

METHODS FOR OVERPRODUCING PROTOPORPHYRIN IX IN ALGAE AND COMPOSITIONS THEREFROM

CROSS-REFERENCE TO RELATED APPLICATION(S)

[0001] This application claims the benefit of priority under 35 U.S.C. § 119(e) of US Provisional Application No. 62/865,800, filed June 24, 2019, of US Provisional Application No. 62/850,227, filed May 20, 2019, and of US Provisional Application No. 62/757,534, filed November 8, 2018, the entire content of each of which is hereby incorporated by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 7, 2019, is named 20498-202380_SL.txt and is 208 kilobytes in size.

BACKGROUND

[0003] With the advent of industrialized animal agriculture, the consumption of animal meat has continued to rise. Accounting for more than 18% of the greenhouse gases generated to date, animal agriculture is one of the leading causes of climate change. In addition to land use, animal agriculture also requires a significant amount of fresh water, a finite resource that is becoming increasingly difficult to access. It is estimated that to produce one pound of beef it takes 1,799 gallons of fresh water and to produce one pound of pork it takes 576 gallons of water. This is compared to 216 gallons of fresh water to produce 1 pound of soybean or 108 gallons to produce one pound of corn. The intensity of fresh water needed to produce animal meat is a result of the water needed to grow the plants that the animals consume and the animal's inefficiency at turning the food that it consumes into actual meat.

[0004] To address the sustainability and ethical concerns over animal meat consumption, the food industry has been aggressively trying to develop plant-based alternatives that taste, touch and smell like meat products. However, many of the current plant-based alternatives have not been able to penetrate the larger food and consumer markets. Typically these alternatives are composed of plant based materials that are extruded to generate a firm texture to improve mouth feel and subsequently mixed with various flavors and aroma forming compounds to improve the taste and smell of these products. Unfortunately, these alternatives

have largely appealed to consumers who have already committed to a vegan/vegetarian lifestyle as opposed to consumers who are more accustomed to eating meat. To improve the sustainability of the food ecosystem it is imperative that products are developed that appeal to consumers who currently prefer meat. By producing the next generation of plant-based products the contribution of greenhouse gases and the demand for water resulting from the animal agriculture industry can be drastically reduced.

[0005] Recent advances have demonstrated the potential of using heme-containing proteins, purified from a host organism, to make the flavor and aroma profile of a product closer to that of meat. It is thought that the heme from heme-containing proteins are responsible for imparting a “meaty” flavor and aroma to meat products. However, the available sources of heme-containing proteins are expensive and technically intensive limiting their utility. For example, the heme binding protein, leghemoglobin, has been extracted from soy roots, but this process is proving to be expensive making its incorporation into meat alternatives less economically viable. The yeast, *Pichia pastoris* has been engineered to express heme binding proteins, for instance with an additional 8 enzyme pathways for producing the heme molecule. This process still requires the heme binding protein to be purified away from the expression host before it is incorporated into a finished product, a process that limits the potential positive impacts due to economic constraints. In addition to poor economics, the product is genetically modified making it less appealing to many consumers who have chosen to consume foods that are not a result of genetic engineering. Thus, a need exists for edible products incorporating heme-containing proteins as set forth herein.

SUMMARY OF THE INVENTION

[0006] Provided herein are compositions and processes for producing such compositions that provide new sources for flavor, color, mouth feel, taste, odor, texture and nutrition for food products and food ingredients, as well as other uses such as animal feed. Provided herein are compositions and processes for producing such compositions from an algae that overproduces protoporphyrin IX. Accordingly, in an exemplary aspect, the present invention provides a composition comprising a preparation from an algae strain, wherein the strain overexpresses or accumulates protoporphyrin IX (PPIX). In some embodiments, the preparation is a biomass from the algae strain. In some embodiments, the preparation is a fractionated biomass from the algae strain. In such embodiments, it is contemplated that the

fractionated biomass comprises a PPIX-enriched fraction. Further, in such embodiments, it is also contemplated that the PPIX-enriched fraction further comprises a protein-enriched fraction. In some embodiments, wherein the preparation is an extracellular fraction of the algae culture.

[0007] In some embodiments, the preparation is red or red-like in color. Alternatively and/or additionally, the preparation contains a greater amount of PPIX than of heme. Alternatively and/or additionally, the preparation contains less than about 1%, about 0.5%, about 0.1%, about 0.05%, about 0.01%, about 0.005% or about 0.001% heme. Alternatively and/or additionally, the preparation contains less than about 1%, about 0.5%, about 0.1%, about 0.05%, about 0.01%, about 0.005% or about 0.001% heme-protein.

[0008] In some embodiments, the preparation does not contain a detectable amount of a heme-protein. Alternatively and/or additionally, the preparation does not contain a detectable amount of heme. Alternatively and/or additionally, the preparation does not contain a detectable amount of protein. In some embodiments, the preparation has a protoporphyrin IX content greater than chlorophyll content.

[0009] In some embodiments, the preparation provides at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% of the total protein content to the edible composition. Alternatively and/or additionally, the preparation provides vitamin A, beta carotene or a combination thereof to the composition. In such embodiments, it is preferred that the vitamin A, the beta carotene or the combination thereof is at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% of the daily recommended requirement. In some embodiments, the preparation provides less than about 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.2%, 1.5%, 2%, 5% or 10% of total saturated fat present in the composition. Alternatively and/or additionally, the preparation provides at least about 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 125 mg, 150 mg, 175 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg or 500 mg of omega-3 fatty acids to the composition.

[0010] In some embodiments, the composition has a red or red-like color derived from the preparation. Alternatively and/or additionally, the composition has a meat or meat-like flavor

derived from the preparation. Alternatively and/or additionally, the composition has a meat or meat-like texture derived from the preparation.

[0011] In some embodiments, the algae is a *Chlamydomonas* sp. Optionally, the algae is *Chlamydomonas reinhardtii*. In some embodiments, the *Chlamydomonas* sp. is strain CC-125 deposited to the University of Minnesota *Chlamydomonas* collection center or a derivative thereof. In some embodiments, the algae has reduced or absence of ferrochelatase activity or expression of ferrochelatase.

[0012] Another aspect of the disclosure includes a food product comprising the composition as described herein. In some embodiments, the food product comprises a clean meat, a cultured meat, a synthetic meat, a plant-based meat or a non-animal cell-based meat. Alternatively and/or additionally, the food product is selected from the group consisting of a beef-like food product, a fish-like product, a chicken-like product, a pork-like product and a meat replica. Alternatively and/or additionally, the food product is vegan, vegetarian or gluten-free.

[0013] Another aspect of the disclosure includes an edible ingredient comprising the composition as described herein. In some embodiments, the ingredient is part of a finished product, wherein the finished product has a red or red-like color derived from the ingredient. Alternatively and/or additionally, the ingredient is part of a finished product, wherein the finished product has a meat or meat-like flavor derived from the ingredient. Alternatively and/or additionally, the ingredient is part of a finished product, wherein the finished product has an appearance of blood derived from the ingredient. In some embodiments, the finished product is an ingredient for a burger, a fish-alternative, a sausage, a kebab, a filet, a ground meat-like product or a meatball. In some embodiments, the edible composition is part of a finished product and wherein the finished product is an animal feed.

[0014] In some embodiments, the edible ingredient is combined with a protein source, a fat source, a carbohydrate, a starch, a thickener, a vitamin, a mineral, or any combination thereof. In some embodiments, the protein source is textured wheat protein, textured soy protein, fungal protein or algal protein. In such embodiments, it is contemplated that the finished product is free of animal proteins. In some embodiments, the fat source comprises at least one of refined coconut oil or sunflower oil. In some embodiments, the edible composition further comprises at least one of potato starch, methylcellulose, water, and a

flavor, wherein the flavor is selected at least one of yeast extract, garlic powder, onion powder, and salt.

[0015] Another aspect of the disclosure includes a meat substitute comprising the composition or the edible ingredient as described herein. In some embodiments, the meat substitute further comprises (a) 0.01%-5% (by weight of the meat replica matrix) of a non-animal protoporphyrin IX, (b) a compound selected from glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, and galactose, and any combination thereof, (c) at least 1.5 mM of a compound selected from cysteine, cystine, thiamine, methionine, and any combination thereof, and (d) one or more proteins selected from the group consisting of plant proteins, fungal proteins and algal proteins. Preferably, the meat substitute is a ground beef-like food product that contains no animal product; wherein cooking the ground beef-like food product results in the production of at least two volatile compounds which have a beef-associated aroma.

[0016] Another aspect of the disclosure includes a method of producing a protoporphyrin IX composition. The method includes steps of growing an algae population comprising an algae that is a protoporphyrin IX over-producer; and isolating a protoporphyrin IX composition from the culture. In some embodiments, the step of growing comprises culturing the algae culture in an aerobic fermentation condition. In some embodiments, the algae contains a chloroplast. In such embodiment, biosynthesis of the protoporphyrin IX occurs in the chloroplast.

[0017] In some embodiments, the algae is deficient for its ability to produce chlorophyll. Alternatively and/or additionally, the algae is deficient for the ability to produce a functional Mg-chelatase enzyme. Alternatively and/or additionally, the algae is reduced in or lacks ChlD1, ChlD2 or ChlDH. Alternatively and/or additionally, the algae is reduced in or lacks a functional light dependent protochlorophyllide. Alternatively and/or additionally, the algae is reduced in or lacks a functional light independent protochlorophyllide. Alternatively and/or additionally, the algae is reduced in or lacks ChlB, ChlL, or ChlN. Alternatively and/or additionally, the algae overexpresses one or more of glutamyl-tRNA reductase, glutamate-1-semialdehyde aminotransferase, ALA dehydratase, porphobilinogen deaminase, UPG III synthase, UPG III decarboxylase, CPG oxidase, and PPG oxidase.

[0018] In some embodiments, the algae is generated by mating and wherein the generated strain is red or red-like in color. Alternatively and/or additionally, the algae is generated by mutagenesis. In some embodiments, the algae is red or red-like in color. In some embodiments, the isolated protoporphyrin IX composition is an algae biomass. In such embodiment, it is contemplated that the algae biomass is fractionated. Alternatively and/or additionally, the algae biomass is fractionated to produce a protein-enriched fraction comprising protoporphyrin IX.

[0019] In some embodiments, the isolated protoporphyrin IX composition is isolated from extracellular media of the algae culture. Alternatively and/or additionally, the isolated protoporphyrin IX composition is isolated away from algae protein. In some embodiments, the algae is deficient for carotenoids. In some embodiments, the algae is a *Chlamydomonas* sp. In some embodiments, the algae is a *Chlamydomonas reinhardtii*. In some embodiments, the *Chlamydomonas* sp. is strain CC-125 deposited to the University of Minnesota *Chlamydomonas* collection center or a derivative thereof.

[0020] In some embodiments, progeny of a protoporphyrin IX overexpressing algae strain grows faster than its parental strain following a mating with another algae. Alternatively and/or additionally, the protoporphyrin IX algae is a strain produced by mating a strain deficient for carotenoids with a strain exhibiting a red or red-like color. Alternatively and/or additionally, the protoporphyrin IX overexpressing algae is generated by mutagenesis of a first starting strain and selection of a second strain that grows faster in the dark than the first starting strain. Alternatively and/or additionally, the protoporphyrin IX algae is generated by mutagenesis of a first strain and selection of a second strain that lacks one or more carotenoids from the mutated first strain.

[0021] In some embodiments, the algae lacks a functional ferrochelatase enzyme. Alternatively and/or additionally, the algae is reduced in the amount of, or activity of, a ferrochelatase enzyme. Alternatively and/or additionally, the algae is reduced in the amount of or lacks heme as compared to a wildtype strain.

[0022] In some embodiments, the method further comprises steps of a) culturing the algae strain under a dark condition, wherein the strain does not produce or is reduced in the production of chlorophyll, and (b) collecting a portion of the algae culture that is red or red-like in color to produce the protoporphyrin IX composition. Preferably, the algae is a

Chlamydomonas sp. In some embodiments, the algae is a *Chlamydomonas reinhardtii*. In some embodiments, the algae exhibits a red or red-like color when grown in the dark condition.

[0023] In some embodiments, the collected portion is extracellular media from the algae culture. Alternatively and/or additionally, the collected portion is a biomass or fractionated biomass from the algae culture. In some embodiments, the algae is grown in an aerobic fermentation condition. Alternatively and/or additionally, the algae is grown to a density greater than about 10g/L, 20 g/L, 30 g/L, 40g/L, 50g, L, 75 g/L, 100g/L, 125 g/L, or 150g/L. Alternatively and/or additionally, the algae is grown with acetate as a reduced carbon source. Alternatively and/or additionally, wherein the algae is grown with sugar as a reduced carbon source. Alternatively and/or additionally, the algae culture is supplemented with iron during the culturing step. In some embodiments, the algae culture is inoculated at a density greater than about 0.1 g/L, 1.0 g/L, 5.0 g/L, 10 g/L, 20 g/L, 50 g/L, 80 g/L, or 100 g/L.

[0024] In some embodiments, the method further comprises fractionating the collected portion, wherein the fractionating removes from the collected portion substantially all or most of a component selected from the group consisting of carotenoids, starch, and protein. Alternatively and/or additionally, the method further comprises fractionating the collected portion, wherein the fractionating removes from the collected portion substantially all or most of heme, heme-binding protein or a combination thereof. Alternatively and/or additionally, the method further comprises fractionating the collected portion, wherein the fractionating produces a protein-enriched fraction.

[0025] In some embodiments, the algae lacks or is reduced in one or more of magnesium chelatase, magnesium protoporphyrinogen IX, protochlorophyllide, chlorophyllide, and chlorophyll. Alternatively and/or additionally, the algae lacks or is reduced in ferrochelatase. In some embodiments, the algae is not a transgenic strain.

[0026] Another aspect of the disclosure includes a clean meat product produced by the method described herein, and the process further comprises combining the collected portion with a clean meat manufacturing composition, wherein the collected portion provides a red or red-like color to the clean meat product. In some embodiments, the collected portion is a PPIX-enriched fraction or purified PPIX.

[0027] Another aspect of the disclosure includes an edible ingredient produced by the method described herein, and the protoporphyrin IX composition confers a meat or meat-like flavor, texture, odor or any combination thereof to the edible ingredient. In some embodiments, the edible ingredient is incorporated in a finished product selected from the group consisting of a beef-like food product, a fish-like product, a chicken-like product, a pork-like product and a meat replica. Alternatively and/or additionally, the edible ingredient is vegan, vegetarian or gluten-free. Alternatively and/or additionally, the edible ingredient is free of animal proteins. Alternatively and/or additionally, the edible ingredient does not contain any genetically modified components.

[0028] Another aspect of the disclosure includes a protoporphyrin IX-containing composition produced by the method as described herein. In some embodiments, the composition does not contain a detectable level of heme, heme-binding protein or a combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] **FIG. 1** is a pictorial diagram showing an exemplary pathway for the production of heme in algae. This exemplary pathway can be used by wildtype algae to produce chlorophyll, but it can also be used to generate protoporphyrin IX (PPIX).

[0030] **FIG. 2** is a pictorial diagram showing an exemplary fractionation of algae overexpressing PPIX, showing the rich red color of the extract.

[0031] **FIG. 3** is a pictorial diagram showing burgers created with 0.01g, 0.1 g, 1.0g, and 5.0g of the PPIX enriched algae.

[0032] **FIG. 4** is a pictorial diagram showing ingredient mixes of the plant-based burger ingredients with no heme-enriched algae, with the addition of PPIX-enriched algae, or the ingredients with the addition of heme-enriched algae shaped into a burger before and after cooking.

[0033] **FIG. 5** is a pictorial diagram showing an example of PPIX-enriched meatless “tuna”.

[0034] **FIG. 6** shows a portion of sequence alignments of a wild type green algae and a red-algae with a mutation in CHLH gene (upper sequence (Seq_1) is a partial nucleic acid

sequence (residues 1621-1679 of SEQ ID NO: 27) and a partial amino acid sequence (residues 451-460 of SEQ ID NO: 28) of CHLH gene of green algae, and lower sequence (Seq_2) is a partial nucleic acid sequence (residues 1621-1680 of SEQ ID NO: 129) and partial amino acid sequence (residues 451-460 of SEQ ID NO: 152) of CHLH gene of red algae has a mutation (asterisk)). As shown, the wild-type CHLH nucleic acid sequence (SEQ ID NO: 27) has an insertion of a thiamine at position 1678 resulting in a change of the wild-type CHLH amino acid sequence of SEQ ID NO: 28 of a proline to a serine at amino acid position 560.

DETAILED DESCRIPTION

[0035] Before the present compositions and methods are described, it is to be understood that this invention is not limited to particular compositions, methods, and experimental conditions described, as such compositions, methods, and conditions may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only in the appended claims. As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. Thus, for example, references to “the method” includes one or more methods, and/or steps of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure and so forth. Furthermore, to the extent that the terms “including”, “includes”, “having”, “has”, “with”, or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term “comprising.”

[0036] The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *e.g.*, the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation, per the practice in the given value. Where particular values are described in the application and claims, unless otherwise stated the term “about” should be assumed to mean an acceptable error range for the particular value.

[0037] As used herein, “a deficiency in” or the “lack of”, or “reduction of”, one or more genes and/or enzymes include, for example, mutation or deletion of the gene sequence, a

reduction in or lack in the expression from a gene (RNA and/or protein) and/or a lack of accumulation or stability of a gene product (RNA and/or protein).

[0038] As used herein, “overexpresses” and “overexpression” of an enzyme or gene include, for example, an increase in expression from a gene (RNA and/or protein) and/or an increase in accumulation or stability of a gene product (RNA and/or protein). Such overexpression can include alterations to the regulatory region(s) and/or to the gene sequence, as well as copy number, genomic position and post-translational modifications.

[0039] As used herein, the term “engineered algae” is used to refer to an algae that contains one or more genetic modifications. In some cases, an engineered algae is also a recombinantly modified organism when it incorporates heterologous nucleic acid into its genome through recombinant technology. In other cases, an engineered algae is not a recombinantly modified organism (for example when it is modified through UV, chemical or radiation mutagenesis). In some cases an algae that is not a recombinantly modified organism is referred to as non-GMO, and components from such algae can be referred to as non-GMO components.

[0040] As used herein, the term “genetic modification” is used to refer to any manipulation of an organism’s genetic material in a way that does not occur under natural conditions. A genetic modification can include modifications that are made through mutagenesis (such as with UV light, X-rays, gamma irradiation and chemical exposure). A genetic modification can include gene editing. In some cases, genetic modifications can be made through recombinant technology. As used herein, “recombinantly modified organism” is used to refer to an organism that incorporates heterologous nucleic acid (e.g., recombinant nucleic acid) into its genome through recombinant technology. Methods of performing such manipulations are known to those of ordinary skill in the art and include, but are not limited to, techniques that make use of vectors for transforming cells with a nucleic acid sequence of interest. Included in the definition are various forms of gene editing in which DNA is inserted, deleted or replaced in the genome of a living organism using engineered nucleases, or “molecular scissors.” These nucleases create site-specific double-strand breaks (DSBs) at desired locations in the genome. The induced double-strand breaks are repaired through nonhomologous end-joining (NHEJ) or homologous recombination (HR), resulting in targeted mutations (*i.e.*, edits).

[0041] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described.

[0042] Provided herein are methods to select, grow and to incorporate algae overexpressing the molecule, protoporphyrin IX (PPIX), into food and animal feed ingredients and products. Such products can include non-genetically modified and plant-based alternative foods. Algae are known for producing many compounds that result in these aquatic organisms being various colors. These compounds include, but are not limited to, chlorophyll which makes algae green, beta-carotene which makes algae appear yellow or orange, astaxanthin which makes algae appear red or other various pigments such as phycocyanin which make algae blue. While each of these previously mentioned compounds has been added to food products, there are to date no products that incorporate an algae over-producing PPIX to impart a red color and/or a meaty taste and smell.

[0043] Provided herein are strains, methods and compositions that employ algae overproducing PPIX. In some embodiments, the algae strain when grown is red or red-like in color. As used herein, in some embodiments, red-like color can be any color with a wavelength between 590 nm to 750 nm or any mixture of the color. Alternatively and/or additionally, in some embodiments, red-like color can be defined as any color in RGB (r.g.b) having r value between 255 and 80 with g or b values between 0 and 80. In some embodiments, a preparation made from the algae culture overproducing PPIX, imparts a pink or red color when incorporated into food and other edible products. In some embodiments, a preparation made from the algae culture overproducing PPIX, imparts a “meaty” flavor, smell and/or texture when incorporated into food and other edible products.

[0044] Without being bound by theory, the heme pathway is a biochemical pathway that branches from the chlorophyll biochemical pathway, as shown in FIG. 1. In short, this pathway starts with a glutamate tRNA which is converted to 5-aminolaevulinic acid (ALA) by a GlutRNA reductase and a GSA amino transferase. Next, ALA is converted to porphobilinogen by ALA dehydratase. Next, porphobilinogen is converted to hydroxymethylbilane by porphobilinogen deaminase. Next, hydroxymethylbilane is converted to uroporphyrinogen III by UPG III synthase. Next, uroporphyrinogen III is converted to

coprophyrinogen by UPG III decarboxylase. Next, coprophyrinogen is converted to protoporphyrinogen IX by CPG oxidase. Next, protoporphyrinogen IX is converted to protoporphyrin IX by PPG oxidase. Protoporphyrin IX can be shuttled to the chlorophyll production pathway or towards heme B. Finally, protoporphyrin IX is converted to heme B by the enzyme ferrochelatase which attaches iron to protoporphyrin IX.

[0045] By reducing metabolic flux towards chlorophyll, it is possible to increase metabolic flux towards PPIX, as well as heme B. By reducing or eliminating ferrochelatase, the pathway produces PPIX but the conversion from PPIX to heme is reduced or eliminated.

[0046] In some embodiments herein, the algae strains used in the methods and compositions produced therewith are reduced in metabolic flux towards chlorophyll and increased in metabolic flux towards heme B. In some embodiments herein, an engineered algae strain contains a genetic modification in ferrochelatase, such as in one or more of the nucleotide sequence (e.g., SEQ ID NO: 7), and/or amino acid sequence (e.g., SEQ ID NO: 8), and includes genetic modifications in one or more of the regulatory regions, such as those of SEQ ID NOs: 114, 115, exons, such as those of SEQ ID NOs: 116-122, and introns, such as those of SEQ ID NOs: 123-128.

[0047] In some embodiments, the algae strain is one where chlorophyll and carotenoid synthesis is decreased. In some embodiments, the algae strain is deficient or reduced in the amount of chlorophyll. In some embodiments, the algae strain is deficient or reduced in the function, amount of, or activity of, ferrochelatase by at least 10%, by at least 20%, by at least 30%, by at least 40%, by at least 50%, compared to the wild type algae. In some embodiments, the algae strain is deficient or reduced in the amount of heme B that accumulates, and the strain is increased in accumulation of PPIX. In some embodiments, the algae strain is red or red-like in color.

[0048] In some embodiments, the algae strain is deficient for one or more enzymes in the chlorophyll biosynthesis pathway. Such deficiencies include, but are not limited to, gene deletions, mutations and other alterations that result in a lack expression of the enzyme or a deficiency in the functionality of the enzyme. In some embodiments, the algae strain is deficient in magnesium chelatase which is the first step in converting protoporphyrin IX to chlorophyll. In some embodiments, the algae strain is deficient for light dependent protochlorophyllide which converts protochlorophyllide to chlorophyllide. In some

embodiments, the algae strain is deficient for a light independent protochlorophyllide reductase which converts protochlorophyllide to chlorophyllide in the dark. In some embodiments, the algae strain is deficient for one or more of ChlB, ChlL, or ChlN. In some embodiments, the algae strain is lacking or reduced in one or more of magnesium chelatase, magnesium protoporphyrin IX, protochlorophyllide, chlorophyllide, and chlorophyll.

[0049] In some embodiments, the algae strain is deficient for one or more of the magnesium chelatase subunits CHLD, CHLH and CHLI. These subunits are also referred to by the gene names, CHLD1 (alternatively written as CHID1), corresponding to the CHLD subunit, CHLH1 (alternatively written as CHIH1), corresponding to the CHLH subunit, and CHLI1 and CHLI2, corresponding to the CHLI subunit, encoded by two genes, CHLI1 and CHLI2 (alternatively written as CHI1 and CHI2).

[0050] In some embodiments, the algae strain is deficient for one or more of CHLD1, CHLH1, CHLI1, CHLI2 or portions thereof (including genetic modification in one or more of intron, exon, regulatory regions, or full gene sequences, such as a genetic modification to one or more of SEQ ID NOs: 45-69, 70-88, 89-113, or 130-150. For example, one of the red algae strain has genetic modification in CHLH locus. The modification deletes a single base pair in CHLH as compared to a green strain, causing a frameshift in the CHLH open reading frame and/or generate a stop codon such that the protein is translated into a truncated form. The sequence comparison is shown in FIG. 6 (upper sequence (Seq_1) is a partial nucleic acid sequence (residues 1621-1679 of SEQ ID NO: 27) and a partial amino acid sequence (residues 451-460 of SEQ ID NO: 28) of CHLH gene of green algae, and lower sequence (Seq_2) is a partial nucleic acid sequence (residues 1621-1680 of SEQ ID NO: 129) and partial amino acid sequence (residues 451-460 of SEQ ID NO: 152) of CHLH gene of red algae has a mutation (asterisk)). The nucleic acid sequences of additional genes that may be altered in such algae strains are provided herein.

[0051] In some embodiments, an engineered algae strain for use with the methods and compositions herein includes a genetic modification in a ferrochelatase gene that decreases or is deficient in production of ferrochelatase and also has a modification in one or more enzymes leading from PPIX to chlorophyll (such as CHLD, CHLI1, CHLI2 and/or CHLH).

