleotide can be a non-UDP nucleotide (i.e. ADP, GDP, CDP, or TDP). In another embodiment, the nucleotide is ADP. In a particular embodiment, the reaction can be carried out with ADP at a concentration between 0.01 mM and 10 mM, such as, for example, between 0.01 mM and 0.05 mM, between 0.05 mM and 0.1 mM, between 0.1 mM and 0.5 mM, between 0.5 mM and 1 mM, between 1 mM and 5 mM, or between 5 mM and 10 mM. In a particular embodiment, ADP is used at a concentration of 0.5 mM.

[0055] The reaction can be carried out with a sucrose concentration between 10 mM and 2M, such as, for example, greater than 10 mM, greater than 50 mM, greater than 100 mM, greater

than 250 mM, greater than 500 mM, greater than 1 M, greater than 1.5 M and greater than 2 M. In a particular embodiment, sucrose is used at a concentration of 450 mM.

[0056] In one embodiment, the reaction is run at any temperature. In another embodiment, the reaction is run at a temperature between 10 °C and 80 °C. Such as, for example, between 10°
5 C to 20° C, between 20° C to 30° C, between 30° C to 40° C, between 40° C to 50° C, between 50° C to 60° C, between 60° C to 70° C, between 70° C to 80° C or 80° C. In a particular embodiment, the one-pot reaction is carried out at 60 °C.

[0057] The reaction medium for conversion is generally aqueous, e.g., purified water, buffer, or a combination thereof. In a particular embodiment, the reaction medium is a buffer. Suitable
10 buffers include, but are not limited to, acetate buffer, citrate buffer, HEPES, and phosphate buffer. In a particular embodiment, the reaction medium is phosphate buffer. The reaction medium can have a pH between 4 and 10. In a particular embodiment, the reaction medium has a pH of 6. The reaction medium can also be, alternatively, an organic solvent.

[0058] The step of contacting the starting composition with the glycosyltransferase and sucrose
15 synthase polypeptides can be carried out in a duration of time between 1 hour and 1 week, such as, for example, between 30 minutes and 1 hours, between 1 hour and 4 hours, between 4 hours and 6 hours, between 6 hours and 12 hours, between 12 hours and 24 hours, between 1 day and 2 days, between 2 days and 3 days, 3 days and 4 days, between 4 days and 5 days, between 6 days and 7 days. In a particular embodiment, the reaction is carried out for 6 hours.

[0059] The reaction can be monitored by suitable methods including, but not limited to, high-
20 performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LCMS), thin layer chromatography (TLC), infrared spectrometry (IR), or nuclear magnetic resonance (NMR).

[0060] The target steviol glycoside can be any steviol glycoside. In one embodiment, the target
25 steviol glycoside is steviol-13-O-glucoside, steviol-19-O-glucoside, rubusoside, steviol-1,2-bioside, steviol-1,3-bioside, rubusoside, dulcoside B, dulcoside A, rebaudioside B, rebaudioside G, stevioside, rebaudioside C, rebaudioside F, rebaudioside A, rebaudioside I, rebaudioside E, rebaudioside E2, rebaudioside H, rebaudioside L, rebaudioside K, rebaudioside J, rebaudioside M, rebaudioside AM, rebaudioside D, rebaudioside N, rebaudioside O, rebaudioside Q, a re-
30 baudioside with 7 covalently attached glucose units (e.g. rebaudioside M plus 1 glucose unit), a synthetic steviol glycoside, an isomer thereof, and/or a steviol glycoside composition. In another embodiment, the target steviol glycoside is rebaudioside I, or isomers thereof. In another embodiment, the target steviol glycoside is rebaudioside M, or isomers thereof. In another em-

bodiment, the target steviol glycoside is rebaudioside AM, or isomers thereof. In another embodiment, the target steviol glycoside is rebaudioside E2, or isomers thereof. In still another embodiment, the target steviol glycosides are Reb I, Reb AM, Reb D and Reb M.

[0061] In one embodiment, the conversion of the starting steviol glycoside composition to Reb I is at least 2% complete, as determined by any of the methods mentioned above. In a particular embodiment, the conversion of the starting steviol glycoside composition to Reb I is at least 10% complete, at least 20% complete, at least 30% complete, at least 40% complete, at least 50% complete, at least 60% complete, at least 70% complete, at least 80% complete or at least 90% complete. In a particular embodiment, the conversion of starting steviol glycoside composition to Reb I is at least 95% complete. In some embodiments, wherein at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the starting steviol glycoside composition is converted to Reb I.

[0062] In one embodiment, the conversion of the starting steviol glycoside composition to Reb M is at least 2% complete, as determined by any of the methods mentioned above. In a particular embodiment, the conversion of the starting steviol glycoside composition to Reb M is at least 10% complete, at least 20% complete, at least 30% complete, at least 40% complete, at least 50% complete, at least 60% complete, at least 70% complete, at least 80% complete or at least 90% complete. In a particular embodiment, the conversion of the starting steviol glycoside composition to Reb M is at least 95% complete. In some embodiments, wherein at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the starting steviol glycoside composition is converted to Reb M.

[0063] In one embodiment, the conversion of the starting steviol glycoside composition to Reb AM is at least 2% complete, as determined by any of the methods mentioned above. In a particular embodiment, the conversion of the starting steviol glycoside composition to Reb AM is at least 10% complete, at least 20% complete, at least 30% complete, at least 40% complete, at least 50% complete, at least 60% complete, at least 70% complete, at least 80% complete or at least 90% complete. In a particular embodiment, the conversion of the starting steviol glycoside composition to Reb AM is at least 95% complete. In some embodiments, wherein at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the starting steviol glycoside composition is converted to Reb AM.

[0064] In one embodiment, the conversion of the starting steviol glycoside composition to Reb E2 is at least 2% complete, as determined by any of the methods mentioned above. In a particular embodiment, the conversion of the starting steviol glycoside composition to Reb E2 is at least 10% complete, at least 20% complete, at least 30% complete, at least 40% complete, at

least 50% complete, at least 60% complete, at least 70% complete, at least 80% complete or at least 90% complete. In a particular embodiment, the conversion of the starting steviol glycoside composition to reb E2 is at least 95% complete. In some embodiments, wherein at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the starting steviol glycoside composition is converted to Reb E2.

5 [0065] In one embodiment, the conversion of the starting steviol glycoside composition to Reb I, Reb AM, Reb D and Reb M is at least 2% complete, as determined by any of the methods mentioned above. In a particular embodiment, the conversion of the starting steviol glycoside composition to Reb I, Reb AM, Reb D and Reb M is at least 10% complete, at least 20% complete, at least 30% complete, at least 40% complete, at least 50% complete, at least 60% complete, at least 70% complete, at least 80% complete or at least 90% complete. In a particular embodiment, the conversion of the starting steviol glycoside composition to Reb E2 is at least 95% complete. In some embodiments, wherein at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the starting steviol glycoside composition is converted to Reb I, Reb AM, Reb D and Reb M.

15 [0066] The target steviol glycoside(s) can be in any polymorphic or amorphous form, including hydrates, solvates, anhydrous or combinations thereof.

[0067] Optionally, the method of the present disclosure further comprises separating the target steviol glycoside from the target composition. The target steviol glycoside(s) can be separated by any suitable method, such as, for example, crystallization, separation by membranes, centrifugation, extraction, chromatographic separation or a combination of such methods.

20 [0068] In one embodiment, the separation of target steviol glycosides produces a composition comprising greater than 80% by weight of the target steviol glycoside(s) on an anhydrous basis, i.e., a highly purified steviol glycoside composition. In another embodiment, separation produces a composition comprising greater than 0.5%, greater than 1%, greater than 2%, greater than 3%, greater than 4%, greater than 5%, greater than 10%, greater than 20%, greater than 30%, greater than 40%, greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 85%, greater than 90%, greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, greater than 99%, or greater than 99.6% by weight of the target steviol glycosides. In particular embodiments, the composition comprises greater than 95% by weight of the target steviol glycoside(s) on an anhydrous basis.

30 [0069] Purified target steviol glycosides can be used in consumable products as a sweetener. Suitable consumer products include, but are not limited to, food, beverages, pharmaceutical

compositions, tobacco products, nutraceutical compositions, oral hygiene compositions, and cosmetic compositions.

[0070] Plasmids containing nucleic acids encoding enzymes having SEQ ID NOs: 2-517 are described in **Table 1** below.

5 **Table 1. Designed B13GTs**

SEQ ID	Plasmid ID	Amino Acid ID	SEQ ID	Plasmid ID	Amino Acid ID	SEQ ID	Plasmid ID	Amino Acid ID
2	pA35354	AA29161	174	pA51826	AA40198	346	pA53154	AA41204
3	pA35355	AA29162	175	pA51827	AA40199	347	pA53155	AA41205
4	pA35356	AA29163	176	pA51828	AA40200	348	pA53156	AA41206
5	pA35357	AA29164	177	pA51829	AA40201	349	pA53157	AA41207
6	pA35358	AA29165	178	pA51830	AA40202	350	pA53158	AA41208
7	pA35359	AA29166	179	pA51831	AA40203	351	pA53159	AA41209
8	pA35360	AA29167	180	pA51832	AA40204	352	pA53160	AA41210
9	pA35361	AA29168	181	pA51833	AA40205	353	pA53161	AA41211
10	pA35362	AA29169	182	pA51834	AA40206	354	pA53162	AA41212
11	pA35363	AA29170	183	pA51835	AA40207	355	pA53163	AA41213
12	pA35364	AA29171	184	pA51836	AA40208	356	pA53164	AA41214
13	pA35365	AA29172	185	pA51837	AA40209	357	pA53165	AA41215
14	pA35366	AA29173	186	pA51838	AA40210	358	pA53166	AA41216
15	pA35367	AA29174	187	pA51839	AA40211	359	pA53167	AA41217
16	pA35368	AA29175	188	pA51840	AA40212	360	pA53168	AA41218
17	pA35369	AA29176	189	pA51841	AA40213	361	pA53169	AA41219
18	pA35370	AA29177	190	pA51842	AA40214	362	pA53170	AA41220
19	pA35371	AA29178	191	pA51843	AA40215	363	pA53171	AA41221
20	pA35372	AA29179	192	pA51844	AA40216	364	pA53172	AA41222
21	pA35373	AA29180	193	pA51845	AA40217	365	pA53173	AA41223
22	pA35374	AA29181	194	pA51846	AA40218	366	pA53174	AA41224

23	pA3537 5	AA2918 2	195	pA5184 7	AA4021 9	367	pA5317 5	AA4122 5
24	pA3537 6	AA2918 3	196	pA5184 8	AA4022 0	368	pA5317 6	AA4122 6
25	pA3537 7	AA2918 4	197	pA5184 9	AA4022 1	369	pA5317 7	AA4122 7
26	pA3537 8	AA2918 5	198	pA5185 0	AA4022 2	370	pA5317 8	AA4122 8
27	pA3537 9	AA2918 6	199	pA5185 1	AA4022 3	371	pA5317 9	AA4122 9
28	pA3538 0	AA2918 7	200	pA5185 2	AA4022 4	372	pA5318 0	AA4123 0
29	pA3538 1	AA2918 8	201	pA5185 3	AA4022 5	373	pA5318 1	AA4123 1
30	pA3538 2	AA2918 9	202	pA5185 4	AA4022 6	374	pA5318 2	AA4123 2
31	pA3538 3	AA2919 0	203	pA5185 5	AA4022 7	375	pA5318 3	AA4123 3
32	pA3538 4	AA2919 1	204	pA5185 6	AA4022 8	376	pA5318 4	AA4123 4
33	pA3538 5	AA2919 2	205	pA5185 7	AA4022 9	377	pA5318 5	AA4123 5
34	pA3538 6	AA2919 3	206	pA5185 8	AA4023 0	378	pA5318 6	AA4123 6
35	pA3538 7	AA2919 4	207	pA5185 9	AA4023 1	379	pA5318 7	AA4123 7
36	pA3538 8	AA2919 5	208	pA5186 0	AA4023 2	380	pA5318 8	AA4123 8
37	pA3538 9	AA2919 6	209	pA5186 1	AA4023 3	381	pA5318 9	AA4123 9
38	pA3539 0	AA2919 7	210	pA5186 2	AA4023 4	382	pA5319 0	AA4124 0
39	pA3539 1	AA2919 8	211	pA5186 3	AA4023 5	383	pA5319 1	AA4124 1
40	pA3539 2	AA2919 9	212	pA5186 4	AA4023 6	384	pA5319 2	AA4124 2
41	pA3539 3	AA2920 0	213	pA5186 5	AA4023 7	385	pA5319 3	AA4124 3
42	pA3539 4	AA2920 1	214	pA5186 6	AA4023 8	386	pA5319 4	AA4124 4
43	pA3539 5	AA2920 2	215	pA5186 7	AA4023 9	387	pA5319 5	AA4124 5
44	pA3539 6	AA2920 3	216	pA5186 8	AA4024 0	388	pA5319 6	AA4124 6
45	pA3539 7	AA2920 4	217	pA5186 9	AA4024 1	389	pA5319 7	AA4124 7
46	pA3539 8	AA2920 5	218	pA5187 0	AA4024 2	390	pA5319 8	AA4124 8
47	pA3539 9	AA2920 6	219	pA5187 1	AA4024 3	391	pA5319 9	AA4124 9
48	pA3540 0	AA2920 7	220	pA5187 2	AA4024 4	392	pA5320 0	AA4125 0
49	pA3540 1	AA2920 8	221	pA5187 3	AA4024 5	393	pA5320 1	AA4125 1

50	pA3540 2	AA2920 9	222	pA5187 4	AA4024 6	394	pA5320 2	AA4125 2
51	pA3540 3	AA2921 0	223	pA5187 5	AA4024 7	395	pA5320 3	AA4125 3
52	pA3540 4	AA2921 1	224	pA5187 6	AA4024 8	396	pA5320 4	AA4125 4
53	pA3540 5	AA2921 2	225	pA5187 7	AA4024 9	397	pA5320 5	AA4125 5
54	pA3540 6	AA2921 3	226	pA5187 8	AA4025 0	398	pA5320 6	AA4125 6
55	pA4973 2	AA3589 8	227	pA5187 9	AA4025 1	399	pA5320 7	AA4125 7
56	pA4971 6	AA3589 9	228	pA5188 0	AA4025 2	400	pA5320 8	AA4125 8
57	pA4971 4	AA3590 0	229	pA5188 1	AA4025 3	401	pA5320 9	AA4125 9
58	pA4971 9	AA3590 1	230	pA5188 2	AA4025 4	402	pA5321 0	AA4126 0
59	pA4973 8	AA3590 2	231	pA5206 3	AA4041 5	403	pA5321 1	AA4126 1
60	pA4978 0	AA3590 3	232	pA5206 4	AA4041 6	404	pA5321 2	AA4126 2
61	pA4972 1	AA3590 4	233	pA5206 5	AA4041 7	405	pA5321 3	AA4126 3
62	pA4970 6	AA3590 5	234	pA5206 6	AA4041 8	406	pA5321 4	AA4126 4
63	pA4972 9	AA3590 6	235	pA5206 7	AA4041 9	407	pA5321 5	AA4126 5
64	pA4974 2	AA3590 7	236	pA5206 8	AA4042 0	408	pA5321 6	AA4126 6
65	pA4977 2	AA3590 8	237	pA5206 9	AA4042 1	409	pA5321 7	AA4126 7
66	pA4975 2	AA3590 9	238	pA5207 0	AA4042 2	410	pA5321 8	AA4126 8
67	pA4972 7	AA3591 0	239	pA5207 1	AA4042 3	411	pA5321 9	AA4126 9
68	pA4975 6	AA3591 1	240	pA5207 2	AA4042 4	412	pA5322 0	AA4127 0
69	pA4976 8	AA3591 2	241	pA5207 3	AA4042 5	413	pA5322 1	AA4127 1
70	pA4977 8	AA3591 3	242	pA5207 4	AA4042 6	414	pA5322 2	AA4127 2
71	pA4978 2	AA3591 4	243	pA5207 5	AA4042 7	415	pA5322 3	AA4127 3
72	pA4975 3	AA3591 5	244	pA5207 6	AA4042 8	416	pA5322 4	AA4127 4
73	pA4977 1	AA3591 6	245	pA5207 7	AA4042 9	417	pA5322 5	AA4127 5
74	pA4974 5	AA3591 7	246	pA5207 8	AA4043 0	418	pA5322 6	AA4127 6
75	pA4975 9	AA3591 8	247	pA5207 9	AA4043 1	419	pA5322 7	AA4127 7
76	pA4975 8	AA3591 9	248	pA5208 0	AA4043 2	420	pA5322 8	AA4127 8

