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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 May 2019 (16.05.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/094051 A1

(51) International Patent Classification:
A01N 41/08 (2006.01) *C12N 9/02* (2006.01)
A23K 10/10 (2016.01) *C12N 15/00* (2006.01)
A61K 31/145 (2006.01) *C12N 15/82* (2006.01)

(21) International Application Number:
PCT/US2017/061337

(22) International Filing Date:
13 November 2017 (13.11.2017)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicant: **PLANT SENSORY SYSTEMS, LLC** [US/US]; 1450 S. Rolling Road, bwtech@UMBC, Room 2049, Baltimore, Maryland 21227 (US).

(72) Inventor: **TURANO, Frank J.**; 1450 S. Rolling Road, bwtech@UMBC, Room 2049, Baltimore, Maryland 21227 (US).

(74) Agent: **IHNEN, Jeffrey L.**; 607 14th Street N.W., Suite 800, Washington, District of Columbia 20005 (US).

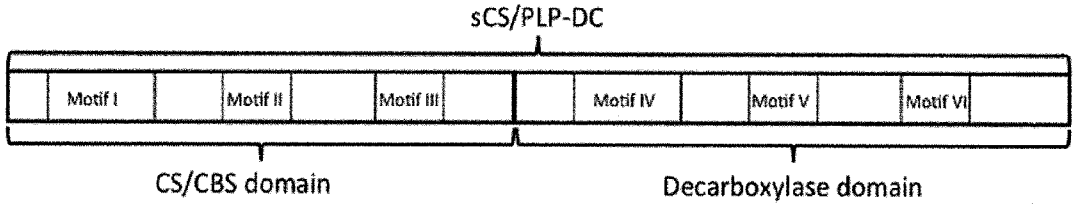
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS,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

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

WO 2019/094051 A1

(54) **Title:** METHODS FOR HIGH TAURINE PRODUCTION USING NOVEL DECARBOXYLASES

FIGURE 1



Motif I = IGxTP{x14-19}KxE{x6}SxKDRI{x7}A{x7}{P/E}G{x6-7}TSGNTG{x3}A{x3}A{x2}G{x11}SxE{x6}AxG

Motif II = P{x6-7}Q{xS}N{x10}EI{x5}G{x9}{S/G}TGxTxxG{x9}{P/G}

Motif III = GxG{K/Y}{x12}D{x21}GxxxGxS{S/A}G{x4-5}A{x13-14}V{x9}YxS

Motif IV = P{x18-25}E{x18-19}PxFxx{Q/F}x{Y/S/F/P}{x9}G{x7}Nx{N/S/V}x{H/F/Y}{x5}P{x5}E{x17-24}G{x6}{S/A}{x9}AR{x7}{K/R}{x11-18}H{Y/S}{x3}K{x5}G

Motif V = {V/T}P{x5}TxGxTxxxAxD{x14}WxHxDxAxxG{x8}{R/N}{x4}GxxRxxSxxWNPHK{x5}Lx{C/Q}{x5}{K/R/H}{x4-5}L

Motif VI = YL{x10-11}DxxDxxIx{C/T}GR{x7}W{x6}G{x30-38}V{x35-38}A{x10}G{x16-18}R

{P/G} = P or G residue x = any amino acid residue {x14-19} = 14 to 19 residues of any amino acid

(57) **Abstract:** The present invention describes methods to produce a synthetic or semi- synthetic cysteine synthase/PLP-dependent decarboxylase (sCs/PLP-DC). More particularly, the invention relates to genetic modification of organisms including eukaryotes and prokaryotes to express a functional sCs/PLP-DC. The invention includes methods to produce taurine in organisms that contain native or heterologous (transgenic) taurine biosynthetic pathways or cells that have taurine by enrichment. The invention also relates to methods to increase taurine levels in the cells and to use the said cells or extracts or purifications from the cells that contain the invention to produce plant growth enhancers, food, animal feed, aquafeed, food or drink supplements, animal-feed supplements, dietary supplements, health supplements or taurine.

[Continued on next page]

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

SPECIFICATION

METHODS FOR HIGH TAURINE PRODUCTION USING NOVEL DECARBOXYLASES

SEQUENCE SUBMISSION

[01] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is entitled SyntheticCSDC.txt, created on October 30, 2017 and is 178 kb in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in their entirety

FIELD OF THE INVENTION

[02] The present invention is in the field of recombinant genes and peptides for the production of taurine or 2-aminoethanesulfonic acid. The present invention relates to methods and materials for making semi-synthetic or synthetic peptides for taurine production. The present invention describes methods to engineer or synthetize peptides and uses thereof for taurine production. The invention includes semi-synthetic peptides made from modifications, replacements or inclusions of polynucleotides to modify amino acids in natural peptides or synthetic peptides made from synthetic polynucleotides or polynucleotides modified by directed evolution or gene shuffling, and their uses for taurine synthesis production. In some embodiments, the invention is aimed to semi-synthesize peptides by fusion and modification of polynucleotides to engineer a peptide for taurine synthesis. In some embodiments, the invention is aimed to synthesize polynucleotides to engineer a peptide that produces taurine. In other embodiments, the invention is aimed to polynucleotides derived by directed evolution to engineer a peptide that produces taurine. The present invention also describes preferred motifs for semi-synthetic or synthetic peptides to make the novel peptides for taurine synthesis. The present invention includes the production of taurine in eukaryotic and unicellular organisms. Unicellular organisms include single cell eukaryotes and prokaryotes, and unicellular organisms include bacteria, microbes, archaea, protozoa, yeast, unicellular algae and unicellular fungi. The invention also relates to methods to increase taurine levels in the cells with the semi-synthetic or synthetic peptides for taurine production by binding taurine or decreasing taurine degradation. The invention includes use in organisms that contain native or heterologous taurine biosynthetic pathways or cells that have taurine by enrichment.

The invention also relates to methods to increase taurine levels in the cells and to use the said cells or extracts or purifications from the cells that contain the invention to produce taurine, plant growth enhancers, food, animal feed, aquafeed, food or drink supplements, animal-feed supplements, dietary supplements, or health supplements.

[03] The publications and other materials used herein to illuminate the background of the invention or provide additional details respecting the practice are incorporated by reference and, for convenience, are respectively grouped in the Bibliography.

BACKGROUND OF THE INVENTION

Taurine is an Essential Compound for Animals

[04] Taurine is essential for human neonatal development (1) and plays an important role in brain development (2, 3). Taurine is involved in the modulation of intracellular calcium homeostasis (4, 5) and may balance glutamate activity, protecting neurons against glutamate excitotoxicity (6, 7). Taurine is also an osmoregulator (8). Taurine is essential for heart function (9), protects the integrity of hepatic tissue (10), and plays a role in photoprotection (11).

Taurine as a Dietary Supplement

[05] Taurine is biosynthesized in most animals and can be found in meat and seafood. Those who do not produce sufficient levels of taurine must acquire it through dietary supplement. Dietary taurine is required for the normal development and growth of cats (12, 13), human infants (14), and carnivorous fish (15-23). Taurine also improves the health and/or growth of other fish species (24-28) and shrimp (29). Taurine is a feed attractant for fish (20, 30).

Taurine as a Pharmaceutical or Therapeutic

[06] Taurine is used as a pharmaceutical and therapeutic. Taurine has been used in the treatment of cardiovascular diseases (31, 32), elevated blood pressure (33), seizure disorders (34), hepatic disorders (35), and alcoholism (36) and may be useful in the treatment of diabetes (37), Alzheimer's disease (38), and ocular disorders (39). Taurine has been shown to prevent obesity (40) and control cholesterol (41, 42). Taurine acts as an antioxidant and protects against toxicity of various substance (43-45). Taurine has been shown to prevent oxidative stress induced by

exercise (46) and is used in energy drinks to improve performance (47). Taurine can also be used in topical applications to treat dermatological conditions (48).

Taurine as a Plant Growth Stimulator

[07] Exogenous application of taurine has been reported to increase crop harvest, yield, and biomass (49). Applications of taurine by foliar spray, soil and roots application, and seed immersion increase crop production and seedling growth (49). Exogenous applications of taurine have also been shown to increase photosynthetic capacity of isolated plant cells (protoplasts and chloroplasts) (49).

Metabolic Pathways that Synthesize Taurine

[08] Several metabolic pathways that synthesize taurine or hypotaurine in animals have been described (50). Agnello *et al.* (51) provided the first data to suggest that prokaryotes may have intact taurine biosynthetic pathways. More recent studies have shown that several algal and microalgal species can synthesize taurine (52), suggesting taurine synthesis is more prevent in nature than what was thought less than a decade ago. The use of polynucleotides and their corresponding peptides and methods for the use of polynucleotides and their corresponding peptides to make taurine in cells have been described in the literature (50, 53, 54).

Methods to Increase Taurine in Cells

[09] The present invention could be combined with other methods or processes to increase taurine in the cell. These methods or processes have been discussed in the literature and in prior inventions (55, 56) and they are described below.

Taurine Enrichment

[10] Other studies have shown that multicellular organisms such as rotifers that contain no or low levels of taurine can be enriched with taurine by diffusion (dissolved method) (57-59), or with liposomes (60). Taurine enrichment methods could also be used with unicellular organisms and in combination with the present invention to increase taurine levels in the cell.

Periplasmic-binding or Taurine-binding Proteins

[11] In bacteria, periplasmic binding proteins or substrate-binding proteins bind specific molecules as part of a multicomponent (peptide) system that is involved in the binding and transportation of specific molecules from the periplasmic space, outside of the bacterium, to the inside of the cell (61-63). In the ABC transporter system, the substrate-binding protein delivers the bound molecule to transporter proteins on the bacterial membrane where the bound molecule is released into the cell in an energy-dependent manner. In the absence of membrane-bound proteins or energy-dependent releasing peptides (ATP-binding proteins) the substrate molecules remain bound to the substrate-binding protein. In the tripartite ATP-independent periplasmic (TRAP) transporter systems, the substrate-binding protein delivers the bound molecule to membrane bound protein complex (with two peptides) and releases the bound molecule into the cell in an ATP-independent process. In the absence of membrane-bound proteins the substrate molecule remains bound to the substrate-binding protein. Methods to increase pools of sulfonic acids, such as taurine, by expressing only the substrate-binding protein from an ABC transporter or TRAP system, TauA or TauK, respectively, in the cells have been described for use in plant tissues (54, 64). The present invention describes methods to express substrate-specific binding proteins in the cell of a unicellular organism to increase taurine in the cell.

Sulfonic Acid or Taurine Degradation

[12] In the absence of sulfur, bacteria utilize the sulfonic acid uptake and degradation pathway or the taurine uptake and degradation pathway to mobilize carbon, nitrogen or sulfur (65-68). Genes and their corresponding peptides involved in the uptake and degradation of taurine are usually on the same operon and are induced in the absence of nitrogen (69, 70) or sulfur (65) or in the presence of taurine (68, 71). The genes for the degradation enzymes and their corresponding gene products are the TauX and TauY genes (70) that encode taurine dehydrogenase (TDH), the TauD gene (65) that encodes taurine dioxygenase (TDO), the Tpa gene (72) that encodes taurine–pyruvate aminotransferase (TPAT) or the SsuDE (SsuD or SsuE) genes (66) that encode the two-component alkanesulfonate monooxygenase (2CASM).

Transcriptional Regulators

[13] Translational regulators, Cbl or TauR, control the expression and induction of the taurine degradation pathways in bacteria (65, 72). Cbl is a LysR-type transcriptional regulator of the sulfonic acid uptake and degradation pathway or the taurine uptake and degradation pathway in several bacteria (73, 74). The Cbl gene is found in Proteobacteria including members of the Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria. In bacteria that lack Cbl or Cbl-like transcriptional regulators there is a MocR subfamily of activators, which include TauR, that control the taurine uptake and degradation system. The TauR is found in Rhizobiales and Rhodobacterales of the Alphaproteobacteria, in Burkholderiaceae and Comamonadaceae of the Betaproteobacteria, in Enterobacteriales, Oceanospirillales and Psychromonadales from the Gammaproteobacteria, and in Rhizobiales and Rhodobacter of the Alphaproteobacteria. This invention describes how to decrease the expression of these genes or decrease the activities of their corresponding proteins in the cell of a unicellular organism to increase taurine in the cell.

SUMMARY OF THE INVENTION

[14] The invention provides methods and compositions for semi-synthetic or synthetic polynucleotides and corresponding peptides to cysteine synthetase/PLP decarboxylase (CS/PLP-DC) for taurine or 2-aminoethanesulfonic acid production (55, 56). The CS/PLP-DC polynucleotide and the resulting peptide increased levels of taurine production in eukaryotic (55) and prokaryotic (56) systems using plants and bacteria, respectively. This invention describes the semi-synthetic and synthetic assembly and use of synthetic CS/PLP-DC (sCS/PLP-DC) polynucleotides and corresponding peptides for taurine synthesis. In addition, the invention describes preferred motifs and their use in sCS/PLP-DC peptides for taurine synthesis.

[15] This invention describes the semi-synthetic assembly of sCS/PLP-DC genes and corresponding peptides from cysteine synthase (CS) and decarboxylase (DC) genes or cystathionine beta-synthase (CBS) and DC genes. This invention describes semi-synthetic methods that include polynucleotide fusion and nucleotide modifications, such as insertions, deletions and substitutes, and methods of directed evolution. CS or CBS genes and a range of known DC genes can be used and include but are not limited to sulfinoalanine decarboxylase (SAD) glutamate

decarboxylase (GAD), aromatic amino acid decarboxylase (AAAD) or 2,4-diaminobutyrate decarboxylase (BABD). This invention describes synthetic methods that include CS/PLP-DC-like genes and corresponding peptides. This invention describes semi-synthetic and synthetic methods that include the use of polynucleotides and corresponding peptides to synthesize an sCS/PLP-DC with conserved domains or motifs necessary for functional CS/PLP-DC or sCS/PLP-DC peptides that increase taurine production (Figure 1). In addition, the invention provides methods for the synthesis of sCS/PLP-DC peptides using directed evolution.

[16] The invention encompasses the use of sCS/PLP-DC polynucleotides in combination with nucleotides and peptides for substrate-binding proteins, such as the TauA or TauK genes, to increase taurine in cells or the use of polynucleotides for peptides that degrade taurine as described in (54, 56). This invention describes methods to use cells with increased taurine pools of the sulfonic acids, such as taurine, by binding taurine in the cell with specific bacterial substrate-binding proteins or by blocking or inhibiting taurine degradation.

[17] This invention also describes the use of the sCS/PLP-DC polynucleotides in combination with methods to block taurine degradation by methods of silencing, mutating or knocking out genes for enzymes in the taurine degradation pathway(s) (56) including the TauX or TauY genes that encode TDH, the TauD gene that encodes TDO, the SsuD or SsuE genes that encode 2CASM, or the Tpa gene that encodes TPAT, or by methods of silencing, mutating or knocking out the Cbl gene that encodes LysR-type transcriptional regulator or the TauR gene that encodes a MocR transcriptional regulator. This invention describes the use of polynucleotides for taurine-binding proteins or taurine degradation proteins and their corresponding peptides in unicellular organisms that are capable of producing taurine due to the presence of endogenous (native) or heterologous (gene transfer) taurine biosynthetic pathways or in cells enriched with taurine.

[18] The invention can be used to increase taurine in eukaryotic and prokaryotic cells and in unicellular organisms that produce taurine through a native or endogenous taurine (52) or heterologous pathway (75) or in cells enriched with taurine (57-60, 75).

[19] The invention provides isolated cells comprising polynucleotides which do not express a functional taurine degradation enzyme. Some isolated cells of the invention comprise: (i) exogenous DNA which disrupts the expression of the endogenous gene or renders the corresponding peptide for the degradation enzyme non-functional, (ii) a basepair mutation that disrupts the expression of the gene or renders the corresponding peptide for the degradation enzyme non-functional, or (iii) a deletion of the entire polynucleotide or a portion of the polynucleotide which disrupts the expression of the gene or renders the corresponding peptide for the degradation enzyme non-functional. The non-functional DNA could be due to changes in the promoter, a portion of the coding region, coding region, or terminator to a polynucleotide which encodes taurine degradation enzyme, that includes TauX, TauY, TauD, Tpa, SsuD, or SsuE or translational activators of those genes including Cbl or TauR genes in a manner where the genes products are not functional. The invention also provides isolated cells comprising non-functional genes or gene products of taurine degradation enzymes from the suppression or decreased accumulation of the corresponding RNA due to antisense RNA or RNA interference.

[20] The invention provides isolated cells comprising exogenous DNA which express enzymes of the sCS/PLP-DC biosynthetic pathway and polynucleotides or methods which result in a non-functional or silenced taurine degradation enzyme. In one embodiment, an isolated cell comprises two separate expression cassettes. A first expression cassette comprises a first promoter operably linked to a first polynucleotide, and a second cassette comprises polynucleotides which result in a non-functional taurine degradation enzyme. In some embodiments, the first polynucleotide encodes sCS/PLP-DC. The second polynucleotide comprises the promoter, a portion of the coding region, coding region, or terminator to genes for a taurine degradation enzyme that results in a non-functional TauX, TauY, TauD, Tpa, SsuD, or SsuE or a non-functional translational activator including Cbl or TauR genes. The genes can be made non-functional in such a manner where the genes are not expressed, the gene products are not functional, or the target polynucleotide is silenced.

[21] The invention also describes how to use the cells, fractions of the cells, or extracts from the cells for the present invention for a variety of purposes, including as an additive, feed ingredient, extract or meal. This invention describes the use of polynucleotides and their corresponding polypeptides that either bind or degrade taurine.

[22] The invention provides methods for increasing taurine in the cells of the invention by growing or treating the cells with an agent that increases sulfur or nitrogen concentration.

[23] The invention also provides nutritional supplements, feed supplements, and pharmaceutical compositions comprising an extract or meal from the cell of the invention,

BRIEF DESCRIPTION OF THE FIGURE

[24] Figure 1 shows a diagram of the sCS/PLP-DC peptide with the CS/CBS and the decarboxylase region domains. Motifs (I-VI) associated with sCS/PLP-DC peptides are indicated. The specific motifs are indicated using standard single letter amino acid abbreviations. X represents any amino acid, and numbers indicate the spaces between conserved residues.

DETAILED DESCRIPTION OF THE INVENTION

[25] The present invention provides methods and materials for the production of taurine (2-aminoethanesulfonic acid) using sCS/PLP-DC. In preferred embodiments, the invention provides methods for the genetic transformation of eukaryotic and prokaryotic cells with sCS/PLP-DC polypeptides and expression of the corresponding sCS/PLP-DC peptides. Another embodiment of the invention is organisms with genes that encode proteins that bind taurine or with silenced or knocked out genes for taurine degradation. The preferred embodiment for eukaryotes is in plants. Another embodiment of the invention is in unicellular organisms. The invention also provides methods of using eukaryotic and prokaryotic cells or unicellular organisms such as algae, microalgae, bacteria, fungi, yeast, or unicellular cellular organisms with increased levels of endogenous taurine or taurine derivatives such as hypotaurine that can be purified or used as a food- or feed-supplement, dietary supplement, as a component of a health supplement or therapy or for plant growth or yield.

[26] The invention describes methods for the use of polynucleotides that encode a functional sCS/PLP-DC peptide using polynucleotides that correspond to CS/PLP-DC, CS, CBS, AAAD, GAD, BABD, or SAD peptides in eukaryotes, prokaryotes or unicellular organisms. The preferred embodiments of the invention are in plants and unicellular organisms but other organisms may be used.

[27] The present invention describes the methods for the synthesis of DNA constructs from polynucleotides and use of vectors and the methods for making transformed eukaryotic organisms, including plants and fungi, and unicellular organisms, including bacteria microbes, fungi, yeast, algae and microalgae that produce taurine due to the presence of sCS/PLP-DC peptide. The present invention describes methods to produce extracts or cells with enhanced taurine production and that result in cells or items with increased nutritional, pharmaceutical, or therapeutic value. The invention can be used in cells enriched with taurine, that contain a native taurine biosynthetic pathway(s), or that contain taurine from the insertion of a heterologous pathway by transformation or gene transfer.

[28] The present invention describes the methods and use of the sCS/PLP-DC constructs alone or in conjunction with polynucleotides and corresponding peptides that bind taurine or with polynucleotides or methods that inhibit taurine degradation. Present invention describes methods to synthesize polynucleotides and vectors and the methods for making transformed organisms including unicellular organisms, microbes, fungi yeast, algae and microalgae. The present invention is unique in that it describes a method to produce taurine that has advantages of enhanced taurine production or hypotaurine and that result in cells with increased nutritional, pharmaceutical, or therapeutic value.

[29] The invention provides methods to develop isolated cells and organisms comprising exogenous DNA that expresses enzymes of sCS/PLP-DC and taurine binding protein. In one embodiment, an isolated cell comprises two separate expression cassettes. A first expression cassette comprises a first promoter operably linked to an sCS/PLP-DC polynucleotide and a second expression cassette comprises another promoter operably linked to a second polynucleotide. The second polynucleotide encodes a taurine binding protein (TauA or TauK).

[30] The present invention describes the methods for the synthesis of sCS/PLP-DC constructs to inhibit taurine degradation from polynucleotides and vectors and the methods for making transformed organisms including unicellular organisms, microbes, fungi yeast, algae and microalgae. The present invention is unique in that it describes a method to produce taurine that has advantages of enhanced taurine production or hypotaurine and that result in cells with increased nutritional, pharmaceutical, or therapeutic value

[31] The present invention describes the insertion of the sCS/PLP-DC polynucleotides in conjunction with polynucleotides that encode functional taurine binding proteins (TauA or TauK) or polynucleotides that silence or knocked-out genes for proteins involved in taurine degradation (TauD, SsuD, SsuE, TauX, TauY, or Tpa) or transcriptional regulators (cbl or TauR) that control taurine degradation in unicellular organisms, or their use in taurine biosynthetic pathway in unicellular organisms where the pathway does not exist or has not clearly been identified.

Enzymes of Taurine Biosynthetic Pathways

[32] Examples of amino acid sequences that can be used for assembly of the sCS/PLP-DC biosynthetic pathways are provided in the sequence listing: SEQ ID NO:2 (CS/PLP-DC), SEQ ID NO:4 (CS/PLP-DC), SEQ ID NO:6 (CS), SEQ ID NO:8 (CS), SEQ ID NO:10 (CS), SEQ ID NO:12 (CBS), SEQ ID NO:14 (CBS), SEQ ID NO:16 (CBS), SEQ ID NO:18 (AAAD), SEQ ID NO:20 (AAAD), SEQ ID NO:22 (GAD), SEQ ID NO:24 (BABD), SEQ ID NO:26 (SAD), SEQ ID NO:28(SAD) and SEQ ID NO:83 (CS/PLP-DC). The invention is not limited to the use of these amino acid sequences. Those of ordinary skill in the art know that organisms of a wide variety of species commonly express and utilize homologous proteins, which include the insertions, substitutions and/or deletions discussed above, and effectively provide similar function. For example, the amino acid sequences for CS/PLP-DC from *Micromonas pusilla, Ostreococcus tauri,* or *Bathycoccus prasinos*; CS from *Brassica oleracea*, *Oryza brachyantha*, or *Escherichia coli;* CBS from *Candidatus kryptonium*, *Chloroflexi bacterium* or *Cyprinus carpio*; AAAD from *Beta vulgaris* or *Lepisosteus oculatus*; GAD from *Danio rerio*; BABD from *Escherichia coli*; or SAD from *Oncorhynchus mykiss* or *Guillardia theta* may differ to a certain degree from the amino acid sequences of CS/PLP-DC, CS, CBS, AAAD, GAD, BABD, or SAD in another species and yet have similar functionality with respect to catalytic and regulatory function. Amino acid sequences comprising such variations are included within the scope of the present invention and are considered substantially or sufficiently similar to a reference amino acid sequence. Although it is not intended that the present invention be limited by any theory by which it achieves its advantageous result, it is believed that the identity between amino acid sequences that is necessary to maintain proper functionality is related to maintenance of the tertiary structure of the polypeptide such that specific interactive sequences will be properly

located and will have the desired activity, and it is contemplated that a polypeptide including these interactive sequences in proper spatial context will have activity.

Substrate Binding Proteins

[33] Examples of amino acid sequences of substrate binding proteins or periplasmic binding proteins that bind taurine are provided in the sequence listing: SEQ ID NO:30 (TauA) and SEQ ID NO:32 (TauK). The invention is not limited to the use of these amino acid sequences. Those of ordinary skill in the art know that organisms of a wide variety of species commonly express and utilize homologous proteins, which include the insertions, substitutions and/or deletions discussed above, and effectively provide similar function. For example, the amino acid sequences for TauA from *Escherichia coli* or TauK from *Roseobacter denitrificans* may differ to a certain degree from the amino acid sequences of TauA or TauK in another species and yet have similar functionality with respect to catalytic and regulatory function. Amino acid sequences comprising such variations are included within the scope of the present invention and are considered substantially or sufficiently similar to a reference amino acid sequence. Although it is not intended that the present invention be limited by any theory by which it achieves its advantageous result, it is believed that the identity between amino acid sequences that is necessary to maintain proper functionality is related to maintenance of the tertiary structure of the polypeptide such that specific interactive sequences will be properly located and will have the desired activity, and it is contemplated that a polypeptide including these interactive sequences in proper spatial context will have activity.

Enzymes of Taurine Degradation Pathways

[34] Examples of amino acid sequences of enzymes in degradation pathways are provided in the sequence listing: SEQ ID NO:34 (TDO), SEQ ID NO:36 (SsuD), SEQ ID NO:38 (SsuE), SEQ ID NO:40 (SsuD), SEQ ID NO:42 (SsuE), SEQ ID NO:44 (TauX), SEQ ID NO:46 (TauY), and SEQ ID NO:48 (Tpa). The invention is not limited to the use of these amino acid sequences. Those of ordinary skill in the art know that organisms of a wide variety of species commonly express and utilize homologous proteins, which include the insertions, substitutions and/or deletions discussed above, and effectively provide similar function. For example, the amino acid sequences for TDO, SsuD or SsuE from *Escherichia coli*, SsuD or SsuE from *Corynebacterium*

glutamicum, TauX, TauY, or Tpa from *Roseobacter denitrificans* may differ to a certain degree from the amino acid sequences of TDO, SsuD, SsuE, TauX, TauY, or Tpa in another species and yet have similar functionality with respect to catalytic and regulatory function. Amino acid sequences comprising such variations are included within the scope of the present invention and are considered substantially or sufficiently similar to a reference amino acid sequence. Although it is not intended that the present invention be limited by any theory by which it achieves its advantageous result, it is believed that the identity between amino acid sequences that is necessary to maintain proper functionality is related to maintenance of the tertiary structure of the polypeptide such that specific interactive sequences will be properly located and will have the desired activity, and it is contemplated that a polypeptide including these interactive sequences in proper spatial context will have activity.

Translational Regulators

[35] Examples of amino acid sequences of translational regulators are provided in the sequence listing: SEQ ID NO:50 or SEQ ID NO:52 (cbl), or SEQ ID NO:54 (TauR). The invention is not limited to the use of these amino acid sequences. Those of ordinary skill in the art know that organisms of a wide variety of species commonly express and utilize homologous proteins, which include the insertions, substitutions and/or deletions discussed above, and effectively provide similar function. For example, the amino acid sequences for cbl from *Escherichia coli*, or cbl from *Corynebacterium glutamicum* or TauR from *Corynebacterium glutamicum* may differ to a certain degree from the amino acid sequences of cbl or TauR in another species and yet have similar functionality with respect to catalytic and regulatory function. Amino acid sequences comprising such variations are included within the scope of the present invention and are considered substantially or sufficiently similar to a reference amino acid sequence. Although it is not intended that the present invention be limited by any theory by which it achieves its advantageous result, it is believed that the identity between amino acid sequences that is necessary to maintain proper functionality is related to maintenance of the tertiary structure of the polypeptide such that specific interactive sequences will be properly located and will have the desired activity, and it is contemplated that a polypeptide including these interactive sequences in proper spatial context will have activity.

[36] Another manner in which similarity may exist between two amino acid sequences is where there is conserved substitution between a given amino acid of one group, such as a non-polar amino acid, an uncharged polar amino acid, a charged polar acidic amino acid, or a charged polar basic amino acid, with an amino acid from the same amino acid group. For example, it is known that the uncharged polar amino acid serine may commonly be substituted with the uncharged polar amino acid threonine in a polypeptide without substantially altering the functionality of the polypeptide. Whether a given substitution will affect the functionality of the enzyme may be determined without undue experimentation using synthetic techniques and screening assays known to one with ordinary skill in the art.

[37] One of ordinary skill in the art will recognize that changes in the amino acid sequences, such as individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is "sufficiently similar" when the alteration results in the substitution of an amino acid with a chemically similar amino acid. Thus, any number of amino acid residues selected from the group of integers consisting of from 1 to 15 can be so altered. Thus, for example, 1, 2, 3, 4, 5, 7 or 10 alterations can be made. Conservatively modified variants typically provide similar biological activity as the unmodified polypeptide sequence from which they are derived. Conserved substitutions of functionally similar amino acids are known by those of ordinary skill in the art. The following three groups each contain amino acids that are conserved substitutions for one another: (1) Alanine (A), Serine (S), and Threonine (T); (2) Aspartic acid (D) and Glutamic acid (E); (3) Asparagine (N) and Glutamine (Q).