[0052] In some embodiments, the algae strain overexpresses one or more enzymes such that the balance of pathways favors PPIX production. In some embodiments, the algae strain

overexpresses one or more of glutamyl-tRNA reductase, glutamyl-l-semialdehyde aminotransferase, ALA dehydratase, porphobilinogen deaminase, UPG III synthase, UPG III decarboxylase, CPG oxidase, and PPG oxidase. In some embodiments, the algae strain overexpresses one or more of such enzymes and is also reduced in the amount or activity of the ferrochelatase enzyme. In some embodiments, the algae strain is improved for its ability to produce ALA, a rate limiting precursor of heme B synthesis, and optionally, is reduced in the amount or activity of the ferrochelatase enzyme. In some embodiments, the algae strain is deficient for its ability to produce a functional ferrochelatase gene, the enzyme responsible for the conversion of protoporphyrin IX to heme B. In some embodiments, the algae strain is improved for its ability to produce UPG III synthase, UPG III decarboxylase, CPG oxidase, or PPG oxidase. In some embodiments, the algae strain has an increased amount of protoporphyrin IX as compared to a wildtype strain.

[0053] In some embodiments, the algae strain produces carotenoids or precursors of carotenoids. Without being bound by theory, carotenoids confer color and can have an impact on the visual appearance of a plant-based alternative. Exemplary carotenoids include, but are not limited to, gamma-carotene, beta-carotene, beta cryptoxanthin, zeaxanthin, atheraxanthin, lutein, polycopene and lycopene.

[0054] In some embodiments, the algae strain is deficient for carotenoids or precursors of carotenoids. Deficiencies in carotenoid biosynthesis can occur due to mutations, such as mutations that impact carotenoid biosynthesis, for example, mutations in the phytoene synthase gene.

[0055] In some embodiments, the algae strain for use in the methods herein and for making PPIX-containing compositions is selected or identified based on one or more phenotypes and/or genotypes. In some embodiments, the algae strain for overproducing PPIX can be created through mating processes. In some embodiments, the algae strain for overproducing PPIX can be created through random mutagenesis, such as ultra violet mutagenesis. In some embodiments, the algae strain for overproducing PPIX can be generated through chemical mutagenesis with a compound that results in DNA alterations.

[0056] In some embodiments, modifications can be created through gene editing such as precisely engineered nuclease targeting to alter the expression of one or more components, such as by CRISPR-CAS nucleases. Such nucleases can be used to create insertions,

deletions, mutations and replacements of one or more nucleotides or regions of nucleotides to modify the expression of one or more pathway enzymes in the pathway to reduce chlorophyll and/or to increase the production or accumulation of PPIX. Subsequent to the creation of the modification, the algae strain is grown and/or mated such that the nuclease and associated guide nucleic acids are removed, and the algae strain that remains does not retain the nuclease and associated editing system. In some embodiments, a nuclease such as the CRISPR-CAS nuclease is used to make a modification to a component of the chlorophyll pathway such that chlorophyll expression and/or accumulation is reduced or abrogated. In some embodiments, a nuclease such as the CRISPR-CAS nuclease is used to make a modification to a component of the chlorophyll pathway such that PPIX expression and/or accumulation is increased. In some embodiments, a nuclease such as the CRISPR-CAS nuclease is used to make a genetic modification in the gene encoding for ferrochelatase, such as a modification in one or more of SEQ ID NOs: 114-128 that reduces expression or abrogates expression of the gene, or a modification that abrogates, truncates or causes a frame shift in the gene encoding the enzyme, such as in SEQ ID NOs: 116-122, and/or a modification that alters or truncates the protein expressed such as an alteration in amino acid SEQ ID NO: 8. In some embodiments, a nuclease such as the CRISPR-CAS nuclease is used to make a modification in one or more of CHLD, CHLI1, CHLI2 or CHLH1 resulting in a PPIX-enriched algae strain. Such modifications are made by designing guide RNAs with modifications to one or more of SEQ ID NOs: 45-113, 130-150 or 153 to include one or more point mutations, insertions, deletions or combinations thereof. In some embodiments, such genetic modifications in more than one target sequence, such as those in a ferrochelatase sequence and in a another chlorophyll-pathway sequence (e.g., CHLD, CHLI1, CHLI2 or CHLH1) are combined either by concurrent or sequential rounds of nuclease engineering and/or by mating engineered algae strains containing the genetic modifications.

[0057] There are several families of engineered nucleases used in gene editing, for example, but not limited to, meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALEN), the CRISPR-Cas system, and ARCUS. However, it should be understood that any known gene editing system utilizing engineered nucleases may be used in the methods described herein. Thus, in some embodiments, the algae strain overproducing PPIX can be genetically modified by using techniques such as a CRISPR-Cas system (e.g., CRISPR-CAS9) or by the use of zinc-finger nucleases.

[0058] CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is an acronym for DNA loci that contain multiple, short, direct repetitions of base sequences. The prokaryotic CRISPR/Cas system has been adapted for use as gene editing (silencing, enhancing or changing specific genes) for use in eukaryotes (see, for example, Cong, *Science*, 15:339(6121):819-823 (2013) and Jinek, et al., *Science*, 337(6096):816-21 (2012)). By transfecting a cell with elements including a Cas gene and specifically designed CRISPRs, nucleic acid sequences can be cut and modified at any desired location. Methods of preparing compositions for use in genome editing using the CRISPR/Cas systems are described in detail in US Pub. No. 2016/0340661, US Pub. No. 2016/0340662, US Pub. No. 2016/0354487, US Pub. No. 2016/0355796, US Pub. No. 2016/0355797, and WO 2014/018423, which are specifically incorporated by reference herein in their entireties.

[0059] Zinc-finger nucleases (ZFNs) are artificial restriction enzymes generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain. Zinc finger domains can be engineered to target specific desired DNA sequences and this enables zinc-finger nucleases to target unique sequences within complex genomes. By taking advantage of endogenous DNA repair machinery, these reagents can be used to precisely alter the genomes of higher organisms. The most common cleavage domain is the Type IIS enzyme FokI. FokI catalyzes double-stranded cleavage of DNA, at 9 nucleotides from its recognition site on one strand and 13 nucleotides from its recognition site on the other. See, for example, U.S. Pat. Nos. 5,356,802; 5,436,150 and 5,487,994; as well as Li *et al. Proc., Natl. Acad. Sci. USA* 89 (1992):4275-4279; Li *et al. Proc. Natl. Acad. Sci. USA*, 90:2764-2768 (1993); Kim *et al. Proc. Natl. Acad. Sci. USA*, 91:883-887 (1994a); Kim *et al. J. Biol. Chem.* 269:31,978-31,982 (1994b), all of which are incorporated herein by reference. One or more of these enzymes (or enzymatically functional fragments thereof) can be used as a source of cleavage domains.

[0060] Methods for selection of algae include, but are not limited to, genetic screening or phenotypic screening for deficiencies, mutations and changes in the chlorophyll biosynthesis pathway and/or chlorophyll accumulation, genetic screening or phenotypic screening for increased expression and/or accumulation of PPIX, PPIX biosynthesis intermediates and heme biosynthesis enzymes. In some embodiments, the algae strain for use in the methods herein and for making PPIX-containing compositions is selected or identified based on its spectral profile and/or its red or red-like color. In some embodiments, the algae for use in the methods herein and for making PPIX-containing compositions is selected or identified based

on its growth rate in dark conditions. In some embodiments, the selection is based on growth rate in dark conditions and the appearance or enhancement of a red or red-like color when grown in dark conditions. In some embodiments, an algae strain is selected which is deficient in or reduced in the amount of carotenoids produced or accumulated.

[0061] In some embodiments, algae strains are mated to combine or enhance characteristics that contribute to PPIX production, PPIX accumulation, reduction in chlorophyll and/or reduction in carotenoids. In some embodiments, an algae strain that has fast growth under dark conditions (e.g., faster than a wildtype strain) is mated with an algae strain that exhibits a red or red-like color. Thus, such algae strain is not a transgenic strain. In some embodiments, an algae strain deficient for carotenoid production or accumulation is mated with an algae strain exhibiting a red or red-like color. It is contemplated that such generated algae is a protoporphyrin IX overexpressing algae strain grows faster than its parental strain following a mating with another algae.

[0062] In some embodiments, an algae strain is mutagenized and then a new strain is selected or identified that exhibits one or more characteristics of increased PPIX production, PPIX accumulation, reduction in chlorophyll and/or reduction in carotenoids. In some embodiments, an algae strain is generated by mutagenesis of a first starting strain and selection of a second strain that grows faster in the dark than the first starting strain. In some embodiments, an algae strain is generated by mutagenesis of a first starting strain and selection of a second strain that lacks one or more carotenoids.

Algae for use in the Compositions and Methods

[0063] In the compositions and methods provided herein for producing PPIX and PPIX-containing compositions, algae strains that have a PPIX biosynthesis pathway are employed. In some embodiments, the algae strain for overproducing PPIX is a Chlorophyta (green algae). In some embodiments, the green algae is selected from the group consisting of *Chlamydomonas*, *Dunaliella*, *Haematococcus*, *Chlorella*, and *Scenedesmeceae*. In some embodiments, the *Chlamydomonas* is a *Chlamydomonas reinhardtii*. In varying embodiments, the green algae can be a *Chlorophyceae*, a *Chlamydomonas*, *C. reinhardtii*, *C. reinhardtii* 137c, or a *psbA* deficient *C. reinhardtii* strain. In some embodiments, the selected host is *Chlamydomonas reinhardtii*, such as in Rasala and Mayfield, *Bioeng Bugs*. (2011) 2(1):50-4; Rasala, *et al.*, *Plant Biotechnol J.* (2011) May 2, PMID 21535358; Coragliotti, *et*

al., *Mol Biotechnol.* (2011) 48(1):60-75; Specht, *et al.*, *Biotechnol Lett.* (2010) 32(10):1373-83; Rasala, *et al.*, *Plant Biotechnol J.* (2010) 8(6):719-33; Mulo, *et al.*, *Biochim Biophys Acta.* (2011) May 2, PMID:21565160; and Bonente, *et al.*, *Photosynth Res.* (2011) May 6, PMID:21547493; US Pub. No. 2012/0309939; US Pub. No. 2010/0129394; and Intl. Pub. No. WO 2012/170125. All of the foregoing references are incorporated herein by reference in their entirety for all purposes.

[0064] In some embodiments, the algae strain for overproducing PPIX is a single-celled algae. Illustrative and additional microalgae species of interest include without limitation, *Achnanthes orientalis*, *Agmenellum*, *Amphiprora hyaline*, *Amphora coffeiformis*, *Amphora coffeiformis lineata*, *Amphora coffeiformis punctata*, *Amphora coffeiformis taylori*, *Amphora coffeiformis tenuis*, *Amphora delicatissima*, *Amphora delicatissima capitata*, *Amphora sp.*, *Anabaena*, *Ankistrodesmus*, *Ankistrodesmus falcatus*, *Boekelovia hooglandii*, *Borodinella sp.*, *Botryococcus braunii*, *Botryococcus sudeticus*, *Carteria*, *Chaetoceros gracilis*, *Chaetoceros muelleri*, *Chaetoceros muelleri subsalsum*, *Chaetoceros sp.*, *Chlamydomonas sp.*, *Chlamydomonas reinhardtii*, *Chlorella anitrata*, *Chlorella Antarctica*, *Chlorella aureoviridis*, *Chlorella candida*, *Chlorella capsulate*, *Chlorella desiccata*, *Chlorella ellipsoidea*, *Chlorella emersonii*, *Chlorella fusca*, *Chlorella fusca var. vacuolata*, *Chlorella glucotropha*, *Chlorella infusionum*, *Chlorella infusionum var. actophila*, *Chlorella infusionum var. auxenophila*, *Chlorella kessleri*, *Chlorella lobophora* (strain SAG 37.88), *Chlorella luteoviridis*, *Chlorella luteoviridis var. aureoviridis*, *Chlorella luteoviridis var. lutescens*, *Chlorella miniata*, *Chlorella minutissima*, *Chlorella mutabilis*, *Chlorella nocturna*, *Chlorella parva*, *Chlorella photophila*, *Chlorella pringsheimii*, *Chlorella protothecoides*, *Chlorella protothecoides var. acidicola*, *Chlorella regularis*, *Chlorella regularis var. minima*, *Chlorella regularis var. umbricata*, *Chlorella reisigii*, *Chlorella saccharophila*, *Chlorella saccharophila var. ellipsoidea*, *Chlorella salina*, *Chlorella simplex*, *Chlorella sorokiniana*, *Chlorella sp.*, *Chlorella sphaerica*, *Chlorella stigmatophora*, *Chlorella vanniellii*, *Chlorella vulgaris*, *Chlorella vulgaris f. tertia*, *Chlorella vulgaris var. autotrophica*, *Chlorella vulgaris var. viridis*, *Chlorella vulgaris var. vulgaris f. tertia*, *Chlorella vulgaris var. vulgaris f. viridis*, *Chlorella xanthella*, *Chlorella zofingiensis*, *Chlorella trebouxii*, *Chlorella vulgaris*, *Chlorococcum infusionum*, *Chlorococcum sp.*, *Chlorogonium*, *Chroomonas sp.*, *Chrysosphaera sp.*, *Cricosphaera sp.*, *Cryptocodinium cohnii*, *Cryptomonas sp.*, *Cyclotella cryptica*, *Cyclotella meneghiniana*, *Cyclotella sp.*, *Dunaliella sp.*, *Dunaliella bardawil*, *Dunaliella bioculata*,

Dunaliella granulate, *Dunaliella maritime*, *Dunaliella minuta*, *Dunaliella parva*, *Dunaliella peircei*, *Dunaliella primolecta*, *Dunaliella salina*, *Dunaliella terricola*, *Dunaliella tertiolecta*, *Dunaliella viridis*, *Dunaliella tertiolecta*, *Eremosphaera viridis*, *Eremosphaera* sp., *Ellipsoidon* sp., *Euglena*, *Franceia* sp., *Fragilaria crotonensis*, *Fragilaria* sp., *Gleocapsa* sp., *Gloeothamnion* sp., *Hymenomonas* sp., *Isochrysis* aff. *galbana*, *Isochrysis galbana*, *Lepocinclis*, *Micractinium*, *Micractinium* (UTEX LB 2614), *Monoraphidium minutum*, *Monoraphidium* sp., *Nannochloris* sp., *Nannochloropsis salina*, *Nannochloropsis* sp., *Navicula acceptata*, *Navicula biskanterae*, *Navicula pseudotenelloides*, *Navicula pelliculosa*, *Navicula saprophila*, *Navicula* sp., *Nephrochloris* sp., *Nephroselmis* sp., *Nitzschia communis*, *Nitzschia alexandrina*, *Nitzschia communis*, *Nitzschia dissipata*, *Nitzschia frustulum*, *Nitzschia hantzschiana*, *Nitzschia inconspicua*, *Nitzschia intermedia*, *Nitzschia microcephala*, *Nitzschia pusilla*, *Nitzschia pusilla elliptica*, *Nitzschia pusilla monoensis*, *Nitzschia quadrangular*, *Nitzschia* sp., *Ochromonas* sp., *Oocystis parva*, *Oocystis pusilla*, *Oocystis* sp., *Oscillatoria limnetica*, *Oscillatoria* sp., *Oscillatoria subbrevis*, *Pascheria acidophila*, *Pavlova* sp., *Phagus*, *Phormidium*, *Platymonas* sp., *Pleurochrysis carterae*, *Pleurochrysis dentate*, *Pleurochrysis* sp., *Prototheca wickerhamii*, *Prototheca stagnora*, *Prototheca portoricensis*, *Prototheca moriformis*, *Prototheca zopfii*, *Pyramimonas* sp., *Pyrobotrys*, *Sarcinoid chrysophyte*, *Scenedesmus armatus*, *Schizochytrium*, *Spirogyra*, *Spirulina platensis*, *Stichococcus* sp., *Synechococcus* sp., *Tetraedron*, *Tetraselmis* sp., *Tetraselmis suecica*, *Thalassiosira weissflogii*, and *Viridiella fridericana*. In some embodiments, the algae is a *Chlamydomonas* species. In some embodiments, the algae is a *Chlamydomonas reinhardtii*. In some embodiments, the algae is a derivative of a green *Chlamydomonas* strain made by mutagenesis or by mating with another algae strain. In some embodiments, the *Chlamydomonas* sp. is strain CC-125 deposited to the University of Minnesota *Chlamydomonas* collection center or a derivative thereof.

Culture methods for overproducing PPIX strains

[0065] Methods for growing algae in liquid media include a wide variety of options including ponds, aqueducts, small scale laboratory systems, and closed and partially closed bioreactor systems. Algae can also be grown directly in water, for example, in an ocean, sea, lake, river, reservoir, etc.

[0066] In some embodiments, the PPIX overproducing algae useful in the methods and compositions provided herein are grown in a controlled culture system, such as a small scale

laboratory system, a large scale system, and/or a closed and partially closed bioreactor system. Small scale laboratory systems refer to cultures in volumes of less than about 6 liters, and can range from about 1 milliliter or less up to about 6 liters. Large scale cultures refer to growth of cultures in volumes of greater than about 6 liters, and can range from about 6 liters to about 200 liters, and even larger scale systems covering 5 to 2500 square meters in area, or greater. Large scale culture systems can include liquid culture systems from about 10,000 to about 20,000 liters and up to about 1,000,000 liters.

[0067] The culture systems for use with the methods for producing the compositions herein include closed structures such as bioreactors, where the environment is under stricter control than in open systems or semi-closed systems. A photobioreactor is a bioreactor which incorporates some type of light source to provide photonic energy input into the reactor. The term bioreactor can refer to a system closed to the environment and having no direct exchange of gases and contaminants with the environment. A bioreactor can be described as an enclosed, and in the case of a photobioreactor, illuminated, culture vessel designed for controlled biomass production of liquid cell suspension cultures.

[0068] In some embodiments, the algae used in the methods and for the compositions provided herein are grown in fermentation vessels. In some embodiments, the vessel is a stainless steel fermentation vessel. In some embodiments, the algae are grown in heterotrophic conditions whereby one or more carbon sources is provided to the culture. In some embodiments, the algae are grown in aerobic and heterotrophic conditions. In some embodiments, the algae are grown to a density greater than or about 10g/L, about 20 g/L, about 30 g/L, about 40g/L, about 50g/L, about 75 g/L, about 100g/L, about 125 g/L or about 150g/L.

[0069] In some embodiments, the algae are inoculated from a seed tank to a starting density of greater than about 0.1g/L, about 1.0g/L, about 5.0g/L, about 10.0g/L, about 20.0g/L, about 50g/L, about 80g/L, or about 100g/L. Once inoculated, the algae are grown heterotrophically using an aerobic fermentation process. During this process, the algae are fed nutrients to maintain their growth. In some embodiments, these nutrients include a reduced carbon source. Exemplary aerobic fermentation process and/or reduced carbon sources include, but are not limited to, acetate, glucose, sucrose, fructose, glycerol and other types of sugars (e.g., dextrose, maltose, galactose, sucrose, ribose, etc.). In some embodiments, the algae culture is supplemented with iron.

[0070] In some embodiments, the algae are cultured under dark conditions. Preferably, the dark condition has a brightness of less than 1000 lux, less than 750 lux, less than 500 lux, less than 400 lux, less than 300 lux, less than 200 lux, less than 100 lux. In some embodiments, the algae cultured under dark conditions lack or are reduced in chlorophyll production at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% compared to the algae cultured under dark conditions. In some embodiments, the algae grown under dark conditions are supplemented with one or more nutrients. In some embodiments, the algae grown under dark conditions are grown in the presence of a reduced carbon source, such as acetate, glucose, sucrose, fructose, glycerol or other types of sugars (e.g., dextrose, maltose, galactose, sucrose, ribose, etc.). In some embodiments, the algae grown under dark conditions are grown in the presence of iron or otherwise supplemented with iron.

PPIX-containing preparations and products

[0071] Algae strains and cultures overproducing PPIX such as described herein can be used in various forms and preparations. In some embodiments, a PPIX-containing composition is prepared from an algae culture overproducing PPIX, where the composition is red or red-like in color.

[0072] In some embodiments, the PPIX-containing composition is prepared from a biomass isolated from cultured algae. In some embodiments, the biomass is further fractionated to remove one or more components. In some embodiments, the biomass is fractionated to remove starch. In some embodiments, the biomass is fractionated to remove protein. In some embodiments, the biomass is fractionated or otherwise treated to remove carotenoids. In some embodiments, the biomass is fractionated or otherwise treated to enrich for certain components. In some embodiments, the fractionated or treated biomass is enriched in PPIX. In some embodiments, the fractionated or treated biomass is enriched in protein or in protein and PPIX. In some embodiments, the fractionation or treatment enhances the red or red-like color of the preparation. The fractionated or treated biomass can be enriched for protein content such that the composition is about 10% protein, greater than about 10% protein, or greater than about 20%, about 30%, about 40% or about 50% protein.

[0073] In some embodiments, the biomass is fractionated or otherwise treated to remove or reduce any heme content and optionally, to enrich for PPIX. Thus, in some embodiments,

the fractionation or composition may include greater amount of PPIX than of heme by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%. Such fractionation can include separation of PPIX from heme. For example, heme-binding proteins and heme associated with proteins can be separated from PPIX which is not a protein-conjugated or protein-associated compound. Both free heme and protein-associated heme can be separated from PPIX based on heme's association with iron. PPIX does not contain an iron moiety and as such, this feature can be used to separate PPIX from a heme-containing fraction. In some embodiments, an algae biomass herein is fractionated or otherwise treated such that the heme content is reduced, such as reduced below 1%, below 0.1%, below 0.05%, below 0.01%, below 0.001% or below a detectable level in a PPIX-containing fraction. Alternatively, an algae biomass herein is fractionated or otherwise treated such that heme-protein content is reduced, such as reduced below 1%, below 0.1%, below 0.05%, below 0.01%, below 0.001% or below a detectable level in a PPIX-containing fraction. In some embodiments, an algae biomass or fractionated biomass is produced from a strain deficient in ferrochelatase or in a strain that does not make or does not accumulate heme such that the biomass or fraction has little to no heme.

[0074] In some embodiments, the PPIX-containing composition is a PPIX-containing liquid prepared from the culture media of the cultured algae. In some embodiments, the PPIX-containing composition is prepared from PPIX found extracellularly in the algae culture (extracellular fraction). In some embodiments, the algae culture is lysed or otherwise treated to release PPIX from the cells. In some embodiments, the PPIX-containing liquid is further fractionated to remove one or more components. In some embodiments, the PPIX-containing liquid is fractionated to remove starch. In some embodiments, the PPIX-containing liquid is fractionated to remove protein. In some embodiments, the PPIX-containing liquid is fractionated or otherwise treated to remove carotenoids. In some embodiments, the PPIX-containing liquid is fractionated or otherwise treated to enrich for certain components. In some embodiments, the fractionated or treated PPIX-containing liquid is enriched in PPIX. In some embodiments, the fractionation or treatment enhances the red or red-like color of the preparation.

[0075] In some embodiments, the PPIX-containing liquid is fractionated or otherwise treated to remove or reduce any heme content and optionally, to enrich for PPIX. Such fractionation can include separation of PPIX from heme. For example, heme-binding proteins

and heme associated with proteins in the liquid can be separated from PPIX which is not a protein-conjugated or protein-associated compound. Both free heme and protein-associated heme can be separated from PPIX based on heme's association with iron. PPIX does not contain an iron moiety and as such, this feature can be used to separate PPIX from a heme-containing fraction. In some embodiments, a PPIX-containing liquid is fractionated or otherwise treated such that the heme content is reduced, such as reduced below 1%, below 0.1%, below 0.05%, below 0.01%, below 0.001% or below a level that is generally detectable in a PPIX-containing fraction. In some embodiments, a PPIX-containing liquid is produced from a strain deficient in ferrochelatase or in a strain that does not make or does not accumulate heme such that the PPIX-containing liquid has little to no heme content.

[0076] In some embodiments, the biomass or PPIX-containing composition is a PPIX-containing liquid, and/or fractionated PPIX-containing composition or a PPIX-containing liquid contains protoporphyrin IX at about 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.5%, 5.0%, 5.5%, 6.0%, 6.5%, 7.0%, 7.5%, 8.0%, 8.5%, 9.0%, 9.5%, 10.0% or more than 10% on a weight per total weight basis. In addition, in some embodiments, the biomass or PPIX-containing composition is a PPIX-containing liquid, and/or fractionated PPIX-containing composition or a PPIX-containing liquid contains protoporphyrin IX greater than chlorophyll content by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, or at least 50%.

[0077] The PPIX-containing compositions, including biomass, liquid and fractionated preparations can be further processed. Such processing can include concentrating, drying, lyophilizing, and freezing. In various embodiments, the PPIX-containing compositions can be combined with additional components and ingredients, for example, to create an edible product. In some embodiments, the PPIX-containing composition confers a red or red-like color to the edible product. In some embodiments, the PPIX-containing composition confers a meat-like characteristic such as a meat-like taste, aroma and/or texture to the edible product. In some embodiments, the PPIX-containing composition provides the appearance of blood to an edible product, such as to a meat replica, a beef-like product, a chicken-like product or the like.

[0078] In some embodiments, PPIX-containing composition is a PPIX-containing liquid, and/or fractionated PPIX-containing composition or a PPIX-containing liquid provides at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% of the protein to the edible composition.

In some embodiments, PPIX-containing composition is a PPIX-containing liquid, and/or fractionated PPIX-containing composition or a PPIX-containing liquid provides greater than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% of the protein in the edible product. In some embodiments, PPIX-containing composition is a PPIX-containing liquid, and/or fractionated PPIX-containing composition or a PPIX-containing liquid provides a daily recommended dosage of omega-3 fatty acids or a portion thereof to the edible product, for example, at least about 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 125 mg, 150 mg, 175 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg or 500 mg of omega-3 fatty acids to the edible composition.