77	pA4977 6	AA3592 0	249	pA5208 1	AA4043 3	421	pA5322 9	AA4127 9
78	pA4973 1	AA3592 1	250	pA5208 2	AA4043 4	422	pA5323 0	AA4128 0
79	pA4970 7	AA3592 2	251	pA5208 3	AA4043 5	423	pA5323 1	AA4128 1
80	pA4972 4	AA3592 3	252	pA5208 4	AA4043 6	424	pA5323 2	AA4128 2
81	pA4976 7	AA3592 4	253	pA5208 5	AA4043 7	425	pA5323 3	AA4128 3
82	pA4973 9	AA3592 5	254	pA5208 6	AA4043 8	426	pA5323 5	AA4128 5
83	pA4975 7	AA3592 6	255	pA5208 7	AA4043 9	427	pA5324 2	AA4129 2
84	pA4973 0	AA3592 7	256	pA5213 2	AA4054 7	428	pA5324 3	AA4129 3
85	pA4976 2	AA3592 8	257	pA5213 3	AA4054 8	429	pA5324 4	AA4129 4
86	pA4976 9	AA3592 9	258	pA5213 4	AA4054 9	430	pA5324 5	AA4129 5
87	pA4971 8	AA3593 0	259	pA5213 5	AA4055 0	431	pA5324 6	AA4129 6
88	pA4974 1	AA3593 1	260	pA5213 6	AA4055 1	432	pA5324 7	AA4129 7
89	pA4973 7	AA3593 2	261	pA5213 7	AA4055 2	433	pA5324 8	AA4129 8
90	pA4974 9	AA3593 3	262	pA5213 8	AA4055 3	434	pA5324 9	AA4129 9
91	pA4970 4	AA3593 4	263	pA5213 9	AA4055 4	435	pA5325 0	AA4130 0
92	pA4971 5	AA3593 5	264	pA5214 0	AA4055 5	436	pA5325 1	AA4130 1
93	pA4973 4	AA3593 6	265	pA5214 1	AA4055 6	437	pA5325 2	AA4130 2
94	pA4974 7	AA3593 7	266	pA5214 2	AA4055 7	438	pA5325 3	AA4130 3
95	pA4976 6	AA3593 8	267	pA5214 3	AA4055 8	439	pA5325 4	AA4130 4
96	pA4973 5	AA3593 9	268	pA5214 4	AA4055 9	440	pA5325 5	AA4130 5
97	pA4970 9	AA3594 0	269	pA5214 5	AA4056 0	441	pA5325 6	AA4130 6
98	pA4971 2	AA3594 1	270	pA5214 6	AA4056 1	442	pA5325 7	AA4130 7
99	pA4972 6	AA3594 2	271	pA5214 7	AA4056 2	443	pA5325 8	AA4130 8
100	pA4970 8	AA3594 3	272	pA5214 8	AA4056 3	444	pA5325 9	AA4130 9
101	pA4974 3	AA3594 4	273	pA5214 9	AA4056 4	445	pA5326 0	AA4131 0
102	pA4975 1	AA3594 5	274	pA5215 0	AA4056 5	446	pA5326 1	AA4131 1
103	pA4976 1	AA3594 6	275	pA5215 1	AA4056 6	447	pA5326 2	AA4131 2

104	pA4978 3	AA3594 7	276	pA5215 2	AA4056 7	448	pA5326 3	AA4131 3
105	pA4970 5	AA3594 8	277	pA5215 3	AA4056 8	449	pA5326 4	AA4131 4
106	pA4975 0	AA3594 9	278	pA5215 4	AA4056 9	450	pA5326 5	AA4131 5
107	pA4973 6	AA3595 0	279	pA5215 5	AA4057 0	451	pA5326 6	AA4131 6
108	pA4977 5	AA3595 1	280	pA5215 6	AA4057 1	452	pA5326 7	AA4131 7
109	pA4973 3	AA3595 2	281	pA5215 7	AA4057 2	453	pA5326 8	AA4131 8
110	pA4972 8	AA3595 3	282	pA5215 8	AA4057 3	454	pA5326 9	AA4131 9
111	pA4977 0	AA3595 4	283	pA5215 9	AA4057 4	455	pA5327 0	AA4132 0
112	pA4977 4	AA3595 5	284	pA5216 0	AA4057 5	456	pA5327 1	AA4132 1
113	pA4976 4	AA3595 6	285	pA5216 1	AA4057 6	457	pA5327 2	AA4132 2
114	pA4977 9	AA3595 7	286	pA5216 2	AA4057 7	458	pA5327 3	AA4132 3
115	pA4972 2	AA3595 8	287	pA5216 3	AA4057 8	459	pA5327 4	AA4132 4
116	pA4972 0	AA3595 9	288	pA5216 4	AA4057 9	460	pA5327 5	AA4132 5
117	pA4972 5	AA3596 0	289	pA5216 5	AA4058 0	461	pA5327 6	AA4132 6
118	pA4971 3	AA3596 1	290	pA5216 6	AA4058 1	462	pA5327 7	AA4132 7
119	pA4976 0	AA3596 2	291	pA5216 7	AA4058 2	463	pA5327 8	AA4132 8
120	pA4971 1	AA3596 3	292	pA5216 8	AA4058 3	464	pA5327 9	AA4132 9
121	pA4977 7	AA3596 4	293	pA5216 9	AA4058 4	465	pA5328 0	AA4133 0
122	pA4978 1	AA3596 5	294	pA5217 0	AA4058 5	466	pA5328 1	AA4133 1
123	pA4976 3	AA3596 6	295	pA5217 1	AA4058 6	467	pA5328 2	AA4133 2
124	pA4974 8	AA3596 7	296	pA5217 2	AA4058 7	468	pA5328 3	AA4133 3
125	pA4974 0	AA3596 8	297	pA5217 3	AA4058 8	469	pA5328 4	AA4133 4
126	pA4971 7	AA3596 9	298	pA5217 4	AA4058 9	470	pA5328 5	AA4133 5
127	pA4977 3	AA3597 0	299	pA5217 5	AA4059 0	471	pA5328 6	AA4133 6
128	pA4974 6	AA3597 1	300	pA5217 6	AA4059 1	472	pA5328 7	AA4133 7
129	pA4971 0	AA3597 2	301	pA5217 7	AA4059 2	473	pA5328 8	AA4133 8
130	pA4974 4	AA3597 3	302	pA5217 8	AA4059 3	474	pA5328 9	AA4133 9

131	pA4975 4	AA3597 4	303	pA5217 9	AA4059 4	475	pA5329 0	AA4134 0
132	pA4975 5	AA3597 5	304	pA5218 0	AA4059 5	476	pA5329 1	AA4134 1
133	pA4972 3	AA3597 6	305	pA5218 1	AA4059 6	477	pA5329 2	AA4134 2
134	pA4976 5	AA3597 7	306	pA5218 2	AA4059 7	478	pA5329 3	AA4134 3
135	pA5178 7	AA4015 9	307	pA5218 3	AA4059 8	479	pA5329 4	AA4134 4
136	pA5178 8	AA4016 0	308	pA5218 4	AA4059 9	480	pA5329 5	AA4134 5
137	pA5178 9	AA4016 1	309	pA5218 6	AA4060 1	481	pA5329 6	AA4134 6
138	pA5179 0	AA4016 2	310	pA5218 7	AA4060 2	482	pA5329 7	AA4134 7
139	pA5179 1	AA4016 3	311	pA5218 8	AA4060 3	483	pA5329 8	AA4134 8
140	pA5179 2	AA4016 4	312	pA5218 9	AA4060 4	484	pA5329 9	AA4134 9
141	pA5179 3	AA4016 5	313	pA5219 0	AA4060 5	485	pA5330 0	AA4135 0
142	pA5179 4	AA4016 6	314	pA5219 1	AA4060 6	486	pA5330 1	AA4135 1
143	pA5179 5	AA4016 7	315	pA5219 2	AA4060 7	487	pA5330 2	AA4135 2
144	pA5179 6	AA4016 8	316	pA5219 3	AA4060 8	488	pA5330 3	AA4135 3
145	pA5179 7	AA4016 9	317	pA5219 4	AA4060 9	489	pA5330 4	AA4135 4
146	pA5179 8	AA4017 0	318	pA5219 5	AA4061 0	490	pA5330 5	AA4135 5
147	pA5179 9	AA4017 1	319	pA5219 6	AA4061 1	491	pA5330 6	AA4135 6
148	pA5180 0	AA4017 2	320	pA5219 7	AA4061 2	492	pA5330 7	AA4135 7
149	pA5180 1	AA4017 3	321	pA5219 8	AA4061 3	493	pA5330 8	AA4135 8
150	pA5180 2	AA4017 4	322	pA5219 9	AA4061 4	494	pA5330 9	AA4135 9
151	pA5180 3	AA4017 5	323	pA5220 0	AA4061 5	495	pA5331 0	AA4136 0
152	pA5180 4	AA4017 6	324	pA5220 1	AA4061 6	496	pA5331 1	AA4136 1
153	pA5180 5	AA4017 7	325	pA5313 3	AA4118 3	497	pA5331 2	AA4136 2
154	pA5180 6	AA4017 8	326	pA5313 4	AA4118 4	498	pA5331 3	AA4136 3
155	pA5180 7	AA4017 9	327	pA5313 5	AA4118 5	499	pA5331 4	AA4136 4
156	pA5180 8	AA4018 0	328	pA5313 6	AA4118 6	500	pA5331 5	AA4136 5
157	pA5180 9	AA4018 1	329	pA5313 7	AA4118 7	501	pA5331 6	AA4136 6

158	pA5181 0	AA4018 2	330	pA5313 8	AA4118 8	502	pA5331 7	AA4136 7
159	pA5181 1	AA4018 3	331	pA5313 9	AA4118 9	503	pA5331 8	AA4136 8
160	pA5181 2	AA4018 4	332	pA5314 0	AA4119 0	504	pA5331 9	AA4136 9
161	pA5181 3	AA4018 5	333	pA5314 1	AA4119 1	505	pA5332 0	AA4137 0
162	pA5181 4	AA4018 6	334	pA5314 2	AA4119 2	506	pA5332 1	AA4137 1
163	pA5181 5	AA4018 7	335	pA5314 3	AA4119 3	507	pA5332 2	AA4137 2
164	pA5181 6	AA4018 8	336	pA5314 4	AA4119 4	508	pA5332 3	AA4137 3
165	pA5181 7	AA4018 9	337	pA5314 5	AA4119 5	509	pA5332 4	AA4137 4
166	pA5181 8	AA4019 0	338	pA5314 6	AA4119 6	510	pA5332 5	AA4137 5
167	pA5181 9	AA4019 1	339	pA5314 7	AA4119 7	511	pA5332 6	AA4137 6
168	pA5182 0	AA4019 2	340	pA5314 8	AA4119 8	512	pA5332 7	AA4137 7
169	pA5182 1	AA4019 3	341	pA5314 9	AA4119 9	513	pA5332 8	AA4137 8
170	pA5182 2	AA4019 4	342	pA5315 0	AA4120 0	514	pA5332 9	AA4137 9
171	pA5182 3	AA4019 5	343	pA5315 1	AA4120 1	515	pA5333 0	AA4138 0
172	pA5182 4	AA4019 6	344	pA5315 2	AA4120 2	516	pA5333 1	AA4138 1
173	pA5182 5	AA4019 7	345	pA5315 3	AA4120 3	517	pA5333 2	AA4138 2

EXAMPLES

Example 1: Computational Design of ADP-Glucose Dependent B13GTs

[0071] Computational designs were conducted to improve and convert the native B13GT from Stevia rebaudiana (Seq ID NO: 1) to an ADP-glycosyltransferase. Structural models of a B13GT variant of SEQ ID NO: 1 were generated and used as the starting point for computational designs. Visual inspection of the structural models identified residues H24 as the catalytic histidine, D123 which activates the histidine, and two sugar binding residues D379 and Q380 (numbering relative to SEQ ID NO: 2). Computational designs incorporating coevolutionary information were conducted to improve the B13GT. 53 of these computational designs were chosen for experimental validation (SEQ ID NOs: 2-54).

[0072] Polynucleotides encoding the amino acid sequences for the designed B13GTs were synthesized (Twist Bioscience) and inserted into the pARZ4 expression vector. The polynucleotides encoded the B13GT enzymes with an N-terminal His tag. Polynucleotides were either ordered as full-length genes or ordered as gene fragments and then assembled using Gibson assembly. The recombinant vectors were used in a heat shock method to transform E. coli HMS174(DE3) (Novagen) or T7 Express lysY/Iq (New England BioLabs), thereby preparing a recombinant microorganism.

[0073] The transformed recombinant microorganisms were inoculated to 1ml LB-kanamycin medium, cultured by shaking at 37°C overnight. The culture was inoculated to 5ml TB-kanamycin medium and grown for 2 hours at 37°C, followed by 25°C for 1 hour. The culture was induced with 50 µl 50 mM IPTG and grown overnight. Finally, the culture was centrifuged at top-speed for 5 minutes and stored at -80°C.

[0074] The microorganisms were dissolved in a lysis buffer (lysozyme, DNaseI, Bugbuster, 300ml 20 mM HEPES pH 7.5, 500mM NaCl, and 20mM Imidazole). Two to three glass beads were added to each well and were disrupted by shaking at 25° C and 220 rpm for 30 minutes. The disrupted liquid was centrifuged at 2200 x g for 6-10 minutes. The obtained supernatant was loaded onto a Ni-NTA plate and shaken for 10 minutes at room temperature. The plate was centrifuged for 4 minutes at 100 x g followed by two washes of 500 µl binding buffer (300 ml 20mM HEPES pH 7.5, 500mM NaCl, 20mM Imidazole) and two-minute centrifugation (500 x g). The proteins were eluted with 150 µl elution buffer (15 ml 20mM HEPES pH 7.5, 500mM NaCl, 500mM Imidazole) and shaken for 1 minute at 0.25 maximum shaking speed followed by centrifugation for 2 minutes at 500 x g. The recovered protein was desalted into a buffer solution for enzyme activity evaluation (50mM HEPES pH 7.5, 50mM NaCl).

[0075] Each B13GT variant was assayed in a one-pot reaction with a B12GT and SuSy enzyme. B12GT and SuSy were used in excess such that in the absence of the B13GT variant, the starting RA50 feed was fully converted to RebD/E. The purified B13GT, B12GT and SuSy were reacted with 100 mg/ml RA50, 450 mM Sucrose, and 0.5 mM ADP in 50 mM pH 6 phosphate buffer, and 50 mM NaCl for 24 hours at 60 °C.

[0076] Steviol glycosides produced and consumed during the reaction, stevioside, Reb A, Reb D, Reb E, Reb I, Reb M and other minor steviol glycosides, were monitored by LCMS using an Agilent 6470 QQQ mass spectrometer (column: Waters ACQUITY UPLC HSS T3 Column, 100 mm x 2.1 mm). The QQQ was run with multi reaction monitoring (MS/MS) to accurately quantitate steviol glycosides of interest. Several designed enzymes expressed well and were able to efficiently produce Reb M (Table 2).

Table 2. Purified Protein Concentration and Reb M Conversion of Successful B13GT Design Variants Expressed in *E.coli*

ID	Reb M Yield (%)	Purified Conc (uM)
AA29162	76.9	12.4
AA29163	50.2	16.5
AA29161	49.2	49.6
AA29165	35.1	75.5
AA29166	29.3	12.9
AA29201	20.7	58.5
AA29164	17.9	39.1
AA29174	14.4	48.6
AA29177	12.9	39.4
AA29181	12.1	20
AA29190	10.1	15.7
AA29172	9.3	73.2
AA29178	8.5	65.4
AA29180	8.5	14.8
AA29204	7.6	17.2
AA29179	7	30.3
AA29173	6.9	25.9
AA29200	6.9	49.4
AA29171	6.8	42.8
AA29184	4.9	36.6
AA29183	4.6	22.8
AA29186	4.3	36.8
AA29167	3.4	10.1
AA29175	3.4	28.4
AA29203	3.4	88.5
AA29213	3.2	28.8
AA29205	3	43.1
AA29187	2.8	14.7
AA29202	2.4	28
AA29176	2.2	19.4
AA29168	2.1	11.5
AA29196	1.9	35.4
AA29197	1.7	27
AA29169	1.7	41.6
AA29185	1.1	13.2
AA29194	1.1	17.8
AA29192	1	13.7
AA29182	0.8	52.7
AA29193	0.8	30.8

AA29189	0.7	22.6
AA29206	0.7	27.6
AA29195	0.6	37.7
AA29170	0.5	65.9
AA29209	0.2	11.8

Example 2: *Pichia pastoris* expression of designed B13GTs and SuSys

[0077] Polynucleotides of the top ten designed B13GTs from Example 1 optimized for *Pichia pastoris* expression were synthesized (Twist Bioscience) and inserted into a *Pichia* shuttle vector. The polynucleotides encoded the B13GT enzymes with a C-terminal His tag instead of the N-terminal His tag used in Example 1. Two unique polynucleotides for each B13GT amino acid sequence were ordered. The vectors were transformed into a commercially available *Pichia pastoris* strain (ATCC). The transformed microorganisms were grown in BMGY (buffered glycerol complex) media and protein expression was induced by feeding of methanol. The *Pichia* cells were lysed with Y-PER (Yeast Protein Extraction Reagent; Thermo Scientific) and the expressed proteins were purified by immobilized metal affinity chromatography (IMAC) and desalted into desalt buffer (20mM KPO4 pH6, 50mM NaCl). The designed B13GTs were solubly expressed (FIG. 3) and enzyme activity was measured, similar to Example 1 (Table 3).