Directed Evolution and Gene Shuffling

[38] The invention is not limited to the fusing of the polynucleotides and corresponding amino acids from CS or CBS with decarboxylases to make a sCS/PLP-DC. Those of ordinary skill in the art know that methods such as directed evolution, gene shuffling, site-directed mutagenesis, saturation mutagenesis, randomized mutagenesis, PCR mutagenesis, sequence saturated mutagenesis and computational modeling can be used to increase catalytic activity or maximize efficiency in specific organisms [see reviews and references therein (76-78)].

Suitable Polynucleotides

[39] Suitable polynucleotides encoding enzymes of taurine biosynthetic and degradation pathways, taurine specific substrate binding proteins, and translational regulators of taurine degradation pathways are described below. The invention is not limited to the use of these sequences, however. In fact, any nucleotide sequence that encodes an enzyme of CS/PLP-DC, or a CS or CBS with a DC including AAAD, GAD, BABD, or SAD can be used in an expression vector to produce recombinant protein with sCS/PLP-DC activity in a eukaryote, prokaryote or unicellular organism alone or in combination with the expression for a taurine-binding protein or an impaired or absent degradation taurine pathway(s) due to suppression, mutation silencing of degradation enzymes or regulators of the degradation taurine pathway.

[40] A suitable polynucleotide for CS/PLP-DC is provided in SEQ ID NO:1, SEQ ID NO: 3, or SEQ ID NO:82. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:82 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:1, SEQ ID NO: 3, or SEQ ID NO:82 when it used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:83 when it is used as a reference for sequence comparison.

[41] A suitable polynucleotide for CS is provided in SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9 when it used as a reference for sequence comparison or polynucleotides that

encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10 when it is used as a reference for sequence comparison.

[42] A suitable polynucleotide for CBS is provided in SEQ ID NO:11, SEQ ID NO:13 or SEQ ID NO:15. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:11, SEQ ID NO:13 or SEQ ID NO:15 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:11, SEQ ID NO:13 or SEQ ID NO:15 when it used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:12, SEQ ID NO:14 or SEQ ID NO:16 when it is used as a reference for sequence comparison.

[43] A suitable polynucleotide for AAAD is provided in SEQ ID NO:17 or SEQ ID NO:19. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:17 or SEQ ID NO:19 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:17 or SEQ ID NO:19 when it used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:18 or SEQ ID NO:20 when it is used as a reference for sequence comparison.

[44] A suitable polynucleotide for GAD is provided in SEQ ID NO:21. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides SEQ ID NO:21 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID

NO:21 when it used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:22 when it is used as a reference for sequence comparison.

[45] A suitable polynucleotide for BABD is provided in SEQ ID NO:23. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides SEQ ID NO:23 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:23 when it used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:24 when it is used as a reference for sequence comparison.

[46] A suitable polynucleotide for SAD is provided in SEQ ID NO:25 or SEQ ID NO:27. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:25 or SEQ ID NO:27 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:25 or SEQ ID NO:27 when it is used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:26 or SEQ ID NO:28 when it is used as a reference for sequence comparison.

[47] Suitable polynucleotides for a taurine-binding protein are provided in SEQ ID NO:29 or SEQ ID NO:31. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:29 or SEQ ID NO:31 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:29 or SEQ ID NO:31 when it is used

as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:30 or SEQ ID NO:32 when it is used as a reference for sequence comparison.

[48] A suitable polynucleotide for TDO is provided in SEQ ID NO:33. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:33 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:33 when it is used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:34 when it is used as a reference for sequence comparison.

[49] Suitable polynucleotides for a SsuD are provided in SEQ ID NO:35 and SEQ ID NO:39. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:35 and SEQ ID NO:39 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:35 and SEQ ID NO:39 when it is used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:36 or SEQ ID NO:40 when it is used as a reference for sequence comparison.

[50] Suitable polynucleotides for a SsuE are provided in SEQ ID NO:37 and SEQ ID NO:41. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:37 and SEQ ID NO:41 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:37 and SEQ ID NO:41 when it is

used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:38 and SEQ ID NO:42 when it is used as a reference for sequence comparison.

[51] A suitable polynucleotide for TauX is provided in SEQ ID NO:43. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:43 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:43 when it is used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:44 when it is used as a reference for sequence comparison.

[52] A suitable polynucleotide for TauY is provided in SEQ ID NO:45. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:45 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:45 when it is used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:46 when it is used as a reference for sequence comparison.

[53] A suitable polynucleotide for Tpa is provided in SEQ ID NO:47. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:47 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:47 when it is used as a reference for sequence comparison or polynucleotides that encode

polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:48 when it is used as a reference for sequence comparison.

[54] Suitable polynucleotides for a cbl are provided in SEQ ID NO:49 and SEQ ID NO:51. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:49 and SEQ ID NO:51 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:49 and SEQ ID NO:51when it is used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:50 or SEQ ID NO:52 when it is used as a reference for sequence comparison.

[55] A suitable polynucleotide for TauR is provided in SEQ ID NO:53. Other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that selectively hybridize to the polynucleotides of SEQ ID NO:53 by hybridization under low stringency conditions, moderate stringency conditions, or high stringency conditions. Still other suitable polynucleotides for use in accordance with the invention may be obtained by the identification of polynucleotides that have substantial identity of the nucleic acid of SEQ ID NO:53 when it is used as a reference for sequence comparison or polynucleotides that encode polypeptides that have substantial identity to amino acid sequence of SEQ ID NO:54 when it is used as a reference for sequence comparison.

[56] Another embodiment of the invention is a polynucleotide (e.g., a DNA construct) that encodes a protein that functions as an sCS/PLP-DC that selectively hybridizes to either SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, or SEQ ID:82. Selectively hybridizing sequences typically have at least 40% sequence identity, preferably 60-90% sequence identity, and most preferably 95% sequence identity with each other.

[57] Another embodiment of the invention is a polynucleotide that encodes an sCS/PLP-DC polypeptide that has substantial identity to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID:83, or SEQ ID:84. Substantial identity of amino acid sequences for these purposes normally means sequence identity of between 50-100%, preferably at least 55%, preferably at least 60%, more preferably at least 70%, 80%, 90%, and most preferably at least 95%.

[58] Another embodiment of the invention is a polynucleotide (e.g., a DNA construct) that encodes a protein that functions as a TauA, TauK, TauD, TauX, TauY, Tpa, or TauR that selectively hybridizes to SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, or SEQ ID NO:53, respectively; or functions as a SsuD that selectively hybridizes to either SEQ ID NO:35 or SEQ ID NO:39; or functions as a SsuE that selectively hybridizes to either SEQ ID NO:37 or SEQ ID NO:41; or functions as a cbl that selectively hybridizes to either SEQ ID NO:49, SEQ ID NO:51. Selectively hybridizing sequences typically have at least 40% sequence identity, preferably 60-90% sequence identity, and most preferably 100% sequence identity with each other.

[59] Another embodiment of the invention is a polynucleotide that encodes a polypeptide that has substantial identity to the amino acid sequence of SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, or SEQ ID NO:54. Substantial identity of amino acid sequences for these purposes normally means sequence identity of between 50-100%, preferably at least 55%, preferably at least 60%, more preferably at least 70%, 80%, 90%, and most preferably at least 100%.

[60] The process of encoding a specific amino acid sequence may involve DNA sequences having one or more base changes (i.e., insertions, deletions, substitutions) that do not cause a change in the encoded amino acid, or which involve base changes which may alter one or more amino acids, but do not eliminate the functional properties of the polypeptide encoded by the DNA sequence.

[61] It is therefore understood that the invention encompasses more than the specific polynucleotides encoding the proteins described herein. For example, modifications to a sequence, such as deletions, insertions, or substitutions in the sequence, which produce "silent" changes that do not substantially affect the functional properties of the resulting polypeptide, are expressly contemplated by the present invention. Furthermore, because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations" and represent one species of conservatively modified variation. Every nucleic acid sequence herein that encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of ordinary skill in the art will recognize that each amino acid has more than one codon, except for methionine and tryptophan that ordinarily have the codons AUG and UGG, respectively. It is known by those of ordinary skill in the art, "universal" code is not completely universal. Some mitochondrial and bacterial genomes diverge from the universal code, e.g., some termination codons in the universal code specify amino acids in the mitochondria or bacterial codes. Thus, each silent variation of a nucleic acid, which encodes a polypeptide of the present invention, is implicit in each described polypeptide sequence and incorporated in the descriptions of the invention.

[62] It is understood that alterations in a nucleotide sequence, which reflect the degeneracy of the genetic code, or which result in the production of a chemically equivalent amino acid at a given site, are contemplated. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a biologically equivalent product.

[63] Nucleotide changes which result in alteration of the amino-terminal and carboxy-terminal portions of the encoded polypeptide molecule would also not generally be expected to alter the activity of the polypeptide. In some cases, it may in fact be desirable to make mutations in the

sequence in order to study the effect of alteration on the biological activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art.

[64] When the nucleic acid is prepared or altered synthetically, one of ordinary skill in the art can take into account the known codon preferences for the intended host where the nucleic acid is to be expressed. For example, although nucleic acid sequences of the present invention may be expressed in different species, sequences can be modified to account for the specific codon preferences and GC-content preferences of the organism, as these preferences have been shown to differ (79-84).

Cloning Techniques

[65] For purposes of promoting an understanding of the principles of the invention, reference will now be made to particular embodiments of the invention and specific language will be used to describe the same. The materials, methods and examples are illustrative only and not limiting. Unless mentioned otherwise, the techniques employed or contemplated herein are standard methodologies well known to one of ordinary skill in the art. Specific terms, while employed below and defined at the end of this section, are used in a descriptive sense only and not for purposes of limitation. The practice of the present invention will employ, unless otherwise indicated, conventional techniques of botany, microbiology, mycology, phycology, tissue culture, molecular biology, chemistry, biochemistry, biotechnology, recombinant DNA technology and synthetic biology, which are within the skill of the art (85-94).

[66] A suitable polynucleotide for use in accordance with the invention may be obtained by cloning techniques using cDNA or genomic libraries, DNA, or cDNA from eukaryotes (i.e. fish, plants, or, mammals), bacteria, algae, microalgae, diatoms, yeast or fungi which are available commercially or which may be constructed using standard methods known to persons of ordinary skill in the art. Suitable nucleotide sequences may be isolated from DNA libraries obtained from a wide variety of species by means of nucleic acid hybridization or amplification methods, such as polymerase chain reaction (PCR) procedures, using as probes or primers nucleotide sequences selected in accordance with the invention.

[67] Furthermore, nucleic acid sequences may be constructed using chemical synthesis or amplified. The product of amplification is termed an amplicon. Moreover, if the particular nucleic acid sequence is of a length that makes chemical synthesis of the entire length impractical, the sequence may be broken up into smaller segments that may be synthesized and ligated together to form the entire desired sequence by methods known in the art. Alternatively, individual components or DNA fragments may be amplified by PCR and adjacent fragments can be amplified together using fusion-PCR (95), overlap-PCR (96) or chemical (de novo) synthesis (97-101) using a vendor (e.g. DNA2.0, GE life technologies, GENEART, Gen9, GenScript) by methods known in the art.

[68] A suitable polynucleotide for use in accordance with the invention may be constructed by recombinant DNA technology, for example, by cutting or splicing nucleic acids using restriction enzymes and mixing with a cleaved (cut with a restriction enzyme) vector with the cleaved insert (DNA of the invention) and ligated using DNA ligase. Alternatively, amplification techniques, such as PCR, can be used, where restriction sites are incorporated in the primers that otherwise match the nucleotide sequences (especially at the 3′ ends) selected in accordance with the invention. The desired amplified recombinant molecule is cut or spliced using restriction enzymes and mixed with a cleaved vector and ligated using DNA ligase. In another method, after amplification of the desired recombinant molecule, DNA linker sequences are ligated to the 5′ and 3′ ends of the desired nucleotide insert with ligase, the DNA insert is cleaved with a restriction enzyme that specifically recognizes sequences present in the linker sequences and the desired vector. The cleaved vector is mixed with the cleaved insert, and the two fragments are ligated using DNA ligase. In yet another method, the desired recombinant molecule is amplified with primers that have recombination sites (e.g. Gateway) incorporated in the primers, that otherwise match the nucleotide sequences selected in accordance with the invention. The desired amplified recombinant molecule is mixed with a vector containing the recombination site and recombinase, the two molecules are fused together by recombination.

[69] The recombinant expression cassette or DNA construct includes a promoter that directs transcription in a specific organism, operably linked to the polynucleotide encoding an sCS/PLP-DC, an sCS/PLP-DC with a TauA, or an sCS/PLP-DC with a TauK. In various aspects of the invention described herein, a variety of different types of promoters are described and used. As

used herein, a polynucleotide is "operably linked" to a promoter or other nucleotide sequence when it is placed into a functional relationship with the promoter or other nucleotide sequence. The functional relationship between a promoter and a desired polynucleotide insert typically involves the polynucleotide and the promoter sequences being contiguous such that transcription of the polynucleotide sequence will be facilitated. Two nucleic acid sequences are further said to be operably linked if the nature of the linkage between the two sequences does not (1) result in the introduction of a frame-shift mutation; (2) interfere with the ability of the promoter region sequence to direct the transcription of the desired nucleotide sequence, or (3) interfere with the ability of the desired nucleotide sequence to be transcribed by the promoter sequence region. Typically, the promoter element is generally upstream (i.e., at the 5′ end) of the nucleic acid insert coding sequence.

[70] While a promoter sequence can be ligated to a coding sequence prior to insertion into a vector, in other embodiments, a vector is selected that includes a promoter operable in the host cell into which the vector is to be inserted. In addition, certain preferred vectors have a region that codes a ribosome binding site positioned between the promoter and the site at which the DNA sequence is inserted so as to be operatively associated with the DNA sequence of the invention to produce the desired polypeptide, i.e., the DNA sequence of the invention in-frame.

Suitable Peptide Linkers

[71] Peptide linkers are known to those skilled in the art to connect protein domains or peptides. In general, linkers that contain the amino acids, glycine and serine, are useful linkers (102, 103). Other suitable linkers that can be used in the invention are known to those of ordinary skill in the art (104-109).

Suitable promoters: Unicellular organisms

[72] A wide variety of promoters are known to those of ordinary skill in the art, as are other regulatory elements that can be used alone or in combination with promoters. A wide variety of promoters that direct transcription in unicellular organisms can be used in connection with the present invention (110-112). The features (binding sites and regulatory elements) necessary for the identification and use of functional bacterial promoters are known to those of ordinary skill

in the art (113-115). For purposes of describing the present invention, promoters are divided into two types, namely, constitutive promoters and non-constitutive promoters (111, 116). Constitutive promoters are classified as providing for a range of constitutive expression. Some are weak constitutive promoters, and others are strong constitutive promoters (117). Other promoters are considered non-constitutive promoters (118-122). A selected promoter can be an endogenous promoter, i.e. a promoter native to the species and or cell type being transformed. Alternatively, the promoter can be a foreign promoter, which promotes transcription of a length of DNA. The promoter may be of viral origin, including a cauliflower mosaic virus promoter (CaMV 35S) (117), and SV40 promoters from viruses have been used to express target genes (123). The promoters may further be selected such that they require activation by other elements known to those of ordinary skill in the art, so that production of the protein encoded by the nucleic acid sequence insert may be regulated as desired. In one embodiment of the invention, a DNA construct comprising a non-constitutive promoter operably linked to a polynucleotide encoding the desired polypeptide of the invention is used to make a transformed unicellular organism that selectively increases the level of the desired polypeptide of the invention in response to a signal. The term "signal" is used to refer to a condition, stress or stimulus that results in or causes a non-constitutive promoter to direct expression of a coding sequence operably linked to it. To make such a unicellular organism in accordance with the invention, a DNA construct is provided that includes a non-constitutive promoter operably linked to a polynucleotide encoding the desired polypeptide of the invention. The construct is incorporated into a unicellular organism to provide a transformed organism that expresses the polynucleotide in response to a signal. It is understood that the non-constitutive promoter does not continuously produce the transcript or RNA of the invention. But in this embodiment the selected promoter for inclusion of the invention advantageously induces or increases transcription of the gene for the desired polypeptide of the invention in response to a signal, such as a chemical or environmental cue or other stress signal including biotic and/or abiotic stresses or other conditions.

Terminators: Unicellular Organisms

[73] Terminators are typically located downstream (3') of the gene, after the stop codon (TGA, TAG or TAA). Terminators play an important role in the processing and stability of RNA as well as in translation and may also control gene expression (124-133). The identification and use of

terminators that are required to express genes in unicellular organisms are known to those of ordinary skill in the art.

Suitable Vectors: Unicellular Organisms

[74] A wide variety of vectors may be employed to transform a unicellular organism with a construct made or selected in accordance with the invention, including high- or low-copy number plasmids, phage vectors and cosmids. Vector systems, expression cassettes, culture methods, and transformation methods are known by those of ordinary skill in the art. The vectors can be chosen such that operably linked promoter and polynucleotides that encode the desired polypeptide of the invention are incorporated into the genome of the unicellular organism. Other vectors that can operably link promoter and polynucleotides that encode the polypeptide of the invention are incorporated into the host but are not incorporated into the host genome. The vector DNA with the clone polynucleotides are autonomously or semi autonomously replicated in the cell. Although the preferred embodiment of the invention is expressed in bacteria, other embodiments may include expression in prokaryotic or unicellular eukaryotic organisms including, but not limited to, yeast, fungi, algae, microalgae, or microbes.

[75] It is known by those of ordinary skill in the art that there exist numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention. There are many commercially available recombinant vectors to transform a unicellular organism. Standard molecular and cloning techniques (89, 92, 134) are available to make a recombinant expression cassette that expresses the polynucleotide that encodes the desired polypeptide of the invention. No attempt will be made to describe in detail the various methods known for the expression of proteins in prokaryotes or eukaryotes. In brief, the expression of isolated nucleic acids encoding a protein of the present invention will typically be achieved by operably linking, for example, the DNA or cDNA to a promoter, followed by incorporation into an expression vector. The vectors can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the DNA encoding a protein of the present invention. To obtain high-level expression of a cloned gene, it is desirable to construct

expression vectors that contain, at the minimum, a strong promoter to direct transcription, a ribosome-binding site for translational initiation, and a transcription/translation terminator.

Expression in Prokaryotes

[76] Protocols for transformation as well as commonly used vectors with control sequences including promoters for transcription initiation (some with an operator), together with ribosome binding site sequences for use in prokaryotes are known to those of ordinary skill in the art. Commonly used prokaryotic control sequences include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences. Commonly used prokaryotic promoters include the beta lactamase (135), lactose (135), and tryptophan (136) promoters. The vectors usually contain selectable markers to identify transfected or transformed cells. Some commonly used selectable markers include the genes for resistance to ampicillin, tetracycline, or chloramphenicol. The vectors are typically a plasmid or phage. Bacterial cells are transfected or transformed with the plasmid vector DNA. Phage DNA can be infected with phage vector particles or transfected with naked phage DNA. The plasmid and phage DNA for the vectors are commercially available from numerous vendors known to those of ordinary skill in the art. Those of ordinary skill in the art know the molecular techniques and DNA vectors that are used in bacterial systems (137-141). In bacteria one messenger RNA can encode for one peptide (referred to as monocistronic) or several independent peptides (referred to as polycistronic). It is known to those of ordinary skill in the art that a portion of a polycistronic messenger RNA can be knocked-out (142) or that heterologous or exogenous genes can be expressed on a monocistronic or polycistronic messenger RNA (140, 141). Genes can be expressed by modification of bacterial DNA (genomic) through the use of knock-in, gene insertion, or by allelic exchange (143-148). Specific gene targeting has been used in bacteria using PCR-based methods (149) and CRISPR/Cas (150-152).

Expression in Algae and Microalgae

[77] Protocols for transformation as well as commonly used vectors with control sequences include promoters for transcription initiation, optionally with an operator, together with ribosome binding site sequences for use in algae and microalgae are known to those of ordinary skill in the

art (111, 153-164). Specific gene targeting systems have been used in algae including ZFNs (165) and transcription activator-like effector nucleases (TALENs) (166).

Transformation of Host Cells: Unicellular Organisms

[78] Transformation of a unicellular organism can be accomplished in a wide variety of ways within the scope of a person of ordinary skill in the art (110, 112, 162, 167). Those of ordinary skill in the art can use different algal, diatom, fungal, yeast and bacteria gene transfer techniques that include, but not limited to, Agrobacterium-mediated (168) glass beads and polyethylene glycol (PEG) (169, 170), electroporation (171-174), microprojectile bombardment or ballistic particle acceleration (175-179), silicon carbide whisker methods (180, 181), viral infection (182, 183), or transposon/transposase complexes (184). Transformation can be targeted to organellular genomes (185). Other methods to edit, incorporate or move genes into bacteria, fungal, or algal genomes include, but are not limited to, Zinc-finger nucleases (ZFNs), transcription activator like effector nucleases (TALENs), or clustered regularly interspaced short palindromic repeats/Cas (CRISPR/Cas).

Suitable Unicellular Organisms

[79] A wide variety of unicellular host cells may be used in the invention, including prokaryotic and unicellular eukaryotic host cells. These cells or organisms may include yeast, fungi, algae, microalgae, microbes, or unicellular photosynthetic organisms. Preferred host cells for this invention are bacteria including archaebacteria and eubacteria. Proteobacteria such as members of Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria can host the invention. Other bacteria including methanotrophs, methylotrophs, or members of the genera *Methylobacterium, Methylobacterium, Methylomonas, Methylobacter, Methylococcus, Methylosinus, Methylocyctis, Methylomicrobium,* and *Methanomonas* (186) can be used with the invention. These include, but are not limited to, *Methylobacterium extorquens, Methylobacterium populi, Methylobacterium radiotolerans, Methylobacterium nodulans,* and *Methylobacterium* spp. Other methylotrophic bacteria *include but are not limited to Methylophilus, Methylobacillus, Methylobacterium, Hyphomicrobium, Xanthobacter, Bacillus, Paracoccus, Nocardia, Arthrobacter, Rhodopseudomonas,* and *Pseudomonas.* Some vectors developed for use in Methylobacterium include, but are not limited

to, pCM62, pCM66, pCM80, pCM160, pHC41, pHC90, pHC91, pHC115, pLC 290, or pLC291. Other bacterial genera that can host the invention include, but are not limited to, Bacillus, *Salmonella*, *Lactococcus, Streptococcus, Brevibacterium* and Coryneform bacteria. Some specific bacterial species that can be used for the invention include, but are not limited to, *Bacillus subtilis*, *Brevibacterium ammoniagene*, *Corynebacterium crenatum*, *Corynebacterim pekinese*, *Corynebacterium glutamicumas*, *Erwinia citreus*, *Erwinia herbicola*, *Escherichia coli*, *Fusarium venenatum, Gluconobacter oxydans, Propionibacterium freudenreicheii*, and *Propionibacterium denitrificans* (187).

[80] Unicellular algae, unicellular photosynthetic organisms, and microscopic algae (microphytes or microalgae) cells may be used in the invention. These include, but are not limited to diatoms, green algae (Chlorophyta), and members of the Euglenophyta, Dinoflagellata, Chrysophyta, Phaeophyta, red algae (Rhodophyta), Heterokontophyta, and Cyanobacteria. The invention can also be used to increase the taurine by binding taurine with a taurine binding protein or knocking out genes for taurine degradation that have been shown to synthesize taurine (52) or may have the capability to synthesize taurine (52). These include but are not limited to *Coccomyxa species, Chlorella species, Trebouxia impressa, Tetraselmis species, Chlamydomonas reinhardtii, Micromonas pusilla, Ostreococcus tauri, Navicula radiosa, Phaeodactylum tricornutum, Pseudo-nitzschia multiseries, Fragilariopsis cylindrus, Thalassiosira weissflogii, Nannochloropsis oceanica, Aureococcus anophagefferens, Saccharina japonica, Sargassum species* and *Bigelowiella natans*.

[81] Protozoa that may be used in the invention include, but are not limited to, ciliates, amoebae and flagellates. Yeast and unicellular fungi that can be used include, but are not limited to, *Ashbya gossypii, Blakeslea trispora, Candida flareri, Eremothecium ashbyii, Mortierella isabellina, Pichia pastoris, Saccharomyces cerevisiae,* and *Saccharomyces pombe*.

Expression in Non-plant Eukaryotes

[82] Protocols for transformation, as well as commonly used vectors, are known to those of ordinary skill in the art. Also known to those of ordinary skill in the art are control sequences that include promoters for transcription initiation and ribosome binding site sequences for use in unicellular eukaryotes. The present invention can be expressed in a variety of eukaryotic expression systems

such as yeast and protozoa. The vectors usually have expression control sequences, such as promoters, an origin of replication, enhancer sequences, termination sequences, ribosome binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and selectable markers (188, 189). There are numerous vectors that can be used with the invention that are known to those of ordinary skill in the art and include, but are not limited to, pREP, pRIP, pD912, pD1201, pD1211, pD1221, pD1231, pYES2/NT, pYSG-IBA, or pESC-TRP. Synthesis of heterologous proteins and fermentation of products in yeast are known to those of ordinary skill in the art (190, 191). Protozoa that can be used include, but are not limited to, ciliates, amoebae and flagellates. Yeast and fungi that can be used with the invention and the molecular protocols for transformation, and the vectors required for expression of genes in these systems, are known to those of ordinary skill in the art (192-197). Also available are plasmid vectors, which may be integrative, autonomously replicating high copy-number vectors, or autonomously replicating low copy number vectors (198, 199). The most common vectors that complement a chromosomal mutation in the host include functional genes such as URA3, HIS3, LEU2, TRP1 and LYS2. Specific gene editing or targeting has been used in unicellular fungi using PCR-based methods (200-202), Zinc-finger nucleases (ZFNs) (203), transcription activator like effector nucleases (TALENs) (204), and clustered regularly interspaced short palindromic repeats/Cas (CRISPR/Cas) (205, 206).

[83] The present invention can be expressed in a variety of eukaryotic expression systems such as yeast, insect cell lines, and mammalian cells which are known to those of ordinary skill in the art. For each host system, suitable vectors are commercially available (e.g., Invitrogen, Startagene, GE Healthcare Life Sciences). The vectors usually have expression control sequences, such as promoters, an origin of replication, enhancer sequences, termination sequences, ribosome binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and selectable markers. Synthesis of heterologous proteins and fermentation of products in yeast are well known to those of ordinary skill in the art (190, 191). Yeast and fungi that can be used include, but are not limited to, *Ashbya gossypii, Blakeslea trispora, Candida flareri, Eremothecium ashbyii, Mortierella isabellina, Pichia pastoris, Saccharomyces cerevisiae* and *Saccharomyces sp.* Molecular protocols for transformation and the vectors required for expression in these systems are known to those of ordinary skill in the art (193, 194, 196, 207, 208).

[84] Insect cell lines that include, but are not limited to, black-fly larvae, mosquito larvae, silkworm, armyworm, moth, and Drosophila cell lines can be used to express proteins of the present invention using baculovirus-derived vectors (209). In addition, mammalian cell lines can be used to express proteins of the present invention (210). A number of suitable host cell lines capable of expressing intact proteins have been developed in the art, and include the HEK293, BHK21, and CHO cell line.

[85] A protein of the present invention, once expressed in any of the non-plant eukaryotic systems, can be isolated from the organism by lysing the cells and applying standard protein isolation techniques to the lysates or the pellets. The monitoring of the purification process can be accomplished by using western blot techniques, radioimmunoassay, or other standard immunoassay techniques.

Expression in Plants

[86] Protocols for plant transformation, as well as commonly used vectors, are known to those of ordinary skill in the art. Also known to those of ordinary skill in the art are control sequences that include promoters for transcription initiation and ribosome binding site sequences for use in plants. A wide variety of promoters are known to those of ordinary skill in the art as are other regulatory elements that can be used alone or in combination with promoters. A wide variety of promoters that direct transcription in plants cells can be used in connection with the present invention. For purposes of describing the present invention, promoters are divided into two types, namely, constitutive promoters and non-constitutive promoters. Constitutive promoters are classified as providing for a range of constitutive expression. Thus, some are weak constitutive promoters, and others are strong constitutive promoters. Non-constitutive promoters include tissue-preferred promoters, tissue-specific promoters, cell-type specific promoters, and inducible-promoters.

Suitable Plant Promoters

[87] Of particular interest in certain embodiments of the present invention are inducible-promoters that respond to various forms of environmental stresses, or other stimuli, including, for example, mechanical shock, heat, cold, salt, flooding, drought, salt, anoxia, pathogens, such as bacteria,

fungi, and viruses, and nutritional deprivation, including deprivation during times of flowering and/or fruiting, and other forms of plant stress. For example, the promoter selected in alternate forms of the invention, can be a promoter that is induced by one or more of the following, but not limited to, abiotic stresses such as wounding, cold, desiccation, ultraviolet-B (211), heat shock (212) or other heat stress, drought stress or water stress. The promoter may further be one induced by biotic stresses including pathogen stress, such as stress induced by a virus (213) or fungi (214, 215), stresses induced as part of the plant defense pathway (216) or by other environmental signals, such as light (217), carbon dioxide (218, 219), hormones or other signaling molecules such as auxin, hydrogen peroxide and salicylic acid (220, 221), sugars and gibberellin (222) or abscisic acid and ethylene (223).