[0079] In some embodiments, PPIX-containing composition is a PPIX-containing liquid, and/or fractionated PPIX-containing composition or a PPIX-containing liquid provides at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% of the daily recommended dosage of vitamin A or at least about 20 µg, 50 µg, 100 µg, 200 µg, 300 µg, 400 µg, 500 µg, 600 µg, 700 µg, 800 µg, 900 µg or 1000 µg of retinol activity equivalents (RAE) for vitamin A. In some embodiments, PPIX-containing composition is a PPIX-containing liquid, and/or fractionated PPIX-containing composition or a PPIX-containing liquid provides no more than about 2,000 µg, 2,500 µg or 3,000 µg of retinol activity equivalents (RAE) for vitamin A. Alternatively and/or additionally, PPIX-containing composition is a PPIX-containing liquid, and/or fractionated PPIX-containing composition or a PPIX-containing liquid provides at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% of the daily recommended dosage of beta-carotene. Alternatively and/or additionally, PPIX-containing composition is a PPIX-containing liquid, and/or fractionated PPIX-containing composition or a PPIX-containing liquid provides about 0.25 mg, 0.5 mg, 1 mg, 1.5 mg, 2 mg, 2.5 mg, 3 mg, 4 mg, 5 mg, 6 mg, 9 mg, 10 mg, 12 mg, or 15 mg of beta-carotene.

[0080] Alternatively and/or additionally, PPIX-containing composition is a PPIX-containing liquid, and/or fractionated PPIX-containing composition or a PPIX-containing liquid provides less than daily recommended limit for saturated fat or a portion thereof to the edible product, for example, no more than about 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%,

40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the daily recommended dosage of saturated fat. Alternatively and/or additionally, PPIX-containing composition is a PPIX-containing liquid, and/or fractionated PPIX-containing composition or a PPIX-containing liquid provides no more than 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.2%, 1.5%, 2%, 5% or 10% of total saturated fat present in the edible composition or in the finished product made from the edible composition.

[0081] In some embodiments, PPIX-containing compositions are combined with additional ingredients to create a meat-like product. Such meat-like products can include clean meat or cultured meat (made from animal cells grown in the laboratory or otherwise outside of an animal), plant-based and non-animal based meats (made from plant ingredients and/or ingredients not from animal sources). In some embodiments, a PPIX-containing composition made from an over-producing algae is combined with additional ingredients to create a meat-like product whereby the addition of the PPIX-containing composition confers a red or red-like color, a meat-like aroma, a meat-like taste and/or a meat-like texture to the meat-like product. In some embodiments, the meat-like features conferred by the PPIX-containing composition are conferred to the raw or uncooked product. In some embodiments, the meat-like features conferred by the PPIX-containing composition is conferred to the cooked product. Alternatively, least one of the features of meat or meat-like flavor or aroma, a meat or meat-like texture, a blood-like appearance, a meat or meat-like color are derived from the algae preparation.

[0082] In some embodiments, whole algae or fractionated algae is combined with an additional protein source in an edible composition. For example, the protein source may be wheat protein, such as wheat protein, textured wheat protein, pea protein, textured pea protein, soy protein, textured soy protein, potato protein, whey protein, yeast extract, a fungus protein such as quorn, or other plant-based protein source or any combination thereof. In some embodiments, whole algae or fractionated algae is combined with an oil or source of fat in an edible composition. For example, the oil or fat source may be coconut oil, canola oil, sunflower oil, safflower oil, corn oil, olive oil, avocado oil, nut oil or other plant-based oil or fat source or any combination thereof. In some embodiments, whole algae or fractionated algae is combined with a starch or other carbohydrate source such as from potato, chickpea, wheat, soy, beans, corn or other plant-based starch or carbohydrate or any combination

thereof. In some embodiments, whole algae or fractionated algae is combined with a thickener in an edible composition. For example, starches such as arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca and their starch derivatives may be used as a thickener; microbial and vegetable gums used as food thickeners include alginin, guar gum, locust bean gum, konjac and xanthan gum; and proteins such as collagen and egg whites may be used as thickeners; and sugar polymers for use as thickeners include agar, methylcellulose, carboxymethyl cellulose, pectin and carrageenan. In some embodiments, whole algae or an algae fraction may be combined with vitamins and minerals in an edible composition, such as vitamin E, vitamin C, thiamine (vitamin B1), zinc, niacin, vitamin B6, riboflavin (vitamin B2), and vitamin B12.

[0083] In some embodiments, whole algae or an algae fraction may be combined with additional ingredients such that the edible composition and/or finished product is vegetarian, vegan or gluten-free, and therefore may conform to the dietary guidelines of Jewish kosher practitioners, and halal practitioners. Thus, in some embodiments, the edible composition and/or finished product may be suitable for consumption by vegetarians, vegans, gluten-free populations, Jewish kosher practitioners, and halal practitioners. In some embodiments, whole algae or an algae fraction may be combined with additional ingredients such that the edible composition and/or finished product is GMO-free and/or does not contain any ingredients derived from genetically engineered organisms or cells.

EXEMPLARY NUMBERED EMBODIMENTS

[0084] The following embodiments recite non-limiting permutations of combinations of features disclosed herein. Other permutations of combinations of features are also contemplated. In particular, each of these numbered embodiments is contemplated as depending from or relating to every previous or subsequent numbered embodiment, independent of their order as listed.

[0085] Embodiment 1. A composition comprising a preparation from an algae strain, wherein the algae strain overexpresses or accumulates protoporphyrin IX (PPIX). 2. The composition of embodiment 2, wherein the preparation is a biomass from the algae strain. 3. The composition of embodiment 2, wherein the preparation is a fractionated biomass from the algae strain. 4. The composition of embodiment 3, wherein the fractionated biomass comprises a PPIX-enriched fraction. 5. The composition of embodiment 4, wherein the PPIX-

enriched fraction further comprises a protein-enriched fraction. 6. The composition of embodiment 1, wherein the preparation is an extracellular fraction of the algae culture. 7. The composition according to any of embodiments 1-6, wherein the preparation is red or red-like in color. 8. The composition according to any of embodiments 1-7, wherein the preparation contains a greater amount of PPIX than of heme. 9. The composition according to any of embodiments 1-8, wherein the preparation contains less than about 1%, 0.5%, 0.1%, 0.05%, 0.01%, 0.005% or 0.001% heme. 10. The composition according to any of embodiments 1-9, wherein the preparation contains less than about 1%, 0.5%, 0.1%, 0.05%, 0.01%, 0.005% or 0.001% heme-protein. 11. The composition according to any of embodiments 1-10, wherein the preparation does not contain a detectable amount of a heme-protein. 12. The composition according to any of embodiments 1-11, wherein the preparation does not contain a detectable amount of heme. 13. The composition according to any of embodiments 1-4, wherein the preparation does not contain a detectable amount of protein. 14. The composition according to any of embodiments 1-13, wherein the preparation has a protoporphyrin IX content greater than chlorophyll content. 15. The composition according to any of embodiments 1-4 and 6, wherein the preparation provides at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% of the total protein content to the edible composition. 16. The composition according to any of embodiments 1-15, wherein the preparation provides vitamin A, beta carotene or a combination thereof to the composition. 17. The composition of embodiment 16, wherein the vitamin A, the beta carotene or the combination thereof is at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% of the daily recommended requirement. 18. The composition according to any of embodiments 1-17, wherein the preparation provides less than about 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.2%, 1.5%, 2%, 5% or 10% of total saturated fat present in the composition. 19. The composition according to any of embodiments 1-18, wherein the preparation provides at least about 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 125 mg, 150 mg, 175 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg or 500 mg of omega-3 fatty acids to the composition. 20. The composition according to any of embodiments 1-19, wherein the composition has a red or red-like color derived from the preparation. 21. The composition according to any of embodiments 1-20, wherein the composition has a meat or meat-like flavor derived from the preparation. 22. The composition according to any of embodiments 1-21, wherein the composition has a meat or

meat-like texture derived from the preparation. 23. The composition according to any of embodiments 1-22, wherein the algae is a *Chlamydomonas* sp. 24. The composition of embodiment 23, wherein the algae is *Chlamydomonas reinhardtii*. 25. The composition of embodiment 23, wherein the *Chlamydomonas* sp. is strain CC-125 deposited to the University of Minnesota *Chlamydomonas* collection center or a derivative thereof. 26. The composition of embodiment 23, wherein the algae has reduced or absence of ferrochelatase activity or expression of ferrochelatase.

[0086] Embodiment 27. A food product comprising the composition according to any of embodiments 1-26. 28. The food product of embodiment 27, wherein the food product comprises a clean meat, a cultured meat, a synthetic meat, a plant-based meat or a non-animal cell-based meat. 29. The food product of any of embodiments 27-28, wherein the food product is selected from the group consisting of a beef-like food product, a fish-like product, a chicken-like product, a pork-like product and a meat replica. 30. The food product of any of embodiments 27-29, wherein the food product is vegan, vegetarian or gluten-free.

[0087] Embodiment 31. An edible ingredient comprising the composition according to any of embodiments 1-25. 32. The edible ingredient of embodiment 31, wherein the ingredient is part of a finished product, wherein the finished product has a red or red-like color derived from the ingredient. 33. The edible ingredient of embodiment 31, wherein the ingredient is part of a finished product, wherein the finished product has a meat or meat-like flavor derived from the ingredient. 34. The edible ingredient of embodiment 31, wherein the ingredient is part of a finished product, wherein the finished product has an appearance of blood derived from the ingredient. 35. The edible ingredient according to any of embodiments 31-34, wherein the finished product is an ingredient for a burger, a sausage, a kebab, a filet, a ground meat-like product or a meatball. 36. The edible ingredient according to any of embodiments 31-34, wherein the edible composition is part of a finished product and wherein the finished product is an animal feed. 37. The edible ingredient according to any of embodiments 31-36, wherein the edible ingredient is combined with a protein source, a fat source, a carbohydrate, a starch, a thickener, a vitamin, a mineral, or any combination thereof. 38. The edible ingredient of embodiment 35, wherein the protein source is textured wheat protein, textured soy protein, fungal protein or algal protein. 39. The edible ingredient according to any of embodiments 37 or 38, wherein the finished product is free of animal proteins. 40. The edible composition of any of embodiments 37-39, wherein the fat source

comprises at least one of refined coconut oil or sunflower oil. 41. The edible composition of any of embodiments 37-40, further comprising at least one of potato starch, methylcellulose, water, and a flavor, wherein the flavor is selected at least one of yeast extract, garlic powder, onion powder, and salt.

[0088] Embodiment 42. A meat substitute comprising the composition according to any of embodiments 1-26 or the edible ingredient according to any of embodiments 31-41. 43. The meat substitute of embodiment 42, further comprising: (a) 0.01%-5% (by weight of the meat replica matrix) of a non-animal protoporphyrin IX; (b) a compound selected from glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, and galactose, and any combination thereof; (c) at least 1.5 mM of a compound selected from cysteine, cystine, thiamine, methionine, and any combination thereof; and (d) one or more proteins selected from the group consisting of plant proteins, fungal proteins and algal proteins, wherein the meat substitute is a ground beef-like food product that contains no animal product; wherein cooking the ground beef-like food product results in the production of at least two volatile compounds which have a beef-associated aroma.

[0089] Embodiment 44. A method of producing a protoporphyrin IX composition, comprising: growing an algae population comprising an algae that is a protoporphyrin IX over-producer; and isolating a protoporphyrin IX composition from the culture. 45. The method of embodiment 44, wherein the growing comprises culturing the algae culture in an aerobic fermentation condition. 46. The method of embodiment 44 or embodiment 45, wherein the algae contains a chloroplast. 47. The method of embodiment 46, wherein biosynthesis of the protoporphyrin IX occurs in the chloroplast. 48. The method according to any of embodiments 44-47, wherein the algae is deficient for its ability to produce chlorophyll. 49. The method according to any of embodiments 44-48, wherein the algae is deficient for the ability to produce a functional Mg-chelatase enzyme. 50. The method according to any of embodiments 44-49, wherein the algae is reduced in or lacks ChlD1, ChlD2 or ChlDH. 51. The method according to any of embodiments 44-50, wherein the algae is reduced in or lacks a functional light dependent protochlorophyllide. 52. The method according to any of embodiments 44-51, wherein the algae is reduced in or lacks a functional light independent protochlorophyllide. 53. The method according to any of embodiments 44-52, wherein the algae is reduced in or lacks ChlB, ChlL, or ChlN. 54. The method according to any of embodiments 44-53, wherein the algae overexpresses one or more of glutamyl-

tRNA reductase, glutamate-1-semialdehyde aminotransferase, ALA dehydratase, porphobilinogen deaminase, UPG III synthase, UPG III decarboxylase, CPG oxidase, and PPG oxidase. 55. The method according to any of embodiments 44-54, wherein the algae is generated by mating and wherein the generated strain is red or red-like in color. 56. The method according to any of embodiments 44-55, wherein the algae is generated by mutagenesis. 57. The method according to any of embodiments 44-56, wherein the algae is red or red-like in color. 58. The method according to any of embodiments 44-57, wherein the isolated protoporphyrin IX composition is an algae biomass. 59. The method of embodiment 58, wherein the algae biomass is fractionated. 60. The method of embodiment 59, wherein the algae biomass is fractionated to produce a protein-enriched fraction comprising protoporphyrin IX. 61. The method according to any of embodiments 44-57, wherein the isolated protoporphyrin IX composition is isolated from extracellular media of the algae culture. 62. The method according to any of embodiments 44-61, wherein the isolated protoporphyrin IX composition is isolated away from algae protein. 63. The method according to any of embodiments 44-62, wherein the algae is deficient for carotenoids. 64. The method according to any of embodiments 44-63, wherein the algae is a *Chlamydomonas* sp. 65. The method according to any of embodiments 44-64, wherein the algae is a *Chlamydomonas reinhardtii*. 66. The method of embodiment 64, wherein the *Chlamydomonas* sp. is strain CC-125 deposited to the University of Minnesota *Chlamydomonas* collection center or a derivative thereof. 67. The method according to any of embodiments 44-66, wherein progeny of a protoporphyrin IX overexpressing algae strain grows faster than its parental strain following a mating with another algae. 68. The method according to any of embodiments 44-66, wherein the protoporphyrin IX algae is a strain produced by mating a strain deficient for carotenoids with a strain exhibiting a red or red-like color. 69. The method according to any of embodiments 44-66, wherein the protoporphyrin IX overexpressing algae is generated by mutagenesis of a first starting strain and selection of a second strain that grows faster in the dark than the first starting strain. 70. The method according to any of embodiments 44-66, wherein the protoporphyrin IX algae is generated by mutagenesis of a first strain and selection of a second strain that lacks one or more carotenoids from the mutated first strain. 71. The method according to any of embodiments 44-70, wherein the algae lacks a functional ferrochelatase enzyme. 72. The method according to any of embodiments 44-70, wherein the algae is reduced in the amount of, or activity of, a ferrochelatase enzyme. 73. The method according to any of embodiments 44-70, wherein the algae is reduced in the amount of or lacks heme as compared to a wildtype strain.

[0090] Embodiment 74. A protoporphyrin IX-containing composition produced by the method according to any of embodiments 44-73. 75. The composition of embodiment 74, wherein the composition does not contain a detectable level of heme, heme-binding protein or a combination thereof. 76. The method according to any of embodiments 44-73, further comprising the steps of: (a) culturing the algae strain under a dark condition, wherein the strain does not produce or is reduced in the production of chlorophyll, and (b) collecting a portion of the algae culture that is red or red-like in color to produce the protoporphyrin IX composition. 77. The method of embodiment 76, wherein the algae is a *Chlamydomonas* sp. 78. The method of embodiment 77, wherein the algae is a *Chlamydomonas reinhardtii*. 79. The method according to embodiment 76, wherein the algae exhibits a red or red-like color when grown in the dark condition. 80. The method of embodiment 76, wherein the collected portion is extracellular media from the algae culture. 81. The method of embodiment 76, wherein the collected portion is a biomass or fractionated biomass from the algae culture. 82. The method of embodiment 76, wherein the algae is grown in an aerobic fermentation condition. 83. The method of embodiment 76, wherein the algae is grown to a density greater than 10g/L, 20 g/L, 30 g/L, 40g/L, 50g, L, 75 g/L, 100g/L, 125 g/L, or 150g/L. 84. The method of embodiment 76, wherein the algae is grown with acetate as a reduced carbon source. 85. The method of embodiment 76, wherein the algae is grown with sugar as a reduced carbon source. 86. The method of embodiment 76, wherein the algae culture is supplemented with iron during the culturing step. 87. The method of embodiment 76, wherein the algae culture is inoculated at a density greater than 0.1 g/L, 1.0 g/L, 5.0 g/L, 10 g/L, 20 g/L, 50 g/L, 80 g/L, or 100 g/L. 88. The method of embodiment 76, further comprising fractionating the collected portion, wherein the fractionating removes from the collected portion substantially all or most of a component selected from the group consisting of carotenoids, starch, and protein. 89. The method of embodiment 76, further comprising fractionating the collected portion, wherein the fractionating removes from the collected portion substantially all or most of heme, heme-binding protein or a combination thereof. 90. The method of embodiment 76, further comprising fractionating the collected portion, wherein the fractionating produces a protein-enriched fraction. 91. The method of embodiment 76, wherein the algae lacks or is reduced in one or more of magnesium chelatase, magnesium protoporphyrinogen IX, protochlorophyllide, chlorophyllide, and chlorophyll. 92. The method of embodiment 76, wherein the algae lacks or is reduced in ferrochelatase. 93. The method according to any of embodiments 44-73 and 76-92, wherein the algae is not a transgenic strain.

[0091] Embodiment 94. A clean meat product produced by the method according to any of embodiments 76-93, wherein the process further comprises combining the collected portion with a clean meat manufacturing composition, wherein the collected portion provides a red or red-like color to the clean meat product. 95. The clean meat product of embodiment 94, wherein the collected portion is a PPIX-enriched fraction or purified PPIX.

[0092] Embodiment 96. An edible ingredient produced by the method according to any of embodiments 44-73 and 76-93, wherein the protoporphyrin IX composition confers a meat or meat-like flavor, texture, odor or any combination thereof to the edible ingredient. 97. The edible ingredient of embodiment 96, wherein the edible ingredient is incorporated in a finished product selected from the group consisting of a beef-like food product, a fish-like product, a chicken-like product, a pork-like product and a meat replica. 98. The edible ingredient of any of embodiments 96-97, wherein the edible ingredient is vegan, vegetarian or gluten-free. 99. The edible ingredient of any of embodiments 96-98, wherein the edible ingredient is free of animal proteins. 100. The edible ingredient of any of embodiments 96-99, wherein the edible ingredient does not contain any genetically modified components.

[0093] Embodiment 101. An engineered algae having a genetic modifications, where the genetic modification results in an accumulation of protoporphyrin IX (PPIX) in the algae as compared to an algae lacking the genetic modification. 102. The engineered algae of embodiment 101, wherein the engineered algae has reduced or absence of chlorophyll production. 103. The engineered algae of embodiments 101 or 102, wherein the algae has red or red-like color. 104. The engineered algae according to any of embodiments 101-103, wherein the algae is capable of growth on glucose as the sole carbon source. 105. The engineered algae according to any of embodiments 101-104, wherein the genetic modification comprises a genetic alteration to chlorophyll synthesis pathway, protoporphyrinogen IX synthesis pathway or heme synthesis pathway. 106. The engineered algae according to any of embodiments 101-105, wherein the genetic modification is associated with a deficiency in the expression of ferrochelatase. 107. The engineered algae according to any of embodiments 101-106, wherein the genetic modification comprises an alteration in one or more of CHLD, CHLI1, CHLI2 or CHLH1. 108. The engineered algae of embodiments 106 or 107, wherein the genetic modification comprises an alteration in an upstream regulatory region, a downstream regulatory region, an exon, an intron or any combination thereof. 109. The engineered algae according to any of embodiments 105-108,

wherein the genetic modification comprises an insertion, a deletion, a point mutation, an inversion, a duplication, a frameshift or any combination thereof.

[0094] Embodiment 110. The engineered algae according to any of embodiments 101-109, wherein the engineered algae has a PPIX content greater than the chlorophyll content. 111. The engineered algae according to any of embodiments 101-109, wherein the engineered algae has a heme content greater than the chlorophyll content. 112. The engineered algae according to any of embodiments 101-111, wherein the engineered algae has reduced production of one or more fatty acids. 113. The engineered algae according to any of embodiments 101-112, wherein the engineered algae further comprises a genetic modification that reduces or eliminates the expression of light independent protochlorophyllide oxidoreductase. 114. The engineered algae of embodiment 113, wherein the genetic modification comprises a mutation or deletion in one or more of ChlB, ChlL or ChlN. 115. The engineered algae according to any of embodiments 101-114, wherein the engineered algae has down-regulated expression of ferrochelatase. 116. The engineered algae according to any of embodiments 101-115, wherein the engineered algae has upregulated expression of protoporphyrinogen IX oxidase.

[0095] Embodiment 117. The engineered algae according to any of embodiments 101-116, wherein the algae contain a recombinant or heterologous nucleic acid. 118. The engineered algae according to any of embodiments 101-117, wherein the engineered algae comprises a *Chlamydomonas* sp. 119. The engineered algae of embodiment 118, wherein the *Chlamydomonas* sp. is *Chlamydomonas reinhardtii*.

[0096] Embodiment 120. An edible composition comprising an algae preparation, wherein the algae preparation comprises an engineered algae of any of embodiments 101-119 or a portion thereof. 121. The edible composition of embodiment 120, wherein the edible composition comprises PPIX derived from the engineered algae. 122. The edible composition of embodiment 120, wherein the algae preparation comprises algae cells. 123. The edible composition of embodiment 120, wherein the algae preparation is a fractionated algae preparation.

EXAMPLES

Example 1: Identification of PPIX-overproducing algae

[0097] Strains of algae (*Chlamydomonas reinhardtii*) overexpressing PPIX were identified by their inability to produce chlorophyll. Additionally, these strains exhibited red, brown, orange or some variation of the listed color. The identified strains exhibit light sensitivity and cannot be grown in direct light greater than $10 \mu\text{E m}^{-2} \text{s}^{-1}$ for extended periods of time.

[0098] One of the identified strains was grown under fed-batch aerobic fermentation conditions where acetate is used as a reduced carbon source of nutrition for the culture. The strain was grown in a fermenter where minimal light can reach the culture. The strain was grown to a density that is greater than 50g/L and harvested via centrifugation. The harvested strain was red in color and can be added to compositions, such as food products, to confer a red, orange or brown color.

[0099] Tables 1-5 show characteristic analysis of one exemplary, identified red heme algae (Strain number: TAI114, Species name: *Chlamydomonas reinhardtii*).

TABLE 1: MICROBIAL ANALYSIS

Quality Measure	Specification	Result	Units	Method	Conclusion
Aerobic Plate Count	$\leq 10,000$	7,250	CFU•g-1	AOAC 990.12	Specification Met
E. coli (Generic)	Negative	Negative	CFU•g-1	AOAC 991.14	Specification Met
Total coliforms	$\leq 1,000$	Negative	CFU•g-1	AOAC 991.14	Specification Met
Salmonella	Negative	Negative	ORG•25g	AOAC 030301	Specification Met
Staphylococcus aureus	Negative	Negative	CFU•g-1	AOAC2003.07	Specification Met
Pseudomonas aeruginosa	Negative	Negative	CFU•g-1	USP	Specification Met

TABLE 2: HEAVY METAL ANALYSIS

Quality Measure	Specification	Result	Units	Method	Conclusion
Arsenic	$\leq 0.01\text{ppm}$	$\leq 0.01\text{ppm}$	ppm	MET-CH-030	Specification Met
Cadmium	$\leq 0.1\text{ppm}$	$\leq 0.01\text{ppm}$	ppm	MET-CH-030	Specification Met
Lead	$\leq 0.01\text{ppm}$	$\leq 0.01\text{ppm}$	ppm	MET-CH-030	Specification Met
Mercury	$\leq 0.005\text{ppm}$	$\leq 0.01\text{ppm}$	ppm	MET-CH-030	Specification Met
Sulfite	$\leq 10 \text{ ppm}$	$\leq 0.01\text{ppm}$	ppm	MET-NHP-018	Specification Met

TABLE 3: BIOMASS ANALYSIS

Quality Measure	Result	Unit
Moisture	10.66	Percent of biomass
Ash	3.19	Percent of biomass
Protein	26.00	Percent of biomass
Fat	4.77	Percent of biomass
Starch	39.5	Percent of biomass
Soluble Dietary Fiber	8.85	Percent of biomass
Insoluble Dietary Fiber	1.15	Percent of biomass

TABLE 4: Porphyrin (Heme) ANALYSIS

Quality Measure	Result	Unit
Heme	0.60	Percent
protoporphyrin IX	4.60	Percent

TABLE 5: AMINO ACID COMPOSITION

Amino Acid	Result	Unit
Alanine	2.25	Percent of biomass
Arginine	2.03	Percent of biomass
Asparagine / Aspartic Acid	2.38	Percent of biomass
Glycine	1.49	Percent of biomass
Cysteine	0.48	Percent of biomass
Glutamine / glutamic acid	2.83	Percent of biomass
Proline	1.63	Percent of biomass
Serine	1.25	Percent of biomass
Tyrosine	1.05	Percent of biomass
Histidine	0.51	Percent of biomass
Isoleucine	1.04	Percent of biomass
Leucine	2.38	Percent of biomass
Lysine	1.78	Percent of biomass
Methionine	0.63	Percent of biomass
Phenylalanine	1.15	Percent of biomass
Threonine	0.83	Percent of biomass
Tryptophan	0.55	Percent of biomass
Valine	1.88	Percent of biomass
Percent Non-Essential Amino Acids	51.1	Percent of protein
Percent Amino Acids	48.9	Percent of protein

Example 2: Fractionation

[0100] Cells from a PPIX overproducing strain of *Chlamydomonas reinhardtii* were harvested from a fermentation culture. Cells were re-suspended in a 10mL solution (8:2 v/v solution of acetone:1.6M HCL) and vortexed for 30 minutes. The cell debris was centrifuged

and the porphyrin layer was separated from the cell debris (FIG. 2). The porphyrin fraction was then diluted 1:10 with H₂O to precipitate the porphyrins from the acetone solution. Samples were further washed with water and can be frozen at that point for further analysis. Shown in FIG. 2 is the PPIX-containing fraction after the final step.