[0078] Polynucleotides encoding the top performing B13GTs were amplified from the *Pichia* shuttle vectors and integrated into the *Pichia* genome. Four integrated *Pichia* strains expressing designed B13GTs were grown in 1L fermentors. The *Pichia* microorganisms were grown with glycerol as the main carbon source for ~24 hours, and then were fed methanol for ~72 hours to express the desired B13GT. The cells were collected and lysed by French press. The expressed protein was purified by immobilized metal affinity chromatography (IMAC) and dialyzed into desalt buffer (20mM KPO4 pH6, 50mM NaCl). All four strains successfully expressed the active B13GT in the fermentations.

Table 3. Purified Protein Concentration and Reb M Conversion of Top B13GT Designs Expressed in *Pichia*

ID	Reb M Yield (%)	Purified Conc (uM)
AA29162	97.4	19.9
AA29162	94.3	28
AA29163	72.8	12.3
AA29166	67.4	25.6
AA29166	67.3	22.4

AA29163	57.3	17.6
AA29164	39.1	15.1
AA29172	20.3	8
AA29177	17.9	9.5
AA29174	17.2	11.8
AA29161	16.4	3.6
AA29177	16.4	9.6
AA29165	16.4	4.9
AA29174	14.2	12.1
AA29172	8.9	5.4
AA29161	6.9	4.2
AA29201	3.1	2.3
AA29201	1.5	2.5

Example 3: Computational Designs of Improved B13GTs

[0079] A previously-developed protein sequence-based deep learning model was fine-tuned with the results from Example 1. The model was used to design improved B13GT enzymes.

- 5 Eighty of these computational designs were chosen for experimental validation (SEQ ID NOs: 55-134). Polynucleotides encoding the B13GT enzymes with a C-terminal His tag were synthesized (Twist Bioscience). *E. coli* strains expressing the designed B13GTs were created as described in Example 1. The designed B13GT enzymes were expressed and purified as described in Example 1.
- 10 [0080] Each B13GT variant was assayed in a one-pot reaction with a B12GT and SuSy enzyme. B12GT and SuSy were used in excess such that in the absence of the B13GT variant, the starting RA50 feed was fully converted to RebD/E. The purified B13GT, B12GT and SuSy were reacted with 71.5 mg/ml RA50, 250 mM Sucrose, and 0.5 mM ADP in 50 mM pH 6 phosphate buffer, and 50 mM NaCl for 24 hours at 60 °C. Steviol glycosides produced and
- 15 consumed during the reaction were measured as described in Example 1. The designed enzymes solubly expressed and were able to efficiently produce Reb M (Table 4).

Table 4. Purified Protein Concentration and Reb M Conversion of Top B13GT Design Variants Expressed in *E. coli*

ID	Reb M Yield (%)	Purified Conc (uM)	ID	Reb M Yield (%)	Purified Conc (uM)
AA35898	94.9	1.1	AA35925	50.1	16.8
AA35921	94.9	0.7	AA35967	49.4	8.0
AA35976	93.6	0.8	AA35971	48.7	2.8

AA35927	92.5	1.0	AA35972	47.9	3.4
AA35908	88.3	24.5	AA35974	47.4	3.6
AA35906	86.6	17.0	AA35952	45.8	4.0
AA35932	85.3	27.7	AA35929	45.1	17.2
AA35926	82.8	14.0	AA35949	42.4	3.7
AA35917	82.3	15.9	AA35911	39.7	23.7
AA35966	81.7	5.3	AA35939	38.9	4.3
AA35915	80.9	10.1	AA35902	37.2	19.9
AA35901	78.5	6.7	AA35934	34	10.8
AA35913	78	20.0	AA35914	32.2	11.7
AA35936	77.9	12.7	AA35938	32	3.1
AA35899	76.7	14.6	AA35942	31.9	2.7
AA35930	76.5	18.2	AA35912	31.6	7.3
AA35940	76	5.2	AA35945	29.9	4.5
AA35935	74.8	19.5	AA35907	29.1	8.1
AA35924	74.3	14.5	AA35944	27.4	4.2
AA35961	72.5	4.2	AA35959	24.8	3.4
AA35937	70.5	12.3	AA35905	24.3	12.3
AA35919	68.2	15.4	AA35956	23.7	3.0
AA35916	68	12.4	AA35969	21	3.4
AA35909	67.3	16.5	AA35962	16	3.3
AA35931	66.9	16.1	AA35918	12.6	11.5
AA35910	65.4	10.2	AA35950	4.4	3.6
AA35928	64.8	12.9	AA35946	3.8	5.1
AA35922	62	15.2	AA35958	3.4	1.4
AA35900	61.7	11.4	AA35968	3.3	0.8
AA35957	61.7	3.9	AA35975	3.2	5.3
AA35923	60.5	10.9	AA35948	1	2.9
AA35920	57.3	17.7	AA35970	0.5	6.8
AA35933	56.6	13.4	AA35941	0.5	5.9
AA35903	56.3	14.2	AA35965	0.4	14.8
AA35951	54	6.0	AA35953	0.4	5.1
AA35947	53	4.9	AA35955	0.4	11.7
AA35954	52.5	2.0	AA35973	0.3	4.6
AA35977	52.5	3.6	AA35963	0.3	6.0
AA35964	52.4	4.2	AA35960	0.2	3.4
AA35904	50.1	14.9			

[0081] Structural models of the top B13GT designs were generated and analyzed. To this end, a structure-based deep learning method trained to predict protein sequences for a given protein backbone was used to design further improved B13GT enzymes. 121 of these computational designs were chosen for experimental validation (SEQ ID NOs: 135-255). The machine learn-

ing model was retrained on crystal structures and structural models of proteins from thermo-
philic organisms. An additional 69 B13GT enzymes were selected for experimental validation
(SEQ ID NOs: 256-324). Polynucleotides encoding the B13GT enzymes (SEQ ID NOs: 135-
324) with a C-terminal His tag were synthesized (Twist Bioscience). *E. coli* strains expressing
5 the designed B13GTs were created as described in Example 1. The designed B13GT enzymes
were expressed and purified as described in Example 1.

[0082] Each B13GT variant was assayed in a one-pot reaction with a B12GT and SuSy en-
zyme. B12GT and SuSy were used in excess such that in the absence of the B13GT variant,
the starting RA50 feed was fully converted to RebD/E. The purified B13GT, B12GT and SuSy
10 were reacted with 71.5 mg/ml RA50, 450 mM Sucrose, and 0.5 mM ADP in 50 mM pH 6
phosphate buffer, and 50 mM NaCl for 24 hours at 60 °C. Steviol glycosides produced and
consumed during the reaction were measured as described in Example 1. The designed en-
zymes solubly expressed and were able to efficiently produce Reb M (Table 5).

15 **Table 5. Purified Protein Concentration and Reb M Conversion of Top B13GT Design
Variants Expressed in *E. coli***

ID	Reb M Yield (%)	Puri- fied Conc (uM)	ID	Reb M Yield (%)	Puri- fied Conc (uM)	ID	Reb M Yield (%)	Puri- fied Conc (uM)
AA40182	93.9	309.6	AA40227	26.6	117.2	AA40608	9.1	180.8
AA40172	86.5	170.5	AA40550	26.0	219.2	AA40572	9.0	19.0
AA40180	83.8	143.8	AA40592	25.3	255.6	AA40249	9.0	111.4
AA40184	80.5	224.6	AA40231	25.0	150.9	AA40211	8.9	26.4
AA40181	76.9	246.7	AA40173	25.0	92.4	AA40237	8.6	29.9
AA40183	73.8	221.0	AA40428	24.9	52.0	AA40238	8.6	26.6
AA40597	66.3	242.6	AA40214	23.0	60.7	AA40229	8.5	14.3
AA40174	60.5	200.3	AA40547	22.4	240.6	AA40423	8.5	6.5
AA40567	58.8	50.0	AA40429	21.9	153.0	AA40252	8.4	146.5
AA40189	57.5	194.6	AA40551	21.9	236.2	AA40248	8.4	42.2
AA40558	53.1	261.9	AA40192	21.4	42.5	AA40575	8.3	176.6
AA40557	52.5	291.2	AA40584	21.0	29.8	AA40218	8.2	43.8
AA40239	51.7	175.0	AA40561	20.4	177.9	AA40598	8.2	17.7
AA40213	50.1	120.7	AA40573	20.3	91.4	AA40198	8.1	7.6
AA40571	49.5	157.6	AA40217	20.1	157.6	AA40225	8.0	25.0
AA40553	49.3	209.0	AA40220	20.0	34.4	AA40416	7.8	5.3
AA40178	47.2	138.4	AA40193	19.8	31.6	AA40425	7.8	8.5
AA40578	46.3	223.8	AA40612	19.6	114.9	AA40615	7.7	203.5
AA40207	45.5	15.9	AA40200	19.4	14.4	AA40212	7.5	34.6
AA40579	44.6	60.5	AA40228	19.0	27.5	AA40235	7.5	19.2
AA40179	44.5	193.9	AA40236	19.0	105.4	AA40240	7.3	78.7

AA40605	43.2	135.6	AA40185	18.9	142.0	AA40588	6.8	39.9
AA40415	43.0	30.2	AA40570	18.7	140.7	AA40548	6.7	30.7
AA40555	43.0	134.4	AA40604	18.6	36.8	AA40253	6.6	46.9
AA40232	41.5	147.9	AA40574	18.6	165.4	AA40609	6.2	72.6
AA40426	40.4	64.5	AA40581	18.5	163.8	AA40576	6.2	203.2
AA40568	39.2	165.0	AA40589	17.5	92.4	AA40422	6.1	5.2
AA40221	38.9	32.0	AA40251	17.4	173.1	AA40190	6.0	7.0
AA40569	38.6	139.3	AA40418	17.1	16.1	AA40595	5.9	107.0
AA40177	38.6	254.2	AA40596	17.1	185.5	AA40556	5.8	16.9
AA40587	37.3	36.8	AA40199	17.1	18.6	AA40206	5.7	19.1
AA40424	37.1	66.3	AA40222	16.8	35.2	AA40226	5.6	18.8
AA40208	37.0	114.5	AA40580	16.6	206.4	AA40583	5.0	13.4
AA40611	37.0	62.9	AA40435	16.1	22.8	AA40603	5.0	81.9
AA40194	36.4	7.5	AA40559	16.1	191.5	AA40562	5.0	10.7
AA40196	35.5	13.9	AA40594	15.9	190.2	AA40614	4.7	317.3
AA40437	35.4	104.1	AA40602	15.5	273.0	AA40168	4.3	23.3
AA40564	35.2	26.6	AA40549	15.4	169.0	AA40244	4.3	31.3
AA40606	35.1	336.0	AA40419	15.2	15.1	AA40203	4.2	7.9
AA40204	34.6	142.7	AA40593	14.8	35.9	AA40563	4.2	8.5
AA40191	34.4	165.1	AA40433	14.7	18.8	AA40170	4.0	24.2
AA40577	34.3	150.6	AA40242	14.3	176.9	AA40169	4.0	7.7
AA40421	33.4	140.0	AA40219	14.1	10.5	AA40175	3.6	72.0
AA40243	32.9	96.2	AA40245	13.9	69.3	AA40201	3.2	4.7
AA40223	32.8	77.6	AA40233	13.9	109.1	AA40241	3.1	12.9
AA40167	32.6	64.5	AA40554	13.3	67.2	AA40163	3.0	13.5
AA40560	32.4	68.8	AA40230	13.3	43.8	AA40613	2.9	10.1
AA40607	32.4	309.6	AA40164	13.2	18.7	AA40187	2.8	62.4
AA40216	31.9	109.9	AA40586	13.1	84.0	AA40585	2.4	7.7
AA40427	31.8	72.3	AA40246	13.1	155.3	AA40161	2.2	18.4
AA40186	31.6	123.4	AA40601	12.6	192.0	AA40159	2.0	82.2
AA40210	31.3	145.4	AA40431	12.3	80.1	AA40166	1.9	8.7
AA40436	30.4	20.1	AA40439	12.2	179.1	AA40176	1.8	104.9
AA40205	30.1	40.0	AA40224	12.1	28.8	AA40434	1.7	10.5
AA40582	29.8	149.0	AA40234	11.2	13.2	AA40165	1.6	68.5
AA40591	28.4	27.8	AA40430	11.1	104.0	AA40162	1.5	24.8
AA40197	28.3	14.6	AA40432	10.9	57.9	AA40552	1.3	15.3
AA40188	28.3	61.2	AA40171	10.7	196.6	AA40616	1.2	131.7
AA40420	28.3	176.1	AA40195	10.6	15.0	AA40250	1.2	26.9
AA40438	28.0	107.1	AA40610	10.6	43.5	AA40247	1.1	5.1
AA40590	27.5	27.5	AA40215	9.4	19.2	AA40599	1.1	197.7
AA40565	27.1	23.3	AA40202	9.3	5.9	AA40254	0.9	12.1
AA40566	26.8	84.4	AA40209	9.3	45.3			
AA40417	26.8	27.9	AA40160	9.2	108.5			

Example 4: Computational Designs of Further Improved B13GTs

[0083] The protein-based deep learning model from Example 3 was fine-tuned with the results from Examples 1 and 3. This sequence-based model was used to design improved B13GT enzymes. One hundred of these computational designs were chosen for experimental validation (SEQ ID NOs: 325-424). Polynucleotides encoding the B13GT enzymes (SEQ ID NOs: 325-424) with a C-terminal His tag were synthesized (Twist Bioscience). *E. coli* strains expressing the designed B13GTs were created as described in Example 1. The designed B13GT enzymes were expressed and purified as described in Example 1.

[0084] Each B13GT variant was assayed in a one-pot reaction with a B12GT and SuSy enzyme. B12GT and SuSy were used in excess such that in the absence of the B13GT variant, the starting RA50 feed was fully converted to RebD/E. The purified B13GT, B12GT and SuSy were reacted with 71.5 mg/ml RA60, 450 mM Sucrose, and 0.5 mM ADP in 50 mM pH 6 phosphate buffer, and 250 mM NaAcetate for 24 hours at 60 °C. Steviol glycosides produced and consumed during the reaction were measured as described in Example 1. The designed enzymes solubly expressed and were able to efficiently produce Reb M (Table 6).

Table 6. Purified Protein Concentration and Reb M Conversion of Top B13GT Design Variants Expressed in *E. coli*

ID	Reb M Yield (%)	Purified Conc (uM)	ID	Reb M Yield (%)	Purified Conc (uM)	ID	Reb M Yield (%)	Purified Conc (uM)
AA41201	17.8	311.8	AA41233	4.9	283.6	AA41240	3.3	194.8
AA41191	16.9	321.1	AA41234	4.9	55.4	AA41269	3.3	239.4
AA41199	15.7	288.3	AA41241	4.7	255.8	AA41245	3.2	202.7
AA41195	15.1	317.7	AA41214	4.6	242.2	AA41232	3.2	93.9
AA41202	14.7	319.9	AA41265	4.6	38	AA41259	3.2	287.9
AA41198	13.9	290	AA41244	4.4	246.7	AA41237	3.1	241.5
AA41200	13.8	296	AA41268	4.4	28.8	AA41204	3.1	196.5
AA41185	13.7	315.6	AA41208	4.3	47.8	AA41223	3.1	20.2
AA41193	13.3	262.9	AA41275	4.3	137.7	AA41203	3	53.9
AA41183	11.9	296.4	AA41258	4.3	307.4	AA41253	3	113.1
AA41194	11.9	297.9	AA41217	4.3	239.4	AA41254	2.9	230
AA41190	11.6	169.6	AA41277	4.3	242.5	AA41212	2.9	255.8
AA41192	11.4	245.5	AA41280	4.2	98.6	AA41228	2.9	95.8
AA41196	10.7	291.6	AA41209	4.2	100.9	AA41205	2.8	77.6
AA41257	10.7	150	AA41242	4.1	17.3	AA41247	2.7	74
AA41186	10.4	251.1	AA41282	4.1	250.5	AA41219	2.7	252
AA41187	9.9	248.9	AA41251	4	205.7	AA41276	2.6	71.7
AA41197	9.5	287.2	AA41235	4	105.4	AA41220	2.6	222.2

AA41184	9.4	75	AA41227	4	85.3	AA41210	2.5	112.9
AA41189	8.7	276.7	AA41236	4	316.4	AA41211	2.5	68.2
AA41188	6.9	279	AA41266	3.9	12.8	AA41238	2.4	8.6
AA41274	6.6	272.5	AA41225	3.9	291.8	AA41215	2.4	157.3
AA41271	6.3	240.2	AA41249	3.8	315.1	AA41248	2.2	35.1
AA41279	5.8	66.4	AA41213	3.8	39.7	AA41207	2.1	202.7
AA41273	5.6	88.7	AA41263	3.8	247.8	AA41252	2.1	58.8
AA41239	5.6	195.4	AA41216	3.8	153.9	AA41255	2.1	80.7
AA41256	5.3	214.5	AA41246	3.8	31.7	AA41270	2	31.8
AA41264	5.3	178.9	AA41226	3.7	87.7	AA41230	1.9	171.4
AA41222	5.3	233.2	AA41221	3.7	210.2	AA41206	1.9	99
AA41260	5.2	212.6	AA41272	3.6	205.2	AA41250	1.8	58.7
AA41267	5.2	125.7	AA41281	3.5	150.4	AA41243	1.7	26.6
AA41218	5.2	117.5	AA41278	3.4	38.4	AA41262	1.7	133.3
AA41261	5.1	31.6	AA41224	3.4	83			
AA41229	5	40.9	AA41231	3.3	36.5			

- [0085] Structural models of the top B13GT designs from Example 3 were generated and analyzed. The structure-based deep learning method trained to predict protein sequences for a given protein backbone was used to generate further improved B13GT enzymes. The method was used to design improved B13GT enzymes. 93 of these computational designs were chosen for experimental validation (SEQ ID NOs: 425-517). Polynucleotides encoding the B13GT enzymes with a C-terminal His tag were synthesized (Twist Bioscience). *E. coli* strains expressing the designed B13GTs were created as described in Example 1. The designed B13GT enzymes were expressed and purified as described in Example 1.
- [0086] Each B13GT variant was assayed in a one-pot reaction with a B12GT and SuSy enzyme. B12GT and SuSy were used in excess such that in the absence of the B13GT variant, the starting RA50 feed was fully converted to RebD/E. The purified B13GT, B12GT and SuSy were reacted with 71.5 mg/ml RA60, 450 mM Sucrose, and 0.5 mM ADP in 50 mM pH 6 phosphate buffer, and 250 mM NaAcetate for 24 hours at 60 °C. Steviol glycosides produced and consumed during the reaction were measured as described in Example 1. The designed enzymes solubly expressed and were able to efficiently produce Reb M (Table 7).