[88] In other embodiments of the invention, tissue-specific promoters are used. Tissue-specific expression patterns as controlled by tissue- or stage-specific promoters that include, but is not limited to, fiber-specific, green tissue-specific, root-specific (224, 225), stem-specific, and flower-specific. Examples of the utilization of tissue-specific expression includes, but is not limited to, the expression in leaves of the desired peptide for the protection of plants against foliar pathogens, the expression in roots of the desired peptide for the protection of plants against root pathogens, and the expression in roots or seedlings of the desired peptide for the protection of seedlings against soil-borne pathogens. In many cases, however, protection against more than one type of pathogen may be sought, and expression in multiple tissues will be desirable.

[89] Of particular interest in certain embodiments of the present invention seed-specific promoters are used. Examples of the utilization of seed-specific promoters for expression includes, but is not limited to, napin (226), sunflower seed-specific promoter (227, 228), phaseolin (229), beta-conglycinin (230), zein (231), and rice glutelin (232).

[90] Although some promoters from dicotyledons have been shown to be operational in monocotyledons and vice versa, ideally dicotyledonous promoters are selected for expression in dicotyledons, and monocotyledonous promoters are selected for expression in monocotyledons. There are also promoters that control expression of genes in green tissue or for genes involved in photosynthesis from both monocotyledons and dicotyledons such as the maize phosphoenol carboxylase gene (233). A promoter selected can be an endogenous promoter, i.e. a promoter

native to the species and or cell type being transformed. Alternatively, the promoter can be a foreign promoter, which promotes transcription of a length of DNA of viral, microbes, bacterial or eukaryotic origin, invertebrates, vertebrates including those from plants and plant viruses. For example, in certain preferred embodiments, the promoter may be of viral origin, including a cauliflower mosaic virus promoter (CaMV), such as CaMV 35S or19S, a figwort mosaic virus promoter (FMV 35S), or the coat protein promoter of tobacco mosaic virus (TMV). The promoter may further be, for example, a promoter for the small subunit of ribulose-1, 3-biphosphate carboxylase. Promoters of bacterial origin (microbe promoters) include the octopine synthase promoter, the nopaline synthase promoter and other promoters derived from native Ti plasmids (234).

[91] The promoters may further be selected such that they require activation by other elements known to those of ordinary skill in the art, so that production of the protein encoded by the nucleic acid sequence insert may be regulated as desired. In one embodiment of the invention, a DNA construct comprising a non-constitutive promoter operably linked to a polynucleotide encoding the desired polypeptide of the invention is used to make a transformed plant that selectively increases the level of the desired polypeptide of the invention in response to a signal. The term "signal" is used to refer to a condition, stress or stimulus that results in or causes a non-constitutive promoter to direct expression of a coding sequence operably linked to it. To make such a plant in accordance with the invention, a DNA construct is provided that includes a non-constitutive promoter operably linked to a polynucleotide encoding the desired polypeptide of the invention. The construct is incorporated into a plant genome to provide a transformed plant that expresses the polynucleotide in response to a signal.

[92] In alternate embodiments of the invention, the selected promoter is a tissue-preferred promoter, a tissue-specific promoter, a cell-type-specific promoter, an inducible promoter or other type of non-constitutive promoter. It is readily apparent that such a DNA construct causes a plant transformed thereby to selectively express the gene for the desired polypeptide of the invention. Therefore, under specific conditions or in certain tissue- or cell-types the desired polypeptide will be expressed. The result of this expression in the plant depends upon the activity of the promoter and in some cases the conditions of the cell or cells in which it is expressed.

[93] It is understood that the non-constitutive promoter does not continuously produce the transcript or RNA of the invention. But in this embodiment the selected promoter for inclusion of the invention advantageously induces or increases transcription of gene for the desired polypeptide of the invention in response to a signal, such as an environmental cue or other stress signal including biotic and/or abiotic stresses or other conditions.

Suitable Plant Terminators

[94] In addition to the selection of a suitable promoter, the DNA constructs require an appropriate transcriptional terminator to be attached downstream of the desired gene of the invention for proper expression in plants. Terminators are typically located downstream (3') of the gene, after the stop codon (TGA, TAG or TAA). Terminators play an important role in the processing and stability of RNA as well as in translation. Most, but not all terminators, contain a polyadenylation sequence or cleavage site. Examples of specific polyadenylation sequences are AAUAAA or AAUAAU. These sequences are known as the near upstream elements (NUEs) (235). NUEs usually reside approximately 30 bp away from a GU-rich region (236-238) which is known as far upstream elements (FUEs). The FUEs enhance processing at the polyadenylation sequence or cleavage site, which is usually a CA or UA in a U-rich region (239). Within the terminator, elements exist that increase the stability of the transcribed RNA (240-242) and may also control gene expression (124, 243).

[95] Several terminators are available and known to persons of ordinary skill in the art. These include, but are not limited to, the tml from CaMV and E9 from rbcS. Another example of a terminator sequence is the polyadenylation sequence from the bovine growth hormone gene. A wide variety of available terminators known to function in plants can be used in the context of this invention. Vectors may also have other control sequence features that increase their suitability. These include an origin of replication, enhancer sequences, ribosome binding sites, RNA splice sites, polyadenylation sites, selectable markers and RNA stability signal. Origin of replication is a gene sequence that controls replication of the vector in the host cell. Enhancer sequences cooperate with the promoter to increase expression of the polynucleotide insert coding sequence. Enhancers can stimulate promoter activity in host cell. RNA splice sites are sequences that ensure accurate splicing of the transcript. Selectable markers usually confer resistance to an

antibiotic, herbicide or chemical or provide color change, which aid the identification of transformed organisms. The vectors also include a RNA stability signal, which are 3′-regulatory sequence elements that increase the stability of the transcribed RNA (241).

Suitable Plant Vectors

[96] A wide variety of vectors may be employed to transform a plant, plant cell or other cells with a construct made or selected in accordance with the invention, including high- or low-copy number plasmids, phage vectors and cosmids. Such vectors, as well as other vectors, are well known in the art. Representative T-DNA vector systems (234, 244) and numerous expression cassettes and vectors and in vitro culture methods for plant cell or tissue transformation and regeneration of plants are known and available (245). The vectors can be chosen such that operably linked promoter and polynucleotides that encode the desired polypeptide of the invention are incorporated into the genome of the plant. Although the preferred embodiment of the invention is expression in plants or plant cells, other embodiments may include expression in prokaryotic or eukaryotic photosynthetic organisms, yeast, fungi, algae, microalgae, microbes, invertebrates or vertebrates.

[97] It is known by those of ordinary skill in the art that there exist numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention. There are many commercially available recombinant vectors to transform a host plant or plant cell. Standard molecular and cloning techniques (89, 92, 134) are available to make a recombinant expression cassette that expresses the polynucleotide that encodes the desired polypeptide of the invention. No attempt to describe in detail the various methods known for the expression of proteins in prokaryotes or eukaryotes will be made. In brief, the expression of isolated nucleic acids encoding a protein of the present invention will typically be achieved by operably linking, for example, the DNA or cDNA to a promoter, followed by incorporation into an expression vector. The vectors can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the DNA encoding a protein of the present invention. To obtain high-level expression of a cloned gene, it is desirable to construct expression vectors that contain, at the minimum, a strong promoter such as

ubiquitin to direct transcription, a ribosome-binding site for translational initiation, and a transcription/translation terminator.

Transit Peptides

[98] A wide variety of plastid transit peptides are known to those of ordinary skill in the art that can be used in connection with the present invention. Suitable transit peptides which can be used to target any sCS/PLP-DC, TauA, or TauK polypeptide to a plastid include, but are not limited to, those described herein and in U.S. Patent Nos. 8,779,237 (246), 8,674,180 (247), 8,420,888 (248), and 8,138,393 (249) and in Lee et al. (250) and von Heijne et al. (251). Identification and use of chloroplast plastid targeting sequences for algae are known to those of ordinary skill in the art (252-255). Cloning a nucleic acid sequence that encodes a transit peptide upstream and in-frame of a nucleic acid sequence that encodes a polypeptide involves standard molecular techniques that are known to those of ordinary skill in the art. In addition, The specific cellular compartments include the apoplast, vacuole, plastids chloroplast, mitochondrion, peroxisomes, secretory pathway, lysosome, endoplasmic reticulum, nucleus or Golgi apparatus. A signal polypeptide or signal sequence is usually at the amino terminus and normally absent from the mature protein due to protease that removes the signal peptide when the polypeptide reaches its final destination. Signal sequences can be a primary sequence located at the N-terminus (256-259), C-terminus (260, 261) or internal (262-264) or tertiary structure (264). If a signal polypeptide or signal sequence to direct the polypeptide does not exist on the vector, it is expected that those of ordinary skill in the art can incorporate the extra nucleotides necessary to encode a signal polypeptide or signal sequence by the ligation of the appropriate nucleotides or by PCR. Those of ordinary skill in the art can identify the nucleotide sequence of a signal polypeptide or signal sequence using computational tools. There are numerous computational tools available for the identification of targeting sequences or signal sequence. These include, but are not limited to, TargetP (265, 266), iPSORT (267), SignalP (268), PrediSi (269), ELSpred (270) HSLpred (271) and PSLpred (272), MultiLoc (273), SherLoc (274), ChloroP (275), MITOPROT (276), Predotar (277) and 3D-PSSM (278). Additional methods and protocols are discussed in the literature (273).

Plant Transformation

[99] Transformation of a plant can be accomplished in a wide variety of ways within the scope of a person of ordinary skill in the art. In one embodiment, a DNA construct is incorporated into a plant by (i) transforming a cell, tissue or organ from a host plant with the DNA construct; (ii) selecting a transformed cell, cell callus, somatic embryo, or seed which contains the DNA construct; (iii) regenerating a whole plant from the selected transformed cell, cell callus, somatic embryo, or seed; and (iv) selecting a regenerated whole plant that expresses the polynucleotide. Many methods of transforming a plant, plant tissue or plant cell for the construction of a transformed cell are suitable. Once transformed, these cells can be used to regenerate transgenic plants (279).

[100] Those of ordinary skill in the art can use different plant gene transfer techniques found in references for, but not limited to, the electroporation, (280-284) microinjection, (285, 286) lipofection, (287) liposome or spheroplast fusions, (288-290) *Agrobacterium*, (291) direct gene transfer, (292) T-DNA mediated transformation of monocots, (293) T-DNA mediated transformation of dicots, (294, 295) microprojectile bombardment or ballistic particle acceleration, (296-299) chemical transfection including $CaCl_2$ precipitation, polyvinyl alcohol, or poly-L-ornithine, (300) silicon carbide whisker methods, (301, 302) laser methods, (303, 304) sonication methods, (305-307) polyethylene glycol methods, (308) vacuum infiltration (309) and transbacter. (310) Other methods to edit, incorporate or move genes into plant genomes include, but are not limited to, Zinc-finger nucleases (ZFNs) (311, 312) transcription activator like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats/Cas (CRISPR/Cas) (313-316).

[101] In one embodiment of the invention, a transformed host cell may be cultured to produce a transformed plant. In this regard, a transformed plant can be made, for example, by transforming a cell, tissue or organ from a host plant with an inventive DNA construct; selecting a transformed cell, cell callus, somatic embryo, or seed which contains the DNA construct; regenerating a whole plant from the selected transformed cell, cell callus, somatic embryo, or seed; and selecting a regenerated whole plant that expresses the polynucleotide.

Suitable Hosts and Plants

[102] A wide variety of host cells may be used in the invention, including prokaryotic and eukaryotic host cells. These cells or organisms may include yeast, fungi, algae, microalgae, microbes, invertebrate, vertebrates or photosynthetic organisms. Preferred host cells are eukaryotic, preferably plant cells, including such as those derived from monocotyledons or dicotyledons, including, acacia, alfalfa, algae, aneth, apple, apricot, artichoke, arugula, asparagus, avocado, banana, barley, beans, beech, beet, bent grass, Bermuda grass, blackberry, blueberry, Blue grass, broccoli, brussel sprouts, bush beans, cabbage, camelina, canola, cantaloupe, carrot, cassava, cauliflower, celery, cherry, chicory, cilantro, citrus, clementines, coffee, corn, cotton, cucumber, duckweed, Douglas fir, eggplant, endive, escarole, eucalyptus, fennel, fescue, figs, forest trees, garlic, gourd, grape, grapefruit, honey dew, jicama, kiwifruit, lettuce, leeks, lemon, lime, Loblolly pine, maize, mango, melon, mushroom, nectarine, nut, oat, okra, onion, orange, ornamental plants, papaya, parsley, pea, peach, peanut, pear, pepper, persimmon, pine, pineapple, plantain, plum, pomegranate, poplar, potato, pumpkin, quince, radiata pine, radicchio, radish, rapeseed, raspberry, rice, rye, rye grass, seaweed, scallion, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugarbeet, sugarcane, sunflower, sweet potato, sweetgum, switchgrass, tangerine, tea, tobacco, tomato, turf, turnip, a vine, watermelon, wheat, yam, and zucchini.

Plastid Transformation: Unicellular Organisms and Plants

[103] The invention can be targeted for transformation into the chloroplast. Chloroplast targeted transformation systems for algae and plants are known by those of ordinary skill in the art (119, 317).

Gene Silencing by Mutagenesis or Using Recombinant Technologies

[104] Genetic modification to silence or inactivate genes or their corresponding gene products of unicellular organisms can be conducted by radiation-, chemical- or UV-based mutagenesis followed by specific screening for biochemical traits or pathways (167, 318-322). Radiation-based mutations can silence or inactive a gene or the corresponding gene product by DNA breakage and repair. Chemical- or UV-based mutations usually result in single DNA basepair changes. Mutations can silence or inactive a gene or the corresponding gene product by one of the following: (1) introduction of a frame-shift mutation; (2) introduction of premature stop

codon; (3) interference with the ability of the promoter region sequence to direct the transcription of the desired nucleotide sequence, (4) interference with the ability of the desired nucleotide sequence to be transcribed by the promoter sequence region, or (5) introduction of an amino acid substitution in the gene product to reduce or inhibit activity (enzymatic or binding) or interfere with the function of the gene product.

[105] Targeted gene silencing or knockouts can be made in unicellular organisms using phage or viruses (116, 323-327), transposons (184, 328-331), PCR-assisted targeting (200-202, 332), recombinases or by allelic exchange (143-148), targeted and random bacterial gene disruptions using a group II intron (Targetron) (333, 334), ZNFs (203), TALENs (204), CRISPER-Cas9 or clustered regularly interspaced short palindromic repeats interference (CRISPi) (150-152, 205, 206, 335, 336). In addition, RNA-mediated methods (337-342), or regulatory RNAs (343-345) have been used to silence or suppress gene expression in unicellular organisms and these techniques and protocols are well known to one with ordinary skill in the art.

[106] One of ordinary skill in the art recognizes that modifications could be made to a protein of the present invention without diminishing its biological activity. Some modifications may be made to facilitate the cloning, expression, targeting or to direct the location of the polypeptide in the host, or for the purification or detection of the polypeptide by the addition of a "tag" as a fusion protein. Such modifications are known to those of ordinary skill in the art and include, for example, a methionine added at the amino terminus to provide an initiation site, additional amino acids (tags) placed on either terminus to create a tag, additional nucleic acids to insert a restriction site or a termination.

[107] In addition to the selection of a suitable promoter, the DNA constructs require an appropriate transcriptional terminator to be attached downstream of the desired gene of the invention for proper expression in unicellular organisms. Several such terminators are available and known to persons of ordinary skill in the art. These include, but are not limited to, the tml from CaMV and E9 from rbcS. A variety of available terminators known to function in unicellular organisms can be used in the present invention. Vectors may also have other control sequence features that increase their suitability. These include an origin of replication, enhancer sequences, ribosome binding sites, RNA splice sites, polyadenylation sites, selectable markers and RNA stability

signal. Origin of replication is a gene sequence that controls replication of the vector in the host cell. Selectable markers usually confer resistance to an antibiotic, herbicide or chemical or provide color change, which aid the identification of transformed organisms. The vectors may also include a RNA stability signal, which are 3′-regulatory sequence elements that increase the stability of the transcribed RNA (240, 241).

[108] In addition, polynucleotides that encode an sCS/PLP-DC can be placed in the appropriate vector used to transform unicellular organisms. The polypeptide can be expressed and then isolated from transformed cells, or metabolites can be synthetized and isolated from the transformed cells. Such transgenic organisms can be harvested, and subjected to large-scale protein or metabolite (taurine) extraction and purification techniques.

[109] The vectors may include another polynucleotide insert that encodes a peptide or polypeptide and used as a "tag" to aid in purification or detection of the desired protein. The additional polynucleotide is positioned in the vector such that upon cloning and expression of the desired polynucleotide a fusion, or chimeric, protein is obtained. The tag may be incorporated at the amino or carboxy terminus. If the vector does not contain a tag, persons with ordinary skill in the art know that the extra nucleotides necessary to encode a tag can be added with the ligation of linkers, adaptors, or spacers or by PCR using designed primers. After expression of the peptide the tag can be used for purification using affinity chromatography, and if desired, the tag can be cleaved with an appropriate enzyme. The tag can also be maintained, not cleaved, and used to detect the accumulation of the desired polypeptide in the protein extracts from the host using western blot analysis. In another embodiment, a vector includes the polynucleotide for the tag that is fused in-frame to the polynucleotide that encodes a functional sCS/PLP-DC, TauA, or TauK to form a fusion protein. The tags that may be used include, but are not limited to, Arg-tag, calmodulin-binding peptide, cellulose-binding domain, DsbA, c-myc-tag, glutathione S-transferase, FLAG-tag, HAT-tag, His-tag, maltose-binding protein, NusA, S-tag, SBP-tag, Strep-tag, and thioredoxin (Trx-Tag). These are available from a variety of manufacturers Clontech Laboratories, Takara Bio Company GE Healthcare, Invitrogen, Novagen Promega and QIAGEN.

[110] The vector may include another polynucleotide that encodes a signal polypeptide or signal sequence ("subcellular location sequence") to direct the desired polypeptide in the host cell, so

that the polypeptide accumulates in a specific cellular compartment, subcellular compartment, or membrane. The specific cellular compartments include the vacuole, chloroplast (not in fungi), mitochondrion, peroxisomes, secretory pathway, lysosome, endoplasmic reticulum, nucleus or Golgi apparatus in fungi or algae. There are specific signal polypeptides or signal sequences to direct peptide transport to the periplasmic space in bacteria.(346-348) A signal polypeptide or signal sequence is usually at the amino terminus and normally absent from the mature protein due to protease that removes the signal peptide when the polypeptide reaches its final destination. Signal sequences can be a primary sequence located at the N-terminus (251, 257-259), C-terminus (260, 261) or internal (262-264) or tertiary structure (264). If a signal polypeptide or signal sequence to direct the polypeptide does not exist on the vector, it is expected that those of ordinary skill in the art can incorporate the extra nucleotides necessary to encode a signal polypeptide or signal sequence by the ligation of the appropriate nucleotides or by PCR. Those of ordinary skill in the art can identify the nucleotide sequence of a signal polypeptide or signal sequence using computational tools. There are numerous computational tools available for the identification of targeting sequences or signal sequence. These include, but are not limited to, TargetP (265, 266), iPSORT (267), SignalP (268), PrediSi (269), ELSpred (270) HSLpred (271) and PSLpred (272), MultiLoc (273), SherLoc (274), ChloroP (275), MITOPROT (276), Predotar (277) 3D-PSSM (278) and PredAlgo. (255) Additional methods and protocols are discussed in the literature (273).

[111] One of ordinary skill to the art recognizes that modifications could be made to a protein of the present invention without diminishing its biological activity. Some modifications may be made to facilitate the cloning, expression, targeting or to direct the location of the polypeptide in the host, or for the purification or detection of the polypeptide by the addition of a "tag" as a fusion protein. Such modifications are well known to those of skill in the art and include, for example, a methionine added at the amino terminus to provide an initiation site, additional amino acids (tags) placed on either terminus to create a tag, additional nucleic acids to insert a restriction site or a termination.

[112] In addition, polynucleotides that encode a sCS/PLP-DC can be placed in the appropriate plant expression vector used to transform plant cells. The polypeptide can then be isolated from plant callus or the transformed cells can be used to regenerate transgenic plants. Such transgenic

plants can be harvested, and the appropriate tissues can be subjected to large-scale protein extraction and purification techniques.

[113] The vectors may include another polynucleotide insert that encodes a peptide or polypeptide used as a "tag" to aid in purification or detection of the desired protein. The additional polynucleotide is positioned in the vector such that upon cloning and expression of the desired polynucleotide a fusion, or chimeric, protein is obtained. The tag may be incorporated at the amino or carboxy terminus. If the vector does not contain a tag, persons with ordinary skill in the art know that the extra nucleotides necessary to encode a tag can be added with the ligation of linkers, adaptors, or spacers or by PCR using designed primers. After expression of the peptide the tag can be used for purification using affinity chromatography, and if desired, the tag can be cleaved with an appropriate enzyme. The tag can also be maintained, not cleaved, and used to detect the accumulation of the desired polypeptide in the protein extracts from the host using western blot analysis. In another embodiment, a vector includes the polynucleotide for the tag that is fused in-frame to the polynucleotide that encodes a functional sCS/PLP-DC to form a fusion protein. The tags that may be used include, but are not limited to, Arg-tag, calmodulin-binding peptide, cellulose-binding domain, DsbA, c-myc-tag, glutathione S-transferase, FLAG-tag, HAT-tag, His-tag, maltose-binding protein, NusA, S-tag, SBP-tag, Strep-tag, and thioredoxin (Trx-Tag). These are available from a variety of manufacturers Clontech Laboratories, Takara Bio Company GE Healthcare, Invitrogen, Novagen Promega and QIAGEN.

[114] The vector may include another polynucleotide that encodes a signal polypeptide or signal sequence ("subcellular location sequence") to direct the desired polypeptide in the host cell, so that the polypeptide accumulates in a specific cellular compartment, subcellular compartment, or membrane. The specific cellular compartments include the apoplast, vacuole, plastids chloroplast, mitochondrion, peroxisomes, secretory pathway, lysosome, endoplasmic reticulum, nucleus or Golgi apparatus. A signal polypeptide or signal sequence is usually at the amino terminus and normally absent from the mature protein due to protease that removes the signal peptide when the polypeptide reaches its final destination. Signal sequences can be a primary sequence located at the N-terminus (256-259), C-terminus (260, 261) or internal (262-264) or tertiary structure (264). If a signal polypeptide or signal sequence to direct the polypeptide does not exist on the vector, it is expected that those of ordinary skill in the art can incorporate the

extra nucleotides necessary to encode a signal polypeptide or signal sequence by the ligation of the appropriate nucleotides or by PCR. Those of ordinary skill in the art can identify the nucleotide sequence of a signal polypeptide or signal sequence using computational tools. There are numerous computational tools available for the identification of targeting sequences or signal sequence. These include, but are not limited to, TargetP (265, 266), iPSORT (267), SignalP (349), PrediSi (269 04), ELSpred (270) HSLpred (272) and PSLpred (272), MultiLoc (273), SherLoc (274), ChloroP (275), MITOPROT (276), Predotar (277) and 3D-PSSM (278). Additional methods and protocols are discussed in the literature (273).

Transformation of Host Cell

[115] Transformation of a plant can be accomplished in a wide variety of ways within the scope of a person of ordinary skill in the art. In one embodiment, a DNA construct is incorporated into a plant by (i) transforming a cell, tissue or organ from a host plant with the DNA construct; (ii) selecting a transformed cell, cell callus, somatic embryo, or seed which contains the DNA construct; (iii) regenerating a whole plant from the selected transformed cell, cell callus, somatic embryo, or seed; and (iv) selecting a regenerated whole plant that expresses the polynucleotide. Many methods of transforming a plant, plant tissue or plant cell for the construction of a transformed cell are suitable. Once transformed, these cells can be used to regenerate transgenic plants (279).

[116] Those of ordinary skill in the art can use different plant gene transfer techniques found in references for, but not limited to, the electroporation (280-284), microinjection (285, 286), lipofection (287), liposome or spheroplast fusions (288-290), *Agrobacterium* (291), direct gene transfer (292), T-DNA mediated transformation of monocots (293), T-DNA mediated transformation of dicots (294, 295), microprojectile bombardment or ballistic particle acceleration (175, 298, 299), chemical transfection including $CaCl_2$ precipitation, polyvinyl alcohol, or poly-L-ornithine (300), silicon carbide whisker methods (301, 302), laser methods (303, 304), sonication methods (305-307), polyethylene glycol methods (308), and vacuum infiltration (309) and transbacter (310). Other methods to edit, incorporate or move genes into plant genomes are found in references of, but not limited to, Zinc-finger nucleases (ZFNs),

transcription, activator like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats)–Cas (CRISPR/Cas) (311, 312, 350-353).

[117] In one embodiment of the invention, a transformed host cell may be cultured to produce a transformed plant. In this regard, a transformed plant can be made, for example, by transforming a cell, tissue or organ from a host plant with an inventive DNA construct; selecting a transformed cell, cell callus, somatic embryo, or seed which contains the DNA construct; regenerating a whole plant from the selected transformed cell, cell callus, somatic embryo, or seed; and selecting a regenerated whole plant that expresses the polynucleotide.

[118] A wide variety of host cells may be used in the invention, including prokaryotic and eukaryotic host cells. These cells or organisms may include yeast, fungi, algae, microalgae, microbes, invertebrate, vertebrates or photosynthetic organisms. Preferred host cells are eukaryotic, preferably plant cells, such as those derived from monocotyledons or dicotyledons.

[119] One embodiment of the invention (Embodiment Number 1) is a method for the increased production of taurine in an organism by the following steps:

[120] 1. operably link a promoter to the 5' end of the polynucleotide for a functional CS or CBS that is linked in-frame, with no linker, with a polynucleotide for a functional DC (using AAAD, GAD, BABD, or SAD) operably linked to a terminator;

[121] 2. insert the sCS/PLP-DC polynucleotide construct (from Step 1, Embodiment Number 1) into a vector; and

[122] 3. transform the vector containing the sCS/PLP-DC protein (from Step 2, Embodiment Number 1) construct into an organism.

[123] Another embodiment of the invention (Embodiment Number 2) is a method for the increased production of taurine in an organism by the following steps:

[124] 1. operably link a promoter to the 5' end of the polynucleotide for functional CS or CBS that is linked in-frame with a short, 3 to 66, polynucleotide (linker) to the 5' end of the polynucleotide for a functional decarboxylase (using AAAD, GAD, BABD, or SAD) operably linked to a terminator;

[125] 2. insert the sCS/PLP-DC polynucleotide construct (from Step 1, Embodiment Number 2) into a vector; and;

[126] 3. transform the vector containing the sCS/PLP-DC protein construct (from Step 2, Embodiment number 2) into an organism.

[127] Another embodiment of the invention (Embodiment Number 3) is a method for the increased production of taurine in an organism by the following steps:

[128] 1. operably link a promoter to the 5' end of the polynucleotide for a truncated functional Tau-binding protein (using TauA or TauK) operably linked to a terminator;

[129] 2. insert the taurine-binding protein polynucleotide construct (from Step 1, Embodiment Number 2) into a vector containing the functional sCS/PLP-DC protein construct (from Step 2, Embodiment Number 1 or from Step 2, Embodiment Number 2); and

[130] 3. transform the vector containing the sCS/PLP-DC and taurine-binding protein (from Step 2, Embodiment Number 3) constructs into an organism.

[131] Another embodiment of the invention (Embodiment Number 4) is a method for the increased production of taurine in a unicellular organism by the following steps:

[132] 1. knockout the gene for a taurine degradation enzyme using chemical or genetic means by replacement or deletion of a promoter, a portion of the coding region, or terminator to one of the following genes, TauX, TauY, TauD, Tpa, SsuD, or SsuE, using a pSC101ts-*sacB*, allelic exchange or λ-red recombinase method in a unicellular organism; and

[133] 2. transform the vector containing a functional sCS/PLP-DC protein construct (from Step 2, Embodiment Number 1 or from Step 2, Embodiment Number 2) into the unicellular organism with the mutation or knocked-out TauX, TauY, TauD, Tpa, SsuD, or SsuE gene (from Step 1, Embodiment Number 4).

[134] Another embodiment of the invention (Embodiment Number 5) is a method for the increased production of taurine in a unicellular organism by the following step:

[135] 1. transform the vector containing the sCS/PLP-DC and taurine-binding protein construct (from Step 2, Embodiment Number 3) into the unicellular organism with the mutated or knocked-out TauX, TauY, TauD, Tpa, SsuD, or SsuE gene (from Step 1, Embodiment Number 4).

[136] Another embodiment of the invention (Embodiment Number 6) is a method for the increased production of taurine in a unicellular organism by the following steps:

[137] 1. introduce a mutation or knock out the gene for the transcription regulator of the taurine degradation pathway(s) using chemical or genetic means by replacement or deletion of a promoter, a portion of the coding region, or terminator to one of the following genes, cbl, or TauR genes using a pSC101ts-*sacB*, allelic exchange or λ-red recombinase method and select the mutant or knocked-out unicellular organism; and

[138] 2. transform the vector containing the functional sCS/PLP-DC protein construct (from Step 2, Embodiment Number 1 or from Step 2, Embodiment Number 2) into the unicellular organism with the mutated or knocked-out cbl or TauR gene (from Step 1, Embodiment Number 6).

[139] Another embodiment of the invention (Embodiment Number 7) is a method for the increased production of taurine in a unicellular organism by the following step:

[140] 1. transform the vector containing the sCS/PLP-DC protein and taurine-binding protein construct (from Step 2, Embodiment Number 3) into the unicellular organism with the mutated or knocked-out cbl or TauR gene (from Step 1, Embodiment Number 6).