Example 3: Preparation of a PPIX-enriched “meatless” burger

[0101] The PPIX-enriched samples were used to prepare compositions of meat-like products produced from plant based materials and algae rich in PPIX. To create a PPIX-enriched burger, ingredients were mixed in the following proportions and formed into a disc shaped algae-plant based burger: 20% or about 20% Textured wheat protein, 20% or about 20% Refined coconut oil, 3% or about 3% Sunflower oil, 2% or about 2% Potato starch, 0.5% or about 0.5% Kojac gum, 0.5% or about 0.5% Xanthan gum, 45% or about 20% water and 4-9% or about 4-9% Flavors, including yeast extract, garlic powder, onion powder, salt, and PPIX -enriched (“red”) algae. Shown in FIG. 3 are burgers created with 0.01g, 0.1 g, 1.0g, and 5.0g of the PPIX enriched algae.

[0102] In this example, the composition of the PPIX-enriched algae was 4.5% PPIX, 0.5% heme, 0% chlorophyll, 24.4% protein, 9% dietary fiber, 40% starch, 0.8% omega-3-fatty acids, 3.9% other fats, 7.5% moisture, and 8.4% ash.

Example 4: Preparation of a PPIX-enriched plant-based burger

[0103] The PPIX-enriched samples was used to prepare burger compositions from plant based materials and algae rich in PPIX. To create a PPIX-enriched plant-based burger, ingredients were mixed in the following proportions and formed into a disc: 20% or about 20% Textured soy protein, 20% or about 20% Refined coconut oil, 3% or about 3% Sunflower oil, 2% or about 2% Potato starch, 1% or about 1% methylcellulose, 45% or about 45% water and 4-9% or about 4-9% Flavors, including yeast extract, garlic powder, onion powder, salt, and PPIX-enriched (“red”) algae. Shown in FIG. 4 are the ingredient mixes of the plant-based burger ingredients with no PPIX-enriched algae (far left), with the addition of PPIX-enriched algae (second from left), the ingredients with the addition of PPIX-enriched algae shaped into a burger before and after cooking (third from left and far right photos, respectively). As shown, the addition of the PPIX-enriched algae conferred a red/red-like color (resembling a burger with animal blood) to the ingredient mix and to the burger, and this color undergoes a transition when cooked.

[0104] In this example, the composition of the PPIX-enriched algae was 4.5% PPIX, 0.5% heme, 0% chlorophyll, 24.4% protein, 9% dietary fiber, 40% starch, 0.8% omega-3-fatty acids, 3.9% other fats, 7.5% moisture, and 8.4% ash.

Example 5: Preparation of a PPIX-enriched meatless “tuna”

[0105] The PPIX-enriched samples were used to prepare fish-like compositions. To create a PPIX-enriched meatless “fish”, ingredients were mixed in the following proportions: 20% Textured soy protein, 65% water and 10% Flavors and PPIX -enriched 5% (“red”) algae. Shown in FIG. 5 is a square portion of the meatless “tuna” produced.

[0106] In this example, the composition of the PPIX-enriched algae was 4.5% PPIX, 0.5% heme, 0% chlorophyll, 24.4% protein, 9% dietary fiber, 40% starch, 0.8% omega-3-fatty acids, 3.9% other fats, 7.5% moisture, and 8.4% ash.

Example 6: Separation of PPIX from heme and heme-binding proteins

[0107] Separation of PPIX from heme and heme-binding proteins may be accomplished as follows. Algae biomass is mixed in a buffer such as Tris-EDTA buffer (pH 7.2) and stirred for 1 h at room temperature at about 1600 RPM. Samples are then placed on ice and treated with ultrasonication such as for 5 min with 1 s pulse. To the ultrasonicated algae, acetonitrile is added and then the mixture is vortexed for about 5 min and then is subjected to centrifugation such as at 2500×g for 5 min, making a pellet of precipitated proteins. The acetonitrile containing supernatant containing porphyrins (including PPIX), is removed and can be analyzed for porphyrin content and further use.

[0108] Further separation can be achieved as follows: To the pellet from the centrifugation, acetonitrile:1.7 M HCl (8:2, v/v) is added and placed in a shaker for about 20 min, extracting heme from the proteins into the acetonitrile. To create a two phase liquid-liquid system, saturated MgSO₄ and NaCl are added, and the solution is then vortexed for about 5 min and centrifuged at 2500×g for 5 min. The top organic layer can be removed and, if necessary, diluted with pure acetonitrile prior to analysis and further separation by LC/MS-MS. (Fyrestam and Östman, Anal Bioanal Chem (2017) 409:6999–7010 “Determination of heme in microorganisms using HPLC-MS/MS and cobalt(III) protoporphyrin IX inhibition of heme acquisition in Escherichia coli”).

Example 7: Targeted Modification of chlorophyll pathway to create PPIX-enriched strains

[0109] Guide RNAs (sgRNAs) can be designed against the ferrochelatase gene to cause a deletion or an insertion that renders the protein complex non-functional, including a modification to one or more of SEQ ID NOs: 116-122. Once designed sgRNAs can be combined with the Cas9 protein by incubating them at 37°C to form ribonuclear proteins (RNPs). These RNPs carrying the sgRNAs to target ferrochelatase are then electroporated into green algae cultures. 3×10^8 cells are placed into MAX efficiency transformation buffer reagent for algae (Thermo fisher scientific) and placed into a cuvette with a 0.2cm gap. The electroporation voltage is set to 250V and the pulse interval is set to 15ms. Once electroporated cells are recovered in growth media with 40mM sucrose added to improve recovery efficiency. Cells are then plated on growth media containing agar and grown in the dark due to the photosensitivity of chlorophyll-deficient mutants. Once recovered the population can be pulled and struck out for individual colonies. Plates are again placed in the dark for 2 to 3 weeks. Mutants of ferrochelatase can be identified by an increase in their fluorescence at 635nm when they are excited by a light with a wavelength of 420nm when compared to the unmodified green algae.

Example 8: Additional modification of PPIX-enriched strains that are improved for different meat imitations and other uses.

[0110] Strains of algae overexpressing PPIX can be mated with strains that are under or overproducing omega-3s, omega-6s or omega-9s. For imitation fish, more omega oils in strains of algae overexpressing heme are ideal. For imitation beef-like products, less omega oils in strains of algae overexpressing heme are ideal. As such strains of algae that are mutants for either over or underexpressing omega oils can be mated with strains of algae overexpressing heme to form a more ideal algae for various meat-like products.

[0111] Mating can be done by identifying strains of *Chlamydomonas* that are the opposite mating type and then starving them for nitrogen. After nitrogen starvation, strains are re-suspended in water to promote the formation of flagella. The flagella of the different mating types assist in the fusion of algae strains that will result in the formation of a zygote. The mated cultures are then exposed to chloroform to kill strains that did not mate. The chloroform does not kill zygotes. The zygotes are then placed into growth medium and

allowed to propagate. Individual colonies are then identified and screened for an increase in PPIX by measuring for an increase in fluorescence of the precursor protoporphyrin IX or by biochemical assay (Abnova KA1617) as well as those that are overexpressing or under-expressing omega oils.

SEQUENCES

ALA dehydratase (ALAD) nucleic acid sequence (SEQ ID NO: 1):

atgcagatgatgcagcgcaacggttggtggccagcgcccgctcgctggctcccgccgctcgctgggtggttgccaac
gttgccggaggtgacccgcccccggtcagcaccacggcaagcaccggactggtgtgcccggagggaactcccatc
gtcaccctcaggacctgcccctcgccccctcgccgcaaccgcccgcagcgagagcttccgtgcttccgttcgtgag
gtgaacgtgtcgcccgccaacttcattcctgcccgatcttcatccacgaggagagcaaccagaacgtgcccacgccc
tccatgcccgtggcatcaaccgcccgtatggcaagaacgtgattgactacgttgctgaggtcgctcttacggt
gtcaaccaggtcggtggttttcccccaagacgcccgaaccctgaagacgcaaacccgaggaggcggttcaacaag
aacggcctcagccagcgacgatccgcccgtgaaggactcttccctgacctggaggtgtacacggacgtggct
ctggacccctacaactcgacggccacgacggtatcggtgctggacgcccgtgtgatcctgaacgacgagaccatc
gagtaacctgtgcccagggccggtgagccagggcggcggtgcccagctgggtgtcgccctctgacatgatggac
ggccgctggggcccatccgcccgccttggaacggcgagggcttcaccaacgtgtccatcatgtcctacacccgccc
aagtaacctccgctactacggccccttccgtgacgcccgtggcgctccgcccgaagccggccaggcgacccgc
cgcatccccccaagaagacctaaccagatggaccccgccaactaccgcgaggccatccgcgaggccaaggcc
gacgaggccgaggggcgctgacatcatgatggtcaagcccggaatgcccgtacctggacgtggtagcctgctgctg
gagaccagcccgtgcccgtggcggtgtaccacgtgtcgggcgagtagccatgctcaaggcggcgaggagcgc
ggctggctgaacgagaaggatgcccgtgcttgaggccatgacctgcttccgcccgcggcgctgacctcatcctc
acctactacggcatgaggcctccaagtggctggcgggcgagaagtaa

ALA dehydratase (ALAD) amino acid sequence (SEQ ID NO: 2):

MQMQRNVVGVQRPVAGSRRLVAVANVAEVTTPAVSTNGKHRTGVPEGTPIVTPQDLPSRPRNRNRSEFRASVRE
VNVSPANFILPIFIHEESNQNPVPIASMPGINRLAYGKNVIDYVAEARSYGVNVVFPKTPDHLKTQAEAFNK
NGLSQRTIRLLKDSFPDLEVYTDVALDPYNSDGHGDI VSDAGVILNDETIEYLCRQAVSQAEAGADVVSPPSMDMD
GRVGAI RRALDREGFTNVSIMSYTAKYASAYGPFPRDALASAPKPGQAHRRIPPNKTYQMDPANYREAIRAKA
DEAEGADIMMVKPGMPYLDVVRLRLRETSPLPVAVYHVSGEYAMLKAAAEERGWLNEKDAVLEAMTCFRRAGADLIL
TYYGIEASKWLAGKEK

coproporphyrinogen III oxidase (CPX1) nucleic acid sequence (SEQ ID NO: 3):

atggcactgcaagcctcaaccgctcgctccagcagcgccgccccttctcttcggcccagacctccaagcgtgtg
tctgtgaccaaggtccgcccgcagcgctatcgaggcggagaactatgtgaagcaggctcccagtcgctggctccgc
ccgggcatcgacactgaggactctatgcgcgctcgcttcgagaaggatgacccgcaacgcccaggactccatctgc
aatgctatctccgagatcgatggcaagcgttccaccaggacgcccgtggacccgcccggcggtggcgccatc
agccgctgctgcaggacggcaacgtgtgggagaaggccggcgctcaacgtgtccgtggtctacggcaccatgccc
cctgaggcctaccgcccgtgccactggcaacgcccagagaagctgaagaacaagggtgacgggtggcgcgctgcccctt
tcgcccgcggcatctcgctgggtgatgcaccccgcaaccccccactgccccaccatgcacttcaactaccgctac
ttcgagactgaggagtggaaacggcatccccggccagtgggtggttcggcgggcggaaccgacatcacccccagctat
gtggtgcccaggacatgaagcacttccacggcacttacaaggcgggtgtgcgaccgccacgatcccgttactac
gagaagttccgcacctggtgcatgagtaacttccatcaagcaccgcccgcagcggcccgccggtggcgccatc
ttcttcgatgacctgaacgaccgcaaccccgaggacatcctgaagtctcgaccgacgcccgtgaacaacgtgggtg
gaggcatactgccccatcatcaagaagcacatgaacgacccctacaccccgaggagaaggagtggcagcagatc
cgccgcccgcgctacgtggagttaacctggtctatgaccgcccaccaccttcggcctgaagacggcgccgccc
attgagtcgatcctcatgtccatgccccagacggcctcatggctgtacgaccaccagcccaaggccggtcgccc
gaggccgagctgctcgacgctgcccgaaccccgcgctctgggtgtaa

coproporphyrinogen III oxidase (CPX1) amino acid sequence (SEQ ID NO: 4):

MALQASTRSLQRRRAFSSAQTSKRVSVTKVRATAIEAENYVKQAPQSLVRPGIDTEDSMRARFEKVI RNAQDSIC
 NAISEIDGKPFHQDAWTRPGGGGGISRVLQDGNVWEKAGVNVSVVYGTMPPEAYRAATGNAEKLKNKGDGGRVVF
 FAAGISSVMHPRNPHCPTMHFNRYFETEEWNGIPGQWWFGGGTDITPSYVVPEDMKHFHGTYSKAVCDRHPAYY
 EKFRTWCDYFLIKHRGERGLGGIFFDLLNDRNPEDILKFSTDAVNNVVEAYCPIIKKHMNDPYTPPEKEWQQI
 RRGRYVEFNLVYDRGTTFLKTGGRIESILMSMPQTASWLYDHQPKAGSPEAELLDACRNPRVWV

coproporphyrinogen III oxidase (CPX2) nucleic acid sequence (SEQ ID NO: 5):

atgctgaggaagcagattggtggatctggccagcagcggcgccgctccgacgggtgaaccaaggacctgcgctg
 cggcggttggcaccctgcccgtgcccggcccgctgcaaacctcgctcccgccacattcaatgcttctgctg
 gactacattcacggactccagaagaacattctgagcactgctgaggatctggagaacggcgagcgggaagtgtgtt
 gttgacccgtgggagcgcgacgcccagcaaccccaacgcgggtatggcattacgtgctgctgaggaacgggaag
 gtgctggagaaggccgcagccaatatctcagtggtgcccgggacgctgtcgccgagcgcgcagtgcccatgagc
 tcccgcggccgcagcagcatcgaccccaaggcgggcagccctacgcccggcgcccatgagcctagtgttccac
 agcgcgcacccgctcatcccaacgctgcccgcgacgtgcggttgttccaggtggcgatgagggcgtggtacggcg
 gtggctgtgacctgacgcccactacctagacgtggaggactcgagctccttccaccgctactggaaggacgtgtg
 gcggcaagtacaagccgggctgtgtacacccagctcaaggagtggtgacacaggtacttctacatcccgcccgca
 aagagcacccgtggcatggcgccctgttctttagatgacatggccactgcccaggcgggctgcgatgtggaggcgt
 ttgtgcccgaagtgggagatggcatcctgcccctgctggctgcccacgtggcgccgacccgtggccagcccttca
 cggagcagcagcggcaatggcagctgctgcccgcggctcgctacatcgagttcaacctgctgtacgaccgcccga
 tcaagttcggcttggacggcgccgcatcgagagcatcatggtgtcgccgcgcgcgctgatcgctggaagtaaca
 acgtggtgccacagccgggcagccccgaggaggagatgctgaaggtgcttcagcagccccgcgagtgggcctga

coproporphyrinogen III oxidase (CPX2) amino acid sequence (SEQ ID NO: 6):

MLRKQIGSGSQQRAGLRRVNQGPARRRLAPCRVAAPVQTSSSVATFNGFVDYIHGLQKNI LSTAEDLENGERKFV
 VDRWERDASNPAGYGITCVLEDGKVLKAAANISVVRGTL SAQRAVAMSSRGRSSIDPKGGQPYAAAAMSLVFH
 SAHPLIPTLRADVRLFQVGDEAWYGGGCDLTPNYLDVEDS QSFHRYWKDVC GKYPGLYTELKEWC DRYFYI PAR
 KEHRGIGGLFFDMDATAEAGCDVEAFVREVGDGILPCWLP IIVARHRGQPFTEQQRQWQLLRGRYIEFNLLYDRG
 IKFGLDGGRIESIMVSAPPLIAWKYNVVPQPGSPREEMLKVLQQPREWA

Ferrochelatase from *Chlamydomonas reinhardtii* nucleic acid sequence (SEQ ID NO: 7):

atggcgctcgcttggattgatgcaaaggacgggtgactgtccccagcttgtggaggagcgggtgttcgcccggctcgct
 ggctgctctggtcgctggcctgccagttatccagcggcaacggcgctggcgctgtgacgtgccaccaacgggtgtccag
 cgagggcgctgtgctgcccggacggccgcttcgaccgacgtggtctccttctgaggaccccaatgacattagaaaa
 cccgcagcagcagcagctggccctgcccgtggataaggtcggcgcttctgctgttaaaccttggcgggcccggaaaag
 ctgcagcagctcaagccttccctgtataacctattcgccgaccagaaattattcgccctgcccagcggcagctcag
 ttcttgcagcggctgctgcgcgacgatcatctccacgcttcgcccggcgaagagcgcggagggtatgaggccatt
 ggcgggtggttagcccgctgctgtaggattacagacgagcagggcggaggcgctggcggagctctctgcgcgccaagggc
 caacctgcgaacgtgtacgtgggcatgctgctattggcaccctacacggaggaggcgctggagcacattaaagcc
 gacggcgctcacgcgcctggtcatcctcccgtgtaccctcagttctccatctctaccagcggctccagccttcga
 ctgcttgagtcgctctcaagagcgacatcgcgctcaagtcgctgcccgcacacggctcatcccgctcctggtaccag
 cggcggggctacgtgagcgcgatggcggacctgattgttagaggagctgaagaagttccgggacgtgccagcgtg
 gagctgtttttctccgcgcacggcgctgcccgaagtcctacgtggaggaggcgggacccatacaaggaggagatg
 gaggagtgcgctgcccgtcattacggacgaggtcaagcggcgcggttcgccaacacgcacacgctggcctaccag
 agccgcgtggggcccgcggaatggctcaagcgtacacgggatgagtcctcaaggagctgggcaagcgcggcgctc
 aagtcgctgctggcggtgcccacagcttctgacgcgagcacattgagacgttggaggagatcgacatggagtac
 cgcgagctggcggaggagagcggcatccgcaactggggcccgctgcccgcgctgaacaccaacgcgcgcttcatc
 gacgacctggcggaacgggtgatggaggcgctgcccctacgtgggctgctggcggggccgacagactcgctggtg
 ccgctggggcgacctggagatgctgctgcaggcctacgacccgcgagcgcgcgacgctgcccctaccggctggtagt
 tgggagtggggctggaccaagagcgcggagacgtggaacggccgcatgcccattgcccattcatcatcctg
 gcgctggaggcagccagcggccagtcctcctcaaaaacctgttctgcccggagtag

Ferrochelatase from *Chlamydomonas reinhardtii* amino acid sequence (SEQ ID NO: 8):

MASFGLMQRTVHCPQLVEERCSPVAGCSGRGLPVIQRQRRGVCSATNGVQRGRVLRRTAASTDVVSFVDPNDIRK
 PAAAAAGPAVDKVGVLNLLNLGGPEKLDVDPFLYNLFADPEIIRLPAAAQFLQPLLATIIISTLRAPKSAEGYEAI
 GGGSPRRITDEQAEALAESLRAGQGPANVYVGMRYWHPYTEALEHIKADGVTRLVILPLYPQFSISTSGSSLR
 LLESFLKSDIALKSLRHTVIPSQYQRRGYVSAMADLIVEELKKFRDVPVELFFSAHGVPKSYVEEAGDPYKEEM
 EECVRLITDEVKRRGFANTHTLAYQSRVGPAEWLKPYTDESIKELGKRGVKSLLAVPISFVSEHIETLEEIDMEY
 RELAEESGIRNWGRVPALNTNAAFIDDLADAVMEALPYVGCLAGPTDSLVLPLGDLEMLLQAYDRERTLPSPVVW
 EWGWTSAETWNGRIAMIAIIIIILALEAASQSILKNLFLAE

Glutamate-1-semialdehyde aminotransferase (GSA) nucleic acid sequence (SEQ ID NO:**9):**

atgcagatgcagctgaacgccaagaccgtgcagggcgccctcaaggcgagcgccctcgctctgtccgcggaac
 gtggcggtgcgcgcagtgccgctccccctaaagctggtcaccaagcgctccgaggagatcttcaaggaggctcag
 gagctgctgcccgggtggcgtgaactcgcccgctgcgcgctttccgctcggttgggtggcgccccatcgctctcgac
 aggggtcaagggtgctactgctgggacgtcgatggcaacaagtagatcgactacgttggctcttggggccctgct
 atttgcggccacggcaacgacgaggtcaacaacgcctgaaggcgagatcgacaaggcgacctcgctcggtgct
 cctgagctggtgagaacgtgctggccaagatggtgattgaccgctgcccctcggtggagatggtgcgctctcgctg
 tcctcgggcactgagggcgctgctgctcggtgctgcgcctgtagcgcgcatcacccggcgcgagaaggtgctgaag
 ttaccgggtgctaccacggccacgcgactccttctggtgaaggcggtcggtgtagatcacctgggctg
 ccgactcgcccggtggtgcccagagcaccgcccgcgcccaccctgaccgcccacctaacaacacctggactcgctg
 cgcgagctgttcgcccgaacaaggcgagattgcccgtgtagatcctggagcccgtggcggaacagcggtctc
 attgtgcccaccaaaggagttcctgcagggcctgcgcgagatctgcacggctgagggcgccgctgctgtgcttcgat
 gaggtcatgacggcttcgcgattgccaagggtgcgcccaggagcacttcggtatcaccccgacctgaccacc
 atgggcaagggtcatgggtggcgcatgctgtggcgccctacggcgccgaagaaggagatcatgaagatggtcgcc
 ccgcccggccccatgtaccaggccggcaccctttcgggcaaccccatggccatgactgcccgcattcaagacgtg
 gagatcctgggcccggcgccctacgagcactgggagaaggtgaccaagcgctgatcgacggcatcatggcc
 gccgccaaggagcacagccacgagatcacccggcggaacatcagcgccatgtttggctctctctctgcaagggc
 cctgtgacctgcttcgaggacgcctggcgccgacactgccaagttcgcgcgcttcacccggcgcatgctggag
 gaggcgctctacctggctcctcgagttcgaggcggttcacctctctggcccactccgaggcggaagctggat
 gccacgatcgccgcgctcgccgctgttcgcccgcattctaa

Glutamate-1-semialdehyde aminotransferase (GSA) amino acid sequence (SEQ ID NO:**10):**

MQMQLNAKTQGAFAQRPVSVRGVAVRAVAAPPKLVTKRSEEIFKEAQELLPGGVNSPVRFRSVGGGPVFD
 RVKGAYCWDVDGNKYIDYVGSWGPACGHGNDVNNALKAQIDKGTSGFAPCELENVLAKMVIDRVPVEMVRFV
 SSGTEACLSVLRMLRAYTGREKVLKFTGCGYHGHADSLVKAGSGVITLGLPDSGVPKSTAAATLTATYNNLDSV
 RELFAANKGEIAGVILEPVVGNNGFIVPTKEFLQGLREICTAEGAVLCFDEVMTGFRIAKGCAQEHFGITPDLTT
 MGKVIGGMPVGGAYGKKKIMKMPVAPAGPMYQAGTSLGNPMAMTAGIKTLEILGRPGAYEHLKVKTKRLIDGIMA
 AAKEHSHEITGGNISGMFGFFCKGPVTCFEDALAADTAKFARFHRGMLLEGGVYLAPSQFEAGFTSLAHSEADVD
 ATIAAARRVFARI

glutamyl-trna reductase (HEMA) nucleic acid sequence (SEQ ID NO: 11):

atgcagaccactatgcagcagcgtctccaggccgtaacgtggccggggcgagcgtcgctccctcggtccctgcc
 catcgctccttccactcacaccgggtcgccactcaaacgcgtacgatcagcgctgctgctagctcaaccaccaag
 ctgccagcttcgcatctggagagcagcaagaaggcgctggattcgctgaagcagcaggccgctcaatcgctacgcg
 ggtgacaagaagagctccattattgccattggtctcaccattcacacgcacccgtggagctgcccgcgagaagctg
 gctgtgctcgaggtgaatggccgctgctattgaggagctctgccagtccccgcacatcgaggaggccgggtg
 ctgtcgacgtgcaatcgcatggagctctacgttgtcggtctgtcggtggcaccgcccgttcgagaggtggaggag
 tggctgtctcgaccagcgcgctgctctggatgagctgcgcctcactgttctgctgctgcgcgaccgcgacgcc
 acgcaccactgatgcgcgtgtcggtggccttgactcgctggttatggcgagggccagattctcgcccaagtgcg
 cgccaggtctacaaggtcgccagaaactgcccggcttcggtcgccacctgaacggccctgttcaagcaggtatc
 accgctggcaagcgctgctgcccagacctccatctccaccggctccgtctccgtctcatccgcccgcgtcgag
 ctggcgagctcaagctccccaccacaactggctcgacgctaaggtctgcatcatcgccgctggcaagatgtct
 acgctgctggtgaagcactgcagagcaagggtgcaaggaggtgacggtgctcaaccgctctctgcccgcgcgc

caggcgctggcgaggaggttccctgaggtcaagttcaacatccacctgatgccccacctgctgcagtgcggtggag
 gccagcgacgtcatcttcgcccgcctccggctctgaggagatcctcatccacaaggagcatgtcgaggccatgtcc
 aagccatcggacgttggtggctccaagcgccgcttcgctgacatctccgtgccccgcaacatcgcccccgccatc
 aacgagctggagcacggcatcgtctacaacgtcgacgacctgaaggaggttggtggccgccaacaaggagggccgc
 ggcgagcgccgcccaggccgaggtgctgatccgagaggagcagcgcgcttcgaggccctggcgctgactctctg
 gagaccgtgcccaccatcaaggcgctgcgctccaaggccgagaccatccgcgcgcgcgagtttgagaaggccgtg
 tctcgctggcgaggggctatccaagaagcagctcaaggcggtggaggagctcagcaagggcacgtcgaacaag
 ctgctgcacgggcccattgacggcactgcgctgcgacggcaccgatccggatgcccgtgggcccagaccctcggaac
 atggaggccctggagcgcatgttccagctctcgaggtggacgtggccgcgctggcggggcaagcagtaa

glutamyl-trna reductase (HEMA) amino acid sequence (SEQ ID NO: 12):