Table 7. Purified Protein Concentration and Reb M Conversion of Top B13GT Design Variants Expressed in *E. coli*

ID	Reb M Yield (%)	Purified Conc (uM)	ID	Reb M Yield (%)	Purified Conc (uM)	ID	Reb M Yield (%)	Purified Conc (uM)
AA41333	10.1	164.5	AA41306	5.2	14	AA41338	3.8	93.9
AA41342	9.6	202.2	AA41366	5.2	64.1	AA41371	3.8	153.1

AA41345	9.5	124.3	AA41337	5.2	135.6	AA41309	3.8	221.7
AA41346	9.2	217.7	AA41294	5.2	248.1	AA41285	3.7	226.9
AA41343	9.1	243.6	AA41350	5.2	185.6	AA41301	3.7	53.3
AA41304	8.6	274.7	AA41353	5	23.3	AA41341	3.6	13.3
AA41355	8.6	280.7	AA41327	4.9	58.5	AA41364	3.6	129.9
AA41336	8.4	142.4	AA41347	4.9	24.3	AA41307	3.5	244
AA41370	8.1	257	AA41305	4.9	248.8	AA41375	3.5	136.7
AA41349	8	99.9	AA41354	4.9	35.6	AA41283	3.4	197.8
AA41292	8	295.3	AA41328	4.9	79.5	AA41299	3.3	182.2
AA41356	7.4	64.4	AA41361	4.8	53.2	AA41308	3.2	76.7
AA41324	7.4	65.3	AA41331	4.8	70	AA41302	3.1	41.8
AA41330	7.4	114.8	AA41318	4.7	54.6	AA41372	3	212.8
AA41319	7	130.5	AA41373	4.7	202.1	AA41367	2.9	236.8
AA41340	6.9	61.8	AA41293	4.7	199.5	AA41312	2.9	4.7
AA41344	6.9	160.7	AA41317	4.7	64	AA41316	2.9	274.2
AA41365	6.7	37.2	AA41310	4.6	113.8	AA41298	2.7	64.8
AA41352	6.7	237.8	AA41358	4.6	118.8	AA41295	2.5	145.4
AA41362	6.5	53.4	AA41303	4.6	208.1	AA41378	2.4	41.8
AA41348	6.3	70.8	AA41322	4.5	12.9	AA41381	2.2	76.4
AA41315	6.2	31.8	AA41321	4.4	145	AA41368	2.1	64.8
AA41334	6.2	144	AA41351	4.4	142.9	AA41374	1.8	173.9
AA41313	6.1	22.1	AA41300	4.2	247.9	AA41382	1.8	163.4
AA41332	6	131.4	AA41323	4.1	114	AA41379	1.8	227.9
AA41339	6	249.8	AA41363	4.1	137.5	AA41376	1.7	78.2
AA41320	5.9	68.6	AA41297	4	255.1	AA41360	1.7	53.1
AA41325	5.7	104.2	AA41296	4	38.6	AA41380	1.6	94.6
AA41357	5.5	227.3	AA41326	3.9	10.9	AA41359	1.4	102.5
AA41335	5.3	100.3	AA41329	3.9	28	AA41377	1.4	85.8
AA41311	5.3	10.8	AA41369	3.9	86.9	AA41314	1.4	83.8

Example 5: Representing Successful B13GTs Designs with a PSSM

[0087] The successful B13GT designs from Example 1 were used to generate a PSSM (Table 8). The PSSM is a concise way to represent the successful designs and related sequences. Sequences that have a PSSM score greater than 332 are considered related to the active B13GT computational designs described herein. To score a sequence with the PSSM, it must first be aligned with the representative sequence, SEQ ID NO: 2. For example, the following successful designs, AA29162, AA29163, AA29161, AA29165, have the following PSSM scores: 332.2, 335.5, 332.5, 337.3, while the wild-type B13GT SEQ ID NO: 1, has a PSSM score of only 326.7.

[0088] The successful B13GT designs from Example 3 were used to generate a PSSM (Table 9). The PSSM is a concise way to represent successful designs and related sequences. Sequences that have a PSSM score greater than 54.5 are considered related to the active B13GT computational designs described in herein. To score a sequence with the PSSM, it must first be aligned with the representative sequence, SEQ ID NO: 2. For example, the following successful designs, AA40232, AA40239, AA40205, AA40243, have the following PSSM scores: 70.74, 80.53, 78.75, 76.08, while the wild-type B13GT SEQ ID NO: 1, has a PSSM score of only 51.16.

[0089] The successful B13GT designs from Example 4 were used to generate a PSSM (Table 10). The PSSM is a concise way to represent successful designs and related sequences. Sequences that have a PSSM score greater than 58.3 are considered related to the active B13GT computational designs described herein. To score a sequence with the PSSM, it must first be aligned with the representative sequence, SEQ ID NO: 2. For example, the following successful designs, AA41333, AA41342, AA41345, AA41346, have the following PSSM scores: 90.04, 92.68, 89.16, 97.08, while the wild-type B13GT SEQ ID NO: 1, has a PSSM score of only 51.16.

Table 8. Position Specific Scoring Matrix (PSSM) of Successful B13GT Designs

Seq Pos	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	-
1	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
3	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
5	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	1	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	1	0
9	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
10	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0.7	0
15	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0.7	0	0.7	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0

20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	1	0.7	0	0	0	0	0	0	0	0	1	0
26	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	1	0	0	0.7	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
32	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0.7	0
35	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0
37	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
38	0	0.7	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
41	0.7	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	1	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0.7	0	0	1	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
48	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
50	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	0.7	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
56	0	0	1	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
61	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
62	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
63	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0

65	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
71	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0.7	0	0.7	0	0	0	0
73	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
76	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
77	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0.7	0	0	0
82	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
85	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
86	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
87	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
88	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
91	0	0	0	0	0	0.7	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0.7
92	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	1	1	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
97	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
98	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	1	0	0	0.7	0	0	0	0	0	0	0
101	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102	0	1	0	1	0	1	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	1	1	0	0	0.7	1	1	0	0	0	0.7	0	0	0	0	0
107	0.7	0	0	0	0.7	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	1	0	0.7	0	0	0	0	0	0	0	0
109	1	0.7	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0

110	1	0	0	0.7	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0.7	0	0	0	0
112	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
113	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
114	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
115	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
116	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	1	0	0	0	0	0
117	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
118	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
119	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0.7
121	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
122	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0
123	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
124	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
125	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
126	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0
127	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0
128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
129	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
130	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
131	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	1	0	0	0	0
132	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
133	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
134	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
135	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
136	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
137	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
138	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
139	0	1	0	0	0	0	0	0	0	0	0	0.7	0	0	1	0	0	0	0	0	0
140	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
141	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
142	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
143	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
144	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
145	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0
146	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0.7	0	0	0	0
147	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
148	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
149	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
150	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
151	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
152	0	0	0	0	0	0	0	0	0.7	0	1	0	0	0	0	0	0	0	0	0	0
153	0.7	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
154	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0

155	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
156	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
157	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
158	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
159	0	0	0	0	0	1	0	0	0	0	1	0.7	0	0	0	0	0	0	0	0	0
160	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
161	0	0	0	1	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
162	0	0	0	1	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
163	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
164	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
165	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0
166	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
167	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
168	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0.7	0	0	0	0	0	0
169	0	0	0	1	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
170	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
171	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
172	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	1	0	0	0	0
173	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
174	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0.7	0	0	0	0	0	0
175	0	0	0	0.7	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
176	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
177	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
178	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
179	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0.7	0
180	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
181	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
182	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
183	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
184	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
185	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
186	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
187	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
188	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
189	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
190	0	0.7	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
191	0	0.7	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
192	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
193	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0
194	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
195	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0
196	0	0	0	1	0	0	0	0	0	0.7	0	1	0	0	0	1	0	0.7	0	0	0
197	0	0	0	1	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
198	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
199	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0

200	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0
201	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
202	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
203	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
204	0	0	0	0.7	0	0	1	1	0	0	0	0	0.7	0	0	0	0	0	0	0
205	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0
206	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
207	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0.7
208	0	0	0	0	0	0	0.7	0	0	0	0	1	0	0	0	0	0	0	0	0
209	0	0	0	0	0	1	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
210	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0
211	0	0.7	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
212	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
213	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
214	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
215	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0
216	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0.7
217	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
218	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0
219	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
220	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
221	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
222	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0
223	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
224	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
225	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
226	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
227	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
228	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
229	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
230	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
231	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0
232	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0.7
233	0	1	0	0	0	0.7	0	0	0	1	0	0	0	0	0	0	0	0.7	0	0
234	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
235	0	0	0	1	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
236	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
237	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
238	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
239	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
240	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
241	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
242	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
243	0	0	0	0	0	0	0	0	0	1	0.7	0	0	0	0	0	0	0	0	0
244	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0

245	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
246	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
247	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
248	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0
249	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0.7	0	0
250	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
251	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
252	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
253	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
254	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0.7	0	0	0	0
255	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
256	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
257	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
258	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
259	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
260	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
261	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
262	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	1	0	0	0
263	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0.7	0	0	1	0
264	0.7	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
265	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
266	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0
267	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
268	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
269	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
270	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
271	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
272	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
273	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0.7	0	0	0	0	0
274	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
275	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
276	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
277	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0
278	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
279	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	1	0	0	0	0	0
280	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
281	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
282	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
283	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
284	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
285	0	0	0	0	0	1	0.7	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
286	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0.7	0
287	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
288	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
289	0.7	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0

290	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
291	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
292	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
293	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
294	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
295	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
296	0	0.7	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
297	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
298	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
299	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
300	0.7	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
301	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
302	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
303	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
304	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
305	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
306	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
307	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0
308	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
309	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
310	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
311	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
312	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
313	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
314	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
315	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
316	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
317	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0.7	0
318	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	1	0	0	0
319	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0
320	0	0	0	0	0	0	0	0	0	0.7	1	0	0	0	0	0	0	0	0	0	1
321	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
322	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	1	0	0	0	0	0	0
323	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
324	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
325	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
326	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
327	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
328	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
329	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
330	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
331	0	1	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
332	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
333	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
334	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	1

335	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
336	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
337	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
338	0.7	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
339	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
340	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
341	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
342	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
343	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
344	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
345	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
346	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0
347	0	0	0	0	0	0	1	1	0	0	0	0.7	0	0	0	0	0	0	0	0	0
348	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
349	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
350	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
351	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
352	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
353	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0
354	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0
355	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0
356	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
357	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
358	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0
359	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
360	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
361	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0
362	0	0	0	0	0	0	0	0	0	0	0	1	0	0.7	0	0	0	0	0	0	0
363	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
364	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
365	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
366	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
367	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
368	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
369	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
370	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
371	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
372	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
373	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
374	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
375	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
376	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
377	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
378	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
379	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

380	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
381	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
382	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
383	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
384	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
385	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
386	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0
387	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0
388	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0
389	0	0	0	1	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
390	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
391	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
392	0	0	0	0	0	0	0	0	0.7	0	0	1	0	0	0	0	0	0	0	0	0
393	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
394	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
395	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
396	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0
397	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
398	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
399	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
400	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
401	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	1	0	0	0
402	0	0	0	0	0	0	0.7	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
403	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
404	0	0	0	0	0	0	1	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
405	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
406	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
407	1	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0
408	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
409	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
410	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0.7	0
411	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
412	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
413	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
414	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0
415	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
416	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
417	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0
418	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
419	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
420	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
421	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0.7	0
422	0	0	0	0	0	0	0	0	0	1	0	0	0.7	0	0	0	0	0	0	0	0
423	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
424	0	0	0	0	0	0.7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

425	0	1	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
426	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
427	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
428	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	1	0
429	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0
430	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0
431	0	0	0	0.7	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
432	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0
433	1	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0
434	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
435	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
436	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
437	0	0	0	0	0	0	0	0	0	0	0.7	1	0	0	0	0	0	0	0	0
438	0.7	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
439	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0.7	0	0	0	0	0
440	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0
441	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0
442	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
443	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
444	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0
445	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
446	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
447	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
448	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
449	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
450	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0
451	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0
452	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
453	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0
454	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0	0	0
455	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
456	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0
457	0	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0	0	0	0	0	0

Table 9. Position Specific Scoring Matrix (PSSM) of Successful B13GT Designs

Seq Pos	Amino Acid																			
	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
1	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0
2	0	.11	.11	0	0	.11	.11	.11	.11	0	.11	.11	.11	.11	.11	0	0	0	0	.11
3	0	0	.11	0	0	0	0	0	0	0	.11	0	.11	.11	0	.11	0	0	0	0
4	0	0	.11	0	0	0	0	0	0	0	0	.11	0	0	.11	.11	.11	0	0	0
5	.11	0	.11	0	0	.11	0	0	.11	0	.11	0	.11	0	0	.11	.11	0	0	0
6	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	.11	0	1	0	0	0
7	.11	0	0	0	0	.11	0	0	0	0	.11	0	0	0	.11	0	.11	0	0	0
8	0	0	0	.11	0	0	0	0	0	0	.11	0	0	0	0	0	.11	0	0	1
9	.11	1	0	0	0	0	0	.11	0	0	.11	.11	.11	0	0	.11	0	0	0	.11
10	0	.11	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0
11	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
12	0	.11	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0	0	0	0
13	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
14	0	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0	0	0	0	0	.11
15	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	1
16	0	0	0	0	0	0	0	0	0	0	.11	0	.11	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
19	0	0	0	0	0	.11	0	0	0	0	.11	0	0	0	0	0	0	0	0	.11
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
22	.11	0	0	0	0	.11	0	0	.11	0	.11	0	0	0	.11	0	0	0	0	0
23	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0
25	.11	0	0	0	0	.11	0	0	.11	.11	.11	0	.11	0	0	.11	0	1	0	.11
26	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	.11	0	0	.11	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	.11	0	.11	0	0	0	0	0	0	0
30	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0
32	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11
35	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
37	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0
38	0	.11	0	0	0	.11	0	0	0	0	0	.11	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
41	.11	1	0	0	0	0	.11	0	0	0	0	0	0	0	0	.11	0	0	0	0

42	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	.11	0	0	1	0
44	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	.11	0
45	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	.11	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	.11	0	0	1	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0
48	0	1	.11	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
49	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
50	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	.11	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
53	0	.11	0	0	.11	.11	.11	0	0	0	.11	.11	0	0	0	0	0	0	0	.11	0
54	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	.11	0	0	.11
55	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	.11	.11	.11	0	.11	0
56	.11	0	.11	.11	0	0	0	0	.11	0	.11	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
59	0	0	0	0	0	.11	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
61	0	0	0	.11	0	.11	.11	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0
62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
63	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
64	0	0	0	0	0	1	1	0	0	0	.11	0	.11	.11	.11	.11	1	0	0	0	0
65	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0
66	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
67	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	.11	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0
69	.11	0	.11	.11	0	.11	0	0	0	0	0	0	.11	0	0	.11	.11	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	.11	.11	0	0	0	0	0
71	0	.11	0	0	0	.11	0	.11	0	0	.11	0	0	0	0	0	.11	0	0	.11	0
72	0	0	0	.11	0	0	.11	0	0	0	0	0	0	0	0	.11	.11	.11	0	0	0
73	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	.11	.11	.11	0	0	0
74	0	.11	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0	0
75	0	0	0	0	0	0	0	.11	0	.11	.11	0	.11	0	0	0	0	0	0	0	0
76	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0
77	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
78	0	.11	0	0	0	.11	.11	.11	0	.11	.11	.11	.11	.11	.11	.11	0	0	0	0	.11
79	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	.11	.11	0	0	0	.11
80	.11	0	0	.11	0	.11	0	0	0	0	.11	0	0	0	0	.11	0	.11	0	0	0
81	.11	0	0	0	.11	0	0	0	.11	0	.11	0	.11	0	0	.11	.11	0	0	.11	0
82	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
83	.11	0	.11	.11	0	0	0	0	0	0	0	.11	0	0	.11	.11	.11	0	0	0	0
84	.11	0	.11	.11	0	0	0	.11	.11	0	.11	0	0	0	0	0	0	0	0	.11	0
85	.11	0	0	0	0	0	.11	0	0	0	.11	0	0	0	0	0	.11	0	0	.11	0
86	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0