[141] Once transformed, the organism may be treated with other "active agents" either prior to or during the growth to further increase production of taurine. "Active agent," as used herein, refers to an agent that has a beneficial effect on the taurine or amino acid production by the unicellular organism. Some of these agents may be precursors of end products for the reaction catalyzed by sCS/PLP-DC. These compounds could promote growth, development, biomass and yield, and change in metabolism. A host of molecules could be used to activate sCS/PLP-DC. These include but are not limited to (1) the twenty amino acids that are involved in protein synthesis, (2) the sulfur containing compounds such as sulfite, sulfide, hydrogen sulfide, sulfate, taurine, hypotaurine, cysteate, 2-sulfacetaldehyde, homotaurine, homocysteine, cystathionine, N-acetyl thiazolidine 4 carboxylic acid (ATCA), glutathione, or bile, (3) other non-protein amino acids, such as GABA, citrulline and ornithine, and (4) other nitrogen containing compounds such as polyamines. Depending on the type of gene construct or recombinant expression cassette, other metabolites and nutrients may be used to activate sCS/PLP-DC. These include, but are not

limited to, sugars, carbohydrates, lipids, oligopeptides, mono- (glucose, arabinose, fructose, xylose, and ribose) di- (sucrose and trehalose) and polysaccharides, carboxylic acids (succinate, malate and fumarate) and nutrients such as phosphate, molybdate, or iron.

[142] In some embodiments, properties of a transgenic unicellular organism are altered using an agent which increases sulfur concentration in the cell, such as sulfur, sulfite, sulfide, hydrogen sulfide, sulfate, taurine, hypotaurine, homotaurine, cysteate, 2-sulfacetaldehyde, N-acetyl-thiazolidine-4-carboxylic acid (ATCA), glutathione, and bile. In other embodiments, the agent increases nitrogen concentration. Amino acids either naturally occurring in proteins (e.g., cysteine, methionine, glutamate, glutamine, serine, alanine, or glycine) or which do not naturally occur in proteins (e.g., GABA, citrulline, or ornithine) and/or polyamines can be used for this purpose.

Pharmaceutical Compositions

[143] The invention provides pharmaceutical compositions that comprise extracts of one or more transgenic organisms described above. Extracts containing hypotaurine or taurine can be used to synthesize or manufacture taurine derivatives (354, 355), taurine-conjugates (356) or taurine-polymers (357) that may have a wide range of commercial and medicinal applications (358). Some taurine derivatives can function as organogelators (359) or dyes (360) and can be used in nanosensor synthesis (361). Some taurine derivatives have anticonvulsant (354) or anti-cancer (362) properties. Other taurine derivatives are used in the treatment of alcoholism (363, 364). Taurine-conjugated carboxyethylester-polyrotaxanes increase anticoagulant activity (365). Taurine-containing polymers may increase wound healing (366, 367). Taurine linked polymers such as poly gamma-glutamic acid-sulfonates are biodegradable and may have applications in the development of drug delivery systems, environmental materials, tissue engineering, and medical materials (368). Extracts from taurine-containing cells may be used in pharmaceutical or medicinal compositions to deliver taurine, hypotaurine, taurine-conjugates, or taurine-polymers for use in the treatment of congestive heart failure, high blood pressure, hepatitis, high cholesterol, fibrosis, epilepsy, autism, attention deficit-hyperactivity disorder, retinal degeneration, diabetes, and alcoholism. It is also used to improve mental performance and as an antioxidant.

[144] Pharmaceutically acceptable vehicles of taurine, taurine derivatives, taurine-conjugates, or taurine-polymers are tablets, capsules, gel, ointment, film, patch, powder or dissolved in liquid form.

Nutritional Supplements and Feeds

[145] Transgenic cells containing hypotaurine or taurine may be consumed or used to make extracts for nutritional supplements. Transgenic cells that contain hypotaurine or taurine may be used for human consumption. Extracts from transgenic cells containing hypotaurine or taurine may be used as nutritional supplements, as an antioxidant or to improve physical or mental performance. The extracts may be used in the form of a liquid, powder, capsule or tablet.

[146] Transgenic cells containing hypotaurine or taurine may be used as fish or animal feed or used to make extracts for the supplementation of animal feed. Transgenic cells that contain hypotaurine or taurine may be used as animal or fish feed. Extracts from transgenic cells containing taurine may be used as feed supplements in the form of a liquid, powder, capsule or tablet.

Enhancer of Plant growth or Yield

[147] Transgenic cells that contain hypotaurine or taurine may be used as an enhancer for plant growth or yield. Extracts from transgenic cells containing hypotaurine or taurine may be used as plant enhancers in the form of a liquid, powder, capsule or tablet.

Fermentation and Taurine Purification

[148] Taurine could be purified from the cells or from extracts of the cells or from media from which the cells were grown. The extracted taurine could be used as a food or feed additive, nutrient, pharmaceutical or an enhancer of plant growth or yield. Prokaryotic or eukaryotic cells with the invention can be grown in culture or by fermentation to produce hyptotaurine or taurine. Methods to produce chemical compounds by batch fermentation, fed-batch fermentation, continuous fermentation or in tanks or ponds are well known to one with ordinary skill in the art (187, 369-379).

[149] Methods such as centrifugation, filtration, crystallization, ion exchange, electrodialysis, solvent extraction, decolorization or evaporation to purify or separate chemical compounds from cells or from liquids or media that grew cells are well known to one with ordinary skill in the art. These methods can be used by one with ordinary skill in the art to purify or separate taurine from cells with the invention, or from liquids or media from which cell suspensions or cell cultures containing the invention were grown (370, 372, 373, 380-383).

DEFINITIONS

[150] The term "polynucleotide" refers to a natural or synthetic linear and sequential array of nucleotides and/or nucleosides, including deoxyribonucleic acid, ribonucleic acid, and derivatives thereof. It includes chromosomal DNA, self-replicating plasmids, infectious polymers of DNA or RNA and DNA or RNA that performs a primarily structural role. Unless otherwise indicated, nucleic acids or polynucleotide are written left to right in 5′ to 3′ orientation, Nucleotides are referred to by their commonly accepted single-letter codes. Numeric ranges are inclusive of the numbers defining the range.

[151] The terms "amplified" and "amplification" refer to the construction of multiple copies of a nucleic acid sequence or multiple copies complementary to the nucleic acid sequence using at least one of the nucleic acid sequences as a template. Amplification can be achieved by chemical synthesis using any of the following methods, such as solid-phase phosphoramidate technology or the polymerase chain reaction (PCR). Other amplification systems include the ligase chain reaction system, nucleic acid sequence based amplification, Q-Beta Replicase systems, transcription-based amplification system, and strand displacement amplification. The product of amplification is termed an amplicon.

[152] As used herein "promoter" includes reference to a region of DNA upstream from the start of transcription and involved in recognition and binding of RNA polymerase, either I, II or III, and other proteins to initiate transcription. Promoters include necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as far as several thousand base pairs from the start site of transcription. In bacteria, the promoter includes a Shine-Dalgarno or ribosomal binding site that can include the sequence

AGGAGG (-35 box) and a Pribnow box or RNA polymerase binding site that can include the sequence TATAAT (-10 box).

[153] The term "algal promoter" refers to a promoter capable of initiating transcription in algal cells.

[154] The term "foreign promoter" refers to a promoter, other than the native, or natural, promoter, which promotes transcription of a length of DNA of viral, bacterial or eukaryotic origin, including those from microbes, plants, plant viruses, invertebrates or vertebrates.

[155] The term "microbe" refers to any microorganism (including both eukaryotic and prokaryotic microorganisms), such as bacteria, fungi, yeast, bacteria, algae and protozoa, as well as other unicellular organisms such as yeast, unicellular algae and unicellular fungi.

[156] The term "constitutive" refers to a promoter that is active under most environmental and developmental conditions, such as, for example, but not limited to, the CaMV 35S promoter.

[157] The term "inducible promoter" refers to a promoter that is under chemical (including biomolecules such as sugars, organic acids or amino acids) or environmental control.

[158] The terms "encoding" and "coding"" refer to the process by which a polynucleotide, through the mechanisms of transcription and translation, provides the information to a cell from which a series of amino acids can be assembled into a specific amino acid sequence to produce a functional polypeptide, such as, for example, an active enzyme or ligand binding protein.

[159] The terms "polypeptide," "peptide," "protein" and "gene product" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. Amino acids may be referred to by their commonly known three-letter or one-letter symbols. Amino acid sequences are written left to right in amino to carboxy orientation, respectively. Numeric ranges are inclusive of the numbers defining the range.

[160] The terms "residue," "amino acid residue," and "amino acid" are used interchangeably herein to refer to an amino acid that is incorporated into a protein, polypeptide, or peptide. The amino

acid may be a naturally occurring amino acid and may encompass known analogs of natural amino acids that can function in a similar manner as the naturally occurring amino acids.

[161] The term "degradation" in reference to the "taurine degradation pathway", "taurine degradation enzymes", "taurine degradation system", and "taurine degradation proteins" refers to the process of breakdown, catabolism, or dissimilation of taurine.

[162] The terms "sulfinoalanine decarboxylase" and "SAD" refer to the protein (4.1.1.29) that catalyzes the following reaction:

$$\text{3-sulfinoalanine} = \text{hypotaurine} + CO_2$$

[163] NOTE: SAD is another name for cysteine-sulfinate decarboxylase, L-cysteine sulfinic acid decarboxylase, cysteine-sulfinate decarboxylase, CADCase/CSADCase, CSAD, cysteic decarboxylase, cysteine sulfinic acid decarboxylase, cysteine sulfinate decarboxylase, sulfoalanine decarboxylase, sulphinoalanine decarboxylase, and 3-sulfino-L-alanine carboxy-lyase.

[164] NOTE: the SAD reaction is also catalyzed by GADL1 (4.1.1.15) (glutamic acid decarboxylase like 1). Although called GADL1 the enzyme has been shown to catalyze the SAD reaction (384, 385).

[165] Other names for hypotaurine are 2-aminoethane sulfinate, 2-aminoethylsulfinic acid, and 2-aminoethanesulfinic acid.

[166] Other names for taurine are 2-aminoethane sulfonic acid, aminoethanesulfonate, L-taurine, taurine ethyl ester, and taurine ketoisocaproic acid 2-aminoethane sulfinate.

[167] The terms "taurine-pyruvate aminotransferase" and "TPAT" refer to the protein (EC 2.6.1.77) that catalyzes the following reaction:

$$\text{taurine} + \text{pyruvate} = \text{L-alanine} + \text{2-sulfoacetaldehyde}$$

[168] TPAT is another name for taurine transaminase or taurine transaminase aminotransferase

[169] The term "Tpa" refers to the gene that encodes TPAT.

[170] The terms "taurine dehydrogenase" and "TDH" refer to the protein (EC:1.4.99.2) that catalyzes the following reaction:

taurine + water = ammonia + 2-sulfoacetaldehyde

[171] TDH is another name for taurine:oxidoreductase, taurine:ferricytochrome-c oxidoreductase,

[172] The term "TauX" or "TauY" refers to the genes that encode for the small and large subunits of TDH, respectively.

[173] The terms "taurine dioxygenase" and "TDO" refer to the protein (EC:1.14.11.17) that catalyzes the following reaction:

taurine + 2-oxoglutarate + O_2 = sulfite + aminoacetaldehyde + succinate + CO_2

[174] TDO is another name for 2-aminoethanesulfonate dioxygenase, alpha-ketoglutarate-dependent taurine dioxygenase, taurine, or 2-oxoglutarate:O_2 oxidoreductase.

[175] 2-oxoglutarate is another name for alpha-ketoglutarate.

[176] The term "TauD" refers to the gene that encodes TDO.

[177] The term "two-component alkanesulfonate monooxygenase" or "2CASM" that catalyzes the following reaction:

taurine + O_2 + $FMNH_2$ = Aminoacetaldehyde + SO_3^2 + H_2O + FMN

or

taurine + O_2 + $Thioredoxin_{red}$ = Aminoacetaldehyde + SO_3^2 + H_2O + $Thioredoxin_{ox}$

[178] The term "SssuDE", "SsuD" or "SsuE" refers to the genes that encode the two-component alkanesulfonate monooxygenase (2CASM).

[179] The terms "cysteine synthetase" and "CS" refer to the protein (EC:2.5.1.47) that catalyzes the following reaction:

O3-acetyl-L-serine + hydrogen sulfide = L-cysteine + acetate

[180] The terms "cystathionine-β-synthase" and "CBS" refer to the protein (EC:4.2.1.22) that catalyzes the following reaction:

L-serine + L-homocysteine = L-cystathionine + H_2O

[181] The terms "cysteine synthetase/PLP decarboxylase" and "CS/PLP-DC" refer to the protein that that forms taurine from: sulfur containing precursors including but not limited to sulfate, sulfite, hypotaurine, cysteamine, 3-sulfinoalanine, cysteine, sulfoacetaldehyde, cysteate, homocysteine or cystathionine.

[182] The term "functional" with reference to sCS/PLP-DC refers to peptides, proteins or enzymes made synthetically, by gene fusion, gene shuffling or directed evolution that catalyzes the CS/PLP-DC reaction.

[183] The term "decarboxylase" refers to enzymes that that catalyzes the removal of a carboxyl group from an organic molecule or the decarboxylation of a particular organic molecule examples of decarboxylases include but are not limited to; sulfinoalanine decarboxylase, glutamate decarboxylase, aromatic amino acid decarboxylase, 2,4-diaminobutyrate decarboxylase, acetoacetate decarboxylase,S-adenosylmethionine decarboxylase, aconitate decarboxylase, aminocarboxymuconate-semialdehyde decarboxylase, aminobenzoate decarboxylase, arginine decarboxylase, aspartate (1 or 4) decarboxylase, dopa decarboxylase, tryptophan decarboxylase, and ornithine decarboxylase.

[184] The term "recombinant" includes reference to a cell or vector that has been modified by the introduction of a heterologous nucleic acid. Recombinant cells express genes that are not normally found in that cell or express native genes that are otherwise abnormally expressed, underexpressed, or not expressed at all as a result of deliberate human intervention, or expression of the native gene may have reduced or eliminated as a result of deliberate human intervention.

[185] The term "recombinant expression cassette" refers to a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements, which permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can

be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid to be transcribed, and a promoter.

[186] The term "transgenic" includes reference to a unicellular, which comprises within its genome a heterologous polynucleotide. Generally, the heterologous polynucleotide is integrated within the genome such that the polynucleotide is passed on to successive generations. The heterologous polynucleotide may be integrated into the genome alone or as part of a recombinant expression cassette. "Transgenic" is also used to include any cell the genotype of which has been altered by the presence of heterologous nucleic acid including those cells altered or created by budding or conjugation propagation from the initial transgenic cell.

[187] The term "vector" includes reference to a nucleic acid used in transfection or transformation of a host cell and into which can be inserted a polynucleotide.

[188] The term "selectively hybridizes" includes reference to hybridization, under stringent hybridization conditions, of a nucleic acid sequence to a specified nucleic acid target sequence to a detectably greater degree (e.g., at least 2-fold over background) than its hybridization to non-target nucleic acid sequences and to the substantial exclusion of non-target nucleic acids. Selectively hybridizing sequences typically have about at least 40% sequence identity, preferably 60-90% sequence identity, and most preferably 100% sequence identity (i.e., complementary) with each other.

[189] The terms "stringent conditions" and "stringent hybridization conditions" include reference to conditions under which a probe will hybridize to its target sequence, to a detectably greater degree than other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences can be identified which can be up to 100% complementary to the probe (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Optimally, the probe is approximately 500 nucleotides in length, but can vary greatly in length from less than 500 nucleotides to equal to the entire length of the target sequence.

[190] Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide or Denhardt solution. Low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulfate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. High stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C. Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated (386), where the T_m = 81.5°C + 16.6 (log M) + 0.41 (%GC) - 0.61 (% form) - 500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m, hybridization and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with $\geq$90% identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3 or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9 or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15 or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m, those of ordinary skill in the art will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. An extensive guide to the hybridization of nucleic acids is found in the scientific literature. (134, 387) Unless otherwise stated, in the

present application high stringency is defined as hybridization in 4X SSC, 5X Denhardt solution (5 g Ficoll, 5 g polyvinypyrrolidone, 5 g bovine serum albumin in 500ml of water), 0.1 mg/ml boiled salmon sperm DNA, and 25 mM Na phosphate at 65°C, and a wash in 0.1X SSC, 0.1% SDS at 65°C.

[191] The following terms are used to describe the sequence relationships between two or more nucleic acids or polynucleotides or polypeptides: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity".

[192] The term "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence.

[193] The term "comparison window" includes reference to a contiguous and specified segment of a polynucleotide sequence, where the polynucleotide sequence may be compared to a reference sequence and the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) when it is compared to the reference sequence for optimal alignment. The comparison window is usually at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100 or longer. Those of ordinary skill in the art understand that the inclusion of gaps in a polynucleotide sequence alignment introduces a gap penalty, and it is subtracted from the number of matches.

[194] Methods of alignment of nucleotide and amino acid sequences for comparison are well known to those of ordinary skill in the art. The local homology algorithm, BESTFIT (388), can perform an optimal alignment of sequences for comparison using a homology alignment algorithm called GAP (389), search for similarity using Tfasta and Fasta (390), by computerized implementations of these algorithms widely available on-line or from various vendors (Intelligenetics, Genetics Computer Group). CLUSTAL allows for the alignment of multiple sequences (391-393) and program PileUp can be used for optimal global alignment of multiple sequences (394). The BLAST family of programs can be used for nucleotide or protein database similarity searches. BLASTN searches a nucleotide database using a nucleotide query. BLASTP searches a protein database using a protein query. BLASTX searches a protein database using a translated nucleotide query that is derived from a six-frame translation of the nucleotide query sequence

(both strands). TBLASTN searches a translated nucleotide database using a protein query that is derived by reverse-translation. TBLASTX search a translated nucleotide database using a translated nucleotide query.

[195] GAP (389) maximizes the number of matches and minimizes the number of gaps in an alignment of two complete sequences. GAP considers all possible alignments and gap positions and creates the alignment with the largest number of matched bases and the fewest gaps. It also calculates a gap penalty and a gap extension penalty in units of matched bases. Default gap creation penalty values and gap extension penalty values in Version 10 of the Wisconsin Genetics Software Package are 8 and 2, respectively. The gap creation and gap extension penalties can be expressed as an integer selected from the group of integers consisting of from 0 to 100. GAP displays four figures of merit for alignments: Quality, Ratio, Identity, and Similarity. The Quality is the metric maximized in order to align the sequences. Ratio is the quality divided by the number of bases in the shorter segment. Percent Identity is the percent of the symbols that actually match. Percent Similarity is the percent of the symbols that are similar. Symbols that are across from gaps are ignored. A similarity is scored when the scoring matrix value for a pair of symbols is greater than or equal to 0.50, the similarity threshold. The scoring matrix used in Version 10 of the Wisconsin Genetics Software Package is BLOSUM62 (395).

[196] Unless otherwise stated, sequence identity or similarity values refer to the value obtained using the BLAST 2.0 suite of programs using default parameters (396). As those of ordinary skill in the art understand that BLAST searches assume that proteins can be modeled as random sequences and that proteins comprise regions of nonrandom sequences, short repeats, or enriched for one or more amino acid residues, called low-complexity regions. These low-complexity regions may be aligned between unrelated proteins even though other regions of the protein are entirely dissimilar. Those of ordinary skill in the art can use low-complexity filter programs to reduce number of low-complexity regions that are aligned in a search. These filter programs include, but are not limited to, the SEG (397, 398) and XNU (399).

[197] The terms "sequence identity" and "identity" are used in the context of two nucleic acid or polypeptide sequences and include reference to the residues in the two sequences, which are the same when aligned for maximum correspondence over a specified comparison window. When

the percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conserved substitutions, the percent sequence identity may be adjusted upwards to correct for the conserved nature of the substitution. Sequences, which differ by such conservative substitutions, are said to have "sequence similarity" or "similarity." Scoring for a conservative substitution allows for a partial rather than a full mismatch (400), thereby increasing the percentage sequence similarity.

[198] The term "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise gaps (additions or deletions) when compared to the reference sequence for optimal alignment. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[199] The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has between 50-100% sequence identity, preferably at least 50% sequence identity, preferably at least 60% sequence identity, preferably at least 70%, more preferably at least 80%, more preferably at least 90%, and most preferably at least 95%, compared to a reference sequence using one of the alignment programs described using standard parameters. One of ordinary skill in the art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of between 50-100%. Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each low stringency conditions, moderate stringency conditions or high stringency conditions. Yet another indication that two nucleic acid sequences

are substantially identical is if the two polypeptides immunologically cross-react with the same antibody in a western blot, immunoblot or ELISA assay.

[200] The terms "substantial identity" in the context of a peptide indicates that a peptide comprises a sequence with between 55-100% sequence identity to a reference sequence preferably at least 55% sequence identity, preferably 60% preferably 70%, more preferably 80%, most preferably at least 90% or 95% sequence identity to the reference sequence over a specified comparison window. Preferably, optimal alignment is conducted using the homology alignment algorithm (389). Thus, a peptide is substantially identical to a second peptide, for example, where the two peptides differ only by a conserved substitution. Another indication that amino acid sequences are substantially identical is if two polypeptides immunologically cross-react with the same antibody in a western blot, immunoblot or ELISA assay. In addition, a peptide can be substantially identical to a second peptide when they differ by a non-conservative change if the epitope that the antibody recognizes is substantially identical.

[201] All patents, patent applications, and references cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

*Development of s*CS/PLP-DC *without the transit peptide from a CS and decarboxylase using chemical synthesis*

[202] Step 1: Use chemical synthesis to make a DNA construct that contains an sCS/PLP-DC gene from a CS gene fused in-frame with a decarboxylase gene, codon optimized for expression in the target organism.

[203] The CS portion of the sCS/PLP-DC gene is derived as follows from the group below:

[204] a. from SEQ ID NO:1 by removing nucleotides 4 through 234 (corresponding to the native transit peptide) and 1444 through 2958 (corresponding to the decarboxylase region), encoding the CS portion of the peptide from *Micromonas pusilla* (SEQ ID NO:2

minus amino acids 2 through 78 and minus amino acids 472 to 984, corresponding to the transit and decarboxylase domains, respectively);

[205] b. from SEQ ID NO:3 by removing nucleotides 4 through 69 (corresponding to the native transit peptide) and 1414 through 2727 (corresponding to the decarboxylase region), encoding the CS portion of the peptide from *Ostreococcus tauri* (SEQ ID NO:4 minus amino acids 2 through 23 and minus amino acids 480 to 905, corresponding to the transit and decarboxylase domains, respectively);

[206] c. from SEQ ID NO:5 without the stop codon, encoding the CS peptide from *Brassica oleracea* (SEQ ID NO:6);

[207] d. from SEQ ID NO:7 without the stop codon, encoding the CS peptide from *Oryza brachyantha* (SEQ ID NO:8);

[208] e. from SEQ ID NO:9 without the stop codon, encoding the CS peptide from *Escherichia coli* (SEQ ID NO:10); or

[209] f. from SEQ ID NO:82 by removing nucleotides 4 through 99 (corresponding to the native transit peptide) and 1723 through 3291 (corresponding to the decarboxylase region), encoding the CS portion of the peptide from *Bathycoccus prasinos* (SEQ ID NO:83 minus amino acids 2 through 33 and minus amino acids 575 to 1096, corresponding to the transit and decarboxylase domains, respectively).

[210] The DC portion of the sCS/PLP-DC gene is derived as follows from the group below:

[211] a. from SEQ ID NO:1 by removing nucleotides 1 through 1413 (corresponding to the native transit and cysteine synthetase peptide regions), encoding a decarboxylase peptide from *Micromonas pusilla* (SEQ ID NO:2 minus amino acids 1 through 471);

[212] b. from SEQ ID NO:17 encoding the DC peptide from *Beta vulgaris* (SEQ ID NO:18);

[213] c. from SEQ ID NO:19 encoding the DC peptide from *Lepisosteus oculatus* (SEQ ID NO:20);

[214] d. from SEQ ID NO:21 encoding the DC peptide from *Danio rerio* (SEQ ID NO:22);

[215] e. from SEQ ID NO:23 encoding the DC peptide from *Escherichia coli* (SEQ ID NO:24);

[216] f. from SEQ ID NO:25 encoding the DC peptide from *Oncorhynchus mykiss* (SEQ ID NO:26);

[217] g. from SEQ ID NO:27 encoding the DC peptide from *Guillardia theta* (SEQ ID NO:28);

[218] h. from SEQ ID NO:82 by removing nucleotides 1 through 1723 (corresponding to the native transit and cysteine synthetase peptide regions), encoding a decarboxylase peptide from *Bathycoccus prasinos* (SEQ ID NO:83 minus amino acids 1 through 574).

[219] Clone sCS/PLP-DC (from Step 1, EXAMPLE 1) into the vector pCAMBIA1105, pET11, pKK223-3, or pSF-Tac, transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants, and confirm the presence of the DNA construct.

EXAMPLE 2

*Development of s*CS/PLP-DC *without the transit peptide from a CBS and decarboxylase using chemical synthesis*

[220] Step 1: Use chemical synthesis to make a DNA construct that contains an sCS/PLP-DC gene from a CBS gene fused in-frame with a decarboxylase gene, codon optimized for expression in the target organism.

[221] The CBS portion of the sCS/PLP-DC gene is derived as follows from the group below:

[222] a. from SEQ ID NO:11 without the stop codon, encoding the CBS peptide from *Candidatus kryptonium* (SEQ ID NO:12);

[223] b. from SEQ ID NO:13 without the stop codon, encoding the CBS peptide from *Chloroflexi bacterium* (SEQ ID NO:14); or

[224] c. from SEQ ID NO:15 without the stop codon, encoding the CBS peptide from *Cyprinus carpio* (SEQ ID NO:16).

[225] The DC portion of the sCS/PLP-DC gene is derived as follows from the group below:

[226] a. from SEQ ID NO:1 by removing nucleotides 1 through 1413 (corresponding to the native transit and cysteine synthetase peptide regions), encoding a decarboxylase peptide from *Micromonas pusilla* (SEQ ID NO:2 minus amino acids 1 through 471);

[227] b. from SEQ ID NO:17 encoding the DC peptide from *Beta vulgaris* (SEQ ID NO:18);

[228] c. from SEQ ID NO:19 encoding the DC peptide from *Lepisosteus oculatus* (SEQ ID NO:20);

[229] d. from SEQ ID NO:21 encoding the DC peptide from *Danio rerio* (SEQ ID NO:22);

[230] e. from SEQ ID NO:23 encoding the DC peptide from *Escherichia coli* (SEQ ID NO:24);

[231] f. from SEQ ID NO:25 encoding the DC peptide from *Oncorhynchus mykiss* (SEQ ID NO:26); or

[232] g. from SEQ ID NO:27 encoding the DC peptide from *Guillardia theta* (SEQ ID NO:28);

[233] h. from SEQ ID NO:82 by removing nucleotides 1 through 1723 (corresponding to the native transit and cysteine synthetase peptide regions), encoding a decarboxylase peptide from *Bathycoccus prasinos* (SEQ ID NO:83 minus amino acids 1 through 574).

[234] Clone sCS/PLP-DC (from Step 1, EXAMPLE 2) into the vector pCAMBIA1105, pET11, pKK223-3, or pSF-Tac, transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants and confirm the presence of the DNA construct.

EXAMPLE 3

*Development of an s*CS/PLP-DC *gene using gene evolution or gene shuffling*

[235] Step 1. Use chemical synthesis to make a DNA construct that contains an sCS/PLP-DC gene (from Step1 EXAMPLE 1 or Step 1 EXAMPLE 2) and subject the sCS/PLP-DC polynucleotide to directed evolution as described by making a mutant library with a non-proof reading taq polymerase for PCR amplification (401, 402) or by DNA shuffling, a method that makes new point mutations and recombines existing mutations (403).

[236] Step 2. Transform vectors with mutated or shuffled sCS/PLP-DC genes into *E. coli*.

[237] Step 3. Grow cultures, harvest cells and supernatant from cultures extract amino acids and subject them to HPLC or GC-MS analysis to validate taurine production.

[238] Step 4. Isolate the sCS/PLP-DC containing vector and sequence the polynucleotide. The resulting sCS/PLP-DC polynucleotide can be used for expression of a CS/PLP-DC peptide similar to SEQ ID NO:84 in other systems.

EXAMPLE 4

Development of sCS/PLP-DC *with a transit peptide using chemical synthesis*

[239] Step1. Use chemical synthesis to make a DNA construct that contains a plastid transit peptide fused in-frame with an sCS/PLP-DC gene (from Step 1 EXAMPLE 1; Step 1 EXAMPLE 2; or Step 4 EXAMPLE 3), codon optimized for expression in the target organism.

[240] The plastid transit peptide nucleotide sequence is SEQ ID NO:55 and encodes the peptide SEQ ID NO:56.

[241] The sCS/PLP-DC gene (from Step 1 EXAMPLE 1; Step 1 EXAMPLE 2; or Step 4 EXAMPLE 3) minus the start codon.

[242] Step2. Clone the transit peptide containing sCS/PLP-DC (TP_sCS/PLP-DC) gene (from Step 1 EXAMPLE 4) into the vector pCAMBIA1105, pET11, pKK223-3, or pSF-Tac, transform *E. coli*, select for antibiotic resistance, conduct PCR identification of cloned DNA constructs in transformants and confirm the presence of the DNA construct.