MQTTMQRLQGRNVAGRSVAPSPAHRSFHSRAATQTATISAAASSTTKLPASHLESSKKALDSLKQQAVNRYA
 GDKKSSIIAIGLTIHNAPVELREKLAVPEAEWPRAIEELCQFPHIEEAVALSTCNRMELYVVGLSWHRGVREVEE
 WLSRTSGVPLDELFPYLFLLRDRDATHHLMRVSGGLDSLVMGEQILAQVRQVYKVGQNCPGFGRHLNGLFKQAI
 TAGKRVRAETSI STGSVSVSSAAVELAQLKLPTHNWSDAKVCII GAGKMSTLLVKHLQSKGCKEVTVLNRSLPRA
 QALAEFFPEVKFNIHLMFDLLQCVESDVI FAASGSEBILIHKEHVEAMSKPSDVVGSKRFRVDISVPRNIAPAI
 NELEHGI VYNVDLKEVVAANKEGRAQAAAAEVL IREEQRAFEAWRDSLETVPTIKALRSKAETIRAAEFKAV
 SRLGEGLSKKQLKAVEELSKGIVNKLHGPMTALRCGTDPDVAGQTLANMEALERMFQLSEVDVAALAGKQ

Light independent protochlorophyllide reductase subunit N (ch1N) nucleic acid

sequence (SEQ ID NO: 13):

atgttatactcacaatttaaacattcgggtgcctttaggcgctaagtctcccccttctttcagggggcccccccttct
 ggggggtcgccccacaacggctgcctcaggcctaggtcgcaacgtggccgtaagaattgggaccccggtgggcttt
 gcccttcgggcccaggtaattatggcagctgcgggcaatactagcgggtgcgcgcaccccgtaggggagtcaccag
 cctgcgttctgccagggtgattctcaacttgaattgagtgtaaaacaggaaattaccatactttttgcccaatt
 agttgtgtttcttggttataccaaaaaattgaagatagttttttcttagttattgggtacaaaaacgtgtgggtat
 tttttacaaaatgctttaggggttatgatttttgccgaacctcgttacgctatggcggaatttagaagaaagcgat
 atttcggcgcaattaaatgattacaaagaattaaaaacgtctatgtttacaaaattaaacaagaccgtaacccaagt
 gttattgtgtggattggcacatgcacaaccgaaatttataaaatggatttagaaggtagtgccaccgaaactagaa
 gctgaaatcggtattccaattgtggttagcacgcgcaaatggacttgattatgcttttacacaagggtgaagatact
 gtttttagctgcgatggttcaaaaatgcccggaattaggcgctattccagctattgtacacctcagattccttctgac
 tctcgtacacttagccaactatctgtagcggcttcggtacccgaaaacagtgcgctctgggcccagaaggggagcct
 tcactagcccagaaggggaatggatttctaagttaacaacaactctccatgcccagtagattctgtctcagaattct
 accccgcgctttcctggacgtgctccgcacgtcgggaaaagtactcctcaaaatttagttttatttggttcatta
 cctagcacgatggcgaatcaactggagtttgaattaaaacgccaaggtattaatgttactgggtggttacctgcg
 gctcgtattcatctttacctgcattaggtgaaaacgtgtatgtttgtgggattaatccatttttaagtcgaact
 gctacttctttaatgctcgtcgttaaatgcaaatatttcagctcctttcccaattgggtccagatggtacaaaa
 gcttggtcgaaaaaatttgtaattgtttcggtgtttacaccaactgggttagaagatcgtagacgtcttggttg
 gaaggtttaaaagattatttaaatctcgtaaaagggaaatctgtttctttatgggtgataatctgttagaaatt
 tcattagcccggttttttaattcgctgtggtatgaccgtttatgaaatcggtattccgtacatggaccaacgattt
 caagctggggaattagaattattaaaaaaaacatgcatggaaatgaacgtgccccctaccgcgtattgttgaaaaa
 cctgataattactatcaaattcaacgtatttaagaattacaaccagatttagttattaccggcatggcccatgca
 aaccactggaagcgcgccgattactacgaaatggcgttgaaattacgttttcgcaaaattcatgggttggc
 aacgcacgtgatatttagaatttagttacaaaacggttacgtcgtataaaaaatctatctaaatatcaatttccg
 ttagatagctgggacaagcctgcttcctgtagcgctcacgaactgtcgccctaa

Light independent protochlorophyllide reductase subunit N (ch1N) amino acid sequence

(SEQ ID NO: 14):

MLYSQFKHSVPLGRKSPLLSGGPPSGGRPTTASGLGRNVAVRIGTPLGFALRAQVIMAAAGNTSGAPHVVGESQ
 PALSQVDSQLVIECTGNYHTFCPI SCVSWLYQKIEDSFFLVIGTKTCGYFLQNALGVMI FAEPYMAAELEESD
 ISAQ LNDYKELKRLCLQIKQDRNPSVIVWIGTCTTEII KMDLEGMAPKLEAEIGIPIVVARANGLDYAFTQGEDT
 VLAAMVQKCP ELGAIPAIVPQIPSDSRTLSQLSVAASVPENSAGSGPEGEPSLAQKGMDSKLTNNSPCRVDSVSES
 TPAFPGRAPHVKGSTPQNLVLFGLPSTMANQLEFELKRQGINVTGWLPAARYSSLPALGENVYVCGINPFLSRT
 ATSLMRRRKCKLISAPFPIGPDGTKAWVEKICNVFGVTPTGLEDRERLVWEGLKDYLNFKVKGKSVFMGDNLLBI

SLARFLIRCGMTVYEIGIPYMDQRFQAGELELLKKTCEMMNVPLPRIVEKPDNYYQIQRIKELQPDLVITGMAHANPLEARGITTTKWSVEFTFAQIHGFGNARDILELVTKPLRRNKNLSKYQFPLDSWDKPAVSGAHELSEA

Light Independent protochlorophyllide subunit B (ch1B) nucleic acid sequence (SEQ ID

NO: 15):

atgaaattagcgtattggatgtatgcgggacgggctcatattggaacattacgagttgcaagctcggttcgaaatgtgcatgctattatgcatgctcccttaggcgatgattattttaacgtaatgcgttcaatgttagaacgtgaacgtgattttacgccagtgacggcaagatattgttgatcgtcatgttttagctcgtgggtcacaaagaaaagtgtgtgaaacattcaacgaaaagataaagaagaatgtccggatttaattttattaacaccaacatgtacctcaagttttgcaagaagattttacaaaattttgtaaaatcgccggcggaagtagcaaagcgttcggatgttttattagctgacgttaaccattaccgagtgaaatgaattacaagcggctgaccgtacgttagagcaaaattgtacgcttttatttagaaaaagaagtaaaacttcacgcgaggttaggcggccttaaaaaacgcttcgctttgcccagcgtacccaaaagccgtctgccaatatttttaggcattgtttacactaggtttccataatcaacatgactgtcgtgaattaaaaacggtttattaatgatttaggtatcgaagtcaatgaagtgattcctgaaggtagttttgtacatggattaaaaaatttaccaaaaacgctgggttttaacatcgctccggtatcgtgaagttgggttaattgacggcaatttttagaaaaagaatttggcattgccttatacctcaatcacgccaatgggcattattgacacccggcggtttattcgtgaaattgcggccattttagtcaaataggcacttcacaggcattctacaaactcaactgaaggactccagaggggagaaaatgtcagtttaactgaaactaattcgattattttaataaagcaaaaatatagaacaatacattaatcaacaaacgcattttgtttctcaagcagcttggttttcaagttctattgactgtcaaaatttaaccggtaaaaaaacggttggtttggatgcaactcagcggaagtatgacgaaaattcctgtgcgcgaaaatgggtattcatgtgttttgcgcgggcagctattgtaaacatgatgcagattgggttagagagcaagtttcaggtttttgtgatcaagttttaattacagatgatcacagccaaattgcggaaatcattgctcaaatggaacctgcagccatttttggtacacaaatggaacgtcatgttgggaaaaggttagatattcctgtgggtttattctgcaccggtacataattcaaaacttcccactaggttttagaccgttttaggttatgaaaggtactaatcaaatttccgatttagtttataattcgttttagtttaggtatggaagatcacttactagaaattttcaacgggtcatgacaataaagaagttattacacgttcgtattcttcagaaactgatttagaatggacaaaaagagcattagatgaactagctcgtgttcctgggtttgttcggtcaaaagttaaacgtaatactgaaaaatttgcgcgtacaaaataaaaatcaagttattactattgaagttatgtacgcagctaaagaagcggatcagcgtaa

Light Independent protochlorophyllide subunit B (ch1B) amino acid sequence (SEQ ID

NO: 16):

MKLAYWMYAGPAHIGTLRVASSFRNVHAIMHAPLGDDYFNVMSMLERERDFTPTASIVDRHVLARGSQEKVVENIQRKDEECPDILLLPTCTSSILQEDLQNFVNRAAEVAKRSDVLLADVNHVRVNELQAADRTLEQIVRFYLEKEVNKLHAEGLGGLKKPLRFAQRTQKPSANILGMFTLGFHNQHDRELKRLNLDLGIENVNEIPEGSFVHGLKNLPKAWFNIVPYREVGLMTAIYLEKEFGMPYTSITPMGIIDTAAFIREIAAICSQISTSQASTNSTEGLQRGENVSLTETNSIIFNKAKYEQYINQTHFVSQAAWFSRSIDCQNLTKGKTVVFGDATHAASMTKILVREMGIHVVCAGTYCKHDADWFREQVSGFCDQVLI TDDHSQIAEIIAQIEPAATFGTQMERHVGKRLDIPCGVISAPVHIQNFLGFRPFLGYEGTNQISDLVYNSFSLGMEDHLLLEIFNGHDNKEVITRSYSSETDLEWTKEALDELARVPGFVRSKVKRNTKFA RTNKNQVITIEVMYAAKEAVSA

Light independent protochlorophyllide reductase subunit L (ch1L) nucleic acid

sequence (SEQ ID NO: 17):

atgaaattagcagtttatggcaaaaggtggtattggtaaatccacaacaagttgtaacatttcaattgcattagcaaaacgtggcaaaaaagttacaaaattgggtgtgatccaaaacacgatagttttacattaaacgggtttttatattccaacaattattgatacttttacaagtaaaagattatcattacgaagatgtttggccggaagatgttatttaccaaggctacgggagtggtgattgtgtgtaagcaggtggcccgccagccggcgccggtgtggtgggtatgtgttggtgaaacagttaaattattaaaagaattaaatgcattttatgaatatgatgttattctgtttgatgttttaggggatgttgatgtggtgggtttgctgcacctttaaattacgcgcgactattgcattattgtcacagataatggctttgatgcgttatttgcgcgcaaacggtattgctgcttcagtgccgcaaaaagcgcgcatcaccattacgttttagctgggttaattgggaatcgtacagccaaacgcgatttaatcgataaaatcgttgaagcgtgcccgatgccagtttagaggtattaccggttaattgaagacattcgtgtgtcacgcgtaaaagggtaaaacattatttgaatggcagaacatgattcatcattacactacatttgtgacttttatttaaatattgcggatcaattattaaactgaaccagaaggtgtgttccgcgcgcaatttagcagaccgtgaattatttactctattatcagattttctatttaaactgctgggactcctagcctagtggtatcgtgagttcggctcaggcgcccttagcggaacgagcggcgaaacagctcccggtaatatgggtcagcacatgagtaaacgcagtaaaaaacgaacaggaaatgaatttctttctgtgttaa

Light independent protochlorophyllide reductase subunit L (chlL) amino acid sequence (SEQ ID NO: 18):

MKLAVYKGGIGKSTTSCNISIALAKRGKKVLQIGCDPKHDSFTLTGFLIPTIIDTLQSKDYHYEDVWPEDVIY
QGYGSVDCVEAGPPAGAGCGGYVVGETVKLLKELNAFYEDVILFDVLGDVVCGGFAAPLNYADYCIIVTDNGF
DALFAANRIAASVREKARIHPLRLAGLIGNRTAKRDLIDKYVEACMPFVLEVLPLIEDIRSVRVKGTFLFEMAEH
DSSLHYTCDFYLNADQLLTEPEGVVPRELADRELFTLLSDFYLNAGTSPSPSGSEFGSGALSGTSGETAPNGMQ
HMSNAVKTNEQEMNFFLV

Magnesium Chelatase subunit H (CHLH2) nucleic acid sequence (SEQ ID NO: 19):

atgcccattgtgctgggtcagcggcctcgagagctttaacgtgggcctgtacaaggatgcccggagctgctgaag
cgctccatgcccacgtcacctccagggttctccgaccgagacctggcctccgacgcccccgcctcccgctg
gaggcggctctggggcgccgcgacatcttctcggatcactgctgttcgactacgaccaggtggagtggctacgg
gcccggctggagcgggtgctgtgctggctagtgtttgagtcggcgttgagctcatgagctgcaacaagggtgggg
tcgttcatgatggggcgccggcgtcccggcgccggcccgccggcaaggcgcccgcccgcccgcccgccgggtgaag
aaggttctctccatgtttggaagcggtcgagaggagacaagatgggcggctcctccaatgtggtggccatgttc
agttacctggtggagacctgatggagccaaagggtgggttatgttgtagtggtggtgtgttatggtggccg
tttcggttgggtgatctgggtggtatctacaacccccctcaacctcacgcctccaggctacgtgcccgcgcct
gtggtggagactccgcactgggtgctcctccacctccgcgcggcgccgctactcgagctcccgcgcgagtac
atgaagtggtagccaggagggcccgctgcccggcagggcgcccggtgggtggcggtgctgctgtaccgcaag
catgtgatcccgaccagcgtacatcccgagctggtcagccagctggaggcgagggggtgctgcccgtgccc
atcttcatcaacggcgtggaggcgacacccgtggttcgcgacctgctgacctccgtgcacgagcaggatctgctt
gcacgcggcgagacgggcgccatcagccccacccctgaagcgggacgcggtcaagggtggacgcggtggtgagcacc
attggcttcccgtggtgggcggcccgccggcaccatggaggggcgccggcaggcgagggtggccaaggccatc
ctgggcgccaaggagcgtgcccgtacacgggtggcgcccgctgcttattcaggacatggagagctggagcaggga
ggcgtggcggtctccagagtgtggtgctgtactcgtgcccggagctggacggcgagtggaacacggtgccactg
ggggggctggtgggggacgacatctacctggtgcccggagcgggtgaagaagctggcgggcggtcgaagtctggtg
cgtacgacacgactaagcatgctctgtttgtgagctccagccctcccccccgctctccctctccacctc
cctctcccttctctcccttctctcactctccacctcttccccctccgcccacataacgaggcgggggctg
ctgggcgccaaggcgccctggagtacccgctgacacctagctagtccaaactccacctcccccaatgcccgaat
agcttccggagatgagcac
cgccaccacacgac
ctgctgacgtaccccgcgactggggcccgccgagtggggcccgtgcccctacctgcccgaaccccgacgtgctg
gttcgcgcgcatggaggcgagctggggcgagctgcgagcctaccgcgccctcaacacctcggcgcgccgcatgttc
caggagtacggggtgacgtggttctgcacttcggcatgcacggcaccgtggagtgggtgctggggcgccgctg
gggaacaaaggcctcagctggagcgagctgctgctcggcgagctgccaaacgtgtacgtgtacgtgccaaac
ccctccgagctccatcgtggcaaggcgccgctacggcaccatcgtcagccacaacgtgcccgcgtacggcgcg
gccccgtctgtacaagcagcttccagcctcaaggagacgcttcaggagtagccgagggccgagccgagccgacgt
gcccagcaggagccagc
agtggtggagctgcccggcggtggcaccggtgttcgacgcctacactgaccgctgtatgcctacctgcagctg
ctggaggggcggtgttcagcgaggggctacacgtactgggagcgccgcccggcgccgaggtgggtggtttt
cccgcgagcttccaacggtaccgtaaactgcccactgcccacttctccccaaacacaggaggctgtcaagatc
cggaaacctgctcatgcagaacacgcaggagctggacgggctgctcaaggccctgggtggcggttacgtgcttccc
gaggcggtggcgacccgtgctgcccagcgggtcgggcggtgctgcccacggcgccgaacatccacgactggacccc
taaccgcatgcccctcccccgcccatggcccggtggggcgcggtggcgcgcccatctctgagcagcaccggcg
gctaacagcggggcggtggcccagacctgcgcgctcaacctgtgggggtggactccatcaagagcaaggcgag
agtggtgggggtggtgctggcgctggtggggcggtgcccgtgcccggaggtacgggcccgtcgcgcgcttccaa
ctgggtgcccgtgtcagagttgggcccggcgctgtggagctgcttgttaacatgagcggcatcttcgcgactcc
ttccagaacgtggtggagctgctcgacgacctgttgaaggggcgccgcccggctgacgagccagatgacatg
aacttcatcgccaaacacgcccagccatggagaagcaggccgtgcccgaacctcgcccgccctgttctccaaac
ccggctggcgactacgggtcgatggtcaacgagcagtggggcaggcgagctgggccaacggcgagcagctgggt
gacacgtgggcggcccgcaacgccttcagctacggcgaggcaaggagcagggcacggcgcgccgaggtgctg
caggcgctgctcaagaccagggaccggtatcgtgacgagatcgacagtggtgagtagggcctgacagacatccag
gagtagtagccaaacagggcgccctcaagagagccgcccaggtggccaaaggcgaccggggcccggtggccgg
cgcccgcgctgggggtgttccattgtggaggccttggcgcgcgggcgcgggcgcgggcgcgcggtggagcg
ggcggtgcccgcgctcgcgagctggaggaggtgctgcccctggagtaccgctcgaagctgctcaaccccaagtgg
gcccggccatggcgcgcgcgccgagcggcgccctacgagatcagtcagcgcatgacggcggtgtgtgggtgg
ggcgccaccacgatttcaggagggggtgggtggtgggacccaggcgccatggacacgtatgtggcgatgaggag
atggccagcaagctcaagaagaacaccccgaggccttggccaaactgctgcccgcgcatgctggaggcgcgggc

cgcgccatgtggagccccaacaaggaccagctggcacagctcaagtcgctgtacagcgagatggacgaccagctg
gaggggggtgacg

Magnesium Chelatase subunit H (CHLH2) amino acid sequence (SEQ ID NO: 20):

MRIVLVSGFESFNVGLYKDAEELLKRSMPNVTLQVFSDRDLASDATRSRLEAALGRADIFFGSLLFDDYDQVEWLR
ARLERVPVRLVFESALELMSCNKVGSFMMGGGGPGGGPPGKAPGPPPAVKKVLMSFGSGREEDKMGGSSNVVAMF
SYLVETLMEPTGGLFSGSWWLCYGWPFRLGDLGWYLQPPSTLTPPGYVPPPVVETPALGCLHPSAPGRYFESPAEY
MKWYAREGRLRGTAAPVGVLLYRKHVIDTQPYIPQLVSLQEAEGLLVPVIFINGVEAHTVVRDLLTSVHEQDLL
ARGETGAI SPTLKRDAVKVDAVVSTIGFPLVGGPAGTMEGGRQAEVAKAILGAKDVPYTVAAPLLIQDMESWSRD
GVAGLQSVVLYSLPELDGAVDTVPLGGLVGDDIYLVPERVKKLAGRLKSWRTTRTKHASVCDVQPLPPPSPLSTL
PLPSSPFLSLSTLFLRPNITRRGLLGASGPWSTRCDLASPTPPIQCRNSFPEMSTHTHTHTHTHTHTHTHT
RHPRTHHTHTHAPPARHTPIPPHPQELLTYPADWGAEWGGLPYLPDPLVRRMEAQWGE LRAYRGLNTSARGMF
QEYGADVVLHFGMHGTVEWLPGAPLGNGLSWSDVLLGELPNVYVYAANNPSESIVAKRRGYGTIVSHNVPPYGR
AGLYKQLSSSLKETLQEYREAAQAARARAGASSSSSSSSSSSSSSSSSSSVELRAALAPVFDAYTDRLYAYLQL
LEGRLFSEGLHVLGAPPAPPQVGGFPASQRYRKLNCPTSPQTQEAVKIRNLLMQNTQELDGLLKLGLGGRYVLP
EAGDILLRDGSGVLPTGRNIHALDPYRMPSPAAMARGAAVAAAILEQHRAANSQWGPETCAVNLWGLDSIKSKGE
SVGVVLLAVGAVPVREGTGRVARFQLVPLSELGRPRVDVLCNMSGIFRDSFQNVVELLDDLFARAAAAADEPDDM
NFIAXHARAMEKQGLSATSARLFSNPAGDYGSVMNERVGGSWANGDELGDTWAARNAFSYGRGKERGTARPEVL
QALLKTTDRIVQQIDSVEYGLTDIQEYYANTGALKRAAEVAKGDPGPGGRRPRVGCSTIVEAFGGAGAGAGGAGGA
GVPPPREELEVLRLEYRSKLLNPKWARAMAAQSGGAYEISQRTALVWGATTDFREGVWVDPGAMDTYVGDDE
MASKLKKNNPQAFANVLRRLMEAAGRGMWSPNKDQLAQLKSLYSEMDDQLEGVT

**Magnesium Chelatase subunit 1 (CHL1) *Chlamydomonas reinhardtii* nucleic acid
sequence (SEQ ID NO: 21):**

atggccctgaacatgcgtgtttcctcttcccaaggtcgctgcccaagcagcagggccgcgcatctccgcggtgccggtt
gtgtcgagcaaggtggcctcctccgcccgcgtggccccctccagggcgctcccgtggccgcgcagcgcgctgct
ctgctggtgcgcgcgcgtgccgctactgaggtcaaggctgctgagggccgcactgagaaggagctgggcccaggcc
cgccccatcttccccttcacccgcatcggtgggcccaggatgagatgaagctggcgctgattctgaacgtgatcgac
cccaagatcggtggtgtcatgatcatgggcgaccgtggcactggcaagtccaccaccattcgtgccctggcggt
ctgctgcccagatgcaggtggttgccaacgacccctttaactcggacccccaccgacccccgagctgatgagcgag
gaggtgcgcaaccgcgtcaaggccggcgagcagctgcccggtcttccaagaagattcccatgggtggacctgccc
ctgggcccactgaggaccgcgtgtgcggcaccatcgacatcgagaaggcgctgaccgaggggtgtcaaggcggttc
gagccggcgctgtggccaaaggccaaccgcggcatcctgtacgtggatgaggtcaacctgctggacgaccacctg
gtcgatgtgctgctggactcggccgcctccggctggaaacaccgtggagcgcgaggggtatctccatcagccacccc
gcccgttcatcctggtcggtcggtgcaaccccgaggaggggtgagctgcgccccagctgctggatcgcttcggc
atgcacgcccagatcggcaccgtcaaggacccccgcctgctgctgcagatcggtgcgcagcgctcgacctcgac
gagaaccccgccgcttccgcaaggactacgaggccggccagatggcgctgaccagcgcatcggtggacgcgcgc
aagctgctgaagcagggcgaggtcaactacgaacttccgcgtcaagatcagccagatctgctcggacctgaacgtg
gacggcatccgcgccgacatcggtaccaaccgcgcgcgcaaggccctggccgcttcgagggccgcaccgaggtg
acccccgaggacatctaccgtgtcatctccctgtgctgcccaccgcctccgaaagacccccctggctgagatc
gacgacgggtgaccgcgtgctgagatcttcaagcaggtgttcggcatggagtaa

**Magnesium Chelatase subunit 1 (CHL1) *Chlamydomonas reinhardtii* amino acid
sequence (SEQ ID NO: 22):**

MALNMRVSSSKVAAKQQGRI SAVPVSSKVVASSARVAPFQGAPVAAQRAALLVRAAAATEVKAAEGRTEKELGQA
RPIFPFTAIVGQDEMKLALILNVIDPKIGVMIMGDRGTGKSTTIRALADLLPEMQVVANDPFNSDPTDPELMSE
EVRNRVKAGEQLPVSSKKIPMVDLPLGATEDRVCCTIDIEKALTEGVKAFEPGLLAKANRGI LYVDEVNLLDDHL
VDVLLD SAASGWN TVEREGISISHPARFILVSGSNPEEGELRPQLLDRFGMHAQIGTVKDPRLRVQIVSQRSTFD
ENPAAFRKDYEAGQMAL TQRIVDARKLLKQGEVNYDFRVKISQICSDLNVDGIRGDIVTNRAAKALAAFEGRTEV
TPEDIYRVIPLCLRHRLRKDPLAEIDGDRVREIFKQVFGME

sequence (SEQ ID NO: 23):

Magnesium Chelatase sunubit1 (CHLI2) *Chlamydomonas reinhardtii* amino acid

MQSLQGQRAFTAVRQGRAGFLRTRLVVRSSVALPSTKAAKPNFPFVKIQGQEMKLALLLNVDPNIGGVLIMG
DRGTAKSVAVRALVMDLMDLPDIDVVEGDAFNSSPTDPKFMGPDTLQFRNGEKLPTVMRMTPLVLPGLATEDIRCG
TIDIEKALTQGIKAYEPGLLAKNAGRIJLYVDENVLLDGLVDVLDLSSAGSLNTEVERGVSVIHPARFIMTGTSGN
PQEGELRPQLLDRFGMSVNVATLQDTKQRTQLVLDRLAYEADPDFAFVDSCKAEQTALTDKLEAARQLRSVKISE
ELQILISDIKICSLRLDGLRGDINVIRAAKALVAFEGREVTNTDNERVVISGCLNHLRLKDPDLPIDNGTKVAILF
KRMTDPTSMKREEEAKKREEEAAKAKGADRPAGAKAGAWGLPPRR

sequence (SEQ ID NO: 25):