87	.11	.11	0	0	0	.11	.11	0	.11	0	.11	.11	.11	0	0	0	0	.11	0	0	0
88	.11	.11	0	0	.11	0	0	0	.11	.11	.11	.11	.11	0	0	0	.11	0	0	.11	0
89	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	.11	.11	.11	0	0	0	0
91	0	.11	.11	0	0	.11	0	0	0	.11	.11	0	0	0	0	0	.11	0	0	.11	0
92	0	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0	0	0	0	0	0	0
93	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	0	.11	0	0	.11	0	.11	0	0	0	0	.11	0	0	0	0	0	0	0	0	0
95	0	0	.11	0	0	0	0	0	.11	0	.11	0	0	0	0	0	.11	0	0	0	0
96	0	0	0	0	.11	0	0	.11	0	0	0	.11	0	0	0	0	0	0	0	0	0
97	.11	0	0	0	0	0	0	0	0	0	0	1	0	0	0	.11	0	0	0	.11	0
98	1	0	0	.11	0	0	.11	0	0	0	0	0	0	0	.11	.11	.11	0	0	0	0
99	0	1	0	0	0	1	.11	0	0	0	.11	0	0	0	.11	.11	0	0	0	.11	0
100	0	0	0	0	0	0	0	0	0	0	.11	0	0	.11	0	0	0	0	0	0	0
101	0	.11	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
102	1	.11	0	0	0	1	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0
103	1	0	0	0	0	0	.11	0	0	1	1	1	1	0	0	0	1	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
105	.11	0	0	0	0	0	.11	0	0	0	1	1	0	0	0	0	0	0	0	0	0
106	0	0	0	.11	0	1	1	.11	0	.11	.11	1	0	0	0	.11	.11	0	.11	0	0
107	.11	0	0	0	.11	0	1	0	0	1	.11	0	0	0	0	0	.11	0	1	.11	0
108	0	0	0	0	.11	0	0	0	.11	1	0	0	.11	0	0	0	0	0	0	0	0
109	1	.11	1	0	0	1	1	0	0	0	.11	0	0	0	0	.11	0	.11	0	0	0
110	.11	0	0	.11	0	0	0	.11	0	0	0	0	0	0	0	.11	0	0	0	0	0
111	0	0	0	0	.11	0	0	.11	0	0	0	0	0	0	0	.11	.11	0	0	0	0
112	.11	0	.11	0	0	0	.11	0	0	0	0	0	0	0	.11	.11	0	0	0	0	0
113	0	0	0	.11	0	0	.11	.11	0	0	0	0	0	0	.11	.11	0	0	0	0	0
114	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
115	0	.11	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
116	0	.11	0	.11	0	0	.11	0	0	0	0	0	0	0	0	.11	1	0	0	0	0
117	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	.11	0
118	.11	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	.11	.11	0	0	.11	0
119	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	.11	0
121	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0
122	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0
123	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
124	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
125	0	0	0	0	0	0	0	0	0	0	.11	0	.11	0	0	0	0	0	0	0	0
126	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0
127	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0
128	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0
129	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	1	0	0	0	0
130	0	0	0	0	0	.11	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
131	.11	0	0	1	0	0	1	0	0	0	0	0	0	0	.11	.11	0	0	0	.11	0

132	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
133	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
134	0	0	0	.11	0	0	.11	.11	0	0	0	1	0	0	0	.11	0	0	0	0	0
135	.11	0	0	0	0	0	1	0	0	0	0	1	0	0	0	.11	0	0	0	0	0
136	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
137	0	0	.11	0	0	0	0	.11	0	0	0	.11	0	0	0	0	0	0	0	0	0
138	0	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0	0	0	0	0	.11	0
139	.11	.11	.11	0	0	0	0	0	0	0	0	.11	0	0	.11	.11	0	0	0	0	0
140	0	.11	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
141	0	0	0	0	0	0	0	0	0	0	.11	0	.11	0	0	0	0	0	0	0	0
142	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
143	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
144	0	0	0	0	0	.11	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0
145	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0
146	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	.11	0	0	0	0	0
147	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
148	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
149	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
150	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
151	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0
152	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0
153	.11	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	.11	.11	0	0	.11	0
154	.11	0	0	0	0	.11	0	0	.11	0	.11	0	0	0	0	0	0	0	0	.11	0
155	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	.11	0
156	.11	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
157	0	0	0	0	0	.11	0	.11	0	0	.11	0	0	0	0	0	0	0	0	0	0
158	0	0	.11	.11	0	.11	.11	0	0	0	0	0	0	0	.11	0	.11	0	0	0	0
159	0	.11	.11	0	0	.11	1	0	0	0	.11	.11	.11	0	0	0	0	0	0	0	0
160	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0
161	0	0	0	.11	0	0	.11	0	0	1	.11	0	0	0	0	0	0	0	0	0	0
162	.11	1	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
163	.11	.11	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0	0	0	0	0	0
164	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
165	0	0	0	0	0	0	0	0	0	1	.11	0	0	0	0	0	0	0	.11	0	0
166	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
167	0	0	0	.11	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
168	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0	.11	0	0	0	0
169	0	0	1	.11	.11	.11	.11	.11	0	0	.11	0	0	0	0	.11	.11	0	0	.11	0
170	.11	0	1	.11	.11	0	0	.11	.11	0	0	0	0	0	.11	.11	.11	0	0	.11	0
171	.11	0	0	.11	0	.11	.11	0	0	0	.11	.11	0	0	.11	.11	.11	0	0	.11	0
172	0	0	.11	0	0	0	1	0	.11	0	0	1	0	0	0	.11	.11	1	0	0	0
173	1	.11	.11	1	0	0	0	.11	0	.11	.11	.11	0	0	.11	.11	0	0	0	0	0
174	0	0	0	1	0	0	.11	0	0	0	.11	0	.11	0	.11	.11	0	0	0	0	0
175	0	0	.11	.11	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
176	1	0	0	0	0	0	.11	0	0	0	0	1	0	0	0	0	0	0	0	0	0

177	0	0	0	0	0	.11	0	0	0	0	0	.11	0	0	.11	.11	0	0	0	0	0
178	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
179	.11	0	0	0	0	0	1	0	0	0	.11	0	0	0	1	.11	0	0	0	.11	0
180	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
181	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0
182	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
183	0	0	0	0	0	1	1	0	1	.11	.11	0	.11	0	.11	0	0	1	1	0	0
184	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
185	0	.11	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0
186	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
187	1	1	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0
188	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
189	0	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0	0	0	0	0	0	0
190	0	.11	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0
191	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
192	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
193	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	.11	0	0
194	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0	0
195	0	0	.11	0	0	.11	0	.11	.11	0	0	0	0	0	0	0	0	0	0	0	0
196	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	.11	0	0	0
197	0	.11	0	0	0	.11	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0
198	0	0	0	.11	0	0	.11	0	0	.11	0	0	.11	0	0	.11	.11	0	0	.11	0
199	.11	0	0	.11	0	.11	.11	.11	.11	0	.11	.11	.11	0	0	.11	0	0	.11	0	0
200	0	.11	.11	0	0	0	0	0	0	0	0	.11	0	0	0	.11	0	0	0	0	0
201	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	.11	0	.11	0	0	0	0
202	0	0	0	0	0	.11	0	0	.11	.11	0	0	0	0	0	0	.11	0	0	.11	0
203	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
204	0	0	0	.11	0	0	1	.11	0	0	0	0	.11	0	0	0	.11	0	0	0	0
205	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	.11	0	0	0	0	0	0
206	0	0	0	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0	0	0	0	0
207	0	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0	0	0	0	0	.11	0
208	.11	0	.11	.11	0	0	.11	.11	0	.11	.11	.11	0	0	0	0	.11	.11	0	.11	0
209	.11	0	0	0	0	.11	.11	.11	0	0	0	0	0	0	0	0	.11	0	.11	0	0
210	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0
211	0	.11	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
212	.11	1	1	0	0	0	1	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
213	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
214	0	.11	0	0	0	0	0	0	0	0	.11	.11	0	0	0	.11	0	0	0	0	0
215	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
216	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
217	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0
218	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0
219	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
220	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
221	.11	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0

222	.11	.11	.11	0	0	.11	0	0	.11	0	0	.11	0	0	.11	0	0	0	0	0	0
223	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
224	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
225	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
226	0	.11	0	0	0	0	.11	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0
227	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0
228	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
229	0	0	0	0	0	.11	0	0	0	0	.11	0	.11	0	0	0	0	0	0	0	0
230	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
231	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0
232	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	.11	0
233	.11	.11	0	0	.11	.11	0	.11	0	.11	.11	.11	.11	0	0	.11	.11	.11	0	.11	0
234	.11	.11	.11	0	0	.11	.11	0	.11	0	0	0	0	0	0	.11	.11	0	0	0	0
235	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
236	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
237	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
238	.11	0	0	0	0	0	0	.11	0	0	0	.11	0	0	0	0	0	0	0	0	0
239	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
240	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
241	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0
242	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
243	0	0	0	0	.11	0	0	0	0	.11	.11	0	.11	0	0	0	0	0	.11	.11	0
244	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	.11	0	0	0	0	0	0
245	0	0	0	0	0	0	0	.11	0	0	.11	0	0	0	0	0	0	0	0	0	0
246	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
247	.11	0	0	0	.11	0	0	0	0	0	.11	.11	0	0	0	0	0	0	0	0	0
248	0	0	0	0	.11	0	0	0	.11	0	.11	0	0	0	0	0	0	.11	0	0	0
249	0	0	.11	0	0	0	.11	0	.11	.11	.11	0	.11	0	.11	.11	.11	0	.11	0	0
250	.11	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0
251	.11	0	0	0	0	0	0	0	0	.11	.11	0	.11	.11	0	0	0	0	0	0	0
252	.11	0	.11	0	0	.11	.11	0	0	0	0	0	0	0	.11	.11	0	0	0	0	0
253	.11	0	.11	.11	0	0	.11	.11	0	0	0	0	0	0	.11	.11	.11	0	0	.11	0
254	.11	0	0	0	0	.11	0	.11	0	0	0	0	.11	0	.11	.11	.11	0	0	.11	0
255	0	0	.11	0	0	0	0	0	0	0	.11	0	0	0	0	.11	.11	0	0	0	0
256	.11	0	0	.11	0	0	0	.11	0	0	.11	0	.11	0	.11	0	.11	.11	0	0	0
257	.11	0	0	0	0	.11	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
258	0	0	.11	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
259	0	0	.11	0	0	.11	.11	.11	.11	0	0	0	0	0	.11	.11	.11	0	.11	0	0
260	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
261	.11	.11	0	0	0	0	0	0	.11	0	.11	.11	0	0	.11	.11	0	.11	0	0	0
262	.11	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	.11	.11	0	0	0	0
263	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	.11	0
264	.11	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0
265	.11	0	0	1	0	.11	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0
266	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	.11	0	0	0

267	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0
268	.11	0	0	.11	0	0	0	.11	.11	0	0	0	0	0	0	0	0	0	0	.11	0
269	0	.11	.11	0	0	.11	0	1	0	0	.11	0	.11	0	0	0	.11	0	0	0	0
270	0	0	0	0	0	.11	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0
271	1	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
272	0	0	0	.11	0	0	1	0	0	0	0	.11	0	0	.11	.11	0	0	0	0	0
273	.11	.11	0	0	0	0	1	1	.11	0	0	.11	0	0	0	.11	0	0	0	0	0
274	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
275	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
276	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
277	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	.11	0	0
278	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
279	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	.11	0	0	0	0	0
280	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0
281	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
282	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0
283	0	0	0	0	0	.11	0	0	0	.11	0	0	0	0	0	0	.11	0	0	0	0
284	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0
285	0	0	0	0	0	.11	.11	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
286	0	0	0	0	0	0	0	0	0	1	.11	0	0	0	0	0	0	0	0	.11	0
287	0	0	0	.11	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
288	0	1	0	0	0	0	.11	0	0	0	1	0	0	0	.11	0	0	0	0	0	0
289	.11	.11	.11	.11	0	0	1	.11	.11	0	0	.11	0	0	0	.11	.11	0	0	0	0
290	0	0	0	.11	0	0	.11	0	0	0	0	0	0	0	0	0	1	0	0	0	0
291	1	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0
292	0	0	0	0	0	0	0	0	0	0	.11	1	0	0	.11	0	0	0	0	0	0
293	1	1	1	0	0	0	.11	0	0	0	0	1	0	0	0	0	1	0	0	0	0
294	0	0	0	0	0	0	0	0	0	.11	1	0	0	0	0	0	0	0	0	0	0
295	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
296	.11	.11	0	1	0	.11	1	0	.11	0	0	0	0	0	0	0	0	0	0	.11	0
297	.11	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
298	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
299	0	0	0	0	0	0	0	0	0	0	0	1	.11	0	0	0	0	0	0	.11	0
300	.11	1	0	.11	0	.11	.11	.11	0	0	1	1	0	0	0	.11	0	0	0	0	0
301	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
302	0	.11	0	0	0	0	0	.11	0	0	0	.11	0	0	0	0	0	0	0	0	0
303	0	0	0	0	.11	.11	0	0	0	1	1	0	0	0	0	0	0	0	0	.11	0
304	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	.11	.11	0	0	0	0	0
305	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0
306	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	.11	0
307	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0
308	0	.11	.11	0	.11	.11	0	0	0	0	.11	0	0	.11	0	.11	.11	0	.11	.11	0
309	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
310	0	.11	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
311	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0

312	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
313	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	.11	0	0	0	0	0
314	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
315	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	.11	.11	0	0	0	0
316	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
317	0	.11	0	0	0	1	1	0	1	0	.11	0	0	0	0	.11	0	0	.11	0	0
318	1	0	0	1	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0	0	0	0
319	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0
320	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	1	0	0	.11	0
321	0	0	0	.11	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
322	.11	.11	0	0	0	0	0	.11	0	1	.11	.11	.11	1	.11	.11	.11	0	0	.11	0
323	0	0	0	0	0	0	0	0	0	1	.11	0	0	0	0	0	0	0	0	0	0
324	0	0	0	.11	0	0	0	.11	0	0	0	0	0	0	0	.11	0	0	0	0	0
325	0	0	0	.11	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
326	.11	0	.11	0	0	0	.11	.11	0	0	0	0	0	0	0	.11	0	0	0	0	0
327	0	0	0	0	0	0	0	0	0	0	.11	0	0	.11	0	0	0	0	.11	0	0
328	0	0	0	0	0	0	0	0	0	0	.11	0	0	1	0	0	.11	0	0	0	0
329	0	0	0	0	.11	0	0	.11	0	0	0	0	0	0	0	.11	0	0	0	0	0
330	0	0	0	.11	0	0	.11	.11	0	0	0	0	0	0	1	.11	.11	0	0	0	0
331	0	.11	0	0	.11	1	0	0	0	0	1	.11	0	0	0	0	0	0	0	0	0
332	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
333	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
334	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0
335	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
336	0	.11	0	0	0	.11	.11	0	0	0	0	.11	.11	0	0	0	0	0	.11	0	0
337	0	0	.11	0	0	0	0	0	0	0	0	0	0	.11	0	.11	0	.11	0	0	0
338	.11	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
339	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
340	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
341	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
342	0	.11	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	.11	0	0	0
343	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0
344	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
345	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
346	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0
347	0	.11	0	.11	0	0	0	.11	.11	0	0	.11	0	0	.11	0	0	0	0	0	0
348	.11	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
349	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0
350	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
351	.11	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
352	0	0	0	0	0	.11	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0
353	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	.11	0	0
354	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0
355	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0
356	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	.11	0	0	0	0	0

357	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
358	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0
359	0	0	.11	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
360	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	.11	0	0	0	0	0
361	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0
362	0	0	0	0	0	0	0	0	0	0	.11	0	.11	0	0	0	.11	0	0	0	0
363	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
364	.11	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	.11	0	0	0	0	0
365	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0
366	.11	0	0	0	.11	0	0	0	0	0	.11	0	0	0	0	.11	.11	0	0	.11	0
367	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
368	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
369	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	.11	0
370	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
371	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0
372	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0
373	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0
374	0	.11	0	0	0	0	0	0	0	0	0	.11	0	0	0	.11	.11	0	0	0	0
375	.11	0	0	.11	0	0	0	0	0	0	0	0	0	0	.11	0	.11	0	0	.11	0
376	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0
377	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
378	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
379	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
380	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
381	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0
382	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
383	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
384	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
385	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
386	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0
387	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0
388	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	.11	0	0	.11	0
389	.11	0	1	.11	0	0	.11	0	0	0	.11	1	0	0	0	.11	0	0	0	0	0
390	0	.11	0	0	0	0	0	0	0	.11	0	1	0	0	0	0	0	0	0	.11	0
391	0	.11	0	0	.11	0	0	0	0	0	.11	1	0	0	0	0	0	0	0	0	0
392	0	0	0	.11	0	.11	0	0	.11	0	0	.11	0	0	0	0	0	0	0	0	0
393	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0
394	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
395	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0
396	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	.11	.11	0	0
397	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
398	0	1	0	1	0	0	.11	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
399	0	1	.11	.11	0	.11	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0	0
400	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
401	0	0	0	0	0	0	0	0	0	0	.11	0	0	.11	0	0	0	.11	0	0	0