EXAMPLE 5

Development of a transgenic bacterium with a TauD knockout *that expresses* sCS/PLP-DC *without a transit peptide using chemical synthesis*

[243] Step 1: Use PCR to amplify the TauD (SEQ ID NO:33) using 500 ng of DNA from *E. coli* strain K12 and the primers for SEQ ID NO:57 and SEQ ID NO:58. Use the PCR-amplified fragment to knock out TauD with λred-mediated recombination as described by Datsenko and Wanner (332) and Baba *et al.* (142).

[244] Step 2: Use chemical synthesis to make a DNA construct that contains a functional sCS/PLP-DC gene (from Step 1 EXAMPLE 1; Step 1 EXAMPLE 2; or Step 4 EXAMLE 3) without the transit peptide codon optimized for expression in *E. coli*. Clone the sCS/PLP-DC gene into a bacterial expression vector, such as pET11, pKK223-3, or pSF-Tac, so it is functional.

[245] Step 3: Transform the vector with the functional sCS/PLP-DC construct (from Step 2, EXAMPLE 5) into the TauD knockout *E. coli* strain (from Step 1, EXAMPLE 5) and confirm the presence of the DNA construct.

EXAMPLE 6

Development of a transgenic bacterium with a TauD knockout that expresses an sCS/PLP-DC without a transit peptide and expresses a taurine binding peptide without a transit peptide using chemical synthesis

[246] Step 1: Use chemical synthesis to make a DNA construct that contains a taurine binding protein (SEQ ID NO:29 or SEQ ID NO:31) without the transit peptide optimized for expression in *E. coli*. Clone the taurine binding protein into a bacterial expression vector, such as pET11, pKK223-3, or pSF-Tac, so it is functional.

[247] The taurine-binding protein gene is derived as follows from the group below:

[248] a. from SEQ ID NO:29 by removing nucleotides 4 through 66 (corresponding to the periplasmic transit peptide) and encoding a truncated taurine-binding peptide from *E. coli* (SEQ ID NO:30 minus amino acids 2 through 22); or

[249] b. from SEQ ID NO:31, by removing nucleotides 4 through 93, (corresponding to the periplasmic transit peptide), optimized for expression in *E. coli* and encoding a truncated taurine-binding peptide from *Roseobacter denitrificans* (SEQ ID NO:32 minus amino acids 2 through 31).

[250] Step 2: Transform the DNA vector with the taurine-binding protein (from Step 1, EXAMPLE 6) into the TauD knockout *E. coli* strain that contains the vector with the pCS/PLP-DC (from Step 3, EXAMPLE 5). Select for antibiotic resistance, and confirm the presence of the DNA constructs.

EXAMPLE 7

Development of a transgenic bacterium with a cbl knockout that expresses sCS/PLP-DC without transit peptide using chemical synthesis

[251] Step 1: Use PCR to amplify the cbl (SEQ ID NO:49) using 500 ng of DNA from *E. coli* strain K12 and the primers for SEQ ID NO:59 and SEQ ID NO:60. Use the PCR-amplified fragment to knockout cbl with λ red-mediated recombination as described by Datsenko and Wanner (332) and Baba *et al.* (142)

[252] Step 2: Transform the vector with the functional sCS/PLP-DC construct (from Step 2, EXAMPLE 5) into the cbl knockout *E. coli* strain (from Step 1, EXAMPLE 7) and confirm the presence of the DNA construct.

EXAMPLE 8

Development of a transgenic bacterium with a cbl knockout that expresses sCS/PLP-DC *without a transit peptide and expresses a taurine binding peptide without a transit peptide using chemical synthesis*

[253] Step 1: Transform the DNA vector with the taurine-binding protein (from Step 1, EXAMPLE 6) into the cbl knockout *E. coli* strain that contains the vector with the sCS/PLP-DC (from Step 2, EXAMPLE 7) and confirm the presence of the DNA constructs.

EXAMPLE 9

Development of a transgenic bacterium with an SsuD knockout that expresses sCS/PLP-DC *without a transit peptide using chemical synthesis*

[254] Step 1: Use PCR to amplify the SsuD (SEQ ID NO:35) using 500 ng of DNA from *E. coli* strain K12 and the primers for SEQ ID NO:61 and SEQ ID NO:62. Use the PCR-amplified fragment to knock out SsuD with λ red-mediated recombination as described by Datsenko and Wanner (332) and Baba *et al.* (142).

[255] Step 2: Transform the vector with the functional sCS/PLP-DC construct (from Step 2, EXAMPLE 5) into the SsuD knockout *E. coli* strain (from Step 1, EXAMPLE 9) and confirm the presence of the DNA construct.

EXAMPLE 10

Development of a transgenic bacterium with an SsuD knockout that expresses sCS/PLP-DC without a transit peptide and expresses a taurine binding peptide without a transit peptide using chemical synthesis

[256] Step 1: Transform the DNA vector with the taurine-binding protein (from Step 1, EXAMPLE 6) into the SsuD knockout *E. coli* strain that contains the vector with the functional sCS/PLP-DC construct (from Step 2, EXAMPLE 9) and confirm the presence of the DNA constructs.

EXAMPLE 11

Development of a transgenic bacterium with an SsuE knockout that expresses sCS/PLP-DC *without a transit peptide using chemical synthesis*

[257] Step 1: Use PCR to amplify the SsuE (SEQ ID NO:37) using 500 ng of DNA from *E. coli* strain K12 and the primers for SEQ ID NO:63 and SEQ ID NO:64. Use the PCR-amplified fragment to

knockout SsuE with λ red-mediated recombination as described by Datsenko and Wanner (332) and Baba *et al.* (142).

[258] Step 2: Transform the vector with the functional sCS/PLP-DC construct (from Step 2, EXAMPLE 5) into the SsuE knockout *E. coli* strain (from Step 1, EXAMPLE 11) and confirm the presence of the DNA construct.

EXAMPLE 12

Development of a transgenic bacterium with an SsuE knockout that expresses sCS/PLP-DC *without a transit peptide and expresses a taurine binding peptide without a transit peptide using chemical synthesis*

[259] Step 1: Transform the DNA vector with the taurine-binding protein (from Step 1, EXAMPLE 6) into the SsuE knockout *E. coli* strain that contains the vector with the functional sCS/PLP-DC (from Step 2, EXAMPLE 12) and confirm the presence of the DNA constructs.

EXAMPLE 13

Development of another type of transgenic bacterium with an SsuD knockout that expresses sCS/PLP-DC without a transit peptide using chemical synthesis

[260] Step 1: Use overlap PCR to amplify a knockout fragment for SsuD (SEQ ID NO:39) using genome DNA from *Corynebacterium glutamicum* and the pK19mobsacB vector as described by Buchholz *et al.* (404). Generate independent DNA fragments using the primer pairs SEQ ID NO:65 and SEQ ID NO:66 and genome DNA from *C. glutamicum* and SEQ ID NO:67 and SEQ ID NO:68 and genome DNA from *C. glutamicum*. Purify each DNA fragment and mix in equal amounts in an overlap PCR using primers SEQ ID NO:65 and SEQ ID NO:68. Clone the resulting fusion product containing the SsuD gene with an internal deletion of 875 bp (SsuD knockout fragment) into pK19mobsacB. Replace the SsuD1 gene with the SsuD knockout fragment by homologous recombination (404).

[261] Step 2: Use chemical synthesis to make a DNA construct that contains an sCS/PLP-DC gene (from Step1 EXAMPLE 1; Step 1 EXAMPLE 2; or Step 4 EXAMPLE 3) optimized for expression in *C. glutamicum*. Clone the sCS/PLP-DC fragment into a bacterial expression vector, such as pET11, pKK223-3, or pSF-Tac, so it is functional.

[262] Step 3: Transform the vector with the functional sCS/PLP-DC construct (from Step 2, EXAMPLE 13) into the SsuD knockout *C. glutamicum* strain (from Step 1, EXAMPLE 13) and confirm the presence of the DNA construct.

EXAMPLE 14

Development of another type of transgenic bacterium with an SsuD knockout that expresses sCS/PLP-DC without a transit peptide and expresses a taurine binding peptide without a transit peptide using chemical synthesis

[263] Step 1: Use chemical synthesis to make a DNA construct that contains a taurine binding protein (SEQ ID NO:29 or SEQ ID NO:31) without the transit peptide. Clone the taurine binding protein into a bacterial expression vector, such as pET11, pKK223-3, or pSF-Tac, so it is functional.

[264] The taurine binding protein gene is derived as follows from the group below:

[265] a. from SEQ ID NO:29 by removing nucleotides 4 through 66 (corresponding to the periplasmic transit peptide) and encoding a truncated taurine-binding peptide from *C. glutamicum* (SEQ ID NO:30 minus amino acids 2 through 22); or

[266] b. from SEQ ID NO:31 by removing nucleotides 4 through 93, (corresponding to the periplasmic transit peptide), optimized for expression in *C. glutamicum* and encoding a truncated taurine-binding peptide from *Roseobacter denitrificans* (SEQ ID NO:32 minus amino acids 2 through 31).

[267] Step 2: Transform the DNA vector with the taurine-binding protein (from Step 1, EXAMPLE 14) into the SsuD knockout *C. glutamicum* strain that contains the vector with the functional sCS/PLP-DC (from Step 2, EXAMPLE 13) and confirm the presence of the DNA construct.

EXAMPLE 15

Development of another type of transgenic bacterium with an SsuE knockout that expresses sCS/PLP-DC without a transit peptide using chemical synthesis

[268] Step 1: Use overlap PCR to amplify a knockout fragment for SsuE (SEQ ID NO:41) using genome DNA from *Corynebacterium glutamicum* and the pK19mobsacB vector as described by Buchholz *et al.* (404) Generate independent DNA fragments using the primer pairs SEQ ID NO:69 and SEQ ID NO:70 and genome DNA from *C. glutamicum* and SEQ ID NO:71 and SEQ ID NO:72 and genome DNA from *C. glutamicum*. Purify each DNA fragment and mix in equal amounts in an overlap PCR using primers SEQ ID NO:69 and SEQ ID NO:72. Clone the resulting fusion product, containing the SsuE gene with an internal deletion of 735 bp (SsuE

knockout fragment), into pK19mobsacB. Replace the SsuE gene with the SsuE knockout fragment by homologous recombination (404).

[269] Step 2: Transform the vector with the functional sCS/PLP-DC construct (from Step 2, EXAMPLE 13) into the SsuE knockout *C. glutamicum* strain (from Step 1, EXAMPLE 15) and confirm the presence of the DNA construct.

EXAMPLE 16

Development of another transgenic bacterium with an SsuE knockout that expresses an sCS/PLP-DC without a transit peptide and expresses a taurine binding peptide without a transit peptide using chemical synthesis

[270] Step 1: Transform the DNA vector with the taurine-binding protein (from Step 1, EXAMPLE 14) into the SsuE knockout *C. glutamicum* strain with the functional sCS/PLP-DC construct (from Step 2, EXAMPLE 15) and confirm the presence of the DNA construct.

EXAMPLE 17

Development of another transgenic bacterium with a cbl knockout that expresses an sCS/PLP-DC without a transit peptide using chemical synthesis

[271] Step 1: Use overlap PCR to amplify a knockout fragment for cbl (SEQ ID NO:51) using genome DNA from *Corynebacterium glutamicum*, and the pK19mobsacB vector as described by Buchholz *et al.* (404). Generate independent DNA fragments using the primer pairs SEQ ID NO:73 and SEQ ID NO:74 and genome DNA from *C. glutamicum* and SEQ ID NO:75 and SEQ ID NO:76 and genome DNA from *C. glutamicum*. Purify each DNA fragment and mix in equal amounts in an overlap PCR using primers SEQ ID NO:73 and SEQ ID NO:76. Clone the resulting fusion product, containing the cbl gene with an internal deletion of 563 bp (cbl knockout fragment) into pK19mobsacB. Replace the cbl gene with the cbl knockout fragment by homologous recombination (404).

[272] Step 2: Transform the vector with the functional sCS/PLP-DC construct (from Step 2, EXAMPLE 13) into the cbl knockout *C. glutamicum* strain (from Step 1, EXAMPLE 17) and confirm the presence of the DNA construct.

EXAMPLE 18

Development of another transgenic bacterium with a cbl knockout that expresses sCS/PLP-DC without a transit peptide and expresses a taurine binding peptide without a transit peptide using chemical synthesis

[273] Step 1: Transform the DNA vector with the taurine-binding protein (from Step 1, EXAMPLE 14) into the cbl knockout *C. glutamicum* strain with the functional sCS/PLP-DC construct (from Step 1, EXAMPLE 17) and confirm the presence of the DNA construct.

EXAMPLE 19

Development of a transgenic bacterium with a TauR knockout that expresses sCS/PLP-DC without a transit peptide using chemical synthesis

[274] Step 1: Use overlap PCR to amplify a knockout fragment for TauR (SEQ ID NO:53) using genome DNA from *Corynebacterium glutamicum* and the pK19mobsacB vector as described by Buchholz *et al.* (404). Generate independent DNA fragments using the primer pairs SEQ ID NO:77 and SEQ ID NO:78 and genome DNA from *C. glutamicum* and SEQ ID NO:79 and SEQ ID NO:80 and genome DNA from *C. glutamicum*. Purify each DNA fragment and mix in equal amounts in an overlap PCR using primers SEQ ID NO:77 and SEQ ID NO:80. Clone the resulting fusion product containing the TauR gene with an internal deletion of 1052 bp (TauR knockout fragment) into pK19mobsacB. Replace the TauR gene with the TauR knockout fragment by homologous recombination (404).

[275] Step 2: Transform the vector with the functional sCS/PLP-DC construct (from Step 2, EXAMPLE 13) into the TauR knockout *C. glutamicum* strain (from Step 1, EXAMPLE 19) and confirm the presence of the DNA construct.

EXAMPLE 20

Development of a transgenic bacterium with a TauR knockout that expresses an sCS/PLP-DC without a transit peptide and expresses a taurine binding peptide without a transit peptide using chemical synthesis

[276] Step 1: Transform the DNA vector with the taurine-binding protein (from Step 1, EXAMPLE 14) into the TauR knockout *C. glutamicum* strain with the functional sCS/PLP-DC construct (from Step 1, EXAMPLE 19) and confirm the presence of the DNA construct.

EXAMPLE 21

Development of transgenic Methylobacterium that expresses sCS/PLP-DC without a transit peptide using chemical synthesis

[277] Step 1: Use chemical synthesis to make a DNA construct that contains an sCS/PLP-DC gene (from Step 1 EXAMPLE 1, Step 1 EXAMPLE 2, or Step 4 EXAMPLE 3) optimized for expression in *Methylobacterium extorquens*. Clone the sCS/PLP-DC fragment into a bacterial expression vector, such as pCM80, pCM160, pHC90, pHC91, pHC115, pLC 290, or pLC291 so it is functional.

[278] Step 2: Transform the vector with the functional sCS/PLP-DC construct (from Step 1, EXAMPLE 21) into *Methylobacterium extorquens, Methylobacterium populi, Methylobacterium radiotolerans, Methylobacterium nodula* or *Methylobacterium spp.* and confirm the presence of the DNA construct.

EXAMPLE 22

Development of a transgenic alga that expresses an sCS/PLP-DC without a transit peptide using chemical synthesis

[279] Step 1: Use chemical synthesis to make an sCS/PLP-DC gene without a transit peptide (from Step1 EXAMPLE 1; Step 1 EXAMPLE 2; or Step 4 EXAMPLE 3) codon optimized from expression in algae, *Chlamydomonas reinhardtii* or *Ostreococcus tauri*. Clone the sCS/PLP-DC gene without a transit peptide into an algal expression vector, such as pCB740 or pD1-Kan, so it is functional, and confirm the presence of the DNA construct.

[280] Step 2: Transform the DNA vector with the *sCS/PLP-DC* (from Step 1, EXAMPLE 22) into *Chlamydomonas reinhardtii* or *Ostreococcus tauri* and confirm the presence of the DNA constructs.

EXAMPLE 23

Development of a transgenic alga that expresses an sCS/PLP-DC without a transit peptide and expresses a taurine binding protein without a transit peptide using chemical synthesis

[281] Step 1: Use chemical synthesis to make a DNA construct that contains a taurine binding protein (SEQ ID NO:29 or SEQ ID NO:31) without the transit peptide. Clone the taurine binding protein into an algal expression vector, such as pCB740 or pD1-Kan, so it is functional.

[282] The taurine binding protein gene is derived as follows from the group below:

[283] a. from SEQ ID NO:29 by removing nucleotides 4 through 66 (corresponding to the periplasmic transit peptide), optimized for expression in *Chlamydomonas reinhardtii* or *Ostreococcus tauri* and encoding a truncated taurine-binding peptide from *E. coli* (SEQ ID NO:30 minus amino acids 2 through 22); or

[284] b. from SEQ ID NO:31 by removing nucleotides 4 through 93, (corresponding to the periplasmic transit peptide), optimized for expression in *Chlamydomonas reinhardtii* or *Ostreococcus tauri* and encoding a truncated taurine-binding protein from *Roseobacter denitrificans* (SEQ ID NO:32 minus amino acids 2 through 31).

[285] Step 2: Transform the DNA vector with the taurine-binding protein (from Step 1, EXAMPLE 23) into *Chlamydomonas reinhardtii* or *Ostreococcus tauri* that contains sCS/PLS-DC (from Step 2, EXAMPLE 22) and confirm the presence of the DNA constructs.

EXAMPLE 24

Development of a transgenic alga that expresses an sCS/PLP-DC gene *with a chloroplast transit peptide using chemical synthesis*

[286] Step 1: Use chemical synthesis to make a TP_sCS/PLP-DC gene with a transit peptide (from Step 1, EXAMPLE 4) codon optimized from expression in algae, *Chlamydomonas reinhardtii* or *Ostreococcus tauri*. Clone the TP_sCS/PLP-DC gene into an algal expression vector, such as pCB740 or pD1-Kan, so it is functional, and confirm the presence of the DNA construct.

[287] Step 2: Transform the DNA vector with the TP_sCS/PLP-DC protein (from Step 1, EXAMPLE 24) into *Chlamydomonas reinhardtii* or *Ostreococcus tauri* and confirm the presence of the DNA constructs.

EXAMPLE 25

Development of a transgenic alga that expresses a TP_sCS/PLP-DC gene with a transit peptide and *expresses* a *taurine binding protein with a chloroplast transit peptide using chemical synthesis*

[288] Step 1: Use chemical synthesis to make a DNA construct that contains a taurine binding protein (SEQ ID NO:29 or SEQ ID NO:31) with the plastid transit peptide (SEQ ID NO:55). Clone the taurine-binding protein into an algal expression vector, such as pCB740 or pD1-Kan, so it is functional.

[289] The nucleotide sequence for the plastid transit peptide (SEQ ID NO:55) encodes the peptide SEQ ID NO:56.

[290] The taurine binding protein gene is derived as follows from the group below:

[291] a. from SEQ ID NO:29 by removing nucleotides 1 through 66 (corresponding to the periplasmic transit peptide), optimized for expression in *Chlamydomonas reinhardtii* or *Ostreococcus tauri* and encoding a truncated taurine-binding peptide from *E. coli* (SEQ ID NO:30 minus amino acids 1 through 22); or

[292] b. from SEQ ID NO:31 by removing nucleotides 4 through 93, (corresponding to the periplasmic transit peptide), optimized for expression in *Chlamydomonas reinhardtii* or *Ostreococcus tauri* and encoding a truncated taurine-binding peptide from *Roseobacter denitrificans* (SEQ ID NO:32 minus amino acids 2 through 31).

[293] Step 2: Transform the DNA vector with the taurine-binding protein (from Step 1, EXAMPLE 25) into *Chlamydomonas reinhardtii* or *Ostreococcus tauri* that expresses the TP_sCS/PLP-DC gene with a transit peptide (from Step 2, EXAMPLE 24) and confirm the presence of the DNA constructs.

EXAMPLE 26

Development of a transgenic alga that expresses an sCS/PLP-DC gene *in the chloroplast via chloroplast transformation using chemical synthesis*

[294] Step 1: Make the following construct: an atpA promoter-59UTR (untranslated region) operably linked to sCS/PLP-DC polynucleotide and the atpA terminator (TatpA). Use the chloroplast destination expression for *Chlamydomonas reinhardtii* as described by Oey *et al.* (121). Use chemical synthesis to make a DNA construct that encodes a sCS/PLP-DC protein (from Step 1 EXAMPLE 1; Step 1 EXAMPLE 2; or Step 4 EXAMPLE 3) optimized for expression in an algal chloroplast. Synthesize the sCS/PLP-DC polynucleotide without a transit peptide and with XbaI at the 5' end and a NcoI site at the 3'end. Clone the sCS/PLP-DC polynucleotide into the XbaI/NcoI site (remove the GFP fragment of the Entry vector). Recombine the atpA/sCS/PLP-DC/atpA cassette from the Entry vector into the Destination vector, pC-Dest/psbA.

[295] Step 2: Transform the DNA vector with the Destination vector containing the atpA promoter/sCS/PLP-DC/TatpA (from Step 1, EXAMPLE 26) into *Chlamydomonas reinhardtii* and confirm the presence of the DNA construct.

EXAMPLE 27

Development of a transgenic fungus that expresses an sCS/PLP-DC gene *without the transit peptide using chemical synthesis*

[296] Step 1: Use chemical synthesis to make an sCS/PLP-DC gene without a transit peptide (from Step 1 EXAMPLE 1; Step 1 EXAMPLE 2; or Step 4 EXAMPLE 3) codon optimized for expression in a fungus such as yeast, and clone into a fungal expression vector such as pESC-TRP, pYES2/NT, or pYSG-IBA, so it is functional.

[297] Step 2: Transform the DNA vector with the sCS/PLP-DC gene (from Step 1, EXAMPLE 27) into the yeast strain and confirm the presence of the DNA constructs.

EXAMPLE 28

*Development of a transgenic fungus that expresses an s*CS/PLP-DC *without a transit peptide and expresses a taurine binding protein without a transit peptide using chemical synthesis*

[298] Step 1: Use chemical synthesis to make a DNA construct that contains a taurine binding protein gene without the transit peptide. Clone the taurine binding protein fragment into a fungal expression vector, such as pESC-TRP, pYES2/NT, or pYSG-IBA vector, so it is functional.

[299] The taurine-binding protein gene is derived as follows from the group below:

[300] a. from SEQ ID NO:29 by removing nucleotides 4 through 66 (corresponding to the periplasmic transit peptide), optimized for expression in yeast, and encoding a truncated taurine-binding peptide (SEQ ID NO:30 minus amino acids 2 through 22); or

[301] b. from SEQ ID NO:31, by removing nucleotides 4 through 93, (corresponding to the periplasmic transit peptide), optimized for expression in yeast, and encoding a truncated taurine-binding peptide from *Roseobacter denitrificans* (SEQ ID NO:32 minus amino acids 2 through 31).

[302] Step 2: Transform the DNA vector with the taurine-binding protein (from Step 1, EXAMPLE 28) into the yeast strain that contains the vector with sCS/PLP-DC (from Step 2, EXAMPLE 27) and confirm the presence of the DNA constructs.

EXAMPLE 29

Development of a transgenic bacterium with TauX suppressed and that expresses an sCS/PLP-DC *without the transit peptide using chemical synthesis*

[303] Step 1: Use chemical synthesis to make an antisense construct to silence or suppress TauX (SEQ ID NO: 43) and clone into the pBAD vector as described by Stefan *et al.* (405). To make the TauX antisense, fuse the polynucleotides for SEQ ID NO:81 to polynucleotides 1 through 360 of SEQ ID NO:43. Clone the TauX antisense fragment into the bacterial expression vector, pBAD, so the TauX antisense fragment can be expressed.

[304] Step 2: Transform the vector with the TauX antisense construct (from Step 1, EXAMPLE 29) into *Roseobacter denitrificans* and confirm the presence of the DNA construct.

[305] Step 3: Use chemical synthesis to make a DNA construct that contains an sC/PLP/DC gene (from Step 1 EXAMPLE 1; Step 1 EXAMPLE 2; or Step 4 EXAMPLE 3) without the transit peptide, optimized for expression in *Roseobacter denitrificans*. Clone the sCS/PLP-DC fragment into a bacterial expression vector, such as pET11, pKK223-3, or pSF-Tac, so it is functional.

[306] Step 4: Transform the vector with the functional sCS/PLP-DC construct (from Step 3, EXAMPLE 29) into the TauX knockdown *Roseobacter denitrificans* strain (from Step 2, EXAMPLE 29) and confirm the presence of the DNA construct.

EXAMPLE 30

Development of a transgenic bacterium with a TauY suppressed and that expresses an sCS/PLP-DC without the transit peptide using chemical synthesis

[307] Step 1: Use chemical synthesis to make an antisense construct to silence or suppress TauY (SEQ ID NO: 45) and clone into the pBAD vector as described by Stefan *et al.* (405). To make the TauY antisense, fuse the polynucleotides for SEQ ID NO:81 to polynucleotides 1 through 360 of SEQ ID NO:45. Clone the TauY antisense fragment into a bacterial expression vector, pBAD, so the TauY antisense fragment can be expressed.

[308] Step2: Transform the vector with the TauY antisense construct (from Step 1, EXAMPLE 30) into *Roseobacter denitrificans* and confirm the presence of the DNA construct

[309] Step 3: Transform the vector with the functional sCS/PLP-DC construct (from Step 3, EXAMPLE 29) into the TauX knockdown *Roseobacter denitrificans* strain (from Step 2, EXAMPLE 30) and confirm the presence of the DNA construct.

EXAMPLE 31

Development of a transgenic bacterium with a Tpa suppressed and that expresses an sCS/PLP-DC without the transit peptide using chemical synthesis

[310] Step 1: Use chemical synthesis to make an antisense construct to silence or suppress Tpa (SEQ ID NO: 47) and clone into the pBAD vector as described by Stefan *et al.* (405). To make the Tpa antisense, fuse the polynucleotides for SEQ ID NO:81 to polynucleotides 1 through 360 of SEQ ID NO:47. Clone the Tpa antisense fragment into the bacterial expression vector, pBAD, so Tpa antisense fragment can be expressed.

[311] Step2: Transform the vector with the Tpa antisense construct (from Step 1, EXAMPLE 31) into *Roseobacter denitrificans* and confirm the presence of the DNA construct.

[312] Step 3: Transform the vector with the functional sCS/PLP-DC construct (from Step 3, EXAMPLE 29) into the Tpa knockdown *Roseobacter denitrificans* strain (from Step 2, EXAMPLE 31) and confirm the presence of the DNA construct.

EXAMPLE 32

Develop bacteria with taurine

[313] Grow bacteria (*E. coli*: EXAMPLES 5-12; *C. glutamicum* EXAMPLES 13-20; or a member of the *Methylobacterium* EXAMPLE 21) with s*CS/PLP-DC* and induce gene expression with the appropriate inducer associated with the vector. Collect the cells and confirm that the cells express the *sCS/PLP-DC* peptide (~96.6 kDa) using western blot analysis. Harvest cells and supernatant from cultures. Extract amino acids and subject them to HPLC or GC-MS analysis to validate taurine production.

EXAMPLE 33

Develop aquafeed using bacterial cells with taurine

[314] Grow bacteria with *sCS/PLP-DC* (such as from EXAMPLE 32) and induce gene expression with the appropriate inducer associated with the vector. Collect the cells and process for use as an additive to feed.

EXAMPLE 34

Develop an E. coli strain that produces taurine

[315] This example demonstrates the use of a *TauD* knockout that expresses an sCS/PLP-DC (such as from EXAMPLE 5) to produce taurine in *E. coli*. Confirm *E. coli* transformation by selection and PCR analysis. Grow *E. coli* in ZYP media (406) and induce using autoinduction with an 8:1 lactose to glucose ratio. Extract free amino acids from 24, 48, or 72 hour culture to determine the level of taurine using high-performance liquid chromatography (HPLC). Separate bacteria from the supernatant by centrifugation, extract the amino acids, and determine the level of taurine in the pellet and supernatant. Taurine levels should be at least 0.25% of the total extracted free amino acids for the pellet or supernatant.

EXAMPLE 35

Development of a transgenic plant that constitutively expresses an sCS/PLP-DC without a transit peptide

[316] Step 1: Use chemical synthesis to make an *sCS/PLP-DC* DNA construct that contains a constitutive promoter, such as 35S, fused with the nucleotide sequence for the *sCS/PLP-DC* gene and a NOS terminator. Clone the DNA construct into a binary vector, such as pCambia1300, pCambia2300, or pCambia3200.

[317] The *sCS/PLP-DC* gene is derived from Step 1 EXAMPLE 1; Step 1 EXAMPLE 2; or Step 4 EXAMPLE 3, optimized for expression in a dicot (such as *Arabidopsis* or soybean) or a monocot (such as corn).

[318] Step 2: Transform the DNA construct (Step 1, EXAMPLE 35) into *Agrobacterium tumefaciens*, select for antibiotic resistance, and confirm the presence of the DNA construct.

[319] Step 3: Transform plant (*Arabidopsis*, soybean, corn, wheat, sugar beet, rice, camelina or canola), select for antibiotic resistance, and confirm the presence of the DNA construct in the transgenic plant.