46

ctgaaccgcatgagcgccgccaagggcgctgcatgcgcctgctggctgagtcgtacaccagccgcgaccaggtg
 tgccctcatcccccttctacggcgacaagggcgaggtgctgctgcccgcctccaagtccatcgccatggcccgccgc
 cgccctggactcgctgcccctgcccggcgggctcgccccttgccgacggcctgtccacggcggtacgtgtgggcatg
 caggccagccagggcgaggtggcgccgctcatgatggtgctcatcacggacggccgcgccaacgtcagcctg
 gccaaagtcacaagaggaccccgagggcgctcaagcccgcacgcgcccagcccacggccgactcgctgaaggacgag
 gtgcgcgacatggccaagaagggcgctcgccggcatcaacgtgcttgctcattgacacggagacaagttcgtg
 agcacccggctttgcccggagagatctccaaggcagcgccagggcaagtactactacctgccccacggcagcgcc
 gccatcgccggcgccgctcgccgcccattggccggcgccaagggcggtactag

Magnesium Chelatase subunit D (CHLD) *Chlamydomonas reinhardtii* amino acid

sequence (SEQ ID NO: 26):

MKSLCHELAGPSVTGCGRRSLRKAFSGAKIAQVSRPAVLNSVQRQRLACSAVAELSAELRAMKVSEEDSKGFD
 ADVSTRILARSYPAAVVGQDNIKQALLLGAVDTGLGGTAIAGRRGTAKSIMARGLHALLPPIEVVEGSI CNADPE
 DPRSWEAGLAEKYAGGPVKTKMRSAPFVQIPLGVTEDRLVGTVDIEASMKEGKTVFPGLLAEAHRGILYVDEIN
 LDDGIANLLLSILSDGVNVVEREGTISISHPCRPLLIATYNPEEGPLREHLLDRIAIGLSADVPSTSDERVKAID
 AAIRFQDKPQDTIDDTAELTDALRTSVILAREYLKDVITIAPEQVTYIVEEARRGGVQGHRAELYAVKCAKACAAL
 EGRERVNKDDLRLQAVQLVILPRATILDQPPPEQEQLPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPP
 EPEIPQEFMFSEGVIMDSPILMFAQQQQRAGRSRAKTLIFSDDRGRIYKPMPLPKGDKVKRLAVDATLRAAAP
 YQKIRRQQAISEGKVQRKYVVDKPDMSKRLARKAGALVIFVVDASGSMALNRMSAAKGACMRLLAESYTSRDQV
 CLIPFYGDKAIEVLLPPSKSIAMARRRLDSLPCGGGSLAHGLSTAVRVMQASQAGEVGRVMMVLI TDGRANVSL
 AKSNEDPEALPKPADPKPTADSLKDEVDRMAKKAASAGINVLVIDTENKFVSTGFABEISKAAQGYKYYLPNASDA
 ATAAAASGAMAAAKGGY

Magnesium Chelatase subunit H (CHLH1) *Chlamydomonas reinhardtii* nucleic acid

sequence (SEQ ID NO: 27):

atgcagacttctctcgcttcttgccggcgccacggcccccacccggctgcccggcgccagcccaagccgggttgccccc
 tcgcccgcgctggctagcaccgcgaggtcgctgcaatgtggcgactggaccccgccgcccattgaccaccttc
 accgggtggcaacaagggccctgctaacgagcaggtgtcgctggatctgcgcgacgagggcgctggcatgttcacc
 agcaccagcccggagatgcccgtgtcgctccctgacgatgtgaagggctcgcgtaaggtgaaggttggtgacgtg
 gtgctggaggccagtagcagtcggccatcagcgctcggtgaagaacatcaacgccaagaactccaaggtgtgc
 ttcgaggtggtgggctacctgctggaggagctgctgaccagaagaacctcgatagctcaaggaggatgtggcc
 tctgccaaacatcttcacggctcgctcatcttcattgaggagcttgccgagaagattgtggaggcggtgagcccc
 ctgcccgcgagaagctggacgcgtgctgatcttcccgtccatgcggcggtcatgaagctgaacaagctgggcacg
 ttttcgatggctcagctgggccagtcgaagtcgggtgttctcgaggttcacaaagtctgctcgcaagaacaacgac
 aacttcgaggagggttgctgaagctggtgcccacccctgcctaaggtgctgaagtatctgcctcggacaaggcg
 caggacgccaaagaacttcgtgaacagcctgcagtaactggcgcggttaactcggacaacactggagaacctgtg
 ctgaacaccgtcagcaactacgtgcccgtctgaaggcggtggaacttcagcggtggtgagccccacggcctacccc
 gatgtgggtatctggccacccctctggcctcgggcatgtacgaggacctgaaggagtacctgaactggtacgacacc
 cgcaaggacatggtcttcgcccaaggacgcccccgctcattggcctggtgctgcagcgctcgcaacctggtgactggc
 gatgaggggccactacagcggcggtggtcgctgagctggagagccgggtgctaaagtcacccccgtcttgccgggt
 ggccctggaacttctccgcccccgtaagaagtcttctacgacccccctgggctctggccgcacgttcgtggacacc
 gtgtgtgctgctgacgggttcgctggtggggcgcccccgcgccaggaacgcccgaaggccattgaggcgctg
 aagaacctgaacgtgcccctacctggtgtcgctgcccgtggtgttcagaccactgaggagtggtcggacagcgag
 ctggcgctgcaccccgctcaggtggtctgacaggttgcctgcccagctggatggtgcatggagcccatcgctg
 ttcgctggcggtgactcgaacaccggcaagtgcactcgctgcccgcacgcacatcgcttcgtgtgctgctcgccgc
 gtgaactggggccaacctgcgcaagaagcgcaacggcgagaagaagctggccgtcacctgttcagcttccccct
 gacaagggaacgtcgccactgcccctacctgaacgtgttcggctccatctaccgctgctgaagaacctgcag
 cgcgagggtacgacgtggcgccctgcgcgcctcgaggaggatctgatccagtcgggtgctgacccagaaggag
 gccaaagttcaactcgaccgacctgcacatcgctacaagatgaaggtggacgagtagaccagaagctgtgcccctac
 gccgaggcgctggaggagaactggggcaagcccccgccacccctgaacaccaacggccaggagctgctggtgtac
 ggccgcccagtagggcaacgtcttcacggcgctgcagcccaccttcggctacgaggggcagcccgatgcccgtgctg
 ttctcgaaagtcggccagccccaccacggcttcgcccgcctactacaccttctcggagaagatcttcaaggccgac
 gccgtgctgcaacttcggcaccacggctcgctggagttcatgcccggcaagcaggtcgccatgtcggtgtgtgc
 taccggcagctcgctgacggcaccatccccacctctactactacgcgcgccaacaacccgtctgaggccaccatc
 gccaggcgccgctcgtagcccaacaccatttcgtacctgacgcgcgctgcgagaaacgcggccgtgtacaagggc
 ctgaaggagctgaaggagctgatcagctcgtagccagggcatgctgagctggccgcgcccagcagatctgcgcc
 accatcatgtgagaccgccaagctgtgcaacctggaccgcgacgtgacctgcccgcgctgacgccaaggacctg

accatggacatgcgcgacagcggttggtgggcccaggtgtaccgcaagctgatggagattgagtcgccgctgctgcc
 tgcggcctgcacgtggtgggctgcccgcacccgcccagggagggcgtggccaccctgggtcaacatcgctgagctg
 gaccgcccggacaacaacccccccatcaagggcatgcccggcatcctggcccgccgcatattggtcgcgacatcgag
 tcgatttacagcggcaacaacaagggcgctcctggctgacgttgaccagctgcagcgcatcaccgaggcctccgc
 acctgctgctgcgcgagttcgtgaaggaccgcacccggcctgaacggccgcatcggcaccacactggatcaccacactg
 ctcaagttcaccggcttctacgtggaccctgggtgcgcggcctgcagaacggcgagtcgccagcgccaaccgc
 gaggagctgatcaccctgttcaactacctggagttctgctgacccaggtggtcaaggacaacgagctgggcgc
 ctggtagaggcgctgaacggccagtagctcgagcccgcccgccgggtgaccccatccgcaacccccaacgtgctg
 cccaccggcaagaacatccacgccttgaccctcagtcgattcccactcaggcccgctgaagagcgcccgctg
 gtgggtggaccgctgctggaccgagcgagcgacaacaacggcggaagtaccccgagaccatcgcgctgggtgctg
 tggggcactgacaacatcaagacctacggcgagtcgctggcccaggtcatgatgatggtcggtgtcaagcccgctg
 gccgacgcccacggcgctgaacaagctggaggtgatccctctggaggagctgggcgcgcccggcgctggacgtg
 gttgtcaactgctcggtgtgttccgcgacctgttcgtgaaccagatgctgctgctggaccgcccacatcaagctg
 gccggccgagcaggacgagcccgatgagatgaacttcgtgcgcaagcacgccaagcagcagcgccggagctgggc
 ctgcagagcctgcgcgacgcccacccgtgtgttctccaacagctcgggctcctactcgtccaacgtcaacctg
 gcggtggagaacacagctggagcgacgagtcgcagctgcaggagatgtacctgaagcgcaagtctacgccttc
 aactcggaacgccccggcgctggcgagatgcagcgcgacgtgttcgagacggccatgaagaccgtggacgtg
 accttccagaacctggactcgtccgagatctcgtgacccgatgtgtcgactacttcgactccgacccaccaaag
 ctgggtggcgctcgtgcgcaacgacggccgaccccccaacgcctacatcgccgacaccaccacggccaacgcgcag
 gtccgcactctgggtgagaccgctgcgcctggacgcccgcaccaagctgctcaacccccaaagtggtagcggggcatg
 ctgcctcgggctacgagggcgctgcgcgagatccagaagcgcatgaccaacaccatgggctggcgccacctcg
 ggcatggtggacaactgggtgtacgacgagggccaactcgaccttcacgaggatgcggccatggccgagcgctg
 atgaacaccaacccccaacgcttcgcgaagctgggtggccaccttcctggaggccaacggcgcggtactgggac
 gccaaagcccgagcagctggagcgctgcgcagctgtacatggacgtggaggacaagattgagggcgctggaataa

Magnesium Chelatase subunit H (CHLH1) *Chlamydomonas reinhardtii* amino acid

sequence (SEQ ID NO: 28):

MQTSLLGRRTAHPAAGATPKPVAPSPRVASTRQVACNVATGPRPMTTFTGNGKPAKQQVSLDLRDEGAMFT
 STSPEMRRVVPDDVKGRVKVKKVYVLEAQYQSAISAAVKNINAKNSKVCFEVVGYLLEELRDQKNLMDLKEDVA
 SANIFIGSLIFIEELAELIVEAVSPLREKLDACLI FSPMPAVMKLNKLGTFSMALGQSKSVFSEFIKSARKNND
 NFEEGLLKLVRTLPKVLKYLPSDKAQDAKNFVNSLQYWLGGNSDNLENLLNTVSNYVPALKGVDVFAEPTAYP
 DVGIWHPLASGMYEDLKEYLNWYDTRKDMVFAKDAPVIGLVLRSHLVTGDEGHYSGVVAELSRGAKVIVFAG
 GLDFSAPVKKFFVDPLGSGRTFVDTVVSLTGFALVGGPARQDAPKAI EALKNLNVPLYVSLPLVFQTEEWLDE
 LGVHPVQVALQVALPELDGAMEPIVFAGRDSNTGKSHSLPDRIASLCARAVNWANLRKKRKAELKLAVTVFSFPP
 DKGNVGTAAAYLNVFGSIYRVLKNLQREGYDVGALPPSEEDLIQSVLTQKEAKFNSTDLHIAYKMKVDEYQKLC
 PYAEALEENWGKPPGTLNTNGQELLVYGRQYGNVFIGVQPTFGYEGDPMRLLFSSKASPHHGFAAYYTFLEKIFKAD
 AVLHFGTHGSLEFMFGKQVGMGSGVCPDLSLIGTIPNLYYAANNPSEATIAKRYSYANTISYLTPPAENAGLYKG
 LKELKELISSYQGMRESGRAEQICATIIETAKLCNLRDVTLPDADAKDLTMDMRDSVVGQVYRKLMBIESRLP
 CGLHVVGCPPTAEAEAVATLVNIAELDRPDNPPKGMPI LARAIGRDIESIYSGNNGVLADVDQLQRITEASR
 TCVREFVKDRGTGLNGRIGTNWITNLLKFTGFYVDPWVRGLQNGEFASANREELITLFNYLEFCLTQVVKDNELGA
 LVEALNGQYVEPGGDPINPNVLP TGKNIHALDPQSIPTQAALKSARLVVDRLDRDRDNNGGKYPETIALVL
 WGTDNIKTYGESLAQVMMVGVKPVADALGRVKNLEVIPLLEELGRPRVDVVNCSGVFRDLFVNQMLLLDRAIKL
 AAQDEPDENMFVRKHAKQQAELGLQSLRDAATRVFSNSSGSYSSNVNLAVENSSWSDESQLEMYLKRKSYAF
 NSDRPGAGGEMQRDVFETAMKTVDVTFQNLDSSEISLTDVSHYFSDPTKLVASLRNDGRTPNAYIADTTTANAQ
 VRTLGETVRDLARTKLLNPKWYEGMLASGYEGVREIQKRMTNTMGWSATSGMVDNWWYDEANSTFIEDAAMAERL
 MNTNPNSPFRKLVAFLFLEANGRGYWDAPKEQLERLRQLYMDVEDKIEGVE

Photochlorophyllide reductase subunit B (ch1B) nucleic acid sequence (SEQ ID NO:

29):

atgaaattagcttatttgatgtacgcaggtcccgcctcatatcggtgtgttgctgttagcagctcttttaaaaaat
 gtacatgccattatgcatgctcctttaggagatgattattttaaatgtaatgcgttccatgttagaacgtgaacgt
 gattttacaccagtaacagccagtagttagatcgatggttttagcaagaggatcgcaagaaaaagtgggtgaa
 aatattacgcgaaaaaataaagaagaaactcctgatttaattttattaactcctacttgtagctcaagcatttta
 caagaagatttacacaattttgtgaatcgccattagctaaaccagtagcaaatagatgaacatgcagaccataaaa
 gtaactcaacaaagtgactttcaagtgtagtcccccttactaccgcttgaaagaaaatacattaatagtagaag
 ctagataagaagcttagcccgcttagcaagttgcatattaatatgcccaatatttgtagtcccggaaggagaaggg
 gaaggggagcagactaaaaaattcaatttttgttaaatctgcaactttaacaaatttgtagaagagggaactatta

aatcaagaacatcataccaaaacaagaaatcactctgacggtatttttagctgatgtaaaccattatcgtgtaaat
gaattacaagctgcagatcgtactcttgaacaaattgtacggtattatatttctcaagcacaaaaacaaaattgt
ttaaacattactaaaacagccaaaccatctgtaaatattattgggtatttttactttgggttttcataatcaacat
gattgtcgtgaattaaaacggtttatttaattgatttaggtattcaaatcaatgaaatcatacctgaaggcggaaat
gtacacaaacttaaaaaaattaccccaagcttggtttaattttgtgccctaccgtgaaattggcttaattgactgct
atgtatttaaaatccgagtttaatatgccttacgtcgcaattactcctatgggattaattgatacggctgctgtg
attcgttcaattttgtaaaaatcattacaactcaattattaaatcagacggctacagtgaggagccatcaaaattt
atttaccggaaggcgagctcattagaacaaaccaatatttctcgaaacctctcaaaaagaaactattcttaagac
aatccagatagcggaaataccctttctacaactgtagaagaaattgaaactttatttaataaatatcgcacaa
caaaactcgtttttgtttcccaagcagccttggtttcacgttctattgactgtcaaaatttaacaggtaaaaaagcc
gtagttttcggagatgctacacattcagctgccatgacaaaattattagcacgtgaaatgggtattaaaggtttca
tgcgctggaaacttattgcaaacacgatgaggattgggtttagagagcaagttagtggtttttgtgatcaagtttta
attaccgatgatcacacacaagtaggggatattggtgcacaattagaacctgcagccattttgggacacaaatg
gaacgtcacgttggttaaacgttttagatatccatgtggtgttatatctgctcctgtgcataattcaaaactttccg
ttaggttatcgaccttttttaggttatgaaggtacaaatcaaatagctgatttagtgataaattcatttaattctt
ggaatggaagaccattattacaaaatttttgagggtcatgattcagaaaacaattcgtcaattgcaacgcatctg
aatacaaataacgcaataaatttagcgccaggatattacctgagggaaggaagcagtagtagaacttcaaatgta
gtgtctacaatttctagtgaaaaaaagccattgtatggtctccagaaggttttagcagaattaaataaagtccca
ggatttctcgaggaaaagttaaacgtaatacgggaaaaatatgctttacaaaaaaattgttcgatgattactgta
gaagttatgtatgcagcaaaaagaagctttgtcggcttaa

Photochlorophyllide reductase subunit B (ch1B) amino acid sequence (SEQ ID NO: 30):

MKLAIFYMYAGPAHIGVLRVSSSFKNVHAIMHAPLGDDYFNVMSMLERERDFTPVTAIVDRHVLARGSQEKVVE
NITRKNKEETPDILLTPTCTSSILQEDLHNFVESALAKPVQIDHADHKVTQQSALSSVPLPLEENTLIVSE
LDKKLSPSSKLHINMPNICIPEGEGEQTKNSIFVKSATLNLSEEEELNQEHTKTRNHSDVILADVNHVRVN
ELQAADRTLEQIVRYIISQAQKQCNLNIKTAKPSVNIIGIFTLGFHNQHDRELKRLFNDLGIQINBIIPEGN
VHNLKKLPQAWFNFPYREIGLMTAMYLKSEFNMPYVAITPMGLIDTAACIRSI CKIITQQLNQTATVQEPSKF
IYPKATSLQETNILETSQKETILKDNPDGNTLSTTVEEIIETLFNKYIDQQTRFVSQAAWFSRSIDCQNLTKKA
VVFGDATHSAAMTKLLAREMGIKVSCAGTYCKHDADWFREQVSGFCDQVLI TDDHTQVGDMAQLEPAAI FGTQM
ERHVGKRLDIPCGVISAPVHIQNFPLGYRPLGYEGTNQIADLVYNSFNLGMDHLLQIFGGHDSENNSSIATHL
NTNNAINLAPGYLPEGEGSSRTSNVVSTISSEKKAIVWSPEGLAELNKVPGFVRGKVKRNTKEYALQKNCSMITV
EVMYAAKEALSA

Photochlorophyllide reductase subunit L (ch1L) nucleic acid sequence (SEQ ID NO:

31):

atgaaattagctgtttacggaaaaggtgggtattggaaaatcaacgacaagttgtaatatatttcgattgctttacga
aaacgtggtaaaaaagtggtacaaattgggtgtgatcctaaacatgatagtaactttacattgacaggggttttta
attccaaccattattgatacattaagttctaaagattatcattatgaagatatttggcccgaaagatgttatttac
ggaggttatgggggtgtagattgtgttgaagctggaggaccacctgccggtgagggtgtggtgggttatggtgta
gggtgaaacggtaaaactttttaaagagttaaatgcttttttcgaatacagatgttattttatttgatgttttaggt
gatgtgtgttgtggtggctttgctgctccattaaactacgctgattattgtattattgtaactgataatgggtttt
gatgctttatttgcgtgcaaatcgtattgcagcttcagttcgtgaaaaagcacgtacacatccattgcgttttagcg
ggtttaatcggaatcgtaacataaaacgtgatttaattgataaatatgtagaagcttgcctatgccagattata
gaagttttaccattaatgaagaaattcgtatttcacgtgttaaaggcaaaactttatttgaatgtcaaataaa
aataatagacttcggctcatatggatggctctaaaggtgacaattctacagtaggagtgtagaaaactccatcg
gaagattatatttgtaatttttatttaaatattgctgatcaattattaacagaaaccagaaggaggtattccacgt
gaattagcagataaagaactttttactctttatcagatcttctatcttaaaatttaa

Photochlorophyllide reductase subunit L (ch1L) amino acid sequence (SEQ ID NO: 32):

MKLAVYKGGIGKSTTSCNISIALRKRGKKVLQIGCDPKHDSTFTLTGFLIPTIIDTLSSKDYHYEDIWPEVDIY
GGYGGVDCVEAGGPPAGAGCGGYVGETVKLLKELNAFFEYDILFDVLGDVVCGGFAAPLNYADYCIIVTDNGF
DALFAANRIAASVREKARTHPLRLAGLIGNRTSKRDLIDKYVEACMPVLEVLPLIEIRISRVKGTFLFEMSNDK
NNMTSAHMDGSKGDNSTVGVSETPSSEDIYICNFYLNIAQQLLTPGVIIPRELADKELFTLLSDFYLKI

Photochlorophyllide reductase subunit N (ch1N) nucleic acid sequence (SEQ ID NO: 33):

atggttagatgggtgccacaacgatttttaattttaaatagtttttttgaatgtgaaactggcaattatcatactttt
tgcccgatttagctgtgtagcttgggttatatcaaaaaatcgaagatagcttttttttagtaattgggacaaaaaca
tgtgggtattttttacaaaatgcccttggagttatgatttttgccgaacctaggtatgctatggcagaattagaa
gaaagtgtatatttcagcacaaattaaacgattataaagaattaaaacgtttatgtttacaaattaaacaagataga
aatcccagcggttatgtttggattggaacttgtacaactgaaattatcaaaatggatttagaagggatggctcca
cgtttagaaaactgaaatcggcataccattgtttagcacgctgaatgggttagattatgcttttacacaaggt
gaagacacagttttatcagcaatggccttagcatccttaaaaaagatgttccttttttagtaggtaatactggg
ttaacaaacaaccagcttctccttgaaaaatcaacttcttcagttaatgggacagacggaaggaattacttaaa
aaatctctgtattatgttgggtccgtaccaagtacagttactacacaattaaacttagaattaaaaaaaggaaggt
attaatgtatctggatggcttccatctgctaattataaagatttacctacttttaataaagatacacttgtatgt
gggtataaatccttttttaagtgaacagctaccacgttaatgcgtcgtagtaagtgcacattaatgtgacccc
tttccaatagggcccgatggcacaagagtttgattgaaaaaatttgtgggtgcttttggcattaatcctagtctt
aatccaattactggtaataactaatttatatgatcgtagacaaaaaattttcaacgggctagaagattatttaaaa
ttattacgtggaaaatctgtattttttatgggtgataatttattagaaatttcttttagcacgttttttaacacgt
tgtgggtatgattgtttatgaaatcgggaattccatatttagataaacgatttcaagcagcagaattagctttatta
gaacaaacttgtaagaaaatgaatgtaccaatgccgcgcattgtagaaaaaccagataattattatcaaattcga
cgtatacgtgaattaaaacctgatttaacgattactggaatggcacatgcaaatccattagaagctcgaggtatt
acaacaaaatggcagttgaatttacttttgcacaaattcatggatttactaatcacagtgaaaattttagaatta
gtaacacagcctcttagacgcaatctaattgtcaaatcaatctgtaaatgctattttcttaa

Photochlorophyllide reductase subunit N (ch1N) amino acid sequence (SEQ ID NO: 34):

MLDGATTILNLNSFFECETGNYHTFCPI SCVAVLYQKIEDSFFLVIGTKTCGYFLQNALGVMI FAEPRYAMAELE
ESDISAQLNDYKELKRLCLQIKQDRNP SVI VWIGTCTTEII KMDLEGMARLETEIGIPIVVARANGLDYAFTQG
EDTVLSAMALASLKKDVPFLVNGTGLTNNQLLEKSTSSVNGTDGKELKKSLVLFSGVSPSTVTTQLTLELKKBG
INVSGWLPSANYKDLPTFNKDTLVCGINPFLSRTATFLMRRSKCTLI CAPFPPIGDGTRVWIEKICGAFGINPSL
NPITGNTNLYDREQKIFNGLEDYLKLLRGKSVFFMGDNLLEISLARFLTRCGMIVYEIGI PYLDKRFQAAELALL
EQTCCKEMNVMPRI VEKPDNYQIRRI RELKPDLTITGMAHANPLEARGITTKWSVEFTFAQIHGFTNTREILEL
VTQPLRRNLMSNQSVNAIS

Porphobilinogen deaminase (PBGD1) nucleic acid sequence (SEQ ID NO: 35):

atgcagcagtgcggtggcccgctccgctccgcgctccgctccagcagggcggtcgcgcccaaggtcgctggcgctcg
gtcagcgcccgctgtgcccgcgtctatgcctccgctgttgctaccaagacgggtgaagattggcacgcgcggctcg
cccttggtcttgcccaggttacatgactcgcgacctgctgaagaagagcttccttgagctgagcagaggagggt
gctctggagatcgatgatcatcaagaccacgggtgacaaaatcctgaaccagccccctggctgacatcggtggcaag
ggctctgtttaccaaggagatcgatgatgctctgctgagcggcaagattgacatcgccgtgcactccatgaaggac
gtgcccacctacctgcccaggggacccatcctgcctgcaacctgcccgcgaggatgtgcccgatgtgttcac
tcgcctgtcgccaaggacctgagcgagctgcccgcggcgccatttgtgggtcgccctcgctgcgcgctcaggcc
cagatcctggccaagtacccccacctcaaggtggagaacttccgcggcaacgtgcagaccgcctgcgcaagctg
aacgagggcgccctgctccgccacctgctggctctggccggtctgaagcgccctggacatgactgagcacatcacc
aagacctcagcatgacgagatgctgcccgcgctgagccagggcgccattggcatgctgcccaccgacgac
ggcgccagcgcaacctgctggccgcccctgaaccacgaggagacccgcacgcgcgtgggtgctgagcgcgccctc
ctgacgcgcccggagcggtcttgcgcgacccccattgcgggctacgcgcacaagggcgccgacggcatgctgcac
ttcagcgccctggtggccaccccgacggcaagcagatcatgcgcgctagccgcgtgggtgcccttcacggaggcg
gatgcccgtcaagtgcggcgaggaggccggcaaggagctcaaggccaacggcccaaggagctgttcattgactaa