402	0	0	1	1	0	0	.11	0	0	.11	.11	0	0	0	0	0	0	0	0	1	0
403	0	.11	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	.11	0
404	1	0	0	1	0	0	1	.11	0	0	0	0	.11	0	0	0	.11	0	0	.11	0
405	.11	1	0	.11	0	.11	.11	0	0	0	0	1	0	0	.11	.11	.11	0	0	0	0
406	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0
407	.11	0	0	0	.11	0	.11	0	0	.11	0	0	0	0	0	0	.11	0	0	.11	0
408	1	.11	.11	.11	.11	0	1	0	0	0	0	.11	0	0	0	.11	.11	0	0	0	0
409	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
410	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0
411	0	.11	0	0	0	.11	.11	0	.11	0	0	.11	0	0	0	.11	.11	0	0	0	0
412	.11	.11	0	0	0	0	1	0	.11	0	1	.11	0	0	0	.11	.11	0	1	0	0
413	0	0	0	0	0	0	0	0	0	0	.11	0	.11	0	0	0	0	0	0	.11	0
414	0	0	0	0	0	0	0	0	0	0	.11	0	.11	0	0	0	0	0	0	0	0
415	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	.11	0
416	1	0	0	.11	0	0	.11	0	0	0	.11	0	0	0	0	.11	0	0	0	0	0
417	.11	.11	0	0	0	.11	.11	0	0	0	0	0	0	0	.11	0	0	0	0	.11	0
418	1	1	0	.11	0	0	.11	0	0	0	0	1	0	0	0	1	0	0	0	0	0
419	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
420	.11	.11	0	0	0	0	.11	.11	0	0	0	1	0	0	0	0	0	0	0	0	0
421	0	0	0	0	0	0	1	0	0	.11	0	0	0	0	1	0	0	0	.11	.11	0
422	0	0	0	0	0	.11	0	0	0	.11	0	0	.11	0	0	0	0	0	1	0	0
423	0	.11	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	.11	0	0	0	0
424	.11	0	1	0	0	.11	.11	0	0	0	0	1	0	0	0	0	0	.11	0	0	0
425	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
426	.11	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	.11	.11	0	0	.11	0
427	.11	.11	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0	0	0	0	0	0
428	0	.11	.11	0	0	.11	.11	.11	0	.11	.11	.11	0	.11	0	.11	.11	0	.11	.11	0
429	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	.11	0	0	0
430	0	.11	0	0	0	.11	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0
431	1	.11	0	.11	0	.11	.11	0	0	0	0	0	.11	0	0	0	0	0	0	0	0
432	0	0	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0	.11	0	0	0	0
433	.11	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
434	0	0	.11	.11	0	0	.11	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
435	.11	.11	.11	.11	.11	.11	.11	.11	0	.11	.11	.11	.11	0	0	.11	.11	0	.11	.11	0
436	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
437	0	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0	0	.11	0	0	.11	0
438	.11	.11	0	0	0	.11	.11	0	0	0	0	0	.11	0	0	.11	0	0	0	0	0
439	.11	.11	.11	.11	0	.11	.11	.11	.11	0	0	.11	.11	0	.11	0	0	.11	0	.11	0
440	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
441	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0
442	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	.11	.11	0	0	0	0
443	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0
444	.11	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0	0
445	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0	0	0	0
446	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	.11	0	0	0	0	0

447	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0
448	0	0	0	0	0	.11	.11	0	.11	0	0	.11	0	0	0	0	0	0	0	0	0
449	0	.11	0	0	0	0	0	.11	0	0	.11	0	0	0	0	.11	0	0	0	0	0
450	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	0
451	0	0	0	0	0	.11	.11	0	0	0	0	0	0	0	0	0	0	0	0	.11	0
452	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	.11	0	0	0	0
453	0	.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.11	0	0
454	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0	.11	0
455	0	.11	0	0	0	0	0	0	0	0	0	0	.11	0	0	.11	0	0	0	0	0
456	0	.11	0	0	0	0	0	0	0	0	0	.11	0	0	0	.11	0	0	0	0	0
457	0	0	0	0	0	0	0	0	0	0	0	.11	0	0	0	0	0	0	0	0	0

Table 10. Position Specific Scoring Matrix (PSSM) of Successful B13GT Designs

Seq Pos	Amino Acid																			
	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
1	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
2	0	.12	.12	0	0	.12	.12	.12	.12	0	.12	.12	.12	.12	.12	0	0	0	0	.12
3	0	0	.12	0	0	0	0	0	0	0	.12	0	.12	.12	0	.12	0	0	0	0
4	0	0	.12	0	0	0	0	0	0	0	0	.12	0	0	.12	.12	.12	0	0	0
5	.12	0	.12	0	0	.12	0	0	.12	0	.12	0	.12	0	0	.12	.12	0	0	0
6	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	0	1	0	0	0
7	.12	0	0	0	0	.12	0	0	0	0	.12	0	0	0	.12	0	.12	0	0	0
8	0	0	0	.12	0	0	0	0	0	0	.12	0	0	0	0	0	.12	0	0	1
9	.12	1	0	0	0	0	0	.12	0	0	.12	.12	.12	0	0	.12	0	0	0	.12
10	0	.12	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0
11	0	.12	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
12	0	.12	0	1	0	.12	0	0	0	0	0	0	0	0	0	.12	0	0	0	0
13	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
14	0	0	0	0	0	0	0	0	0	.12	.12	0	0	0	0	0	0	0	0	.12
15	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	1
16	0	0	0	0	0	0	0	0	0	0	.12	0	.12	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
19	0	0	0	0	0	.12	0	0	0	0	.12	0	0	0	0	0	0	0	0	.12
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
22	.12	0	0	0	0	.12	0	0	.12	0	.12	0	0	0	.12	0	0	0	0	0
23	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0
25	.12	0	0	0	0	.12	0	0	.12	.12	.12	0	.12	0	0	.12	0	0	0	.12
26	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	.12	0	0	.12	0	0	0	0	0	0	0

29	0	0	0	0	0	0	0	0	0	0	0	.12	0	.12	0	0	0	0	0	0	0
30	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
32	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12	0
35	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0
37	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
38	0	.12	0	0	0	.12	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
41	.12	0	0	0	0	0	.12	0	0	0	0	1	0	0	0	.12	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	.12	0	0	0	0
44	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12	0
45	0	0	0	0	0	0	0	0	0	0	.12	0	0	.12	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	.12	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0
48	0	1	.12	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
49	.12	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
50	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	.12	1	0	0	0	0	0	0	0	0	1	.12	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
53	0	.12	0	0	.12	.12	.12	0	0	0	.12	.12	0	0	0	0	0	0	0	.12	0
54	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	.12	0	0	.12	0
55	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	.12	.12	0	.12	0
56	.12	0	.12	.12	0	0	0	0	.12	0	.12	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
59	0	0	0	0	0	.12	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
61	0	0	0	.12	0	.12	.12	0	0	0	0	0	0	0	0	.12	.12	0	0	0	0
62	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
63	0	.12	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
64	0	0	1	0	0	0	1	0	0	0	.12	0	.12	.12	.12	.12	1	0	0	0	0
65	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12	0
66	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
67	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	0	1	.12	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
69	.12	0	.12	.12	0	.12	1	0	0	0	0	0	.12	0	0	.12	.12	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	.12	.12	0	0	0	0	0
71	0	.12	0	0	0	.12	0	.12	0	0	.12	0	0	0	0	0	.12	0	0	.12	0
72	0	0	0	.12	0	0	.12	0	0	0	0	0	0	0	.12	.12	.12	0	0	0	0
73	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	.12	.12	0	0	0	0

74	0	.12	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	.12	0	0	0
75	0	0	0	0	0	0	0	.12	0	.12	.12	0	.12	0	0	0	0	0	0	0	0
76	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	.12	0	0	0	0
77	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
78	0	.12	0	0	0	.12	.12	.12	0	.12	.12	.12	.12	.12	.12	0	0	0	0	.12	0
79	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	.12	.12	0	0	0	.12	0
80	.12	0	0	.12	0	.12	0	0	0	0	.12	0	0	0	.12	0	.12	0	0	0	0
81	.12	0	0	0	.12	0	0	0	.12	0	.12	0	.12	0	0	.12	.12	0	0	.12	0
82	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
83	.12	0	.12	.12	0	0	0	0	0	0	0	.12	0	0	.12	.12	.12	0	0	0	0
84	.12	0	.12	.12	0	0	0	.12	.12	0	.12	0	0	0	0	0	0	0	0	.12	0
85	.12	0	0	0	0	0	.12	0	0	0	.12	0	0	0	0	0	.12	0	0	.12	0
86	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
87	.12	.12	0	0	0	.12	.12	0	.12	0	.12	.12	.12	0	0	0	0	.12	0	0	0
88	.12	.12	0	0	.12	0	0	0	.12	.12	.12	.12	.12	0	0	0	.12	0	0	.12	0
89	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	.12	.12	.12	0	0	0	0
91	0	.12	.12	0	0	.12	0	0	0	.12	.12	0	0	0	0	0	.12	0	0	.12	0
92	0	0	0	0	0	0	0	0	0	.12	.12	0	0	0	0	0	0	0	0	0	0
93	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	0	.12	0	0	.12	0	.12	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
95	0	0	.12	0	0	0	0	0	.12	0	.12	0	0	0	0	0	0	.12	0	0	0
96	0	0	0	0	.12	0	0	.12	0	0	0	.12	0	0	0	0	0	0	0	0	0
97	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	.12	0
98	1	0	0	.12	0	0	.12	0	0	0	0	0	0	0	.12	.12	.12	0	0	0	0
99	0	1	0	0	0	1	.12	0	0	0	.12	1	0	0	.12	.12	0	0	0	.12	0
100	0	0	0	0	0	0	0	0	0	0	.12	0	0	.12	0	0	0	0	0	0	0
101	0	.12	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
102	1	.12	0	0	0	1	1	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
103	1	0	0	0	0	0	.12	0	0	1	1	1	0	0	0	0	1	0	0	1	0
104	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
105	.12	0	0	0	0	0	.12	0	0	0	0	1	0	0	0	0	0	0	0	0	0
106	1	1	1	.12	0	1	1	.12	0	.12	.12	1	0	0	0	.12	.12	0	.12	0	0
107	.12	0	0	0	.12	0	0	0	0	1	.12	0	0	1	0	0	.12	0	1	.12	0
108	0	0	0	0	.12	0	0	0	.12	1	1	0	.12	0	0	0	0	0	0	0	0
109	1	.12	0	0	0	0	1	0	0	0	.12	0	0	0	0	.12	0	.12	0	0	0
110	.12	0	0	.12	0	0	0	.12	0	0	0	0	0	0	0	.12	0	0	0	0	0
111	0	0	0	0	.12	0	0	.12	0	0	0	0	0	0	0	.12	.12	0	0	0	0
112	.12	0	.12	0	0	0	.12	0	0	0	0	0	0	0	.12	.12	0	0	0	0	0
113	0	0	0	.12	0	0	.12	.12	0	0	0	0	0	0	.12	.12	0	0	0	0	0
114	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
115	0	.12	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
116	0	.12	0	.12	0	0	.12	0	0	0	0	1	0	0	0	.12	1	0	0	0	0
117	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	.12	0
118	.12	1	0	0	.12	0	0	0	0	0	0	0	0	0	0	.12	.12	0	0	.12	0

119	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	.12
121	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
122	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0
123	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
124	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
125	0	0	0	0	0	0	0	0	0	0	.12	0	.12	0	0	0	0	0	0	0
126	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0
127	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0
128	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
129	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	1	0	0	0
130	0	0	0	0	0	.12	1	0	0	0	1	0	0	0	0	0	0	0	0	0
131	.12	0	0	1	0	0	1	0	0	0	0	0	0	0	.12	.12	0	0	0	.12
132	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12
133	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
134	0	0	0	.12	0	0	.12	.12	0	0	0	1	0	0	0	.12	0	0	0	0
135	.12	1	0	0	0	0	1	0	0	0	0	1	0	0	0	.12	0	0	0	0
136	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
137	0	0	.12	0	0	0	0	.12	0	0	0	.12	0	0	0	0	0	0	0	0
138	0	0	0	0	0	0	0	0	0	.12	.12	0	0	0	0	0	0	0	0	.12
139	.12	.12	.12	0	0	0	0	0	0	0	0	.12	0	0	.12	.12	0	0	0	0
140	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
141	0	0	0	0	0	0	0	0	0	0	.12	0	.12	0	0	0	0	0	0	0
142	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12
143	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
144	0	0	0	0	0	.12	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
145	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0
146	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	0	0	0	0
147	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0
148	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
149	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
150	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
151	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
152	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0
153	.12	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	.12	.12	0	0	.12
154	.12	0	0	0	0	.12	0	0	.12	0	.12	0	0	0	0	0	0	0	0	.12
155	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	.12
156	.12	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0
157	0	0	0	0	0	.12	0	.12	0	0	.12	0	0	0	0	0	0	0	0	0
158	0	0	.12	.12	0	.12	.12	0	0	0	0	0	0	0	.12	0	.12	0	0	0
159	0	.12	.12	0	0	.12	1	0	1	0	.12	.12	.12	0	0	0	0	0	0	0
160	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
161	0	0	0	.12	0	0	.12	0	0	0	.12	0	0	0	0	0	0	0	0	0
162	.12	0	0	0	0	1	.12	0	0	0	0	1	0	0	0	0	0	0	0	0
163	.12	.12	0	0	0	1	0	0	0	0	.12	.12	0	0	0	0	0	0	0	0

164	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
165	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	0	0
166	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
167	0	0	1	.12	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
168	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12	0	.12	0	0	0	0
169	1	0	1	.12	.12	.12	.12	.12	0	0	.12	0	0	0	0	.12	.12	0	0	.12	0
170	.12	0	1	.12	.12	0	0	.12	.12	0	0	0	0	0	.12	.12	.12	0	0	.12	0
171	.12	0	1	.12	0	.12	.12	0	0	0	.12	.12	0	0	.12	.12	.12	0	0	.12	0
172	0	0	.12	0	0	0	1	0	.12	0	0	1	0	0	0	.12	.12	0	0	0	0
173	0	.12	.12	1	0	0	0	.12	0	.12	.12	.12	0	0	.12	.12	0	0	0	0	0
174	0	0	0	0	0	0	.12	0	0	0	.12	0	.12	0	.12	.12	0	0	0	0	0
175	0	0	.12	.12	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
176	1	0	0	0	0	0	.12	0	0	0	0	1	0	0	0	0	0	0	0	0	0
177	0	0	0	0	0	.12	0	0	0	0	0	.12	0	0	.12	.12	0	0	0	0	0
178	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0
179	.12	0	0	0	0	0	1	0	0	0	.12	0	0	0	1	.12	0	0	0	.12	0
180	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
181	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
182	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
183	0	0	0	0	0	0	1	0	0	.12	.12	0	.12	0	.12	0	0	1	1	0	0
184	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
185	0	.12	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
186	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0
187	0	1	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
188	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
189	0	0	0	0	0	0	0	0	0	.12	.12	0	0	0	0	0	0	0	0	0	0
190	0	.12	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
191	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
192	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
193	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	.12	0	0
194	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	.12	.12	0	0	0	0	0
195	0	0	.12	0	0	.12	0	.12	.12	0	0	0	0	0	0	0	0	0	0	0	0
196	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	0	0	0
197	0	.12	0	0	0	.12	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
198	0	0	0	.12	0	0	.12	0	0	.12	0	0	.12	0	0	.12	.12	0	0	.12	0
199	.12	0	0	.12	0	.12	.12	.12	.12	0	.12	.12	.12	0	0	.12	0	0	.12	0	0
200	0	.12	.12	0	0	0	0	0	0	0	0	.12	0	0	0	.12	0	0	0	0	0
201	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	0	.12	0	0	0	0
202	0	0	0	0	0	.12	0	0	.12	.12	0	0	0	0	0	0	.12	0	0	.12	0
203	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
204	0	0	0	.12	0	0	1	.12	0	0	0	0	.12	0	0	0	.12	0	0	0	0
205	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	.12	0	0	0	0	0	0
206	0	0	0	0	0	0	0	0	0	0	0	.12	.12	0	0	0	0	0	0	0	0
207	0	0	0	0	0	0	0	0	0	.12	.12	0	0	0	0	0	0	0	0	.12	0
208	.12	0	.12	.12	0	0	.12	.12	0	.12	.12	.12	0	0	0	0	.12	.12	0	.12	0