EXAMPLE 36

Development of a transgenic plant that constitutively expresses a TP_CS/PLP-DC protein with a plant plastid transit peptide

[320] Step 1: Use chemical synthesis to make a DNA construct that contains a constitutive promoter, such as 35S, fused with the nucleotide sequence for a plastid transit peptide (SEQ ID NO: 55), sCS/PLP-DC and a NOS terminator. Clone the DNA construct into a binary vector, such as

pCambia1300, pCambia2300, or pCambia3200. The TP_CS/PLP-DC gene is derived from Step 1, EXAMPLE 4, optimized for expression in a dicot (such as *Arabidopsis* or soybean) or a monocot (such as corn).

[321] Step 2: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance, and confirm the presence of the DNA construct.

[322] Step 3: Transform plant (*Arabidopsis*, soybean, corn, wheat, sugar beet, rice, camelina or canola), select for antibiotic resistance, and confirm the presence of the DNA constructs in the transgenic plant.

EXAMPLE 37

Development of a transgenic plant that constitutively expresses an sCS/PLP-DC without a transit peptide and expresses a taurine binding protein without a transit peptide using chemical synthesis

[323] Step 1: Use chemical synthesis to make a DNA construct that contains a taurine binding protein (SEQ ID NO:29 or SEQ ID NO:31) without the transit peptide. Clone the DNA construct into a binary vector, such as pCambia1300, pCambia2300, or pCambia3200.

[324] The taurine binding protein gene is derived as follows from the group below:

[325] a. from SEQ ID NO:29 by removing nucleotides 4 through 66 (corresponding to the periplasmic transit peptide), optimized for expression in a dicot (such as *Arabidopsis* or soybean) or a monocot (such as corn) and encoding a truncated taurine-binding peptide from *E. coli* (SEQ ID NO:30 minus amino acids 2 through 22); or

[326] b. from SEQ ID NO:31 by removing nucleotides 4 through 93, (corresponding to the periplasmic transit peptide), optimized for expression in a dicot (such as *Arabidopsis* or soybean) or a monocot (such as corn) and encoding a truncated taurine-binding protein from *Roseobacter denitrificans* (SEQ ID NO:32 minus amino acids 2 through 31).

[327] Step 2: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance, and confirm the presence of the DNA construct

[328] Step 3: Transform the DNA vector with the taurine-binding protein without a transit peptide (from Step 1, EXAMPLE 37) into a plant (*Arabidopsis*, soybean, corn, wheat, sugar beet, rice, camelina or canola), that contains sCS/PLS-DC without the transit peptide (from Step 3, EXAMPLE 35) and confirm the presence of the DNA constructs.

EXAMPLE 38

Development of a transgenic plant that constitutively expresses a TP_CS/PLP-DC protein with a plant plastid transit peptide and expresses a taurine binding protein with a transit peptide using chemical synthesis

[329] Step 1: Use chemical synthesis to make a DNA construct that contains a constitutive promoter, such as 35S, fused with the nucleotide sequence for a plastid transit peptide (SEQ ID NO: 55), taurine binding protein and a NOS terminator. Clone the DNA construct into a binary vector, such as pCambia1300, pCambia2300, or pCambia3200.

[330] The nucleotide sequence for the plastid transit peptide (SEQ ID NO:55) encodes the peptide SEQ ID NO:56.

[331] The taurine binding protein gene is derived as follows from the group below:

[332] a. from SEQ ID NO:29 by removing nucleotides 1 through 66 (corresponding to the periplasmic transit peptide), optimized for expression in a dicot (such as *Arabidopsis* or soybean) or a monocot (such as corn) and encoding a truncated taurine-binding peptide from *E. coli* (SEQ ID NO:30 minus amino acids 1 through 22); or

[333] b. from SEQ ID NO:31 by removing nucleotides 4 through 93, (corresponding to the periplasmic transit peptide), optimized for expression in a dicot (such as *Arabidopsis* or soybean) or a monocot (such as corn) and encoding a truncated taurine-binding peptide from *Roseobacter denitrificans* (SEQ ID NO:32 minus amino acids 2 through 31).

[334] Step 2: Transform the DNA construct into *Agrobacterium tumefaciens*, select for antibiotic resistance, and confirm the presence of the DNA construct.

[335] Step 3: Transform the DNA vector with the taurine-binding protein with the transit peptide (from Step 1, EXAMPLE 38) into (*Arabidopsis*, soybean, corn, wheat, sugar beet, rice, camelina or canola), that contains sCS/PLS-DC with the transit peptide (from Step 3, EXAMPLE 36) and confirm the presence of the DNA constructs.

EXAMPLE 39

Development of a transgenic plant that expresses an sCS/PLP-DC gene *in the chloroplast via plastid transformation using chemical synthesis*

[336] Step 1: Use chemical synthesis to make a DNA construct that contains a functional sCS/PLP-DC gene (Step 1 EXAMPLE 1; Step 1 EXAMPLE 2; or Step 4 EXAMPLE 3) without the transit peptide, codon optimized for expression in *Glycine max* (soybean) chloroplast. Clone the

sCS/PLP-DC gene into the soybean plastid transformation vector pCLT312 as described by Dufourmantel *et al.* (317) so it is functional.

[337] Step 2: Transform the DNA vector with the sCS/PLP-DC-containing soybean plastid transformation vector (from Step 1, EXAMPLE 39) into soybean and confirm the presence of the DNA construct.

[338] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[339] Embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context. Embodiments of this invention are described herein, including the best mode known to the

inventors for carrying out the invention. Variations of those embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

BIBLIOGRAPHY

1. Sturman JA (1988) Taurine in development. *J Nutr* 118(10):1169-1176.
2. Sturman JA & Hayes KC (1980) The biology of taurine in nutrition and development. *Adv Nutr Res* 3:231-299.
3. Chen XC, Pan ZL, Liu DS, & Han X (1998) Effect of taurine on human fetal neuron cells: Proliferation and differentiation. *Adv Exp Med Biol* 442:397-403.
4. El Idrissi A & Trenkner E (1999) Growth factors and taurine protect against excitotoxicity by stabilizing calcium homeostasis and energy metabolism. *J Neurosci* 19:9459-9468.
5. El Idrissi A & Trenkner E (2003) Taurine regulates mitochondrial calcium homeostasis. *Adv Exp Med Biol*526:527-536.
6. Trenkner E (1990) Possible role of glutamate with taurine in neuron-glia interaction during cerebellar development. *Progr Clin Biol Res* 351:133-140.
7. Wu H, *et al.* (2005) Mode of action of taurine as a neuroprotector. *Brain Res* 1038:123-131.
8. Schaffer S, Takahashi K, & Azuma J (2000) Role of osmoregulation in the actions of taurine. *Amino Acids* 19:527-546.
9. Chapman RA, Suleiman MS, & Earm YE (1993) Taurine and the heart. *Cardiovascular Res* 27:358-363.
10. Tabassuma H, Rehmana H, Banerjeeb BD, Raisuddina S, & Parvez S (2006) Attenuation of tamoxifen-induced hepatotoxicity by taurine in mice. *Clinica Chimica Acta* 370:129-136.
11. Rocket N, *et al.* (2007) The osmolyte taurine protects against ultraviolet B radiation-induced immunosuppression. *J Immunol* 179:3604-3612.
12. Knopf K, Sturman JA, Armstrong M, & Hayes AC (1978) Taurine: An essential nutrient for the cat. *J Nutr* 108:773-778.

13. Morris JG, Rogers QR, & Pacioretty LM (1990) Taurine: an essential nutrient for cats. *J Small Anim Pract* 31(10):502-509.

14. Chesney R, *et al.* (1998) The Role of Taurine in Infant Nutrition. *Taurine 3, Advances in Experimental Medicine and Biology*, eds Schaffer S, Lombardini J, & Huxtable R (Springer US), Vol 442, pp 463-476.

15. Gibson GT, *et al.* (2007) Supplementation of taurine and methionine to all-plant protein diets for rainbow trout (Oncorhynchus mykiss). *Aquaculture* 269:514-524.

16. Buentello A, Jirsa D, Barrows FT, & Drawbridge M (2015) Minimizing fishmeal use in juvenile California yellowtail, *Seriola lalandi*, diets using non-GM soybeans selectively bred for aquafeeds. *Aquaculture* 435(0):403-411.

17. Rossi W, Moxely D, Buentello A, Pohlenz C, & Gatlin DM (2013) Replacement of fishmeal with novel plant feedstuffs in the diet of red drum *Sciaenops ocellatus*: an assessment of nutritional value. *Aquaculture Nutr* 19:72-81.

18. Watson AM, Buentello A, & Place AR (2014) Partial replacement of fishmeal, poultry by-product meal and soy protein concentrate with two non-genetically modified soybean cultivars in diets for juvenile cobia, *Rachycentron canadum*. *Aquaculture* 434(0):129-136.

19. Takagia S, *et al.* (2008) Taurine is an essential nutrient for yellowtail *Seriola quinqueradiata* fed non-fish meal diets based on soy protein concentrate. *Aquaculture* 280:198-205.

20. Lunger AN, McLean E, Gaylord TG, Kuhn D, & Craig SR (2007) Taurine supplementation to alternative dietary proteins used in fish meal replacement enhances growth of juvenile cobia (*Rachycentron canadum*). *Aquaculture* 271:401-410.

21. Watson AM, Barrows FT, & Place AR (2013) Taurine supplementation of plant derived protein and n-3 fatty acids are critical for optimal growth and development of cobia, *Rachycentron canadum*. *Lipids* 48(9):899-913.

22. Watson AM, Barrows FT, & Place AR (2013) Taurine supplemented plant protein based diets with alternative lipid sources for juvenile gilthead sea bream, *Sparus aurata*. *J Fish Aquaculture* 4:59-66.

23. Park GS, Takeuchi T, Yokoyama M, & Seikai T (2002) Optimal dietary taurine level for growth of juvenile Japanese flounder *Paralichthys olivaceus*. *Fish Sci* 68:824–829.

24. Gaylord TG, Teague AM, & Barrows FT (2006) Taurine supplementation of all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *J World Aquaculture Soc* 37:509-517.

25. Salze GP & Davis DA (2015) Taurine: a critical nutrient for future fish feeds. *Aquaculture* 437:215-229.

26. Yang H, Tian L, Huang J, Liang G, & Liu Y (2013) Dietary taurine can improve the hypoxia-tolerance but not the growth performance in juvenile grass carp *Ctenopharyngodon idellus*. *Fish Physiol Biochem* 39(5):1071-1078.

27. Kuz'mina VV, Gavrovskaya LK, Rusanova PV, Kulivatskaya EA, & Ryzhova OV (2011) Effect of taurine on the glycemia level and the activity of hydrolases in the intestinal mucosa in carp (*Cyprinus carpio L.*). *Inland Water Biol* 4(2):242-248.

28. Al-Feky SSA, El-Sayed AFM, & Ezzat AA (2016) Dietary taurine enhances growth and feed utilization in larval Nile tilapia (*Oreochromis niloticus*) fed soybean meal-based diets. *Aquaculture Nutr* 22(2):457-464.

29. Yue Y-R, *et al.* (2012) The effect of dietary taurine supplementation on growth performance, feed utilization and taurine contents in tissues of juvenile white shrimp (*Litopenaeus vannamei*, Boone, 1931) fed with low-fishmeal diets. *Aquaculture Res* DOI: 10.1111/j.1365-2109.2012.03135.x.

30. Brotons Martinez J, Chatzifotis S, Divanach P, & Takeuchi T (2004) Effect of dietary taurine supplementation on growth performance and feed selection of sea bass *Dicentrarchus labrax* fry fed with demand-feeders. *Fish Sci* 70(1):74-79.

31. Milei J, *et al.* (1992) Reduction of reperfusion injury with preoperative rapid intravenous infusion of taurine during myocardial revascularization. *Amer Heart J* 123:339-345.

32. Militante JD & Lombardini JB (2002) Treatment of hypertension with oral taurine. *Endocrinol* 147:3276-3284.

33. Fujita T, Ando K, Noda H, Ito Y, & Sato Y (1987) Effects of increased adrenomedullary activity and taurine in young patients with borderline hypertension. *Circulation* 75:525-532.

34. McCown TJ, Givens BS, & Breese GR (1987) Amino acid influences on seizures elicited within the inferior colliculus. *Pharmacol Exp Ther* 243:603-608.

35. Matsuyama Y, Morita T, Higuchi M, & Tsujii T (1983) The effect of taurine administration on patients with acute hepatitis. *Progr Clin Biol Res* 125:461-468.

36. Ikeda H (1977) Effects of taurine on alcohol withdrawal. *Lancet* 2:509.

37. Franconi F, Di Leo MAS, Bennardini F, & Ghirlanda G (2004) Is taurine beneficial in reducing risk factors for diabetes mellitus? *Neurochem Res* 29:143-150.

38. Paula-Lima AC, De Felice FG, Brito-Moreira J, & Ferreira ST (2005) Activation of GABAA receptors by taurine and muscimol blocks the neurotoxicity of [beta]-amyloid in rat hippocampal and cortical neurons. *Neuropharmacol* 49:1140-1148.

39. Nakamori K, *et al.* (1993) Quantitative evaluation of the effectiveness of taurine in protecting the ocular surface against oxidant. *Chem Pharm Bull* 41:335-338.

40. Zhang M, *et al.* (2004) Beneficial effects of taurine on serum lipids in overweight or obese non-diabetic subjects. *Amino Acids* 26:267-271.

41. Yokogoshi H, *et al.* (1999) Dietary taurine enhances cholesterol degradation and reduces serum and liver cholesterol concentrations in rats fed a high-cholesterol diet. *J Nutr* 129:1705-1712.

42. Yamamoto K, *et al.* (2000) Dietary taurine decreases hepatic secretion of cholesterol ester in rats fed a high-cholesterol diet. *Pharmacol* 60:27-33.

43. Green TR, Fellman JH, Eicher AL, & Pratt KL (1991) Antioxidant role and subcellular location of hypotaurine and taurine in human neutrophils. *Biochim Biophys Acta* 1073:91-97.

44. Gürer H, Ozgünes H, Saygin E, & Ercal N (2001) Antioxidant effect of taurine against lead-induced oxidative stress. *Arch Environ Contam and Toxicol* 41:397-402.

45. Das J, Ghosh J, Manna P, & Sil PC (2008) Taurine provides antioxidant defense against NaF-induced cytotoxicity in murine hepatocytes. *Pathophysiol* 15:181-190.

46. Zhang M, *et al.* (2004) Role of taurine supplementation to prevent exercise-induced oxidative stress in healthy young men. *Amino Acids* 26:203-207.

47. Williams M (2005) Dietary supplements and sports performance: Amino acids. *J Int Soc Sports Nutr* 2:63-67.

48. da Silva DLP, *et al.* (2008) Penetration profile of taurine in the human skin and its distribution in skin layers. *Pharm Res* 25:1846-1850.

49. Suzuki A, Kajita T, & Furushima M (1989) US Patent No. 4,877,447.

50. Turano FJ, Turano KA, Carlson PS, & Kinnersley AM (2012) US 9,267,148 (February 23, 2016).

51. Agnello G, Chang LL, Lamb CM, Georgiou G, & Stone EM (2013) Discovery of a substrate selectivity motif in amino acid decarboxylases unveils a taurine biosynthesis pathway in prokaryotes. *ACS Chem Biol* 8(10):2264-2271.

52. Tevatia R, *et al.* (2015) The taurine biosynthetic pathway of microalgae. *Algal Res* 9:21-26.

53. Honjoh KI, *et al.* (2010) Enhancement of menadione stress tolerance in yeast by accumulation of hypotaurine and taurine: co-expression of cDNA clones, from *Cyprinus carpio*, for cysteine dioxygenase and cysteine sulfinate decarboxylase in *Saccharomyces cerevisiae*. *Amino Acids* 38:1173-1183.

54. Turano FJ, Price MB, & Turano KA (2014) US Patent Application No. 20170283821.

55. Turano FJ (2016) International Patent Application No. PCT/US2016/026465.

56. Turano FJ (2016) International Patent Application No. PCT/US2016/028958.

57. Matsunari H, *et al.* (2005) Effect of feeding rotifers enriched with taurine on growth performance and body composition of Pacific cod larvae *Gadus macrocephalus*. *Aquaculture Sci* 53(3):297-304.

58. Salze G, McLean E, & Craig SR (2012) Dietary taurine enhances growth and digestive enzyme activities in larval cobia. *Aquaculture* 362–363:44-49.

59. Takahashi T, Amano T, & Takeuchi T (2005) Establishment of direct enrichment method of taurine to rotifer. *Aquaculture Sci* 53(2):121-126.

60. Hawkyard M, Laurel B, Barr Y, Hamre K, & Langdon C (2015) Evaluation of liposomes for the enrichment of rotifers (*Brachionus sp.*) with taurine and their subsequent effects on the growth and development of northern rock sole (*Lepidopsetta polyxystra*) larvae. *Aquaculture* 441:118-125.

61. Higgins CF (2001) ABC transporters: physiology, structure and mechanism – an overview. *Res Microbiol* 152:205–210.

62. Berntsson RPA, Smits SHJ, Schmitt L, Slotboom D-J, & Poolman B (2010) A structural classification of substrate-binding proteins. *FEBS Lett* 584(12):2606-2617.

63. Mulligan C, Fischer M, & Thomas GH (2011) Tripartite ATP-independent periplasmic (TRAP) transporters in bacteria and archaea. *FEMS Microbiol Rev* 35(1):68-86.

64. Turano FJ & Turano KA (2011) US 8,742,204 (Issued June 3, 2014).

65. van der Ploeg JR, *et al.* (1996) Identification of sulfate starvation-regulated genes in *Escherichia coli*: a gene cluster involved in the utilization of taurine as a sulfur source. *J Bacteriol* 178(18):5438-5446.

66. van der Ploeg JR, Cummings NJ, Leisinger T, & Connerton IF (1998) *Bacillus subtilis* genes for the utilization of sulfur from aliphatic sulfonates. *Microbiol* 144(9):2555-2561.

67. Brüggemann C, Denger K, Cook AM, & Ruff J (2004) Enzymes and genes of taurine and isethionate dissimilation in *Paracoccus denitrificans*. *Microbiol* 150(4):805-816.

68. Denger K, Ruff J, Schleheck D, & Cook AM (2004) *Rhodococcus opacus* expresses the xsc gene to utilize taurine as a carbon source or as a nitrogen source but not as a sulfur source. *Microbiol* 150(6):1859-1867.

69. Denger K, Smits THM, & Cook AM (2006) Genome-enabled analysis of the utilization of taurine as sole source of carbon or of nitrogen by *Rhodobacter sphaeroides* 2.4.1. *Microbiol* 152(11):3197-3206.

70. Krejcik Z, Schleheck D, Hollemeyer K, & Cook AM (2012) A five-gene cluster involved in utilization of taurine-nitrogen and excretion of sulfoacetaldehyde by *Acinetobacter radioresistens* SH164. *Arch Microbiol* 194(10):857-863.

71. Gorzynska AK, Denger K, Cook AM, & Smits THM (2006) Inducible transcription of genes involved in taurine uptake and dissimilation by Silicibacter pomeroyi DSS-3T. *Arch Microbiol* 185(5):402-406.

72. Novak RT, Gritzer RF, Leadbetter ER, & Godchaux W (2004) Phototrophic utilization of taurine by the purple nonsulfur bacteria Rhodopseudomonas palustris and Rhodobacter sphaeroides. *Microbiol* 150(6):1881-1891.

73. van der Ploeg JR, Iwanicka-Nowicka R, Kertesz MA, Leisinger T, & Hryniewicz MM (1997) Involvement of CysB and Cbl regulatory proteins in expression of the tauABCD operon and other sulfate starvation-inducible genes in Escherichia coli. *J Bacteriol* 179(24):7671-7678.

74. van der Ploeg JR, Iwanicka-Nowicka R, Bykowski T, Hryniewicz MM, & Leisinger T (1999) The Escherichia coli ssuEADCB Gene Cluster Is Required for the Utilization of Sulfur from Aliphatic Sulfonates and Is Regulated by the Transcriptional Activator Cbl. *J Biol Chem* 274(41):29358-29365.

75. Feinberg LF & Marx CJ (2014).

76. Jäckel C & Hilvert D (2010) Biocatalysts by evolution. *Curr Opin Biotechnol* 21(6):753-759.

77. Lane MD & Seelig B (2014) Advances in the directed evolution of proteins. *Current opinion in chemical biology* 22(Supplement C):129-136.

78. Packer MS & Liu DR (2015) Methods for the directed evolution of proteins. *Nature* 16:379.

79. Bennetzen JL & Hall BD (1982) Codon selection in yeast. *J Biol Chem* 257(6):3026-3031.

80. Gouy M & Gautier C (1982) Codon usage in bacteria: correlation with gene expressivity. *Nucleic Acids Res* 10(22):7055-7074.

81. Campbell WH & Gowri G (1990) Codon Usage in Higher Plants, Green Algae, and Cyanobacteria. *Plant Physiol* 92(1):1-11.

82. Douglas ES & Penny LS (The Plastid Genome of the Cryptophyte Alga, Guillardia theta: Complete Sequence and Conserved Synteny Groups Confirm Its Common Ancestry with Red Algae. *J Mol Evol* 48(2):236-244.

83. Yoon HS, Müller KM, Sheath RG, Ott FD, & Bhattacharya D (2006) Defining the major lineages of red algae (rhodophyta). *Journal of Phycology* 42(2):482-492.

84. Fletcher SP, Muto M, & Mayfield SP (2007) Optimization of Recombinant Protein Expression in the Chloroplasts of Green Algae. *Transgenic Microalgae as Green Cell Factories*, eds León R, Galván A, & Fernández E (Springer New York, New York, NY), pp 90-98.

85. Langenheim JH & Thimann KV (1982) *Botany: Plant Biology and its Relation to Human Affairs* (John Wiley & Sons Inc., New York).

86. Vasil IK (1984) *Cell Culture and Somatic Cell Genetics of Plants: Laboraory Procedures and Their Applications* (Academic Press, Orlando).

87. Stanier R, Ingrahm J, Wheelis M, & Painter P (1986) *The Microbial World* (Prentice-Hall, New Jersey) 5 Ed.

88. Dhringra OD & Sinclair JB (1985) *Basic plant pathology methods* (CRC Press, Boca Raton, FL).

89. Maniatis T, Fritsch EF, & Sambrook J (1985) *Molecular Cloning: A Laboratory Manual: DNA Cloning* (Cold Spring Harbor, New York).

90. Gait (1984) *Oligonucleotide Synthesis-A Practical Approach* (IRL Press, Washington, D.C.).

91. Hames DD & Higgins SJ (1984) *Nucleic Acid Hybridization: A Practical Approach* (IRL Press, Washington D.C.).

92. Watson JD, Gilman M, Witowski J, & Zoller M (1992) *Recombinant DNA* (Scientific American Books, New York).

93. Chen YY, Galloway KE, & Smolke CD (2012) Synthetic biology: advancing biological frontiers by building synthetic systems. *Genome Biol* 13(2):240.

94. Ceroni F, Carbonell P, François J-M, & Haynes KA (2015) Editorial – Synthetic Biology: Engineering Complexity and Refactoring Cell Capabilities. *Front Bioeng Biotechnol* 3:120.

95. Szewczyk E, *et al.* (2006) Fusion PCR and gene targeting in Aspergillus nidulans. *Nature Protocols* 1:3111-3121.

96. Ho SN, Hunt HD, Horton RM, Pullen JK, & Pease LR (1989) Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene* 77:51-59.

97. Fuhrmann M, Oertel W, & Hegemann P (1999) A synthetic gene coding for the green fluorescent protein (GFP) is a versatile reporter in *Chlamydomonas reinhardtii. Plant J* 19:353-361.

98. Mandecki W & Bolling TJ (1988) FokI method of gene synthesis. *Gene* 68:101–107.

99. Stemmer WP, Crameri, A., Ha, K.D., Brennan,T.M. and Heyneker,H.L. (1995) Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides. *Gene* 164:49–53.

100. Gao X, Yo P, Keith A, Ragan TJ, & Harris TK (2003) Thermodynamically balanced inside-out (TBIO) PCR-based gene synthesis: a novel method of primer design for high-fidelity assembly of longer gene sequences. *Nucleic Acids Res* 31:e143.

101. Young L & Dong Q (2004) Two-step total gene synthesis method. *Nucleic Acids Res* 32:e59.

102. Trinh R, Gurbaxani B, Morrison SL, & Seyfzadeh M (2004) Optimization of codon pair use within the (GGGGS) 3 linker sequence results in enhanced protein expression. *Mol Immunol* 40(10):717-722.

103. Chang TW & Yu L (1999) Genetic engineering. (Google Patents).

104. Kuusinen A, Arvola M, & Keinanen K (1995) Molecular dissection of the agonist binding site of an AMPA receptor. *EMBO J* 14(24):6327-6332.

105. Robinson CR & Sauer RT (1998) Optimizing the stability of single-chain proteins by linker length and composition mutagenesis. *PNAS* 95(11):5929-5934.

106. Armstrong N & Gouaux E (2000) Mechanisms for activation and antagonism of an AMPA-sensitive glutamate receptor: crystal structures of the GluR2 ligand binding core. *Neuron* 28(1):165-181.

107. Arai R, Ueda H, Kitayama A, Kamiya N, & Nagamune T (2001) Design of the linkers which effectively separate domains of a bifunctional fusion protein. *Protein Eng* 14(8):529-532.

108. Wriggers W, Chakravarty S, & Jennings PA (2005) Control of protein functional dynamics by peptide linkers. *Biopolymers* 80(6):736-746.

109. Reddy Chichili VP, Kumar V, & Sivaraman J (2013) Linkers in the structural biology of protein-protein interactions. *Protein Sci: Pub Protein Soc* 22(2):153-167.

110. Rosano GL & Ceccarelli EA (2014) Recombinant protein expression in microbial systems. *Front Microbiol* 5:341.

111. Hlavova M, Turoczy Z, & Bisova K (2015) Improving microalgae for biotechnology--From genetics to synthetic biology. *Biotechnol Adv* 33:1194-1203.

112. Çelik E & Çalık P (2012) Production of recombinant proteins by yeast cells. *Biotechnol Adv* 30(5):1108-1118.

113. de Jong A, Pietersma H, Cordes M, Kuipers OP, & Kok J (2012) PePPER: a webserver for prediction of prokaryote promoter elements and regulons. *BMC Genomics* 13:299.

114. Lee DJ, Minchin SD, & Busby SJW (2012) Activating Transcription in Bacteria. *Annu Rev Microbiol* 66(1):125-152.

115. Meysman P, *et al.* (2014) Structural properties of prokaryotic promoter regions correlate with functional features. *PLoS ONE* 9(2):e88717.

116. Fujiwara T, Ohnuma M, Yoshida M, Kuroiwa T, & Hirano T (2013) Gene targeting in the red alga *Cyanidioschyzon merolae*: Single- and multi-copy insertion using authentic and chimeric selection markers. *PLoS ONE* 8(9):e73608.

117. Mikami K, Hirata K, Takahashi M, Uji T, & Saga N (2011) Transient transformation of red algal cells: Breakthrough toward genetic transformation of marine crop porphyra species. *Genetic Transformation*, ed Alvarez M (InTech).

118. Manuell AL, *et al.* (2007) Robust expression of a bioactive mammalian protein in Chlamydomonas chloroplast. *Plant Biotechnol J* 5(3):402-412.

119. Cui Y, Qin S, & Jiang P (2014) Chloroplast Transformation of Platymonas (Tetraselmis) subcordiformis with the bar Gene as Selectable Marker. *PLoS ONE* 9(6):e98607.

120. Oey M, *et al.* (2013) RNAi Knock-Down of LHCBM1, 2 and 3 Increases Photosynthetic H2 Production Efficiency of the Green Alga *Chlamydomonas reinhardtii*. *PLoS ONE* 8(4):e61375.

121. Oey M, Ross IL, & Hankamer B (2014) Gateway-assisted vector construction to facilitate expression of foreign proteins in the chloroplast of single celled algae. *PLoS ONE* 9(2):e86841.

122. Wang B, Wang J, Zhang W, & Meldrum DR (2012) Application of synthetic biology in cyanobacteria and algae. *Front Microbiol* 3:344.

123. Wang J, Jiang P, Cui Y, Guan X, & Qin S (2010) Gene transfer into conchospores of *Porphyra haitanensis* (Bangiales, Rhodophyta) by glass bead agitation. *Phycologia* 49(4):355-360.

124. Ingelbrecht IL, Herman LM, Dekeyser RA, Van Montagu MC, & Depicker AG (1989) Different 3′ end regions strongly influence the level of gene expression in plant cells. *Plant Cell* 1:671-680.

125. Zaret KS & Sherman F (1982) DNA sequence required for efficient transcription termination in yeast. *Cell* 28:563-573.

126. Helden Jv, Rios AF, & Collado-Vides J (2000) Discovering regulatory elements in non-coding sequences by analysis of spaced dyads. *Nucleic Acids Res* 28(8):1808-1818.

127. Graber JH (2003) Variations in yeast 3'-processing *cis*-elements correlate with transcript stability. *Trends Genet* 19(9):473-476.

128. Wodniok S, Simon A, Glöckner G, & Becker B (2007) Gain and loss of polyadenylation signals during evolution of green algae. *BMC Evol Biol* 7(1):1-12.

129. Shen Y, Liu Y, Liu L, Liang C, & Li QQ (2008) Unique Features of Nuclear mRNA Poly(A) Signals and Alternative Polyadenylation in *Chlamydomonas reinhardtii*. *Genetics* 179(1):167-176.

130. Schlackow M, *et al.* (2013) Genome-wide analysis of poly(A) site selection in *Schizosaccharomyces pombe*. *RNA (New York, N.Y.)* 19(12):1617-1631.