Porphobilinogen deaminase (PBGD1) amino acid sequence (SEQ ID NO: 36):

MQQCVGRSVRAPSSRAVAPKVAGARVSRRCRVYASAVATKTVKIGTRGSPLALAQAYMTRDLLKKSFPELSEEG
ALEIVI IKTGDKILNQPLADIGGKGLFTKEIDDALLSGKIDIAVHSMKDVPTYLPEGTIILPCNLPRDVRDVF
SPVAKDLSLELPAGAI VGSASLRRQAQILAKYPHLKVENFRGNVQTRLRLKLNAGAC SATLLALAGLKR LDMTEHT
KTL SIDEMLPVQSQAIGIACRTDDGASRNLLAALNHEETRIAVVCERAFALTALDGS CRTPIAGYAHKGADGMLH
FSGLVATPDGKQIMRASRVVPFTEADAVKCGEEAGKELKANGPKELFMY

Prophobilinogen deaminase (PBGD2) nucleic acid sequence (SEQ ID NO: 37):

atgcatcgatctgctcaaggctcaagtggcctcatgtcagttttcgcgcacgtcgaaggctctggagactggcg
 ccgggttctgacagacgacggtgtcggggcctcactcggacacgcactgcgcggccccccaccagcgagcccgcc
 ccgcatccagcagcggaagagcgggaacgacactcgtgatagccacgcgcccatctaagcttgcaaaggag
 cagacgcggcaggtgcagcagctgctgctggcggcgcgagctcaaggacgagcagctgcagctgagcaccctg
 gaactggcgtctaggggcgacacgactcagggtgtgtcgtgcgcagctctgggctcggggccttcaccgaggag
 ctggaccaggctgtgctgtcgggcgtgcccacatgtcgggtgcacagcctgaaggactgccccgcggcctggcg
 ccgggctgctgctggccgctgctgcccggggcgacccccgggagctcctcatcgcgcggcggccacctcg
 ctgggcgagctggtgcccggcgagcgtgtgggcaccagcagcagcgcgcggcgcgagatcaagcactccttc
 cccacctgcaggttgtgcagctgcgcggcaatgtggactcgcggctggggcgcatccgcagccgcgacatcggc
 gccacagtgtggcgggcggggctcaagcggctgggtgtgatgaactcggacgaggggtgacactaccgctacg
 ggcccgctgggggtggtgtgcagggcagacgatgagtggtgtcggcctgctggacgccatctcgcaaccgcgcg
 acggcctggaggtggcgggcgagcggcgctgctggcagcgtgctggcgggcgggcgcgctgcccagcgttca
 gcgttcccgacatgtcgtgggcctgccacacgcggcagcagcccgacagcaacacaatggacctggattgcctg
 gtggcgagcctggagggaaggagctcttcaggtacacggagttctacggccggctcattgacgaggtggacgcg
 gtgtcgtggggcgctgtacggcagcctgtgcgcagtgatggcgccaccaggcgcgccccctgttggcagcta
 ccttctcgcggcattag

Prophobilinogen deaminase (PBGD2) amino acid sequence (SEQ ID NO: 38):

MRSYLLKAQVASCQFSRTSKVWRLAPGSDRRRCRGLTRTPHCAAPTSEPAPPSSSGKSGQRPLVIATRPSKLAK
 QTRQVQQLLLAAQLKDEQLQLSTLELASRGDTTQGVSLRSLGSGAFTEELDQAVLSGAADMSVHSLKDCPAALA
 PGLLLAACLPADPRDVLIAPEATSLGELVPGSRVGTSSSRRAQIKHSFPHLQVQLRGNVDSRLGRIRSRDIG
 ATVLAAGLKRLGVMNSDEGDTTATGAVGVVCRADDEWVVLDAISHRGTALEVAERACLAALLGGGGACQRS
 AFPDIAWACHTRHPDSNTMDLDCLVADLEGKELFRYTEFYRPVIDEVDVSLGSLYGSLLRMMAPPGAAPCQWL
 PSSRH

Protoporphyrinogen oxidase (PPX1) nucleic acid sequence (SEQ ID NO: 39):

atgatgttgaccagactcctgggacgcgcaaggcttctagccggcggtcgcagatccgctcggctgcgcacgtc
 tcgcgaaggctcgcgcctcggccacgccttctcggctcgcgagccccgcgacgcgtgcgagccccgcgacgcg
 gcggccgcgcacactccaccgcactgctgcggcgccactgggtgctcccacggcgtccggagccggcgctgcgc
 aagacgctcgacaatgtgtatgacgtgatcgtgggtggaggtctctcgggcctggtagccggccaggccctg
 gcggctcagcacaattcagaactccttgttacggaggctcgcgagcgcgtcggcggaacattacgtccatg
 tcggggatggctacgtgtgggagggggccgaacagcttccagcccaacgatagcatgctgcagattgcggtg
 gactctggctgcgagaaggacctgtgtcgggtgacccacggctccccgcttcgtgtggtaggggcaagctg
 cgccccgtgccctcgggcctggacgccttcaccttcgacctcatgtccatccccggcaagatccgcgcgggctg
 ggcgccatcggcctcatcaacggagccatgcccctcttcgaggagagtgtaggagcagtcacccgcgcaacctg
 ggcatgaggtgttcttcgcctgatcgagcccttctgctccggcggtgtagcgggcgacccctccaagctgtcc
 atgaaggcgcccttcaacaggatctggattctggagaagaacggcgggcagcctggtgggaggtgccatcaagctg
 ttccaggaacgcagctccaacccggccccgcgcgggacccgcgcctgcccgcgaagcccaagggccagacgggtg
 ggctcgttcgcgaaggccctgaagatgctgcgggacgccattgagcgcaacatccccgacaagatccgcgtgaac
 tggagctggtgtctctgggcgcgagggcgacggcggtacgggctggtgtacgacacgcccagggccgctgtc
 aaggtgtttgcccgcgccgtggctctgacccgcgccagctacgtgggtggcgacctggtcaaggagcaggcgccc
 gccgcgcgcgagccctgggctccttcgactaccgcgcgggtgggcgcgctgacgtgtcgtacccgctgagcgcc
 gtgcgggaggagcgcaaggcctcggacgggtccgtgcggggcttcggtcagctgcaccgcgcacgcagggcatc
 accactctgggcaccatctacagctccagcctgttccccggccgcgcgcgcgagggccacatgctgtgctcaac
 tacatcggcgccaccaccaacccgcggcatcgtcaaccagaccacgcgagcagctggtggagcaggtggacaaggac
 ctgcgcaacatggtcatcaagcccgacgcgcgccaagcccgctgtggtagggcgctgcgcgtgtggcgcgccatc
 ccgagttcaacctgggcccactggagcagctggacaaggcgcgcaaggcgctggacgcggcggggctgcagggc
 gtgcacctggggggcaactacgtcagcggtgtggccctgggcaagggtgtaggacacggctacagctccgcagcc
 aacctggccaagagcgtgtccaaggccgcagtcaggccctaa

Protoporphyrinogen oxidase (PPX1) amino acid sequence (SEQ ID NO: 40):

MLLTQTPGTATASSRRSQIRSAHVSAKVAPRPTPFVSPATASPATAAARRTLHRTAAATGAPTASGAGVA
 KTLDNVYDVI VVGGLSLVTGQALAAQHKTQNLVTEARERVGNI TMSGDGYVWEEGPNFQPNDSMLQIAV
 DSGCEKDLVFGDPTAPRFVWWEGLRPVPSGLDAFTFDLMSIPGKIRAGLGAIGLINGAMPSEESVEQFIRNLL
 GDEVFFRLIEPFCSGVYAGDPSKLSMKAAFNRIWILEKNGSLVGGAIKLFQERQSNPAPPRDRLPKPKGQTV

Uroporphyrinogen III decarboxylase (UROD1) nucleic acid sequence (SEQ ID NO: 41):

Uroporphyrinogen III decarboxylase (UROD1) amino acid sequence (SEQ ID NO: 42):

Uroporphyrinogen III synthase (HEM4) nucleic acid sequence (SEQ ID NO: 43):

Uroporphyrinogen III synthase (HEM4) amino acid sequence (SEQ ID NO: 44):

CHLD 5' untranslated region (regulatory region) (SEQ ID NO: 45):

CHLD 3' untranslated region (regulatory region) (SEQ ID NO: 46):

52

gcctggaacgcttgcgatcagatggtcgagctcaagaagatttggttgagccgttgggtcgtcgtcatattatg
gcttgcatcttcggggagcggcaagaaacggactccaatgcaggccctcgggcgagaagattgggcgtgtccg
gggtgcattctcgcgcgtggggtgcataaatttcgttgagtcccttcccgaggagggggggcggtagt
caaccccatcatcgtaggggggttgtaaatgccagccaaactaaa

CHLD Exon 1 (SEQ ID NO: 47):

atgaagtctctctgccatgagctcgctggccccagcgtaactgggtgcggccggcgaaagcctccggaaggcttct
agcgggtgccaaagtgcgcaggtctctgcggcgctgtgcttaacagcgtgcagcgccaaacagcgtctcgctgt
ctgcccgtggccgagctctccgctgctgagctgcgcg

CHLD Exon 2 (SEQ ID NO: 48):

ccatgaagggtgtctgaggaggactccaagggtctcgatgcggatgtgtcgacccgcctggccgcctcgtacctc
tggcggcggtggtggccaggacaacatcaagcaggcgctgctgtgggcgcctggacaccgggctggcgggca
tcgccatcgccggtcgccggtaccgccaagtcacatcatggtctcgccgctgcacgctctgctgccgccattg
aggtggtggagggcagcatctgcaacgcgcaccccgaggaccccgctcctgggag

CHLD Exon 3 (SEQ ID NO: 49):

gctggcctggctgagaagtatgcgggcggccctgtgaagaccaagatgcgcctcgggcgcggtttgtgcagatccct
ctgggtgtgactgaggaccgcttggtgggcactgtggacattgagggcgtccatgaag

CHLD exon 4 (SEQ ID NO: 50):

gagggcaagactgtgtccagcccggtgctggctgaggcgaccgcggcatcctgtacgtggacgagatcaac
ctgctggatgacggcattgccaacctgctgctgtccatcctgtcggacggagtcaacgtggtggagcgcgagggc
atctccatcagccacc

CHLD exon 5 (SEQ ID NO: 51):

ctgcgcggcgcgtgctgattgccacctacaaccccgaggagggccctctgcgtgagcacctgctggaccgcacgc
cattggcctcagcgccgagctcccagcaccagcgacgagcgcgctcaaggc
cattgacgcagccatccgcttcaggacaagccgcag

CHLD exon 6 (SEQ ID NO: 52):

gacactattgacgacacc gcggagctcaccgacgccctgcgcacctcg

CHLD exon 7 (SEQ ID NO: 53):

gtcatcctggctcgcgagtacctgaaggacgtgaccatcgcgcggagcaggtgacctacattgtggaggaggcg
cgccgcggcgagtcacaggggcacc gcgcggagctgtacgggtcaag

CHLD exon 8 (SEQ ID NO: 54):

tgtgccaaaggcgtgtgcggctctggaagggccgtgagcgtgtgaacaaggatgacctgcccaggccgtgcagctg
gtcatctctgccgcgcgccaccatcctggaccagccccgcccagcaggacagcccccgccgcgcccccgccc
cctccccgcgcgcgcgcg

CHLD exon 9 (SEQ ID NO: 55):

gaccaaattggaggacgaggaccaggaggagaaggaggacgagaaggaggaggaggagaaggagaaacgaggaccag
gacgagcccgag

CHLD Intron 6 (SEQ ID NO: 64):

gtgagtgaggggggccgtgcgtttgtttgttgcgtgggctggctggctggctttgttggatgagggcgctgctca
ccactcatctctttgaatccccacttatccagtgcctgcatgaaaccccgctgactcactccccaccatcctg
taccgcttttccaaacatccttgcaaccatcccgccatccccaccgcag

CHLD Intron 7 (SEQ ID NO: 65):

gtgaggagttggagggggaagggcgaggggatgacgacagaagcgagggcgaggggagccgggtgggttgttgc
aagtgtcgtgaattatagaatgacccccaaaagcgccggcccaacagggcctattacttgcgagtcattccaaccc
ctgatatagggagaatgggtagaggtcgtatcacgacagcaaggatgtacagtgggccttgggggtgggaggtta
cagggaaaaaggagaggacatgggggttgggttaagcggggaataacaaatatacaccagcgtttatggaagtggg
agatggaaacggggcgagcaacaggaacagggccggatggaggggctatgggggcatgggtgggtgggggtac
ggcgcggggcagagcaggggtcttgggtgaatgggcaagatgctgatgcttgggatgaagacactatgagcaaaaga
aatgggtgttgacgattgcatgatcatcgagtgggggagggcggggtggcaatacgggcagtcacagttgggg
tgcatcaagattgattggagtaccagcagtgccgggatctggctgacgtgtctcgagcaggttgctgggggtgg
caaggagatgcaggggcagacgacgttggtgcgaccacacttacacacatttccctcccccttgcgtgtgtccgtgc
gcctgtgctccag

CHLD Intron 8 (SEQ ID NO: 66):

gtacgtaaacgtatttgattgctcaggtgggttagccttgggtggctgctggttgacttgtgcagctgtctttgt
gtacatgttccacaacccgtactccccatattccgccccattccag

CHLD Intron 9 (SEQ ID NO: 67):

gtgagagggcgccggcggttgcgggcgaaggcgggggcgggcgagggaatgcggccgcgcatggccagca
acggaagggctggctatcaacacggcgagcgacgatattcatataagagtgccatcgtgcaatgctgaatactt
gcgccaaccggatctcgtgctccgttccaccggactgctttctcatctctcccttcacctgtgtgatcca
cag

CHLD Intron 10 (SEQ ID NO: 68):

gtgagtgcccaggtgggtgggtggtgaattggggcacgagggatgtggggcctaaggagctgaatggggcatgt
tttcttctgagcatcacggtcagagcttgacctgtctctcccgctgtaccccgctgcacggtcgcacacag

CHLD Intron 11 (SEQ ID NO: 69):

gtgagtacagcgcatcccgccgaatcattgggcctagttactgctgcaggactcgtgtgctcttaagggtggc
agctgtcagaagctctactcctcgcaactgaccaactgtgcctttctctccttccctctcctccccgcacccctc
ctccacttctcaacag

CHLI2 5' - untranslated region (regulatory region) (SEQ ID NO: 70):

gcagacttccataaagctcttgtaacgctgtaccaactagtaagcgggtacaattcgctgagcccgagcaacgcg
acctttctgtctgtggtatcttgataatctaaccagacaaaaccttttactaatctaggcaaca

CHLI2 3' - untranslated region (regulatory region) (SEQ ID NO: 71):

aaaaggctgggtgtaggcctgtcgggtcgtgttaaagggttgcgtgacgtgtaagtgtgacagtggtccggta
tgtgtgtgtatacatgtgttgcgggtgtgcttttggcggtacatggtgatgactgagcgggtgggacagagcac
gggttaactgacgagggcagtcctgctgcgagacggacgtttttagccgaggtgcaaggactgatgacgggctaag
ctgctgggagacttgaggttgagagtgcaggtggatcgacgggttctctaaaggagtatgaataggcaggagggctg
gagacatttgggggtgcaaggagggcggtagtatgggagatgtccatgggcggatttggcctctgtaacttcttaa
cgccca

CHLI2 Exon 1 (SEQ ID NO: 72):

atgcagagtctccagggtcagcgcgcgttcactgcggtgcccagggtcgggcgggtccctgaggactcgctg
gtcgtgcgctcgtctgttgccttgccatccacgaaagccgcgaagaagccgaacttccggtcgtcaagattcag
ggccaggaggagatgaagcttgactgctgctgaacgtggtcgaccccaacatcggcggagtgcctattatgggt
gaccgcggcactgccaagtcggtcgcg

CHLI2 Exon 2 (SEQ ID NO: 73):

gtccgcgccttggtggatatgcttcccgacattgacgtgggtgagggcgacgccttcaacagctccccaccgac
cccaagttcatgggccccgacacctgcagcgttccgcaacggcgagaagctgccaccgtccgcatgcggacc
ccctg

CHLI2 Exon 3 (SEQ ID NO: 74):

gtggagctgcctctgggcgccaccgaggaccgcatctcgggcaccatcgacatcgagaaggcgtgacgcagggc
atcaaggcctacgagccccgcctgctg

CHLI2 Exon 4 (SEQ ID NO: 75):

gccaaaggccaaccgcggcatcctgtatgtggacgaggtgaacctgctggatgatggcctg

CHLI2 Exon 5 (SEQ ID NO: 76):

gttgatgtcgtgctggactcgtcggttagcggcctgaacactgtggagcgtgaggggtgtgtccattgtgcacct
gcccgttcatcatgattggctcaggcaacccccag

CHLI2 Exon 6 (SEQ ID NO: 77):

gagggtagctgcgcgcgcagctgctggatcgcttcggcatgagcgtcaacgtggccacgtgcaggacaccaag
cagcgacgcagctggtgctggaccg

CHLI2 Exon 7 (SEQ ID NO: 78):

gcttgctacgagggcgaccctgacgcatttgtggactcgtgcaaggccgagcagacggcgctcacggacaagct
ggagggcgcccgccagcgctgcggtccgtcaagatcagcgaggagctgcag

CHLI2 Exon 8 (SEQ ID NO: 79):

atcctgatctcgacatttgcctcgccctggatgtggatggcctgcgcggtgacattgtgatcaaccgcgcgcgc
aaggcgcttgtggccttcgagggccgcaccgaggtgaccacgaatgacgtggagcgcgtcatctcggtgcctc
aaccaccg

CHLI2 Exon 9 (SEQ ID NO: 80):

cctgcgcaaggaccgcgtggacccattgacaacggcaccaaggtggccatcctgttcaagcgcgatgaccgaccc
cgagatcatgaagcgcgaggaggagggccaagaagaagcgcgagggaggcgccgccaaggccaaggcggagggcaa
ggcggaccgccccacgggcgccaaggctggcgctgggctggcttgcctcctcgtcggtaa

CHLI2 Intron 1 (SEQ ID NO: 81):

gtaggtaacacaagcaattatggggcgaagatctaggctccgctgatccgggcgggcaatcggcacgtcggtgc
aacgctggggcgctctgtgcaccccttgcgtggtgccaggtgcctgactgcctgcattcctgtaccgagccacat
tggctgctttgcagcgtgcatgggacgggtgtaggataagcgtatgtatgcgatagcgcgggtgcacggcttg
gcatggcaaggttgccgggtgcacatgcgtgccagcgtccctcagcatcagagtctggatctaagggtcagcg
gcttccctgcgcatgtgggtccttgcgtagtgtacgaagccttataattaaagctcatgtattgagtggtccggg
tttggggcactagttagtcaggaggcgcgtgccaggtgatagagcatatcagcaccgcttccctgcgaaacg

cttcggttgctcccttccccaccacctccccgctcatacccatacatatggctatccgtcctctcattgcttg
ccctacag

CHLI2 Intron 2 (SEQ ID NO: 82):

gtgagcgggacctacttctgaagacagtcttacgtgttgactgcagcgggtgtgcgcacctctgcttttgctg
cgccgggaagcgcggattgcggcctcacagatcaagcccgaaacgcttgtgtttccagcgggtggcacacacg
cgcgcgcgacagtgacacctcacggcgcgctgcctgcag

CHLI2 Intron 3 (SEQ ID NO: 83):

gtgcgtagtgcattggggagaggggacgaggggaggaggcagggccataaaccgaaccccaagtcacgagaca
cagaaccgataatagctcccagatcgccaaggggtgaggcgggaagccaaggatgatgcgttggccgcatgctg
tggtgacgtcaggcttacacagggctgactggctgtgcttgggggttggcacgcttcttgactggccccgtacg
catgctgcag

CHLI2 Intron 4 (SEQ ID NO: 84):

gtgagtggtggtggtttctgggtcagcagaggacttctgtagtaggtaatgtgggccagggaagtgtggctaaca
tgccaaacacgggggagcaccagtgcagctgcattcgctgacgtgcacgggtgcaatgggtgcaaggcgaaactg
caatcgcggtgcacagttgccagggtgcgctcacgcttgagtgtctgcacacgcactgcag

CHLI2 Intron 5 (SEQ ID NO: 85):

gtgcgtagcgtgcgcgcattgtacttgtctcccttgctcatgttgggaaaggctcggtccccagcctgcttgcaagat
gcggccgggtcagcagctgcggacgggtcagcacctacgtgccgagggtgtgttaacatgaatggcgttggggcgcc
gacctgccacaagctgaactgcgaccagcaaggcagctgccagcaacgcacacccgacgtgctacacgcttggtg
tttgacctcctaacaacaccccgcccgctgtctgtcacgtccacag

CHLI2 Intron 6 (SEQ ID NO: 86):

gtaagcggcggcgcgcggggacacggaggacatttcgcgagcatgggttgaggagtcgggaggattcggtggc
tggccggagtcgggagtcggagtcgcgagtcggaagtcgaagcttctggcggcttcgtgctgtcgggtgcgctcgc
catgatggcgctgaccggagggtgcagctgtgtatgtgggcgcgcag

CHLI2 Intron 7 (SEQ ID NO: 87):

gtacggggcgtagcagcggggcggtgcacggggccagtgaccgacagggcagcacgcggctggcgaaagagcgcac
aaagtgcaggggtgaccaagaccgggtgatgccacgagaggggcgcgggagccgtgcattgggtcgaggaggag
gaatgcaactttacactgatgcctctgtatacggccgccttccgagccctgcaaaccttcgctttcccccgacgc
acgcag

CHLI2 Intron 8 (SEQ ID NO: 88):

gtgagcgcagcgtgcgggtggatgcggtgcgctgcgggttgccaaactattattttgtacgtggacgcgtggctg
gcgatggcatgtcatggcggaatggatattgggcgaatggataccggtaatggtagcacggggcgagggcct
ggcggtagtggggttgagggggcaggactccagcgcgcgatacatgccatgttcagcatggcccaactgacag
cgcccgctgcctgtgcgccccgctccctccgcgcacccgctcctcctacacag

CHLH1 5' - untranslated region (regulatory region) (SEQ ID NO: 89):

ctagtctagagggaaactagggaggggcaacagagaa

CHLH1 3' - untranslated region (regulatory region) (SEQ ID NO: 90):

ggggcctcccttcattggtagcactagttggcgggttggtgtggactaggcggctagggtatatacctagtagc
ggcggctgcggagtgagggtggcgccagcgcgagggcgtggccttccctcctggaccgagagcgctccgcg

aggagacggcgagtgagataggcagcagcgagcggagatcgatttgtgaacagttttgtggcgggatcccatagc
ggatgcagagaagaccttagagcagcttcctcgggtggagtgaacgagccagagcggagggaaaggcgcatgagggg
actgcaggggactggaaactcggggagtgacaggtccgggtgctaggtccgctaacaagtgccggtctacgcctgtgtgt
gaggtgtgcgtgtgtgtgtgagctgtgcgggttttgtgtgcaaagtaggagtgagccgagccgcgcgtactttgt
ggcgtgtttggctgctggcgtgagagccaagagagggtaaacgggtttggtattttatggtgcggggtgaaagc
agccctcgcaggaatggagcagattctgcagcatgatgcacgtgtgcctgcgcgtggatgggtggctgtgatatgg
ctctgccactccggcagcacgcgtacgatacctagcgggtgcctggagtggctctctctgtttgggtgcgtgatgttt
gggtttgccgttttgattctttgtttcgtgctgaatggctgagggcggcaagaccctcgtgccagtgtagagagc
ctcacggctccctcggaccccgctggggacgtccattcccggtggcgggtgtgcctcggcgggtgtaaagcaaaa
aatatttt

CHLH1 Exon 1 (SEQ ID NO: 91):

atgcagacttcctcgcttcttggccggcgacggccccaccggctgcgggcgcgacgccccaaagccg

CHLH1 Exon 2 (SEQ ID NO: 92):

gttgccctcgccccgcgtggctagcaccgcag

CHLH1 Exon 3 (SEQ ID NO: 93):

gtcgcgtgcaatgtggcgactggacccccggcggcccatgaccaccttcaccgggtggcaacaaggccctgctaag
cagcaggtgtcgctggatctgcgcgacgagg

CHLH1 Exon 4 (SEQ ID NO: 94):

gcgctggcatgttcaccagcaccagccccggagatgcgcctgtcgtccctgacgatgtgaagggtcgcgttaagg
tgaaggttggtacgtggtgctggaggccagtagcagtcggccatcagcgtgcggtgaagaacatcaacgcca
agaactccaag

CHLH1 Exon 5 (SEQ ID NO: 95):

gtgtgcttcgaggtgggtgggtacctgctggaggagctgcgtgaccagaagaacctcgatatgctcaaggaggat
gtggcctctgccaacatcttcacggctcgctcatcttcattgaggagcttgccgagaag

CHLH1 Exon 6 (SEQ ID NO: 96):

attgtggaggcgggtgagccccctgcgcgagaagctggacgcgtgcctgatcttcccgtccatgccggcgggtcatg
aagctgaacaagctgggcacgttttcgatggctcagctgggcccagtcgaagtcggtgtctcggagttcatcaag
tctgctcgcaag

CHLH1 Exon 7 (SEQ ID NO: 97):

aacaacgacaacttcgaggagggttgcgtgaagctgggtgcgcacctgcctaaggtgctgaagtatctgccctcg
gacaaggcgcaggaagccaagaacttcgtgaacagcctgcagtagtggctgggaggtaactcggacaacctggag
aacctgctgctgaacaccgtcagcaactacgtgcccgctctgaagggcgtggacttcagcgtggctgagccacc
gcctaccccgatgtgggtatctggcacccctctggcctcgggcatgtacgaggacctgaaggagtacctgaactg