209	.12	0	0	0	0	.12	.12	.12	0	0	0	0	0	0	0	0	.12	0	.12	0	0
210	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0
211	0	.12	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
212	.12	0	0	0	0	0	1	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
213	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
214	0	.12	0	0	0	0	0	0	0	0	.12	.12	0	0	0	.12	0	0	0	0	0
215	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
216	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0
217	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0
218	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0
219	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
220	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
221	.12	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
222	.12	.12	.12	0	0	.12	0	0	.12	0	0	.12	0	0	.12	0	0	0	0	0	0
223	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
224	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
225	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
226	0	.12	0	0	0	0	.12	0	0	0	0	0	0	0	0	.12	.12	0	0	0	0
227	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	.12	0	0	0	0
228	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
229	0	0	0	0	0	.12	0	0	0	0	.12	0	.12	0	0	0	0	0	0	0	0
230	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
231	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0
232	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12	0
233	.12	.12	0	0	.12	.12	0	.12	0	.12	.12	.12	.12	0	0	.12	.12	.12	0	.12	0
234	.12	.12	.12	0	0	.12	.12	0	.12	0	0	0	0	0	0	.12	.12	0	0	0	0
235	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
236	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0
237	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
238	.12	0	0	0	0	0	0	.12	0	0	0	.12	0	0	0	0	0	0	0	0	0
239	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
240	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
241	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
242	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
243	0	0	0	0	.12	0	0	0	0	.12	.12	0	.12	0	0	0	0	0	.12	.12	0
244	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	.12	0	0	0	0	0	0
245	0	0	0	0	0	0	0	.12	0	0	.12	0	0	0	0	0	0	0	0	0	0
246	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
247	.12	0	0	0	.12	0	0	0	0	0	.12	.12	0	0	0	0	0	0	0	0	0
248	0	0	0	0	.12	0	0	0	.12	0	.12	0	0	0	0	0	0	.12	0	0	0
249	0	0	.12	0	0	0	.12	0	.12	.12	.12	0	.12	0	.12	.12	.12	0	.12	0	0
250	.12	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0
251	.12	0	0	0	0	0	0	0	0	.12	.12	0	.12	.12	0	0	0	0	0	0	0
252	.12	0	.12	0	0	.12	.12	0	0	0	0	0	0	0	.12	.12	0	0	0	0	0
253	.12	0	.12	.12	0	0	.12	.12	0	0	0	0	0	0	.12	.12	.12	0	0	.12	0

254	.12	0	0	0	0	.12	0	.12	0	0	0	0	.12	0	.12	.12	.12	0	0	.12	0
255	0	0	.12	0	0	0	0	0	0	0	.12	0	0	0	0	.12	.12	0	0	0	0
256	.12	0	0	.12	0	0	0	.12	0	0	.12	0	.12	0	.12	0	.12	.12	0	0	0
257	.12	0	0	0	0	.12	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
258	0	0	.12	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
259	0	0	.12	0	0	.12	.12	.12	.12	0	0	0	0	0	.12	.12	.12	0	.12	0	0
260	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
261	.12	.12	0	0	0	0	0	0	.12	0	.12	.12	0	0	.12	.12	0	.12	0	0	0
262	.12	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	.12	0	0	0	0
263	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	.12	0
264	.12	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
265	.12	0	0	1	0	.12	1	0	.12	0	0	0	0	0	0	0	0	0	0	0	0
266	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	.12	0	0	0
267	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
268	.12	0	0	.12	0	0	0	.12	.12	0	0	0	0	0	0	0	0	0	0	.12	0
269	0	.12	.12	0	0	.12	0	0	0	0	.12	1	.12	0	0	0	.12	0	0	0	0
270	0	0	0	0	0	.12	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
271	1	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
272	0	0	0	.12	0	0	0	0	0	0	0	.12	0	0	.12	.12	0	0	0	0	0
273	.12	.12	0	0	0	0	1	1	.12	0	0	.12	0	0	0	.12	0	0	0	0	0
274	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
275	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0
276	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
277	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	0	0
278	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0
279	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	0	0	0	0	0
280	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
281	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
282	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	.12	0	0	0	0
283	0	0	0	0	0	.12	0	0	0	.12	0	0	0	0	0	0	.12	0	0	0	0
284	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	.12	0	0	0	0
285	0	0	0	0	0	.12	.12	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
286	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	.12	0
287	0	0	0	.12	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0
288	0	1	0	0	0	0	.12	0	0	0	0	0	0	0	.12	0	0	0	0	1	0
289	.12	.12	.12	.12	0	0	1	.12	.12	0	0	.12	0	0	0	.12	.12	0	0	0	0
290	0	0	0	.12	0	0	.12	0	0	0	0	0	0	0	0	0	1	0	0	0	0
291	1	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
292	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	.12	0	0	0	0	0	0
293	1	1	1	0	0	0	.12	0	0	0	0	1	0	0	0	0	1	0	0	0	0
294	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0
295	.12	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
296	.12	.12	0	1	0	.12	1	0	.12	0	0	0	0	0	0	0	0	0	0	.12	0
297	.12	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
298	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0

299	0	0	0	0	0	0	0	0	0	1	0	0	.12	0	0	0	0	0	.12	0
300	.12	1	0	.12	0	.12	.12	.12	0	0	1	1	0	0	0	.12	0	0	0	0
301	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0
302	0	.12	0	0	0	0	1	.12	0	0	0	.12	0	0	0	0	0	0	0	0
303	0	0	0	0	.12	.12	0	0	0	1	1	0	0	0	0	0	0	0	0	.12
304	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	.12	.12	0	0	0	0
305	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
306	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	.12
307	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0
308	0	.12	.12	0	.12	.12	0	0	0	0	.12	0	0	.12	0	.12	.12	0	.12	.12
309	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12
310	0	.12	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0
311	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
312	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0
313	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	.12	0	0	0	0
314	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	.12
315	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	.12	.12	0	0	0
316	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0
317	0	.12	0	0	0	1	1	0	1	0	.12	1	0	0	0	.12	0	0	.12	0
318	1	1	0	1	0	0	.12	0	0	0	0	0	0	0	0	1	.12	0	0	0
319	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0
320	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12
321	0	0	0	.12	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
322	.12	.12	0	0	0	0	0	.12	0	0	.12	.12	.12	0	.12	.12	.12	0	0	.12
323	0	0	0	0	0	0	0	0	0	1	.12	0	0	0	0	0	0	0	0	0
324	0	0	0	.12	0	0	0	.12	0	0	0	0	0	0	.12	0	0	0	0	0
325	0	0	0	.12	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
326	.12	0	.12	0	0	0	.12	.12	0	0	0	0	0	0	0	.12	0	0	0	0
327	0	1	0	0	0	0	0	0	0	0	.12	1	0	.12	0	0	0	0	.12	0
328	0	0	0	0	0	0	0	1	0	0	.12	0	0	0	0	0	.12	0	0	0
329	0	0	0	0	.12	0	0	.12	0	0	0	0	0	0	.12	0	0	0	0	0
330	0	0	0	.12	0	0	.12	.12	0	0	0	0	0	0	1	.12	.12	0	0	0
331	1	.12	0	0	.12	1	0	0	0	0	0	.12	0	0	0	0	0	0	0	0
332	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0
333	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
334	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12
335	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12
336	0	.12	0	0	0	.12	.12	0	0	0	0	.12	.12	0	0	0	0	0	.12	0
337	0	0	.12	0	0	0	0	0	0	0	0	0	0	.12	0	.12	0	.12	0	0
338	.12	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12
339	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
340	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
341	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	.12
342	0	.12	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	.12	0	0
343	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12

344	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
345	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
346	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0
347	0	.12	0	.12	0	0	0	.12	.12	0	0	.12	0	0	.12	0	0	0	0	0	0
348	.12	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
349	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12	0
350	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
351	.12	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
352	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
353	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	.12	0	0
354	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0
355	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0
356	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	0	0	0	0	0
357	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
358	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0
359	0	0	.12	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
360	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	0	0	0	0	0
361	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0
362	0	0	0	0	0	0	0	0	0	0	.12	0	.12	0	0	0	.12	0	0	0	0
363	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
364	.12	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	0	0	0	0	0
365	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12	0
366	.12	0	0	0	.12	0	0	0	0	0	.12	0	0	0	0	.12	.12	0	0	.12	0
367	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
368	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
369	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	.12	0
370	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
371	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0
372	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0
373	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
374	0	.12	0	0	0	0	0	0	0	0	0	.12	0	0	0	.12	.12	0	0	0	0
375	.12	0	0	.12	0	0	0	0	0	0	0	0	0	0	.12	0	.12	0	0	.12	0
376	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0
377	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
378	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
379	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
380	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
381	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0
382	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
383	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0
384	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
385	1	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
386	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0
387	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0
388	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	.12	0	0	.12	0

389	.12	1	0	.12	0	0	.12	0	0	0	.12	0	0	0	0	.12	0	0	0	0	0
390	0	.12	0	0	0	0	1	0	0	.12	0	1	0	0	0	0	0	0	0	.12	0
391	0	.12	0	0	.12	0	0	0	0	0	.12	1	0	0	0	0	0	0	0	0	0
392	0	1	0	.12	0	.12	0	1	.12	0	0	.12	0	0	0	0	0	0	0	0	0
393	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12	0
394	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
395	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12	0
396	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	.12	.12	0
397	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0
398	0	1	0	1	0	0	.12	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
399	0	1	.12	.12	0	.12	1	0	0	0	0	0	0	0	.12	.12	0	0	0	0	0
400	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
401	0	0	0	0	0	0	0	0	0	0	.12	0	0	.12	0	0	0	.12	0	0	0
402	0	0	1	1	0	0	.12	0	0	.12	.12	0	0	0	0	0	0	0	0	0	0
403	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	.12	0
404	1	0	0	1	0	0	1	.12	0	0	0	0	.12	0	0	0	.12	0	0	.12	0
405	.12	1	0	.12	0	.12	.12	0	0	0	0	1	0	0	.12	.12	.12	0	0	0	0
406	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0
407	.12	0	0	0	.12	0	.12	0	0	.12	0	0	0	0	0	0	.12	0	0	.12	0
408	1	.12	.12	.12	.12	0	0	0	0	0	0	.12	0	0	0	.12	.12	0	0	0	0
409	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0
410	1	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12	0
411	0	.12	1	0	0	.12	.12	0	.12	0	0	.12	0	0	0	.12	.12	0	0	0	0
412	.12	.12	0	0	0	0	0	0	.12	0	1	.12	0	0	0	.12	.12	0	1	0	0
413	0	0	0	0	0	0	0	0	0	0	.12	0	.12	0	0	0	0	0	0	.12	0
414	0	0	0	0	0	0	0	0	0	0	.12	0	.12	0	0	0	0	0	0	0	0
415	0	1	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	.12	0
416	0	0	0	.12	0	0	.12	0	0	0	.12	0	0	0	0	.12	0	0	0	0	0
417	.12	.12	0	0	0	.12	.12	0	0	0	0	0	0	0	.12	0	0	0	0	.12	0
418	1	0	0	.12	0	0	.12	0	0	0	0	1	0	0	0	1	0	0	0	0	0
419	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0
420	.12	.12	0	0	0	1	.12	.12	0	0	0	1	0	0	0	0	0	0	0	0	0
421	0	0	0	0	0	0	0	0	1	.12	0	0	0	0	1	0	0	0	.12	.12	0
422	0	0	0	0	0	.12	0	0	0	.12	0	0	.12	0	0	0	0	0	0	0	0
423	0	.12	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	.12	0	0	0	0
424	.12	1	0	1	0	.12	.12	0	0	0	0	1	0	0	0	0	0	.12	0	0	0
425	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
426	.12	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	.12	.12	0	0	.12	0
427	.12	.12	0	0	0	0	1	0	0	0	.12	.12	0	0	0	0	0	0	0	0	0
428	1	.12	.12	0	0	.12	.12	.12	0	.12	.12	.12	0	.12	0	.12	.12	0	.12	.12	0
429	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	.12	0	0	0
430	0	.12	0	0	0	.12	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
431	0	.12	0	.12	0	.12	.12	0	0	0	0	0	.12	0	0	0	0	0	0	0	0
432	0	0	0	0	0	0	0	0	0	0	.12	.12	0	0	0	.12	0	0	0	0	0
433	.12	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0

434	0	0	.12	.12	0	0	.12	.12	0	0	0	0	0	0	0	0	0	0	0	0
435	.12	.12	.12	.12	.12	.12	.12	.12	0	.12	.12	.12	.12	0	0	.12	.12	0	.12	.12
436	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0
437	0	0	0	0	0	0	0	0	0	.12	.12	0	0	0	0	0	.12	0	0	.12
438	.12	.12	0	0	0	.12	.12	0	0	0	0	0	.12	0	0	.12	0	0	0	0
439	.12	.12	.12	.12	0	.12	.12	.12	.12	0	0	.12	.12	0	.12	0	0	.12	0	.12
440	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0
441	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	0	0	0
442	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	.12	.12	0	0	0
443	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0
444	.12	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12	0
445	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12	0	0	0
446	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0	.12	0	0	0	0
447	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
448	0	0	0	0	0	.12	.12	0	.12	0	0	.12	0	0	0	0	0	0	0	0
449	0	.12	0	0	0	0	0	.12	0	0	.12	0	0	0	0	.12	0	0	0	0
450	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0
451	0	0	0	0	0	.12	.12	0	0	0	0	0	0	0	0	0	0	0	0	.12
452	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	.12	0	0	0
453	0	.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.12	0
454	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0	.12
455	0	.12	0	0	0	0	0	0	0	0	0	0	.12	0	0	.12	0	0	0	0
456	0	.12	0	0	0	0	0	0	0	0	0	.12	0	0	0	.12	0	0	0	0
457	0	0	0	0	0	0	0	0	0	0	.12	0	0	0	0	0	0	0	0	0

Example 6: E. coli Fermentation of Designed B13GTs

[0090] E. coli strains that control B13GT expression with a constitutive promoter were made for the top B13GTs from Example 3. The E. coli strains were grown in 1 liter fermenters with a glycerol feed for 48 hours. The fermentations of strains expressing AA37959, AA40205, AA40232, and AA40243 yielded 171-, 60-, 182-, and 190-grams wet cell mass per liter fermentation. Twenty to thirty grams of cells from each fermentation were resuspended and lysed with a homogenizer. All lysates were tested and confirmed to have B13GT activity. The clarified lysate was purified using IMAC. The resulting purified protein samples of AA37959, AA40205, AA40232, and AA40243 had protein yields of 0.47, 0.15, 1.20 and 1.42 mg/ml, respectively.

INCORPORATION BY REFERENCE

[0091] All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. However, mention of any reference, article, publication, patent, patent publication, and patent application

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CLAIMS:

1. An engineered beta-1,3-glycosyltransferase polypeptide that comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 4-517, 2, and 3.
2. The engineered beta-1,3-glycosyltransferase polypeptide of claim 1, comprising an amino acid sequence that is identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 4-517, 2, and 3.
3. An engineered beta-1,3-glycosyltransferase polypeptide that has a score greater than 332 when scored by the PSSM shown in Table 8, wherein the engineered beta-1,3-glycosyltransferase polypeptide has been aligned to SEQ ID NO: 2 prior to scoring.
4. An engineered beta-1,3-glycosyltransferase polypeptide that has a score greater than 54.5 when scored by the PSSM shown in Table 9, wherein the engineered beta-1,3-glycosyltransferase polypeptide has been aligned to SEQ ID NO: 2 prior to scoring.
5. An engineered beta-1,3-glycosyltransferase polypeptide that has a score greater than 58.3 when scored by the PSSM shown in Table 10, wherein the engineered beta-1,3-glycosyltransferase polypeptide has been aligned to SEQ ID NO: 2 prior to scoring.
6. A polypeptide having the sequence of:
MPNTXXTXXRXXXXXXXXFPVPFPGHXNPMLQLANXLYXXGFXIXIXXXXFNXPK
TSXYPHFXFXXXXDXXPQXXXLRNLPTXGPGXGARIPXINXHGXNXXXXXXLXX
XXXXXXXXDXXXXCIXDALWYFXXXVXXXLXXXXLVLXTXSLFNFXLXXLP
XFXXXGXLXXXXXXXXXXXXXXXXGXPLXXXDIXXAYSHXXEXKPILXKMXXXT
XXXSGXIWNSXKELEESELETXXREIPAPSFLXPLPKHXXASSXSLLDXDXXXXX
WLXXQXXXSVXYVXFGSQXXXXXXXXXXXXXXXXGLXXSXXXLWLVRPGFXKG
XXWXXXXPXGXXGXXXRXVKXPPQEVLAHXAIGAFTWGGWNGTXEXVCE
GVPMIXSXFGLDQPLNAXYMXXXXXXGXLYLXXGXXXXXXXXXXXXXXXXXXXX
GXXXXXXAXLXXKDXSXXXGGSSYESLEXLSYISSL