131. Yamanishi M, *et al.* (2013) A genome-wide activity assessment of terminator regions in *Saccharomyces cerevisiae* provides a "Terminatome" toolbox. *ACS Synth Biol* 2(6):337-347.

132. Chen Y-J, *et al.* (2013) Characterization of 582 natural and synthetic terminators and quantification of their design constraints. *Nat Meth* 10(7):659-664.

133. Leavitt JM & Alper HS (2015) Advances and current limitations in transcript-level control of gene expression. *Curr Opin Biotechnol* 34:98-104.

134. Ausubel FM, *et al.* (1995) *Curr Protoc Mol Biol* (Greene Publishing and Wiley-Interscience, New York).

135. Chan HW & Wells RD (1974) Structural uniqueness of lactose operator. *Nature* 252:205-209.

136. Goeddel DV, *et al.* (1980) Synthesis of human fibroblast interferon by *E. coli Nucleic Acids Res* 8:4057-4074.

137. Marx CJ & Lidstrom ME (2001) Development of improved versatile broad-host-range vectors for use in methylotrophs and other Gram-negative bacteria. *Microbiol* 147:2065-2075.

138. Atomi H, Imanaka T, & Fukui T (2012) Overview of the genetic tools in the Archaea. *Front Microbiol* 3:337.

139. Farkas JA, Picking JW, & Santangelo TJ (2013) Genetic techniques for the archaea. *Annu Rev Genet* 47:539-561.

140. Tan S (2001) A modular polycistronic expression system for overexpressing protein complexes in Escherichia coli. *Protein Expr Purif* 21(1):224-234.

141. Tan S, Kern RC, & Selleck W (2005) The pST44 polycistronic expression system for producing protein complexes in *Escherichia coli*. *Protein Expr Purif* 40(2):385-395.

142. Baba T, *et al.* (2006) Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol Syst Biol* 2:2006.0008-2006.0008.

143. Reyrat JM, Pelicic V, Gicquel B, & Rappuoli R (1998) Counterselectable markers: untapped tools for bacterial genetics and pathogenesis. *Infect Immun* 66(9):4011-4017.

144. Nakashima N & Miyazaki K (2014) Bacterial cellular engineering by genome editing and gene silencing. *Int J Mol Sci* 15(2):2773-2793.

145. Ried JL & Collmer A (1987) An nptI-sacB-sacR cartridge for constructing directed, unmarked mutations in gram-negative bacteria by marker exchange-eviction mutagenesis. *Gene* 57(2-3):239-246.

146. Murphy KC, Campellone KG, & Poteete AR (2000) PCR-mediated gene replacement in *Escherichia coli*. *Gene* 246 (1-2):321-330.

147. Sun W, Wang S, & Curtiss R (2008) Highly efficient method for introducing successive multiple scarless gene deletions and markerless gene insertions into the Yersinia pestis chromosome. *Appl Environ Microbiol* 74:4241–4245.

148. Costantino N & Court DL (2003) Enhanced levels of λ Red-mediated recombinants in mismatch repair mutants. *PNAS* 100(26):15748-15753.

149. Datsenko KA & Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *PNAS* 97(12):6640-6645.

150. Lv L, Ren Y-L, Chen J-C, Wu Q, & Chen G-Q (2015) Application of CRISPRi for prokaryotic metabolic engineering involving multiple genes, a case study: Controllable P(3HB-co-4HB) biosynthesis. *Metab Eng* 29:160-168.

151. Peters JM, *et al.* (2015) Bacterial CRISPR: accomplishments and prospects. *Curr Opin Microbiol* 27:121-126.

152. Selle K & Barrangou R (2015) Harnessing CRISPR–Cas systems for bacterial genome editing. *Trends Microbiol* 23(4):225-232.

153. Rehnstam-Holm A-S & Godhe A (2003) Genetic engineering of algal species. *Biotechnology*, ed Doelle HW (UNESCO, Eolss Publishers, Oxford, UK).

154. Rosa L, Galván-Cejudo A, & Fernández E eds (2007) *Transgenic Microalgae as Green Cell Factories* (Springer Science+Business Media, LLC, New York, NY), Vol 616.

155. Leon R & Fernandez E (2007) Nuclear transformation of eukaryotic microalgae: historical overview, achievements and problems. *Adv Exp Med Biol* 616:1-11.

156. Mikami K, Hirata R, Takahashi M, Uji T, & Saga N (2011) Transient transformation of red algal cells: Breakthrough toward genetic transformation of marine crop *Porphyra Species*. *Genetic Transformation*, ed Alvarez M (InTech).

157. Umen JG & Olson BJ (2012) Genomics of Volvocine Algae. *Advances in botanical research* 64:185-243.

158. Liu L, *et al.* (2013) Development of a new method for genetic transformation of the green alga *Chlorella ellipsoidea*. *Mol Biotechnol* 54(2):211-219.

159. Gimpel JA, Specht EA, Georgianna DR, & Mayfield SP (2013) Advances in microalgae engineering and synthetic biology applications for biofuel production. *Current Opin Chemical Biol* 17(3):489-495.

160. Rasala BA, Chao S-S, Pier M, Barrera DJ, & Mayfield SP (2014) Enhanced genetic tools for engineering multigene traits into green algae. *PLoS ONE*.

161. Rasala BA, *et al.* (2010) Production of therapeutic proteins in algae, analysis of expression of seven human proteins in the chloroplast of *Chlamydomonas reinhardtii*. *Plant Biotechnol J* 8(6):719-733.

162. Potvin G & Zhang Z (2010) Strategies for high-level recombinant protein expression in transgenic microalgae: a review. *Biotechnol Adv* 28(6):910-918.

163. León-Bañares R, González-Ballester D, Galván A, & Fernández E (2004) Transgenic microalgae as green cell-factories. *Trends Biotechnol* 22(1):45-52.

164. Heitzer M & Zschoernig B (2007) Construction of modular tandem expression vectors for the green alga *Chlamydomonas reinhardtii* using the Cre/lox-system. *Biotechniques* 43(3):324, 326, 328 passim.

165. Sizova I, Greiner A, Awasthi M, Kateriya S, & Hegemann P (2013) Nuclear gene targeting in *Chlamydomonas* using engineered zinc-finger nucleases. *Plant J* 73(5):873-882.

166. Daboussi F, *et al.* (2014) Genome engineering empowers the diatom *Phaeodactylum tricornutum* for biotechnology. *Nat Commun* 5.

167. Hlavova M, Turoczy Z, & Bisova K (2015) Improving microalgae for biotechnology--From genetics to synthetic biology. *Biotechnol Adv* 33(6 Pt 2):1194-1203.

168. Pratheesh PT, Vineetha M, & Kurup GM (2013) An efficient protocol for the Agrobacterium-mediated genetic transformation of microalga *Chlamydomonas reinhardtii*. *Molecular Biotechnol*56(6):507-515.

169. Kindle KL (1998) Nuclear Transformation: Technology and Applications. *The Molecular Biology of Chloroplasts and Mitochondria in Chlamydomonas*, eds Rochaix JD, Goldschmidt-Clermont M, & Merchant S (Springer Netherlands, Dordrecht), pp 41-61.

170. Ohnuma M, Yokoyama T, Inouye T, Sekine Y, & Tanaka K (2008) Polyethylene Glycol (PEG)-Mediated Transient Gene Expression in a Red Alga, *Cyanidioschyzon merolae* 10D. *Plant Cell Physiol* 49(1):117-120.

171. Shimogawara K, Fujiwara S, Grossman A, & Usuda H (1998) High-efficiency transformation of *Chlamydomonas reinhardtii* by electroporation. *Genetics* 148(4):1821-1828.

172. Hayashi M, Hirono M, & Kamiya R (2001) Recovery of flagellar dynein function in a Chlamydomonas actin/dynein-deficient mutant upon introduction of muscle actin by electroporation. *Cell Motil Cytoskeleton* 49(3):146-153.

173. van Ooijen G, Knox K, Kis K, Bouget F-Y, & Millar AJ (2012) Genomic transformation of the Picoeukaryote *Ostreococcus tauri*. *J Vis Exp* (65):4074.

174. Vieler A, *et al.* (2012) Genome, functional gene annotation, and nuclear transformation of the Heterokont Oleaginous Alga *Nannochloropsis oceanica* CCMP1779. *PLoS Genetics* 8(11):e1003064.

175. Boynton JE, *et al.* (1988) Chloroplast transformation in *Chlamydomonas* with high velocity microprojectiles. *Science* 240(4858):1534-1538.

176. Apt KE, Kroth-Pancic PG, & Grossman AR (1996) Stable nuclear transformation of the diatom Phaeodactylum tricornutum. *Mol Gen Genet* 252(5):572-579.

177. Dunahay TG, Jarvis EE, & Roessler PG (1995) Genetic transformation of the diatoms cyclotella cryptica and navicula saprophila. *J Phycol* 31(6):1004-1012.

178. Falciatore A, Casotti R, Leblanc C, Abrescia C, & Bowler C (1999) Transformation of Nonselectable Reporter Genes in Marine Diatoms. *Marine Biotechnol (New York, N.Y.)* 1(3):239-251.

179. Zaslavskaia LA, Lippmeier JC, Kroth PG, Grossman AR, & Apt KE (2000) Transformation of the diatom Phaeodactylum tricornutum (*Bacillariophyceae*) with a variety of selectable marker and reporter genes. *J Phycol* 36(2):379-386.

180. Dunahay TG (1993) Transformation of *Chlamydomonas reinhardtii* with silicon carbide whiskers. *Biotechniques* 15(3):452-455, 457-458, 460.

181. Te MR, Lohuis, & Miller DJ (1998) Genetic transformation of dinoflagellates (Amphidinium and Symbiodinium): expression of GUS in microalgae using heterologous promoter constructs. *Plant J* 13(3):427-435.

182. Henry EC & Meints RH (Recombinant viruses as transformation vectors of marine macroalgae. *J Appl Phycol* 6(2):247-253.

183. Van Etten JL & Meints RH (1999) Giant viruses infecting algae. *Annu Rev Microbiol* 53:447-494.

184. Kojima H & Kawata Y (2001) A mini-transposon/transposase complex as a new tool for the genetic transformation of microalgae. *Photosynthetic Microorganisms in Environment Biotechnology*, eds Kojima H & Lee YK (Springer-Verlag, Berlin, Germany), pp 41-61.

185. Lapidot M, Raveh D, Sivan A, Arad SM, & Shapira M (2002) Stable chloroplast transformation of the unicellular red alga Porphyridium species. *Plant Physiol* 129(1):7-12.

186. Jiang H, *et al.* (2010) Methanotrophs: Multifunctional bacteria with promising applications in environmental bioengineering. *Biochem Eng J* 49(3):277-288.

187. Demain AL (2007) The business of biotechnology. *Indust Biotechnol* 3:269-283.

188. Romanos MA, Scorer CA, & Clare JJ (1992) Foreign gene expression in yeast: a review. *Yeast (Chichester, England)* 8(6):423-488.

189. Agmon N, *et al.* (2015) Yeast Golden Gate (yGG) for the Efficient Assembly of S. cerevisiae Transcription Units. *ACS Synth Biol* 4(7):853-859.

190. Sherman F (1991) Getting started with yeast. *Methods in Enzymology, Guide to Yeast Genetics and Molecular Biology*, eds Guthrie C & Fink GR (Acad. Press, New York), Vol 194, pp 3-21.

191. Sherman F, Fink GR, & Hick JB (1982) *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, New York).

192. Olmedo-Monfil V, CortÈs-Penagos C, & Herrera-Estrella A (2004) Three Decades of Fungal Transformation, Vol 267, pp 297-313.

193. Weld RJ, Plummer KM, Carpenter MA, & Ridgway HJ (2006) Approaches to functional genomics in filamentous fungi. *Cell Res* 16(1):31-44.

194. Kawai S, Hashimoto W, & Murata K (2010) Transformation of Saccharomyces cerevisiae and other fungi: Methods and possible underlying mechanism. *Bioengineered Bugs* 1(6):395-403.

195. van den Berg MA & Maruthachalam K eds (2015) *Genetic Transformation Systems in Fungi, Volume 1* (Springer, New York, NY).

196. Rivera AL, Magana-Ortiz D, Gomez-Lim M, Fernandez F, & Loske AM (2014) Physical methods for genetic transformation of fungi and yeast. *Physics of life reviews* 11(2):184-203.

197. Vickers CE, Bydder SF, Zhou Y, & Nielsen LK (2013) Dual gene expression cassette vectors with antibiotic selection markers for engineering in *Saccharomyces cerevisiae*. *Microbial Cell Factories* 12(1):1-11.

198. Sherman F (1997) Yeast genetics. *The Encyclopedia of Molecular Biology and Molecular Medicine*, ed Meyers RA (VCH Publisher, Weinheim, Germany), Vol 6, pp 302-325.

199. Romanos MA, Scorer CA, & Clare JJ (1992) Foreign gene expression in yeast: a review. *Yeast (Chichester, England)* 8.

200. Baudin A, Ozier-Kalogeropoulos O, Denouel A, Lacroute F, & Cullin C (1993) A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. *Nucleic Acids Res* 21(14):3329-3330.

201. Longtine MS, *et al.* (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast (Chichester, England)* 14(10):953-961.

202. Krawchuk MD & Wahls WP (1999) High-efficiency gene targeting in *Schizosaccharomyces pombe* using a modular, PCR-based approach with long tracts of flanking homology. *Yeast (Chichester, England)* 15(13):1419-1427.

203. Epinat J-C, *et al.* (2003) A novel engineered meganuclease induces homologous recombination in yeast and mammalian cells. *Nucleic Acids Res* 31(11):2952-2962.

204. Li T, *et al.* (2011) Modularly assembled designer TAL effector nucleases for targeted gene knockout and gene replacement in eukaryotes. *Nucleic Acids Res.*

205. DiCarlo JE, et al. (2013) Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems. *Nucleic Acids Res* 41(7):4336-43.

206. Jacobs JZ, Ciccaglione KM, Tournier V, & Zaratiegui M (2014) Implementation of the CRISPR-Cas9 system in fission yeast. *Nat Commun* 5.

207. Olmedo-Monfil V, Cortes-Penagos C, & Herrera-Estrella A (2004) Three decades of fungal transformation: key concepts and applications. *Methods Mol Biol* 267:297-313.

208. van den Berg MA & Maruthachalam K (2015) *Genetic Transformation Systems in Fungi, Volume 1* (Springer International Publishing, Cham).

209. van Oers MM, Pijlman GP, & Vlak JM (2015) Thirty years of baculovirus–insect cell protein expression: from dark horse to mainstream technology. *J Gen Virol* 96(1):6-23.

210. Almo SC & Love JD (2014) Better and faster: improvements and optimization for mammalian recombinant protein production. *Curr Opin Struct Biol* 26:39-43.

211. van Der Krol AR, *et al.* (1999) Developmental and wound-, cold-, desiccation-, ultraviolet-B-stress-induced modulations in the expression of the petunia zinc finger transcription factor gene ZPT2-2. *Plant Physiol* 121(4):1153-1162.

212. Shinmyo A, *et al.* (1998) Metabolic engineering of cultured tobacco cells. *Biotechnol Bioeng* 58(2-3):329-332.

213. Sohal AK, Pallas JA, & Jenkins GI (1999) The promoter of a *Brassica napus* lipid transfer protein gene is active in a range of tissues and stimulated by light and viral infection in transgenic *Arabidopsis*. *Plant Mol Biol* 41(1):75-87.

214. Cormack RS, *et al.* (2002) Leucine zipper-containing WRKY proteins widen the spectrum of immediate early elicitor-induced WRKY transcription factors in parsley. *Biochimica et Biophysica Acta* 1576(1-2):92-100.

215. Eulgem T, Rushton PJ, Schmelzer E, Hahlbrock K, & Somssich IE (1999) Early nuclear events in plant defence signalling: rapid gene activation by WRKY transcription factors. *EMBO J* 18(17):4689-4699.

216. Lebel E, *et al.* (1998) Functional analysis of regulatory sequences controlling PR-1 gene expression in *Arabidopsis*. *Plant J*16(2):223-233.

217. Ngai N, Tsai FY, & Coruzzi G (1997) Light-induced transcriptional repression of the pea AS1 gene: identification of cis-elements and transfactors. *Plant J* 12(5):1021-1034.

218. Kucho K, Ohyama K, & Fukuzawa H (1999) CO(2)-responsive transcriptional regulation of CAH1 encoding carbonic anhydrase is mediated by enhancer and silencer regions in *Chlamydomonas reinhardtii*. *Plant Physiol* 121(4):1329-1338.

219. Kucho K, Yoshioka S, Taniguchi F, Ohyama K, & Fukuzawa H (2003) Cis-acting elements and DNA-binding proteins involved in CO2-responsive transcriptional activation of Cah1 encoding a periplasmic carbonic anhydrase in *Chlamydomonas reinhardtii*. *Plant Physiol* 133(2):783-793.

220. Chen W, Chao G, & Singh KB (1996) The promoter of a H2O2-inducible, Arabidopsis glutathione S-transferase gene contains closely linked OBF- and OBP1-binding sites. *Plant J* 10(6):955-966.

221. Chen W & Singh KB (1999) The auxin, hydrogen peroxide and salicylic acid induced expression of the Arabidopsis GST6 promoter is mediated in part by an ocs element. *Plant J* 9(6):667-677.

222. Andrade MA, O'Donoghue SI, & Rost B (1998) Adaptation of protein surfaces to subcellular location. *J Mol Biol* 276.

223. Leubner-Metzger G, Petruzzelli L, Waldvogel R, Vogeli-Lange R, & Meins F, Jr. (1998) Ethylene-responsive element binding protein (EREBP) expression and the transcriptional regulation of class I beta-1,3-glucanase during tobacco seed germination. *Plant Mol Biol* 38(5):785-795.

224. de Framond AJ (1991) A metallothionein-like gene from maize (*Zea mays*). Cloning and characterization. *FEBS Letters* 290(1-2):103-106.

225. Hudspeth RL, Hobbs SL, Anderson DM, Rajasekaran K, & Grula JW (1996) Characterization and expression of metallothionein-like genes in cotton. *Plant Mol Biol* 31(3):701-705.

226. Kridl JC, *et al.* (1991) Isolation and characterization of an expressed napin gene from *Brassica rapa*. *Seed Science Research* 1(04):209-219.

227. Zavallo D, Lopez Bilbao M, Hopp HE, & Heinz R (2010) Isolation and functional characterization of two novel seed-specific promoters from sunflower (*Helianthus annuus L.*). *Plant Cell Rep* 29(3):239-248.

228. Kim MJ, *et al.* (2006) Seed-specific expression of sesame microsomal oleic acid desaturase is controlled by combinatorial properties between negative cis-regulatory elements in the SeFAD2 promoter and enhancers in the 5'-UTR intron. *Mol Genet Genom* 276(4):351-368.

229. Bustos MM, *et al.* (1989) Regulation of beta-glucuronidase expression in transgenic tobacco plants by an A/T-rich, cis-acting sequence found upstream of a French bean beta-phaseolin gene. *Plant Cell* 1(9):839-853.

230. Fujiwara T & Beachy RN (1994) Tissue-specific and temporal regulation of a beta-conglycinin gene: roles of the RY repeat and other cis-acting elements. *Plant Mol Biol* 24(2):261-272.

231. Wienand U, Langridge P, & Feix G (1981) Isolation and characterization of a genomic sequence of maize coding for a zein gene. *Molec Gen Genet* 182(3):440-444.

232. Takaiwa F, Oono K, Wing D, & Kato A (1991) Sequence of three members and expression of a new major subfamily of glutelin genes from rice. *Plant Mol Biol* 17:875-885.

233. Hudspeth RL, Grula JW, Dai Z, Edwards GE, & Ku MS (1992) Expression of maize phosphoenolpyruvate carboxylase in transgenic tobacco: Effects on biochemistry and physiology. *Plant Physiol* 98(2):458-464.

234. Herrera-Estrella L, Depicker A, van Montagu M, & Schell J (1983) Expression of chimaeric genes transferred into plant cells using a Ti-plasmid-derived vector. *Nature* 303:209-213.

235. Nagaya S, Kawamura K, Shinmyo A, & Kato K (2010) The HSP terminator of Arabidopsis thaliana increases gene expression in plant cells. *Plant Cell Physiol* 51(2):328-332.

236. Mogen BD, MacDonald MH, Graybosch R, & Hunt AG (1990) Upstream sequences other than AAUAAA are required for efficient messenger RNA 3'-end formation in plants. *Plant Cell* 2(12):1261-1272.

237. Mogen BD, MacDonald MH, Leggewie G, & Hunt AG (1992) Several distinct types of sequence elements are required for efficient mRNA 3' end formation in a pea rbcS gene. *Mol Cell Biol* 12(12):5406-5414.

238. Rothnie HM, Reid J, & Hohn T (1994) The contribution of AAUAAA and the upstream element UUUGUA to the efficiency of mRNA 3'-end formation in plants. *EMBO J* 13(9):2200-2210.

239. Bassett CL (2007) *Regulation of Gene Expression in Plants: The Role of Transcript Structure and Processing* (Springer Press, New York).

240. Ohme-Takagi M, Taylor CB, Newman TC, & Green PJ (1993) The effect of sequences with high AU content on mRNA stability in tobacco. *PNAS* 90(24):11811-11815.

241. Newman TC, Ohme-Takagi M, Taylor CB, & Green PJ (1993) DST sequences, highly conserved among plant SAUR genes, target reporter transcripts for rapid decay in tobacco. *Plant Cell* 5(6):701-714.

242. Gutiérrez RA, Macintosh GC, & Green PJ (1999) Current perspectives on mRNA stability in plants: multiple levels and mechanisms of control. *Trends Plant Sci* 4:429-438.

243. An G, *et al.* (1989) Functional analysis of the 3' control region of the potato wound-inducible proteinase inhibitor II gene. *Plant Cell* 1:115-122.

244. An G, Watson BD, Stachel S, Gordon MP, & Nester EW (1985) New cloning vehicles for transformation of higher plants. *EMBO J* 4:277-284.

245. Gruber MY & Cosby WL (1993) Vectors for plant transformation. *Methods in Plant Molecular Biology and Biotechnology*, eds Glick BR & Thompson JE (CRC Press, Baco Raton, Fla.), pp 89-119.

246. Hatzfeld Y (2014) US 8779237.

247. Franklin S, Somanchi A, Espina K, Rudenko G, & Chua P (2014) US Patent No. 8,674,180.

248. Feng PCC, Malven M, & Flasinski S (2013) US Patent No. 8,420,888.

249. Manjunath S, *et al.* (2012) US Patent No. 8,138,393.

250. Lee DW, *et al.* (2008) Arabidopsis nuclear-encoded plastid transit peptides contain multiple sequence subgroups with distinctive chloroplast-targeting sequence motifs. *Plant Cell* 20(6):1603-1622.

251. von Heijne G, *et al.* (1991) CHLPEP: a database of chloroplast transit peptides. *Plant Mol Biol Rep* 9:104-126.

252. Waller RF, Reed MB, Cowman AF, & McFadden GI (2000) Protein trafficking to the plastid of *Plasmodium falciparum* is via the secretory pathway. *EMBO J* 19(8):1794-1802.

253. Minge MA, *et al.* (2010) A phylogenetic mosaic plastid proteome and unusual plastid-targeting signals in the green-colored dinoflagellate *Lepidodinium chlorophorum*. *BMC Evol Biol* 10(1):1-11.

254. Li H-m & Teng Y-S (2013) Transit peptide design and plastid import regulation. *Trends Plant Sci* 18(7):360-366.

255. Tardif M, *et al.* (2012) PredAlgo: A New Subcellular Localization Prediction Tool Dedicated to Green Algae. *Mol Biol Evol* 29(12):3625-3639.

256. von Heijne G (1986) Mitochondrial targeting sequences may form amphiphilic helices. *EMBO J* 5:1335–1342.

257. Swinkels BW, Gould SJ, Bodnar AG, Rachubinski RA, & Subramani S (1991) A novel, cleavable peroxisomal targeting signal at the amino-terminus of the rat 3-ketoacyl-CoA thiolase. *EMBO J* 10(11):3255-3262.

258. Rusch SL & Kendall DA (1995) Protein transport via amino-terminal targeting sequences: Common themes in diverse systems. *Mol Membrane Biol* 12(4):295-307.

259. Soll J & Tien R (1998) Protein translocation into and across the chloroplastic envelope membranes. *Plant Mol Biol* 38:191–207.

260. Gould SJ, Keller GA, & Subramani S (1988) Identification of peroxisomal targeting signals located at the carboxy terminus of four peroxisomal proteins. *J Cell Biol* 107(3):897-905.

261. Gould SJ, Keller GA, Hosken N, Wilkinson J, & Subramani S (1989) A conserved tripeptide sorts proteins to peroxisomes. *J Cell Biol* 108(5):1657-1664.

262. McCammon MT, McNew JA, Willy PJ, & Goodman JM (1994) An internal region of the peroxisomal membrane protein PMP47 is essential for sorting to peroxisomes. *J Cell Biol* 124(6):915-925.

263. Cokol M, Nair R, & Rost B (2000) Finding nuclear localization signals. *EMBO Rep* 1(5):411-415.

264. Helenius A & Aebi M (2001) Intracellular functions of N-linked glycans. *Science* 291(5512):2364-2369.

265. Emanuelsson O, Brunak S, von Heijne G, & Nielsen H (2007) Locating proteins in the cell using TargetP, SignalP and related tools. *Nature Protoc* 2(4):953-971.

266. Emanuelsson O, Nielsen H, Brunak S, & von Heijne G (2000) Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. *J Mol Biol* 300(4):1005-1016.

267. Bannai H, Tamada Y, Maruyama O, Nakai K, & Miyano S (2002) Extensive feature detection of N-terminal protein sorting signals. *Bioinformatics* 18(2):298-305.

268. Bendtsen JD, Nielsen H, von Heijne G, & Brunak S (2004) Improved prediction of signal peptides: SignalP 3.0. *J Mol Biol* 340(4):783-795.

269. Hiller K, Grote A, Scheer M, Munch R, & Jahn D (2004) PrediSi: prediction of signal peptides and their cleavage positions. *Nucleic Acids Res* 32(Web Server issue):W375-379.

270. Bhasin M & Raghava GP (2004) ESLpred: SVM-based method for subcellular localization of eukaryotic proteins using dipeptide composition and PSI-BLAST. *Nucleic Acids Res* 32(Web Server issue):W414-419.

271. Garg A, Bhasin M, & Raghava GP (2005) Support vector machine-based method for subcellular localization of human proteins using amino acid compositions, their order, and similarity search. *J Biol Chem* 280(15):14427-14432.

272. Bhasin M, Garg A, & Raghava GP (2005) PSLpred: prediction of subcellular localization of bacterial proteins. *Bioinformatics* 21(10):2522-2524.

273. Hoglund A, Donnes P, Blum T, Adolph HW, & Kohlbacher O (2006) MultiLoc: prediction of protein subcellular localization using N-terminal targeting sequences, sequence motifs and amino acid composition. *Bioinformatics* 22(10):1158-1165.

274. Shatkay H, *et al.* (2007) SherLoc: high-accuracy prediction of protein subcellular localization by integrating text and protein sequence data. *Bioinformatics* 23(11):1410-1417.

275. Emanuelsson O, Nielsen H, & von Heijne G (1999) ChloroP, a neural network-based method for predicting chloroplast transit peptides and their cleavage sites. *Protein Sci* 8(5):978-984.

276. Claros MG & Vincens P (1996) Computational method to predict mitochondrially imported proteins and their targeting sequences. *Eur J Biochem* 241(3):779-786.

277. Small I, Peeters N, Legeai F, & Lurin C (2004) Predotar: A tool for rapidly screening proteomes for N-terminal targeting sequences. *Proteomics* 4(6):1581-1590.

278. Kelley LA, MacCallum RM, & Sternberg MJ (2000) Enhanced genome annotation using structural profiles in the program 3D-PSSM. *J Mol Biol* 299(2):499-520.

279. Shahin EA (1985) Totipotency of tomato protoplasts. *Theor Appl Genet* 69:235-240.

280. Fromm M, Taylor LP, & V. W (1985) Expression of genes transferred into monocot and dicot plant cells by electroporation. *PNAS* 82:5824-5828.

281. Fromm ME, Taylor LP, & Walbot V (1986) Stable transformation of maize after gene transfer by electroporation. *Nature* 319(6056):791-793.

282. Riggs CD & Bates GW (1986) Stable transformation of tobacco by electroporation: evidence for plasmid concatenation. *PNAS* 83(15):5602-5606.

283. D'Halluin K, Bonne E, Bossut M, De Beuckeleer M, & Leemans J (1992) Transgenic maize plants by tissue electroporation. *Plant Cell* 4:1495-1505.

284. Laursen CM, Krzyzek RA, Flick CE, Anderson PC, & Spencer TM (1994) Production of fertile transgenic maize by electroporation of suspension culture cells *Plant Mol Biol* 24:51-61

285. Crossway A, *et al.* (1986) Integration of foreign DNA following microinjection of tobacco mesophyll protoplasts. *Molecular and General Genetics* 202:179-185.

286. Griesbach RJ (1983) Protoplast microinjection. *Plant Mol Biol Rep* 1:32-37.

287. Sporlein B & Koop H-U (1991) Lipofectin: direct gene transfer to higher plants using cationic liposomes. *Theor Appl Genet* 83:1-5.