wherein residue 5 is N or S

wherein residue 6 is P or T

wherein residue 8 is T or V
wherein residue 9 is R or V
wherein residue 11 is R or D or E or H or K or S or T
wherein residue 12 is R or D or L or K
wherein residue 13 is R or H or T or W
wherein residue 14 is I or V
wherein residue 15 is A or I or V
wherein residue 16 is I or L or M
wherein residue 25 is I or L or W or V
wherein residue 34 is L or V
wherein residue 37 is R or S
wherein residue 38 is R or K
wherein residue 41 is A or R or N or D or Q or E or K or S
wherein residue 43 is I or T or V
wherein residue 45 is I or L or F
wherein residue 46 is H or Y
wherein residue 47 is M or T
wherein residue 48 is A or R or N or Q or E or K or S or T
wherein residue 51 is A or R or L or K
wherein residue 56 is N or L
wherein residue 61 is Q or E or T
wherein residue 63 is R or E or I or L or K or V
wherein residue 64 is R or N or D or Q or E or L or F or P or S or T or Y
wherein residue 65 is I or L or F or T or V
wherein residue 66 is I or L
wherein residue 68 is R or N or E
wherein residue 69 is D or E
wherein residue 72 is D or P or T
wherein residue 73 is D or E
wherein residue 74 is W or Y
wherein residue 81 is H or T
wherein residue 85 is A or V
wherein residue 91 is Q or I or L or V
wherein residue 94 is Q or E or K

wherein residue 97 is A or K
wherein residue 98 is A or D or E or K or P
wherein residue 99 is A or R or Q or E or K or P or T or V
wherein residue 100 is L or F
wherein residue 101 is R or E or L or K or Y
wherein residue 102 is A or R or D or Q or E or K
wherein residue 103 is A or E or I or L or K or M or T or V
wherein residue 105 is R or Q or E or L or K or S
wherein residue 106 is A or R or N or D or Q or E or I or L or K or S
wherein residue 107 is A or C or E or I or L or M or F or Y or V
wherein residue 108 is I or L or K or M
wherein residue 109 is A or R or N or D or Q or E or L or K or S
wherein residue 110 is A or N or D or E or S
wherein residue 111 is S or T
wherein residue 112 is A or E or G or K or P
wherein residue 113 is D or E or K or S
wherein residue 115 is E or V
wherein residue 116 is R or Q or E or K or P or T or V
wherein residue 117 is I or L or V
wherein residue 118 is A or R or S
wherein residue 120 is L or F or V
wherein residue 122 is A or T
wherein residue 129 is A or T
wherein residue 130 is A or N or Q or E or G or L or K or M or T or Y
wherein residue 131 is D or E or L or P or S
wherein residue 133 is A or T
wherein residue 134 is R or D or Q or E or K
wherein residue 135 is R or E or K or S
wherein residue 137 is N or G
wherein residue 138 is I or L
wherein residue 139 is R or K or P
wherein residue 140 is R or L or P
wherein residue 144 is Q or M
wherein residue 146 is G or S

wherein residue 152 is H or L
wherein residue 153 is A or C
wherein residue 155 is A or V
wherein residue 156 is C or S
wherein residue 159 is A or R or D or Q or E or H or L or K
wherein residue 161 is R or D or E or I or L
wherein residue 162 is A or R or D or Q or E or K
wherein residue 163 is A or R or D or Q or L or K
wherein residue 165 is I or L or W or Y
wherein residue 167 is N or D
wherein residue 168 is L or P
wherein residue 169 is A or N or D or E or S or T
wherein residue 170 is N or D or S or T
wherein residue 171 is N or Q or K or P or S or T
wherein residue 172 is R or E or H or L or K or S or T or W
wherein residue 173 is A or R or N or D or G or K or P
wherein residue 174 is D or E or L or P
wherein residue 175 is D or E
wherein residue 176 is A or D or Q or E or K or T
wherein residue 177 is Q or P
wherein residue 178 is A or V
wherein residue 179 is E or K or P or S or V
wherein residue 181 is A or L or F or Y
wherein residue 183 is R or Q or E or H or I or L or M or F or W or Y
wherein residue 185 is R or L or K or T
wherein residue 186 is A or R or N or W or V
wherein residue 187 is A or R or G or K
wherein residue 190 is R or L or K
wherein residue 191 is R or K or S
wherein residue 196 is D or I or K or S or W
wherein residue 197 is D or Q
wherein residue 199 is A or S
wherein residue 204 is D or E or G or M or S
wherein residue 207 is I or T or V

wherein residue 208 is E or K or T
wherein residue 209 is A or Q or E or M or T
wherein residue 211 is R or K
wherein residue 212 is A or R or N or Q or E or L or K
wherein residue 213 is A or S
wherein residue 216 is I or V
wherein residue 221 is A or F
wherein residue 232 is I or V
wherein residue 233 is R or Q or I or W
wherein residue 243 is I or L
wherein residue 249 is L or F or Y
wherein residue 250 is R or T
wherein residue 254 is S or T
wherein residue 259 is E or T
wherein residue 261 is R or P
wherein residue 262 is S or T
wherein residue 263 is A or F or T or V
wherein residue 264 is A or F
wherein residue 265 is A or D or Q or E or P
wherein residue 268 is A or D
wherein residue 269 is R or Q or G or K or T
wherein residue 271 is A or P
wherein residue 272 is A or D or E or K or P
wherein residue 273 is R or N or E or G or K or S
wherein residue 276 is L or V
wherein residue 279 is G or S
wherein residue 285 is Q or E or S
wherein residue 286 is I or L or M or F or V
wherein residue 287 is D or E or S or T
wherein residue 288 is A or R or E or L or K or P or V
wherein residue 289 is A or D or E or K
wherein residue 290 is D or Q or E or T
wherein residue 291 is A or F
wherein residue 292 is L or K

wherein residue 293 is A or R or N or E or K or T
wherein residue 294 is I or L
wherein residue 295 is A or L or M or V
wherein residue 296 is R or D or Q or E or H or K
wherein residue 299 is R or I or K or V
wherein residue 300 is A or R or D or E or L or K
wherein residue 302 is E or G or K
wherein residue 303 is Q or E or H or I or L or K or V
wherein residue 304 is R or N or K or P or S
wherein residue 305 is A or F or T or V
wherein residue 314 is I or V
wherein residue 317 is A or R or Q or E or H or L or K or S or Y
wherein residue 318 is A or R or D or E or K or S or T
wherein residue 320 is I or L or T or V
wherein residue 321 is D or E or S
wherein residue 322 is A or D or I or L or F or P or S or V
wherein residue 323 is I or L
wherein residue 325 is D or E or P
wherein residue 327 is R or L or K or F or Y
wherein residue 328 is G or L or M or F
wherein residue 330 is D or E or P
wherein residue 331 is A or R or N or Q or L or K
wherein residue 332 is G or I
wherein residue 334 is I or V
wherein residue 338 is A or C
wherein residue 347 is R or E or G or K or P
wherein residue 362 is L or M or V
wherein residue 364 is A or S
wherein residue 373 is C or F
wherein residue 375 is D or P
wherein residue 385 is A or R or T or V
wherein residue 388 is A or S or T or V
wherein residue 389 is R or N or D or E or K
wherein residue 390 is E or I or K or V

wherein residue 391 is R or L or K
wherein residue 392 is R or N or E or G or H or K
wherein residue 393 is I or V
wherein residue 395 is I or V
wherein residue 398 is R or D or E
wherein residue 399 is R or N or D or E or K
wherein residue 401 is F or W
wherein residue 402 is R or N or D or Q or E or I or K or V
wherein residue 403 is A or R or E or K or P or T or V
wherein residue 404 is A or R or D or Q or E or G or K
wherein residue 405 is A or R or N or D or E or K
wherein residue 406 is I or V
wherein residue 407 is A or I or V
wherein residue 408 is A or R or N or D or E or K
wherein residue 410 is A or I or L or V
wherein residue 411 is R or N or Q or E or H or K
wherein residue 412 is R or E or H or L or K or T or Y
wherein residue 413 is I or V
wherein residue 414 is L or M
wherein residue 415 is R or Q or E or L or K or T or V
wherein residue 416 is A or D or E or S
wherein residue 417 is A or E or K or P
wherein residue 418 is A or R or E or K or F or S
wherein residue 420 is A or R or Q or E or L or K
wherein residue 421 is A or E or G or H or K or P or Y or V
wherein residue 422 is I or M or Y
wherein residue 423 is A or R or K
wherein residue 424 is A or R or N or D or Q or E or K
wherein residue 425 is R or N
wherein residue 427 is A or R or Q or E or L or K
wherein residue 428 is A or R or E or L or K or V
wherein residue 430 is A or K
wherein residue 431 is A or R or D or Q or E
wherein residue 433 is A or L

wherein residue 435 is R or G or V

wherein residue 437 is I or L

wherein residue 438 is A or M

wherein residue 439 is K or P

wherein residue 449 is R or S

wherein residue 451 is Q or V (SEQ ID NO: 518).

7. The polypeptide of any one of claims 1-6, wherein the polypeptide is expressed in a host microorganism.
8. The polypeptide of claim 7, wherein the host microorganism is *E. coli*, *Saccharomyces sp.*, *Aspergillus sp.*, *Pichia sp.*, or *Bacillus sp.*
9. The polypeptide of any one of claims 1-8, wherein the polypeptide is immobilized to a solid support.
10. The polypeptide of claim 9, wherein the solid support is derivatized from cellulose, glass, ceramic, methacrylate, styrene, acrylic, a metal oxide, or a membrane.
11. The polypeptide of claim 9 or 10, wherein the polypeptide is immobilized to the solid support by covalent attachment, adsorption, cross-linking, entrapment, or encapsulation.
12. A method for transferring a sugar moiety to a substrate steviol glycoside, the method comprising contacting a beta-1,3-glycosyltransferase polypeptide, a sucrose synthase and optionally a beta-1,2-glycosyltransferase polypeptide with a starting composition comprising one or more steviol glycosides, a nucleotide diphosphate, and sucrose.
13. The method of claim 12, wherein the beta-1,3-glycosyltransferase polypeptide is an engineered beta-1,3-glycosyltransferase polypeptide that comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 4-517, 2, and 3.

14. The method of claim 12, wherein the sucrose synthase is an engineered sucrose synthase with a polypeptide sequence that is at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a sucrose synthase derived from the species *Acidithiobacillus caldus*, *Nitrosomonas europaea*, *Denitrovibrio acetiphilus*, *Thermosynechococcus elongatus*, *Oryza sativa*, *Arabidopsis thaliana*, or *Coffea arabica*.
15. The method of claim 12, wherein the beta-1,2-glycosyltransferase polypeptide is an engineered beta-1,2-glycosyltransferase polypeptide with a polypeptide sequence that is at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a beta-1,2-glycosyltransferase polypeptide derived from the species *Hordeum vulgare*, *Oryza sativa*, *Solanum lycopersicum*, *Lycium barbarum*, *Solanum tuberosum*, or *Stevia rebaudiana*.
16. The method of claim 12, wherein
- (a) the beta-1,3-glycosyltransferase polypeptide is an engineered beta-1,3-glycosyltransferase polypeptide that comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 4-517, 2, and 3.
 - (b) the sucrose synthase is an engineered sucrose synthase with a polypeptide sequence that is at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a sucrose synthase derived from the species *Acidithiobacillus caldus*, *Nitrosomonas europaea*, *Denitrovibrio acetiphilus*, *Thermosynechococcus elongatus*, *Oryza sativa*, *Arabidopsis thaliana*, or *Coffea arabica*.
 - (c) the beta-1,2-glycosyltransferase polypeptide is an engineered beta-1,2-glycosyltransferase polypeptide with a polypeptide sequence that is at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a beta-1,2-glycosyltransferase polypeptide derived from the species *Hordeum vulgare*, *Oryza sativa*, *Solanum lycopersicum*, *Lycium barbarum*, *Solanum tuberosum*, or *Stevia rebaudiana*.

17. The method of claim 12, wherein the beta-1,3-glycosyltransferase polypeptide is an engineered beta-1,3-glycosyltransferase polypeptide that has a score greater than 332 when scored by the PSSM shown in Table 8, wherein the engineered beta-1,3-glycosyltransferase polypeptide has been aligned to SEQ ID NO: 2 prior to scoring.
18. The method of claim 12, wherein the beta-1,3-glycosyltransferase polypeptide is an engineered beta-1,3-glycosyltransferase polypeptide that has a score greater than 54.5 when scored by the PSSM shown in Table 9, wherein the engineered beta-1,3-glycosyltransferase polypeptide has been aligned to SEQ ID NO: 2 prior to scoring.
19. The method of claim 12, wherein the beta-1,3-glycosyltransferase polypeptide is an engineered beta-1,3-glycosyltransferase polypeptide that has a score greater than 58.3 when scored by the PSSM shown in Table 10, wherein the engineered beta-1,3-glycosyltransferase polypeptide has been aligned to SEQ ID NO: 2 prior to scoring.
20. The method of any one of claims 12 to 19, wherein the substrate steviol glycoside comprises at least one steviol glycoside selected from the group consisting of steviol, steviol-13-O-glucoside, steviol-19-O-glucoside, rubusoside, steviol-1,2-bioside, steviol-1,3-bioside, rubusoside, dulcoside B, dulcoside A, rebaudioside B, rebaudioside G, stevioside, rebaudioside C, rebaudioside F, rebaudioside A, rebaudioside I, rebaudioside E, rebaudioside H, rebaudioside L, rebaudioside K, rebaudioside J, rebaudioside M, rebaudioside D, rebaudioside E2, rebaudioside AM, rebaudioside N, rebaudioside O, rebaudioside Q, an isomer thereof, and a synthetic steviol glycoside.
21. The method of claim 20, wherein the substrate steviol glycoside comprises a mixture of stevioside and rebaudioside A.
22. The method of claim 20, wherein the substrate steviol glycoside comprises stevioside.
23. The method of claim 20, wherein the substrate steviol glycoside comprises one or more selected from the group consisting of: Reb A, RS50, RS60, RS90, and RS99.
24. The method of any one of claims 12 to 23, further comprising producing a target steviol glycoside, wherein the target steviol glycoside comprises at least one steviol glycoside

selected from the group consisting of steviol, steviol-13-O-glucoside, steviol-19-O-glucoside, rubusoside, steviol-1,2-bioside, steviol-1,3-bioside, rubusoside, dulcoside B, dulcoside A, rebaudioside B, rebaudioside G, stevioside, rebaudioside C, rebaudioside F, rebaudioside A, rebaudioside I, rebaudioside E, rebaudioside E2, rebaudioside H, rebaudioside L, rebaudioside K, rebaudioside J, rebaudioside AM, rebaudioside M, rebaudioside D, rebaudioside N, rebaudioside O, rebaudioside Q, an isomer thereof, and a synthetic steviol glycoside.

25. The method of claim 24, wherein the target steviol glycoside comprises at least one steviol glycoside selected from the group consisting of stevioside rebaudioside A, rebaudioside E, rebaudioside E2, rebaudioside I, rebaudioside D, rebaudioside AM, and rebaudioside M.
26. The method of claim 24, wherein the target steviol glycoside comprises at least one steviol glycoside selected from the group consisting of rebaudioside I, rebaudioside D, rebaudioside AM, and rebaudioside M.
27. The method of claim 24, wherein the target steviol glycoside comprises rebaudioside M.
28. The method of claim 24, wherein the target steviol glycoside comprises rebaudioside AM.
29. The method of any of claims 12 to 28, wherein the nucleotide diphosphate comprises one selected from the group consisting of adenosine diphosphate, uridine diphosphate, cytidine diphosphate, thymidine diphosphate, and guanosine diphosphate.
30. The method of any of claims 12 to 28, wherein the nucleotide diphosphate comprises one selected from the group consisting of adenosine diphosphate, guanosine diphosphate, cytidine diphosphate, or thymidine diphosphate.
31. The method of any of claims 12 to 28, wherein the nucleotide diphosphate comprises adenosine diphosphate.
32. The method of any of claims 12 to 31, wherein the starting composition comprises greater than about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about

20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.6% of steviol glycoside by weight on an anhydrous basis.

33. The method of any of claims 12 to 32, further comprising producing a target composition, wherein the target composition comprises greater than about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.6% of the target steviol glycoside by weight on an anhydrous basis.
34. A polynucleotide encoding a polypeptide of any of claims 1 to 6.
35. A host microorganism heterologously expressing a polynucleotide of claim 34.
36. A microorganism of claim 35 where the host microorganism is *E. coli*, *Saccharomyces sp.*, *Aspergillus sp.*, *Pichia sp.*, or *Bacillus sp.*
37. A method for transferring a sugar moiety to a substrate steviol glycoside, the method comprising contacting a beta-1,3-glycosyltransferase polypeptide and a sucrose synthase with one or more steviol glycosides, a nucleotide diphosphate, and sucrose.
38. The polypeptide of claim 1, wherein the polypeptide comprises H at position 24, D at position 123, D at position 379, and Q at position 380, and wherein the position numbers are based on SEQ ID NO: 2.
39. An engineered beta-1,3-glycosyltransferase polypeptide that comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 4, and wherein the polypeptide comprises H at position 24, D at position 123, D at position 379, and Q at position 380.

40. An engineered beta-1,3-glycosyltransferase polypeptide that comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 3, and wherein the polypeptide comprises H at position 24, D at position 123, D at position 379, and Q at position 380.
41. An engineered beta-1,3-glycosyltransferase polypeptide that comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 219, and wherein the polypeptide comprises H at position 24, D at position 123, D at position 379, and Q at position 380.
42. An engineered beta-1,3-glycosyltransferase polypeptide that comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 208, and wherein the polypeptide comprises H at position 24, D at position 123, D at position 379, and Q at position 380.
43. An engineered beta-1,3-glycosyltransferase polypeptide that comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 181, and wherein the polypeptide comprises H at position 24, D at position 123, D at position 379, and Q at position 380.