288. Ohgawara T, Uchimiya H, & Harada H (1983) Uptake of liposome-encapsulated plasmid DNA by plant protoplasts and molecular fate of foreign DNA *Protoplasma* 116:145-148.

289. Deshayes A, Herrera-Estrella L, & Caboche M (1985) Liposome-mediated transformation of tobacco mesophyll protoplasts by an *Escherichia coli* plasmid. *EMBO J* 4(11):2731-2737.

290. Christou P, Murphy JE, & Swain WF (1987) Stable transformation of soybean by electroporation and root formation from transformed callus. *PNAS* 84(12):3962-3966.

291. Horsch RB, *et al.* (1985) A Simple and General Method for Transferring Genes into Plants. *Science* 227:1229-1231.

292. Paszkowski J, *et al.* (1984) Direct gene transfer to plants. *Embo J* 3(12):2717-2722.

293. Hooykaas-Van Slogteren GM, Hooykaas PJ, & Schilperoort RA (1984) Expression of Ti plasmid genes in monocotyledonous plants infected with *Agrobacterium tumefaciens. Nature* 311:763-764.

294. Rogers SG, Horsch, R.B., and Fraley, R.T. 1986. Gene transfer in plants: Production of transformed plants using Ti-plasmid vectors. (1986) Gene transfer in plants: Production of transformed plants using Ti-plasmid vectors. *Methods Enzymol* 118:627-640.

295. Bevan MW & Chilton M-D (1982) T-DNA of the *Agrobacterium* Ti and Ri plasmids. *Annu Rev Genet* 16:357-384.

296. Klein TM, *et al.* (1988) Transfer of foreign genes into intact maize cells with high-velocity microprojectiles. *PNAS* 85(12):4305-4309.

297. Klein TM, Gradziel T, Fromm ME, & Sanford JC (1988) Factors influencing gene delivery into *Zea mays* cells by high-velocity microprojectiles. *Biotechnol* 6:559-563.

298. McCabe DE, Swain WF, Martinell BJ, & Christou P (1988) Stable transformation of soybean (*Glycine max*) by particle acceleration. *Biotechnol* 6:923-926.

299. Sanford JC, Smith FD, & Rushell JA (1993) Optimizing the biolistic process for different biological application. *The Methods in Enzymology*, ed Wu R (Academic Press, Orlando), Vol 217, pp 483-509.

300. Freeman JP, *et al.* (1984) A comparison of methods for plasmid delivery into plant protoplasts. *Plant Cell Physiol* 25:1353-1365.

301. Frame BR, *et al.* (1994) Production of fertile transgenic maize plants by silicon carbide whisker-mediated transformation. *Plant J* 6:941–948.

302. Thompson JA, Drayton P, Frame B, Wang K, & Dunwell JM (1995) Maize transformation utilizing silicon carbide whiskers: a review. *Euphytica* 85:75–80.

303. Guo Y, Liang H, & Berns MW (1995) Laser-mediated gene transfer in rice. *Physiol Plantarum* 93:19–24.

304. Badr YA, Kereim MA, Yehia MA, Fouad OO, & Bahieldin A (2005) Production of fertile transgenic wheat plants by laser micropuncture. *Photochem Photobiol Sci* 4:803-807.

305. Bao S, Thrall BD, & Miller DL (1997) Transfection of a reporter plasmid into cultured cells by sonoporation in vitro. *Ultrasound Med Biol* 23:953-959.

306. Finer KR & Finer JJ (2000) Use of Agrobacterium expressing green fluorescent protein to evaluate colonization of sonication-assisted Agrobacterium-mediated transformation-treated soybean cotyledons. *Lett Appl Microbiol* 30(5):406-410.

307. Amoah BK, Wu H, Sparks C, & Jones HD (2001) Factors influencing Agrobacterium-mediated transient expression of uidA in wheat inflorescence tissue. *J Exp Bot* 52(358):1135-1142.

308. Krens FA, Molendijk L, Wullems GJ, & Schilperoort RA (1982) In Vitro transformation of plant protoplasts with Ti-plasmid DNA. *Nature* 296:72-74.

309. Bechtold N & Pelletier G (1998) In planta Agrobacterium-mediated transformation of adult *Arabidopsis thaliana* plants by vacuum infiltration. *Meth Mol Biol* 82:259-266.

310. Broothaerts W, *et al.* (2005) Gene transfer to plants by diverse species of bacteria. *Nature* 433:629-633.

311. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, & Gregory PD (2010) Genome editing with engineered zinc finger nucleases. *Nature Rev. Genetics* 11(9):636-646.

312. Weinthal D, Tovkach A, Zeevi V, & Tzfira T (2010) Genome editing in plant cells by zinc finger nucleases. *Trends Plant Sci* 15(6):308-321.

313. Gaj T, Gersbach CA, & Barbas CF (2013) ZFN, TALEN and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol* 31(7):397-405.

314. Sprink T, Metje J, & Hartung F (2015) Plant genome editing by novel tools: TALEN and other sequence specific nucleases. *Curr Opin Biotechnol* 32:47-53.

315. Bortesi L & Fischer R (2015) The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnol Adv* 33(1):41-52.

316. Kumar V & Jain M (2015) The CRISPR-Cas system for plant genome editing: advances and opportunities. *J Exp Bot* 66(1):47-57.

317. Dufourmantel N, *et al.* (2004) Generation of fertile transplastomic soybean. *Plant Mol Biol* 55(4):479-489.

318. Miller JH (1992) *A short course in bacterial genetics: a laboratory manual and handbook for Escherichia coli and related bacteria* (Cold Spring Harbor Laboratory Press, Plainview, N.Y.).

319. Parekh S, Vinci VA, & Strobel RJ (2000) Improvement of microbial strains and fermentation processes. *Appl Microbiol Biotechnol* 54(3):287-301.

320. Forsburg SL (2001) The art and design of genetic screens: Yeast. *Nature Rev Genetics* 2(9):659-668.

321. Flynn T, Ghirardi ML, & Seibert M (2002) Accumulation of O2-tolerant phenotypes in H2-producing strains of *Chlamydomonas reinhardtii* by sequential applications of chemical mutagenesis and selection. *Int J Hydrogen Energy* 27(11–12):1421-1430.

322. Doan TTY & Obbard JP (2012) Enhanced intracellular lipid in *Nannochloropsis sp.* via random mutagenesis and flow cytometric cell sorting. *Algal Res* 1(1):17-21.

323. Bernheim AG, Libis VK, Lindner AB, & Wintermute EH (2016) Phage-mediated delivery of targeted sRNA constructs to knock down gene expression in *E. coli.* (109):e53618.

324. Zhang R, *et al.* (2014) High-Throughput genotyping of green algal mutants reveals random distribution of mutagenic insertion sites and endonucleolytic cleavage of transforming DNA. *Plant Cell* 26(4):1398-1409.

325. Dent RM, Haglund CM, Chin BL, Kobayashi MC, & Niyogi KK (2005) Functional genomics of eukaryotic photosynthesis using insertional mutagenesis of *Chlamydomonas reinhardtii*. *Plant Physiol* 137(2):545-556.

326. Colombo SL, *et al.* (2002) Use of the bleomycin resistance gene to generate tagged insertional mutants of *Chlamydomonas reinhardtii* that require elevated CO2 for optimal growth. *Funct Plant Biol* 29(3):231-241.

327. Gonzalez-Ballester D, *et al.* (2011) Reverse genetics in *Chlamydomonas*: a platform for isolating insertional mutants. *Plant Methods* 7(1):1-13.

328. Kleckner N, Bender J, & Gottesman S (1991) Uses of transposons with emphasis on Tn10. *Methods Enzymol* 204:139-180.

329. Wu-Scharf D, Jeong B-r, Zhang C, & Cerutti H (2000) Transgene and transposon silencing in *Chlamydomonas reinhardtii* by a DEAH-Box RNA helicase. *Science* 290(5494):1159-1162.

330. Casas-Mollano JA, *et al.* (2008) Diversification of the core RNA interference machinery in *Chlamydomonas reinhardtii* and the role of DCL1 in transposon silencing. *Genetics* 179(1):69-81.

331. Goryshin IY, Jendrisak J, Hoffman LM, Meis R, & Reznikoff WS (2000) Insertional transposon mutagenesis by electroporation of released Tn5 transposition complexes. *Nat Biotech* 18(1):97-100.

332. Datsenko KA & Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *PNAS* 97.

333. Zhong J, Karberg M, & Lambowitz AM (2003) Targeted and random bacterial gene disruption using a group II intron (targetron) vector containing a retrotransposition-activated selectable marker. *Nucleic Acids Res* 31(6):1656-1664.

334. Minoda A, Sakagami R, Yagisawa F, Kuroiwa T, & Tanaka K (2004) Improvement of culture conditions and evidence for nuclear transformation by homologous recombination in a red alga, *Cyanidioschyzon merolae* 10D. *Plant Cell Physiol* 45(6):667-671.

335. Qi Lei S, *et al.* (2013) Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell* 152(5):1173-1183.

336. Jiang W, Bikard D, Cox D, Zhang F, & Marraffini LA (2013) RNA-guided editing of bacterial genomes using CRISPR-Cas systems. *Nat Biotechnol* 31(3):233-239.

337. Zhao T, Wang W, Bai X, & Qi Y (2009) Gene silencing by artificial microRNAs in *Chlamydomonas*. *Plant J* 58(1):157-164.

338. Si T, HamediRad M, & Zhao H (2015) Regulatory RNA-assisted genome engineering in microorganisms. *Curr Opin Biotechnol* 36:85-90.

339. Meng J, *et al.* (2012) A genome-wide inducible phenotypic screen identifies antisense RNA constructs silencing Escherichia coli essential genes. *FEMS microbiology letters* 329(1):45-53.

340. Xiao H & Zhao H (2014) Genome-wide RNAi screen reveals the E3 SUMO-protein ligase gene SIZ1 as a novel determinant of furfural tolerance in *Saccharomyces cerevisiae*. *Biotechnol Biofuels* 7(1):1-11.

341. Bao Z, *et al.* (2015) Homology-integrated CRISPR–Cas (HI-CRISPR) system for one-step multigene disruption in *Saccharomyces cerevisiae*. *ACS Synth Biol* 4(5):585-594.

342. De Backer MD, *et al.* (2001) An antisense-based functional genomics approach for identification of genes critical for growth of *Candida albicans*. *Nat Biotech* 19(3):235-241.

343. Na D, *et al.* (2013) Metabolic engineering of Escherichia coli using synthetic small regulatory RNAs. *Nat Biotech* 31(2):170-174.

344. Ohnuma M, *et al.* (2009) Transient gene suppression in a red alga, *Cyanidioschyzon merolae* 10D. *Protoplasma* 236(1-4):107-112.

345. Molnar A, *et al.* (2009) Highly specific gene silencing by artificial microRNAs in the unicellular alga *Chlamydomonas reinhardtii*. *Plant J* 58:165-174.

346. Nakai K & Kanehisa M (1991) Expert system for predicting protein localization sites in gram-negative bacteria. *Proteins: Struct, Funct, and Bioinf* 11(2):95-110.

347. Bendtsen JD, Nielsen H, von Heijne G, & Brunak S (2004) Improved prediction of signal peptides: SignalP 3.0. *J Mol Biol* 340(4):783-795.

348. Bendtsen JD, Kiemer L, Fausbøll A, & Brunak S (2005) Non-classical protein secretion in bacteria. *BMC Microbiol* 5(1):1-13.

349. Bendtsen JD, Jensen LJ, Blom N, von Heijne G, & Brunak S (2004) Feature based prediction of non-classical protein secretion. *Protein Eng Des Sel* 17.

350. Gaj T, Gersbach CA, & Barbas CF, 3rd (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol* 31(7):397-405.

351. Kumar V & Jain M (2015) The CRISPR–Cas system for plant genome editing: advances and opportunities. *J Exp Bot* 66(1):47-57.

352. Bortesi L & Fischer R (2015) The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnol Adv* 33(1):41-52.

353. Sprink T, Metje J, & Hartung F (2015) Plant genome editing by novel tools: TALEN and other sequence specific nucleases. *Curr Opin Biotechnol* 32(Supplement C):47-53.

354. Andersen L, Sundman L-O, Inge-Britt Lindèn I-B, Kontro P, & Simo SO (1984) Synthesis and anticonvulsant properties of some 2-Aminoethanesulfonic acid (Taurine) derivatives. *J Pharm Sci* 73:106-108.

355. Herdeis C & Weis CE (1999) US Patent No. 5889183.

356. Tserng K-Y, Hachey DL, & Klein PD (1977) An improved procedure for the synthesis of glycine and taurine conjugates of bile acids. *J Lipid Res* 18:404-407.

357. Fong DW & Hoots JE (1992) US Patent No. 5,128,419.

358. Seeberger S, Griffin RJ, Hardcastle IR, & Golding BT (2007) A new strategy for the synthesis of taurine derivatives using the 'safety-catch' principle for the protection of sulfonic acids. *Org Biomol Chem* 5:132-138.

359. Suzuki M, Nakajima Y, Sato T, Shirai H, & Hanabusa K (2006) Fabrication of TiO2 using L-lysine-based organogelators as organic templates: control of the nanostructures. *Chem Commun* (4):377-379.

360. Mikhalenko SA, Soloveva LI, & Lukyanets EA (2004) Phthalocyanines and related compounds: XXXVIII. Synthesis of symmetric taurine- and choline-substituted phthalocyanines. *Russ J Gen Chem* 74:1775-1800.

361. Capone R, Blake S, Restrepo MR, Yang J, & Mayer M (2007) Designing nanosensors based on charged derivatives of *Gramicidin A*. *J Amer Chem Soc* 129:9737-9745.

362. Gupta RC, Win T, & Bittner S (2005) Taurine analogues; A new class of therapeutics: Retrospect and prospects *Curr Med Chem* 12:2021-2039.

363. Johnson BA (2008) Update on neuropharmacological treatments for alcoholism: Scientific basis and clinical findings. *Biochem Pharmacol* 75:34-56.

364. Tambour S & Quertemont E (2007) Preclinical and clinical pharmacology of alcohol dependence. *Fund Clin Pharmacol*21:9-28.

365. Joung YK, Sengoku Y, Ooya T, Park KD, & Yui N (2005) Anticoagulant supramolecular-structured polymers: Synthesis and anticoagulant activity of taurine-conjugated carboxyethylester-polyrotaxanes. *Sci Tech Adv Materials* 6:484-490.

366. Özmeriç N, *et al.* (2000) Chitosan film enriched with an antioxidant agent, taurine, in fenestration defects. *J Biomed Materials Res Part A* 51:500 - 503.

367. Degim Z, *et al.* (2002) An investigation on skin wound healing in mice with a taurinechitosan gel formulation. *Amino Acids* 22:187-198.

368. Matsusaki M, Serizawa T, Kishida A, Endo T, & Akashi M (2002) Novel functional biodegradable polymer: Synthesis and anticoagulant activity of poly(γ-Glutamic Acid)sulfonate (γ-PGA-sulfonate). *Bioconjugate Chem*13:23-28.

369. Roubos JA, van Straten G, & van Boxtel AJB (1999) An evolutionary strategy for fed-batch bioreactor optimization; concepts and performance. *J Biotechnol*67(2–3):173-187.

370. Oka T (1999) Amino acids, production processes. *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation*, eds Flickinger MC & Drew SW (Wiley, London).

371. Borowitzka MA (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. *J Biotechnol* 70(1–3):313-321.

372. Hermann T (2003) Industrial production of amino acids by coryneform bacteria. *J Biotechnol* 104(1-3):155-172.

373. Ikeda M (2003) Amino acid production processes. *Adv Biochem Eng Biotechnol* 79:1-35.

374. Ikeda M (2005) Towards bacterial strains overproducing l-tryptophan and other aromatics by metabolic engineering. *Appl Microbiol Biotechnol* 69(6):615-626.

375. Richmond A & Hu Q eds (2013) *Handbook of Microalgal Culture: Biotechnology and Applied Phycology* (Wiley-Blackwell, Hoboken, New Jersey), 2nd Ed.

376. Cardozo KH, et al. (2007) Metabolites from algae with economical impact. *Comp Biochem Physiol C Toxicol Pharmacol* 146(1-2):60-78.

377. Milledge JJ (2011) Commercial application of microalgae other than as biofuels: a brief review. *Reviews in Environ Sci Biotechnol*10:31-41.

378. Xu Q, Li S, Huang H, & Wen J (2012) Key technologies for the industrial production of fumaric acid by fermentation. *Biotechnol adv* 30(6):1685-1696.

379. Dufossé L, Fouillaud M, Caro Y, Mapari SAS, & Sutthiwong N (2014) Filamentous fungi are large-scale producers of pigments and colorants for the food industry. *Curr Opin Biotechnol* 26:56-61.

380. Höfler A, *et al.* (1998) US Patent No. 5,840,358

381. Lee I, Lee K, Namgoong K, & Lee Y-S (2002) The use of ion exclusion chromatography as approved to the normal ion exchange chromatography to achieve a more efficient lysine recovery from fermentation broth. *Enzyme Microb Technol* 30(6):798-803.

382. Binder M & Uffmann K-E (2002) US Patent No.6,465,025.

383. Leuchtenberger W, Huthmacher K, & Drauz K (2005) Biotechnological production of amino acids and derivatives: current status and prospects. *Appl Microbiol Biotechnol* 69(1):1-8.

384. Liu P, *et al.* (2012) Role of Glutamate Decarboxylase-like Protein 1 (GADL1) in Taurine Biosynthesis. *J Biol Chem* 287(49):40898-40906.

385. Winge I, *et al.* (2015) Mammalian CSAD and GADL1 have distinct biochemical properties and patterns of brain expression. *Neurochem Int* 90:173-184.

386. Meinkoth J & G. W (1984) Hybridization of nucleic acids immobilized on solid supports. *Anal Biochem*138:267-284.

387. Tijssen P (1993) Overview of principles of hybridization and the strategy of nucleic acid probe assays. *Laboratory Techniques in Biochemistry and Molecular Biology - Hybridization with Nucleic Acid Probes: Part I*, (Elsevier, New York).

388. Smith TF & Waterman MS (1981) Comparison of biosequences. *Adv Appl Math* 2:482–489.

389. Needleman SB & Wunsch CD (1970) A general method applicable to the search for similarities in the amino acid sequence of two proteins. *J Mol Biol* 48:443-453.

390. Pearson WR & Lipman DJ (1988) Improved tools for biological sequence comparison. *PNAS* 85:2444–2448.

391. Higgins DG, Bleasby AJ, & Fuchs R (1992) CLUSTAL V: improved software for multiple sequence alignment. *Comp Appl Biosci* 8(2):189-191.

392. Higgins DG & Sharp PM (1988) CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. *Gene* 73(1):237-244.

393. Higgins DG & Sharp PM (1989) Fast and sensitive multiple sequence alignments on a microcomputer. *Comp Appl Biosci* 5(2):151-153.

394. Feng DF & Doolittle RF (1987) Progressive sequence alignment as a prerequisite to correct phylogenetic trees. *J Mol Evol* 25(4):351-360.

395. Henikoff S & Henikoff J (1989) Amino acid substitution matrices from protein blocks *PNAS* 89:10915-10919.

396. Altschul SF, *et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389-3402.

397. Wootton JC & Federhen S (1993) Statistics of local complexity in amino acid sequences and sequence databases. *Comp Chem* 17:149-163.

398. Wootton JC & Federhen S (1996) Analysis of compositionally biased regions in sequence databases. *Methods Enzymol* 266:554-571.

399. Claverie J-M & States DJ (1993) Information enhancement methods for large scale sequence analysis. *Comp Chem* 17:191–201.

400. Myers EW & Miller W (1988) Optimal alignments in linear-space. *Comp App Biol Sci* 4:11-17.

401. Cadwell RC & Joyce GF (1994) Mutagenic PCR. *PCR Meth Appl* 3(6):S136-140.

402. Lin-Goerke JL, Robbins DJ, & Burczak JD (1997) PCR-based random mutagenesis using manganese and reduced dNTP concentration. *Biotechniques* 23(3):409-412.

403. Stemmer WP (1994) Rapid evolution of a protein in vitro by DNA shuffling. *Nature* 370(6488):389-391.

404. Buchholz J, *et al.* (2013) Platform engineering of Corynebacterium glutamicum with reduced pyruvate dehydrogenase complex activity for improved production of L-lysine, L-valine, and 2-ketoisovalerate. *Appl Environ Microbiol* 79(18):5566-5575.

405. Stefan A, Schwarz F, Bressanin D, & Hochkoeppler A (2010) Shine-Dalgarno sequence enhances the efficiency of lacZ repression by artificial anti-lac antisense RNAs in Escherichia coli. *J Biosci Bioeng* 110(5):523-528.

406. Studier FW (2014) Stable Expression Clones and Auto-Induction for Protein Production in *E. coli. Structural Genomics: Methods and Protocols, Methods in Molecular Biology*, ed Chen YW (Springer, New York).

CLAIMS

WHAT IS CLAIMED IS:

1. A cell comprising:

(a) a unit expressing a synthetic or semi-synthetic cysteine synthetase/PLP decarboxylase (sCS/PLP-DC), wherein

(i) exogenous DNA comprises a single expression cassette, wherein the single expression cassette comprises a promoter operably linked to a polynucleotide which encodes a cysteine synthetase (CS) fused in-frame with a decarboxylase (DC) or

(ii) exogenous DNA comprises a single expression cassette, wherein the single expression cassette comprises a promoter operably linked to a polynucleotide which encodes a cystathionine beta synthase (CBS) fused in-frame with a DC; or

(b) two units expressed as one polycistronic message, wherein

(i) an exogenous DNA comprises a single expression cassette, wherein the single expression cassette comprises a promoter operably linked to a polynucleotide which encodes a sCS/PLP-DC protein and

(ii) a second polynucleotide which encodes a taurine-binding protein,

wherein the expression units are expressed in the cell and wherein the cell produces taurine.

2. The cell of claim 1, wherein the sCS/PLP-DC polynucleotide encodes a polypeptide with the amino acid sequence SEQ ID NO:84.

3. The cell of claim 1, wherein the sCS/PLP-DC polynucleotide encodes a polypeptide with the amino acid sequence with 90% identity to SEQ ID NO:84.

4. The cell of claim 1, wherein the sCS/PLP-DC polynucleotide encodes a polypeptide with the amino acid sequence with 80% identity to SEQ ID NO:84.

5. The cell of claim 1 is a prokaryotic cell.

6. The prokaryotic of claim 5 wherein the function of the TauD, TauX, TauY, SsuD, SsuE, Tpa, cbl or TauR gene(s) is disrupted by a genetic knock out resulting in negligible expression of the corresponding gene products TDO, TDH, TDH, SsuD, SsuE, Tpa, cbl or TauR identified herein as TauD/KO, TauX/KO, TauY/KO, SsuD/KO, SsuE/KO, Tpa/KO, cbl/KO or TauR/KO.

7. A plant growth or yield enhancer comprising a concentrate, extract or secretion of the prokaryotic cell of claim 5.

8. A pharmaceutical composition comprising a concentrate, extract or secretion of the prokaryotic cell of claim 5.

9. A food or food/nutritional supplement comprising a concentrate, extract or secretion of the prokaryotic cell of claim 5.

10. An animal feed or feed supplement comprising a concentrate, extract or secretion of the prokaryotic cell of claim 5.

11. The bacterial cell of claim 5 wherein the bacteria is selected from the group consisting of Proteobacteria, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria, methanotrophs, *Methylobacterium,* Bacillus, *Salmonella, Lactococcus, Streptococcus, Brevibacterium*, coryneform bacteria, *Bacillus subtilis, Brevibacterium ammoniagene, Corynebacterium crenatum, Corynebacterim pekinese, Corynebacterium glutamicumas, Erwinia citreus, Erwinia herbicola, Escherichia coli, Fusarium venenatum, Gluconobacter oxydans, Propionibacterium freudenreicheii, and Propionibacterium denitrificans.*

12. A cell comprising:

(a) a unit expressing a sCS/PLP-DC, wherein

(i) exogenous DNA comprises a single expression cassette, wherein the single expression cassette comprises a promoter operably linked to a polynucleotide which encodes a cysteine synthetase (CS) fused in-frame with a decarboxylase (DC);

(ii) exogenous DNA comprises a single expression cassette, wherein the single expression cassette comprises a promoter operably linked to a polynucleotide which encodes a cystathionine beta synthase (CBS) fused in-frame with a DC; or

(iii) exogenous DNA comprises a single expression cassette, wherein the single expression cassette comprises a promoter operably linked to a polynucleotide which encodes a transit peptide which is fused in-frame with an sCS/PLP-DC.

wherein the expression units are expressed in the cell and wherein the cell produces taurine.

13. The cell of claim 12 is a eukaryotic cell.

14. A plant growth or yield enhancer comprising a concentrate, extract or secretion of the eukaryotic cell of claim 13.

15. A pharmaceutical composition comprising a concentrate, extract or secretion of the eukaryotic cell of claim 13.

16. A food or food/nutritional supplement comprising a concentrate, extract or secretion of the eukaryotic cell of claim 13.

17. An animal feed or feed supplement comprising a concentrate, extract or secretion of the eukaryotic cell of claim 13.

18. The eukaryotic cell of claim 13 wherein the eukaryotic is selected from the group consisting of algae, yeast, fungi, insects, or plants.

19. The plant cell of claim 18, wherein the plant cell is selected from the group consisting of acacia, alfalfa, aneth, apple, apricot, artichoke, arugula, asparagus, avocado, banana, barley, beans, beech, beet, Bermuda grass, blackberry, blueberry, Blue grass, broccoli, brussel sprouts, cabbage, camelina, canola, cantaloupe, carinata, carrot, cassava, cauliflower, celery, cherry, chicory, cilantro, citrus, clementines, coffee, corn, cotton, cucumber, duckweed, Douglas fir, eggplant, endive, escarole, eucalyptus, fennel, fescue, figs, forest trees, garlic, gourd, grape, grapefruit, honey dew, jatropha, jicama, kiwifruit, lettuce, leeks, lemon, lime, Loblolly pine, maize, mango, melon, mushroom, nectarine, nut, oat, okra, onion, orange, an ornamental plant, palm, papaya, parsley, pea, peach, peanut, pear, pepper, persimmon, pine, pineapple, plantain, plum, pomegranate, poplar, potato, pumpkin, quince, radiata pine, radicchio, radish, rapeseed, raspberry, rice, rye, rye grass, scallion, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugarbeet, sugarcane, sunflower, sweet potato, sweetgum, switchgrass, tangerine, tea, tobacco, tomato, turf, turnip, a vine, watermelon, wheat, yams, and zucchini.

FIGURE 1

Motif I = IGxTP(x14-19)KxE(x6)SxKDRI(x7)A(x7)(P/E)G(x6-7)TSGNTG(x3)A(x3)A(x2)G(x11)SxE(x6)AxG

Motif II = P(x6-7)Q(x5)N(x10)EI(x5)G(x9)(S/G)TGxTxxG(x9)(P/G)

Motif III = GxG(K/Y)(x12)D(x21)GxxxGxS(S/A)G(x4-5)A(x13-14)V(x9)YxS

Motif IV = P(x18-25)E(x18-19)PxFxx(Q/F)x(Y/S/F/P)(x9)G(x7)Nx(N/S/V)x(H/F/Y)(x5)P(x5)E(x17-24)G(x6)(S/A)(x9)AR(x7)(K/R)(x11-18)H(Y/S)(x3)K(x5)G

Motif V = (V/T)P(x5)TxGxTxxxAxD(x14)WxHxDxAxxG(x8)(R/N)(x4)GxxRxxSxxWNPHK(x5)Lx(C/Q)(x5)(K/R/H)(x4-5)L

Motif VI = YL(x10-11)DxxDxxIx(C/T)GR(x7)W(x6)G(x30-38)V(x35-38)A(x10)G(x16-18)R

(P/G) = P or G residue x = any amino acid residue (x14-19) = 14 to 19 residues of any amino acid

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/061337

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 41/08; A23K 10/10; A61K 31/145; C12N 9/02; C12N 15/00; C12N 15/82 (2018.01)

CPC - A23K 10/12; A23K 20/10; A61K 31/145; C12N 9/0069; C12N 9/1085; C12N 9/88; C12N 15/52; C12N 15/8257; C12P 11/00; C12P 13/001; C12Y 113/1102; C12Y 205/01047 (2018.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 426/635; 435/468; 562/30; 562/125 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/184175 A1 (PLANT SENSORY SYSTEMS, LLC) 26 October 2017 (26.10.2017) entire document	1, 5-11
X	WO 2017/176277 A1 (PLANT SENSORY SYSTEMS, LLC) 12 October 2017 (12.10.2017) entire document	12-19
A	AGNELLO et al. "Discovery of a Substrate Selectivity Motif in Amino Acid Decarboxylases Unveils a Taurine Biosynthesis Pathway in Prokaryotes," ACS Chemical Biology, 23 August 2013 (23.08.2013), Vol. 8, Pgs. 2264-2271. entire document	1-19
A	US 2014/0121405 A1 (QIANJIANG YONGAN PHARMACEUTICALS CO., LTD) 01 May 2014 (01.05.2014) entire document	1-19
A	WO 2017/083351 A1 (KNIPBIO, INC.) 18 May 2017 (18.05.2017) entire document	1-19
A	TEVATIA et al. "The Taurine Biosynthetic Pathway of Microalgae," Algal Research, 27 February 2015 (27.02.2015), Vol. 9, Pgs. 21-26. entire document	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 January 2018	14 FEB 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/061337

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NO:84 was searched.