CHLH1 Exon 8 (SEQ ID NO: 98):

gtacgacaccgcgaaggacatggtcttcgccaaggacgccccctcattggcctgggtgctgcagcgtcgcacct
ggtagtgccgatgagggccactacagcggcgtggtcgctgagctggagagccgcgggtgctaaggtcatcccgt
ctttgccg

CHLH1 Exon 9 (SEQ ID NO: 99):

gtggcctggacttctccgcccccgtaagaagtctcttctacgacccccctgggctctggccgcacgttcgtggaca
ccgttgtgtcgctgacccgcttcgcgctggtgggcgcgcgcgcagacgcgcgaaggccattgaggcg
tgaagaacctgaacgtgcctacctgggtgtcgctgccgctgggtgtccagaccactgaggagtggtggacagcg
agctggcgctgcaccccgtaagggtggtctgcag

CHLH1 Exon 10 (SEQ ID NO: 100):

gttgccctgcccagctggatgggtgccatggagcccatcggtgtcgctggccgtgactcgaaacacccgcaagtcg
cactcgctgcccagaccgcatcgcttcgctgtgcgctcgccgctgaactgggccaacctgcgcaagaagcgcaac
gccgagaagaagctggccgtcaccgtgttcagcttccccctgacaagggaacgtcgccactgcccctacctg
aacgtgttcggctccatctaccgctgctgaagaacctgcagcgcgagggtacgacgtggcgccctgccgccc
tcggaggaggatctgatccagtgggtgctgacccagaaggaggccaagttcaactcgaccgacctgcacatcgcc
tacaagatgaagggtggaacgagtagccagaagctgggtccctacgcgcgaggcgctggaggagaactggggcaagccc
cccgccacctgaacaccaaaggccaggagctgctgggtgtacggccgcagtagcgcaacgtcttcacggcgctg
cagcccccttcggctacgaggggcgacccgatgcgcctgctgttctcgaagtcggccagccccaccacggcttc
gccgctactacaccttctggagaagatcttcaaggccgacgcgctgctgcaacttcggcaccacggctcgctg
gagttcatgcccggcaagcaggtcgccatgtcggtgtgtgtgtaccccgactcgctgatcggcaccatccccaac
ctctactactacgcgcgaacaaacccgtctgaggccaccatcgccaagcgccgctcgtagcgaacaccatttcg
tacctgacgcgcctgcccgaacgcggcctgtacaaggccctgaaggagctgaaggagctgatcagctcgctac
cagggcatgctgagctgtggcgcgccgagcagatctgcgccaccatcattgagacggccaagctgtgcaacctg
gacgcgacgtgacctgcccgcgctgacgccaaggacctgacctggacatgcgcgacagcggttggggcag
gtgtaccgcaagctgatggagattgagtcggcctgctgcccctgcggcctgcacgtgggtgggtgcccgcacc
gccgaggaggccgtggccacctggtcaacatcgctgagctggaccgcccggacaacaaccccccatcaagggc
atgcccgccatctgcccgcgcctatgggtcgcgacatcgagtcgatttacagcggaacaacaaggcgctcctg
gctgacgttgaccagctgcagcgcatcaccgaggcctccgcacctgctgcgcgagtgctgaaggaccgcacc
ggcctgaacggccgcctcgccaccaactggatcaccacactgctcaagtcacccgcttctacgtggacccctgg
gtgcgcggcctgcagaacggcgagttcgccagcgccaaccgcgaggagctgatcacctgttcaactacctggag
ttctgcctgaccag

CHLH1 Exon 11 (SEQ ID NO: 101):

gtggtcaaggacaacgagctgggcgcctggtagaggcgctgaacggccagtagctcgagcccgccccggcggt
gacccccatcgcaacccccacgtgtgcccacggcgaagaacatccacgcccctggacctcagtcgattccact
caggcccgctgaagagcgcgcctggtggtggaccgctgctggaccgagcgcgacaacaacggcggaag
taccggagaccatcgcgctggtgtgtggggcactgacaacatcaagacctacggcgagtcgctggcccaggtc
atgatgatggtcggtgtcaagcccggtggccgacgcctgggcccgcgtgaacaagctggaggtgatccctctggag
gagtgggccgcccccgctggacgtggtgtcaactgctcggtgtgttcgcgacctgttcgtgaaccagatg
ctgctgctggaccgcccacatcaagctggcgccgagcaggacgagcccgatgagatgaacttcgtgcgaagcac
gccaagcagcaggcgccgagctgggcctgcagagcctgcgcgacgcggccaccctgtgttctccaacagctcg
ggctcctactcgtccaacgtcaacctggcggtggagaacagcagctggagcgacgagtcgcagctgcaggagatg
tacctgaagcgcaagtcgtacgccttcaactcggaccg

CHLH1 Exon 12 (SEQ ID NO: 102):

ccccggcgccggtggcgagatgcagcgcgacgtgttcgagacggccatgaagacctggacgtgaccttcagaa
cctggactcgtccgagatctcgctgaccgatgtgtcgactacttcgactccgacccccaccaagctggtggcgctc
gtgctgcaacgacggccgcaccccccaacgcctacatcgccgacaccaccgcgaacgcgcaggtccgcactct
gggtgagaccgtgcgcctggacgccccgcaccaagctgtcaacccccaaagtgtacgagggcatgcttgcctcggg
ctacgagggcgctgcgcgagatccagaagcgcatgaccaacaccatgggtggtcgccacctcgggcatggtgga
caactgggtgtacgacgagggccaactcgaccttcatcgaggatgcggccatggccgagcgctgatgaacaccaa
ccccacagcttccgcaagctggtggccaccttcttgaggccaaacggccggcgtactgggacggccaagcccg
gagctggagcgccctgcgcagctgtacatggacgtggaggacaagattgaggcgctcgaataa

CHLH1 Intron 1 (SEQ ID NO: 103):

gtagggtgaattagaaggatcaaaacctagcggcctgatctgggactgacggcctcgcgcttcaatcactctgat
gcag

CHLH1 Intron 2 (SEQ ID NO: 104):

gtaggcacggcagaatgctcaatgaacatgcagctacatatgtttgggatcatggctgatctctgtgcgacgggt
ccgcgcag

CHLH1 Intron 3 (SEQ ID NO: 105):

gtgagcagcggcgaccgagcaagcgtggcgatgcagttggatttgttgttcttgggtcaggcgcctcgctcgatg
gccagcgcgtgtatTTAATGGGATAAGGGTTGAGACAAAGCATCTCTTCGGGTA AAAATCTTAGTTTTCGACAGC
acgttgagaggcatgcaacttgctctttcgag

CHLH1 Intron 4 (SEQ ID NO: 106):

gtgggtaaggagttgcattatcagtggtggcatgggtgttgcgggcgtctggggcgctgcaacagcggcatcggtgcc
gaactgaccgtgcccgggtacccgcgtgcag

CHLH1 Intron 5 (SEQ ID NO: 107):

gtgcgctaggggtgggggtctggaggggtgggatTGCGCCAAGTGCCCTGTTGCCTTGGCGGTCTGCTGTCATGA
TGTGAGGGTGACGTAGTGCACTCAATTGCTGTACGTCAACCACTTTGATGGGCTGGATCTGAGGCAGGTCAGC
TCGGTTCCTGTGTCATCCAGTGTCCTGTGCCTTGACGTTTGACGCTGTTCCCCCTTCGCACTGTCTCGCT
TTGCAG

CHLH1 Intron 6 (SEQ ID NO: 108):

gtgtgggcacgcgttttgggaaggaggcatacatttttggttgcgggttaggctgggcgcggacttggcaactcac
acggtcattgcacactcatgtctcaccttcatttacggtcccttggtccgaactacctacag

CHLH1 Intron 7 (SEQ ID NO: 109):

gtgagcagcatcagggcagagtgcattgaacggattggtggcagtggggaatggaattagacggacacgtctgggc
ggcaatatgttgcgctgcagtttttgggggtgtagtgaactagaaaatagggaagagataggccacataacatccg
aaagctcatatttttgcaaccggcgacattcacagcccacctgaagggttttgtagtcaacgcgtgcaactga
ctagatgtccccttacctgtctgatttcag

CHLH1 Intron 8 (SEQ ID NO: 110):

gtgaggcggggcgcgctgccctcggtagggttgcatatggtgatgggttaaccgaatgcatggccaatggggag
tgaaatcaggaaaggagggttaacacaatgcagggcagcacctgaatcgtgaaggcggagttaggcagggatctg
tcagttcgctgtcacgtggatgggcgcagctgacctttgtggtggttggtgtggcgag

CHLH1 Intron 9 (SEQ ID NO: 111):

gtgagctcagctgggacatgtagggtcggtcgccggagcatcgatgtagaattacgggaggaggggagaggg
gagaggattgcacgaaccgagatgagggcggtggttcgggatttcgggcaaaagctcgtgcggcaagcgttcagt
gactgaagagcagtggtcttcaactgcccctctgtccctcag

CHLH1 Intron 10 (SEQ ID NO: 112):

gtgcgaccgggtgccgctgcgtggccaacagcttgggtgccaccttcctgcgggtgttgatttacactgtgtgcgtgg
atgtgttggtttttcgcaactttagtctgggtccagctctttgccttcattgatcactcgtcttacctcctgcg
ccatcatttgaatacag

CHLH1 Intron 11 (SEQ ID NO: 113):

gtgagccttaatgcaacacgtgtagccgttcgcatgggtggctgggtcatgctatgggtggatcggcgctccgcct
gcttgctactgctgttcggtaccagcggttactgaccccgctgtgccaattcccaccacctaaccctcgctt
gcag

Ferrochelatase 5' – Untranslated region (regulatory region) (SEQ ID NO: 114):

gacagtgatatagcaataaccgatataatagggttggcgggcttcaccttgctccttaccagaatgtggccctgac
agtcgatttccagcccccttgccactcgctccctgatcttctcaatcaactagttgggtcggtttctcgtaagg

Ferrochelatase 3' – Untranslated region (regulatory region) (SEQ ID NO: 115):

gggggcgggtggcgagtaaggcgtatggcggagcagagagatgggctgtggcggtggcgggtgttctttgtgtga
ttggaaacatagacgggtgcggcacgcggcctgactgctgcgcggttggtgtggttgcgggggagcggggtcg
atggggcagcgccacgagttgggtgaaggaggaggccaggcgctgggtacacccatgggttgaggatgctag
tgagtgatgtgtcggggggcatgggtgtgtaccattcagagtcagatgcacgcacgggttgcgtgggagcgttcc
ctgctgtgcatgatgatggcgcccttcgatgaatcatctcttgaagggtccaaatgaaacgtctgaagtctgcagag
gggtgggtgctggacatgccatccaggcggaagtgggcagctgtgtctgactacaaagtaggtcttgtttgtctgg
atagcgtttggctatgtagcgtgtattctgtctcatcaatcacgccaggcgtcagggaactacccatgcaagtccgg
agcgtggctggctcttgaaaagtgttagctgctaggtggcgttggctgggggtgtcatgcatctcggcaggtaggc
ggtagcgttgagacacctctgcagcggagcatgtgcaagaatgtgactgcgcgtatgcacccgtatatgacggcgt
tggcgtcagttgttgagagtgaacagaggagagacgagcgaagctgccatgcccttagtggctgggtgcgagagg
gaagaaagagagaggaaggactttcgggcagtgcacacgcggaggttggggacacgggtcatcaacagggcgcg
gagctgggcggagtggtgtgtgatgggacaggggttcaaggcagggttggcgaggctcggagtggttagaccagtcc
ttcagtgaaggccattagggcatgatgtaagggtgaagcttg

Ferrochelatase Exon 1 (SEQ ID NO: 116):

atggcgctcggttggttgatgcaaggacgggtgcactgtcccagcttggtggaggagcgggtgttcgcccgtcgct
ggctgctctgggtcggtggcctgccagttatccagcggcaacg

Ferrochelatase Exon 2 (SEQ ID NO: 117):

gcgtggcggtgtgcagtgccaccaacgggtgtccagcagggcggtgtgctgcgcggacggccgcttcgaccgacgt
ggctctcttcgtagaccccaatgacattagaaaaaccgcagcagcagcagctggccctgcgggtggataaggctcg
cggtctgctgttaaaccttggcggggcccgaaaagctcgacgacgtcaagcctttcctgtataacctattcgccga
cccagaaattattcgccctgccagcggcagctcagttcctgcagccgctgctcgcgacgatcatctccacgcttcg
cgccccgaagagcgcggagggtatgaggccattggcgggtggtagcccggttgcgtaggattacagacgagcaggc
ggaggcgctggcggagtcctcgcgcgccaaggccaaacctgcgaacgtgtacgtgggcatgcgctattggcacc
ctacacggaggaggcgctggagcacattaaggccgacggcgctcacgcgcctggtcatcctccgctgtacctca
gttctccatctctaccagcggctccagccttcgactgcttgagtcgctcttcaagagcgacatcgcgctcaagtc
gctgcggcacacgggtcatccgctcctggtaccagcggcggggtacgtgagcgcgatggcggacctgattgtaga
g

Ferrochelatase Exon 3 (SEQ ID NO: 118):

gagctgaagaagttccgggacgtgccagcgtggagctgtttttctccgcgcacggcggtgcccaagtcctacgtg
gaggaggcgggcgaccatacaaggaggagatggaggagtgctgcggctcattacggacgag

Ferrochelatase Exon 4 (SEQ ID NO: 119):

gtcaagcggcgcggttcgccaaacgcacacgctggcctaccagagccgcgtgggcccccggaatgggtcaag
ccgtacacggatgagtcacaa

Ferrochelatase Exon 5 (SEQ ID NO: 120):

ggagctgggcaagcgcggcgctcaagtcgctgctggcggtgcccatcagcttctgtcagcgagcacattgagacgtt
ggaggagatcgacatggagtaccgcgagctggcggaggagagcg

Ferrochelatase Exon 6 (SEQ ID NO: 121):

gcataccgaactggggccgcgtgccggcgctgaacaccaacgcgccttcatacgacgacctggcggacgcgggtga
tggaggcgctgcctacgtgggtgcctggcgggcccagacagactcgtggcgctgg

Ferrochelatase Exon 7 (SEQ ID NO: 122):

gcgacctggagatgctgctgcaggcctacgacccgagcgcgcgcacgctgccgtcaccgggtggatgtgggagt
ggggctggaccaagagcgcggagacgtggaaacggccgcattgccatgatggccatcatcatcatcctggcgctgg
aggcagccagcggccagtcctcctcaaaaacctgttcctggcggagtag

Ferrochelatase Intron 1 (SEQ ID NO: 123):

gtgcgataataaatttgcatccttatgaattgctcaatgactaacgagcagcgtccgcgaccacag

Ferrochelatase Intron 2 (SEQ ID NO: 124):

gtgaggggtggcattctgtaaaggagttgtggagttgggcagagcagagtggtttggtcgccagggcgaggatgt
tgccggggcggttggcaggaacagggtgctagggcttgctggccagcagactagggtttcgactggccagcgcgcg
ccggggcgcgcttgccgaagctgcacagccccagcgcttctgtggatcaaatggaaacttctggcagtggtat
gctagcgcttggcgcaagaccaattttagtggatattactgttatctactgtggtagcgggtgggtattcggcggcg
tggttggttggtgcagccccgtgcgactaagacccgctggcaacgacagcaagccgcgcacccaggcatatacggc
ccaccagcaccacgtacacaaccacgtgcctttgcactctacgcaccacagcgcgctgctgccgctcccacctc
ccatcccacggccccctcttacccccacttcacaaccctcctctcacacgcctcctcttccccctcctcttcc
ag

Ferrochelatase Intron 3 (SEQ ID NO: 125):

gtgggcccgggcgcagcggggcgggcgaggaggggcaggaggggcaggaggggaggaaggaggggaggaagggaatgg
aaagctggcgagcggcagcggcgggacaggtagagggcgctgccccagcggcggcaggtgggcatggtgggcgg
gtaggggcgacgcgtgagggactcgtcaggcatccgcatggcggcgacttgctgctcctcaccgctgacgggtgc
atctgctgtgtgcgtaacctggcctggctggcaccgcag

Ferrochelatase Intron 4 (SEQ ID NO: 126):

gtgaggcccgctgggtgggacgcggggagggaacgcggggagggggagacgcgggagcgggacaagggtgaggatac
ggggaggggaataggagaggccatggggagggatggggacacgggaggtatgcacgggacctgggtggagccaggggg
aagtggacgacgagcccggcgggagggagggctgggtagaaggacgcgggaggtggttgggacaggtggacggggg
gtgtggagcatacggcgcaagaagcgggactgagcgggttgcaaggatggatgtaatacaggcaagtaagaaccc
cgagtggggctcagcgtgtcagcctgccttatctttcgcgcaagcgcctgggggttttatttcgctgtacacacgtc
gcgcctttctgcgcag

Ferrochelatase Intron 5 (SEQ ID NO: 127):

gtgaggagggcgccggagtgttgggggaagggtgcccgcgtgaagcgagatggcagggggcgaagggaaggagcggat
gggtggctgggtgcaagcggagaggcgacagagtgagggttttggtggagcgggttggggagagggggcgagcag
ggatgcccgcctggggatggcgggacagaaggaggaaggttgccaagtgaaggggggggtgctcaaggaggaga
gggcgggtggaggttaagacggccgtgctggttatgctgggggttgcaaggcgcattgggcgcattggagccgggggag
tttggctgtggatgggcactgcggatgggcaaggcttgctactcatgtgcggtcgcgggtccgcgggtgtgtcagcc
agccaggacccatcccactgggtcttctcgtgcctgggactgcttgccgccacccacccattcatcaccacca
ctgcgcagacccaccaacacgcgtgcctgaactgctctgactcttggcgtcctcag

gtgagtcgcgcgcgtcgcggttggttcgcggatgcgggttggcggatgacgttcggcggttggcattggggttggg
tttgaggggttggtgggtgaggtcgggattggggtcgggattgggggtcgaagcgtggggctggcgtggaatgatgc
cgtggtctttggaaggggcttggggaggttgcgcgttggaatgcggacagcatgggcgcgcagctggcctatgtgc
atgtgctgtgtcaaacgcttggtgcgttcagttgtctcttcgctgcctccaacgtcagcagcatcccgccgcgc
ctggaccgttagagaccgctacgtgtccgctagcggcctcggcctcagcctaagcgcacgtagcgcacgcgacac
aagcaaacactgtcgctaatggcagcagcggcagcagcagcagtcacgagaatgccgcgggcggggagaaagtgtc
cctagccgggggcccgcgcgtagctggtttcctcagcgcgtggacggtggtgccttcattccgaccaccccaaggcg
cgtccccagtcctcgagctcgctgcctgcttgtggccgccttgaccgcctggcgcaccccggtggctcgcata
acgactcgccttcctgcgttcctcgctgacgctgtccgcctgacgctctgcgcttgactctttgcgccttcctccc
tcttccccag

atgcagacttctctcgcttcttggecggcgcacggccccaccggctggggcgcgacgcccaagccggttgccccc
tcgcccgcgctggctagcaccgcgcaggctcgctgcaatgtggcgactggaccccgccgcgccatgaccaccttc
accggtggcaacaagggccctgctaaagcagcaggtgtcgctggatctgcgcgacgagggcgctggcatgttcacc
agcaccagcccgagatgcccgcgtgctcctcagcatgtgaagggtcgctgaaggtgaaggttgtgtacgtgtg
tgctgtggaggccagctaccagtcggccatcagcgtcggtgaagaacatcaacgcgaagaactccaaggttgtgc
ttcgaggtggtgggctacctgctggaggagctcgctgaccagaagaacctcgatatgctcaaggaggatgtggcc
tctgccaaactcttcacggtcgctcatcttcattgaggagcttgcccgagaagatgtggaggcggtgagcccc
ctgcgcgagaagctggacgcgctgctgatctccccgcatgccggcggtcatgaagctgaacaagctgggca
ttttcgatggctcagctggcgccagtcgaagtcgggtgtctcgaggttcatcaagttcgtcgcaagaacaacgac
aacttcgaggagggttgtgaagctggtgcgcacccctgcctaaggtgctgaagtatctgcccctcggaacaaggcg
caggacgccaaagaactctgtgaacagcctgcagtactggctgggcgggtaactcggacaacctggagaacctgtgc
ctgaacaccgtcagcaactacgtgcccgctctgaagggcgctggacttcagcgtggctgagcccaccgcctacc
gatgtgggtatctggcacccttggtctcggtcctgacgaggacctgaaggatcactgaactggtagcacacg
cgcaaggacatggtcttcgccaaggacgccccgtcattggtcggtgtgcgcagcgtcgcaactggtagtcatggc
gatgaggggccactacagcggcggtggtcgctgagctggagagccgcggtgctaaggtcatccccgtctttgcccgt
ggcctggactcttcgcccccgctcaagaagttcttcacgacccccctgggtctggccgcacgtctgtggacac
gttgtgtcgtgacccgctctcgcgctggtggggcgccccgcgcgcaggacgcgcgaaggccattgaggcgctg
aagaactcgaaactgcccctacctggtgtcgtgcgcgtggtgttcagacacatgaggatggctggacgcgag
ctgggcgtgcaccccgctcagggtggctctgcaggttgccctgcccgagctggatggtgccatggagcccatcgtg
ttcgctggcgctgactcgaaacaccggcaagtcgcactcgctgcccgacccgatcgcttcgctgtgcgctcgcgcc
gtgaactggggcaacctgcgcaagaagcgcaacgcgcgagaagaagctggccgtcacctggttcagcttccccct
gcaaggggcaactcggtcctgcgcctacctgaacgtgttggtctcatctaccgctgctgaagaacctgcag
cgaggggctcagcagctggggcgctgtcgccctcggaaggagatctgatccagtcggtgctgacccagtaagga
ggccaagtccaactcgaccgcacctgcacatcgctacaagatgaaggtggacgagtagccagaagctgtgccctta
cgccgagggcgctggaggagaactggggcaagcccccggcacccctgaacaccaacgcgcaggagctgctggtgta
cgccgcgcagtagcgccaacgctcttcacggcgtgcagcccaactctcggtcacgaggcgccagctgcgcctgct
gttctcgaaagtccggcaagcccccaacggcttgcgcgctactacaactctcgaggagaagatcttcgaaggcga
cgcgctgctgcacttcggcacccacggctcgctggagttcatgcccggaacgaggtcggtatgtcggtgtgtg
ctaccgcgactcgctgatcggcacctatccccaaactctactactacgcgcgccaacaaccgctctgaggccaacct
cgccaagcgcgctcgtagcgcgaacaacaccttctgactgacgcgcgctgcgcgagaacgcgcgctgtacaagg
ctgaaggagctgaaggagctgatcagctctgacagggcatgctgagttggccgcgcgagcagatctgcgc
caccatcattgagacgcgcaagctgtgcaacctggacgcgacgtgaccttgcgcgacgctgacgccaaggacct
gacctggacatgcgcgacagcgttgtgggcccaggtgtaccgcaagctgatggagattgagtcgcgcctgctgcc
ctgcgcgctgcagctggtgggtgcgcgcgccacgcgcgaggagccgctggccacacctggtcaacatcgctgagct
ggacgcgcgcgcaacaacaacccccctcaagggtatgccggcatctggtgcgcgcgctatggtcgcgacatcga
tgcgattacagcgccaacaacaaggcgctctggtgaagttgacgtgacgctgcagcgcacacagaggcttcgcg
cacctgcgtgcgcgagttcgtgaaggaccgcaccggcctgaacggccgcacatcggcaccaactggatcaccaact
gctcaagttcaccggtctctacgtggacccctgggtgcgcggcctgcagaacggcgagttcgccagcgccaacccg
cgaggagctgatcacctgttcaactacctggagttctgctgtgacccaggtggtcaaggacaacgagctggggcg
cctggtagaggcgctgaacggccagtagctgcgacccggccccgcgcggtgacccccatcgcaacccccaaactgct
gcccacggcaagaacatccagccctggacctcagtcgattccactcaggccgcgctgaagagcgcccgct
ggtggtggacgcgctgctggacgcgcgagcgcgacaacaacggcggaagtaccccgagacctcgcgctggtgct
gtggggcactgacaacatcaagcactacggcgagtcgctggccccaggtcatgatggtcggtgtcaagccgct
ggcgacgcgctggcgcgctgaacaagctggaggtcatccctctggaggagctgggcgcgcgcgcgcgctggacgt
ggttgtcaactgctcggttgtgttcgcgacactgtctgtgaaccaagatgctgctgctgagaccgcgcatcaagc

ggcgccgagcaggacgagcccgatgagatgaacttcgtgcgcaagcacgccaagcagcaggcgggcgagctggg
 cctgcagagcctgcgcgacgcccacccgtgtgttctccaacagctcgggctcctactcgtccaacgtcaacct
 ggcgggtggagaacagcagctggagcgacgagtcgcagctgcaggagatgtacctgaagcgcaagtcgtacgcctt
 caactcggaccgccccggcgccgggtggcgagatgcagcgacgtgttcgagacggccatgaagaccgtggacgt
 gaccttccagaacctggactcgtccgagatctcgtgacccgatgtgtcgcaactacttcgactccgaccccadcaa
 gctgggtggcgctcgtgcgcaacgacggccgaccccccaacgcctacatcgccgacaccaccaccgccaacgcgca
 ggtccgcactctgggtgagaccgtgcgcctggacgcccgcaccaagctgctcaaccccaagtggtagaggggcat
 gcttgctcgggctacgagggcggtgcgcgagatccagaagcgcatgaccaacaccatgggctggtagggccacctc
 gggcatggtagaactgggtgtacgacgagggccaactcgaccttcatcgaggatgcccgcattggccgagcgct
 ctgaaacaccaacccccacagcttccgcaagctggtagggccaccttccctggaggccaacggccgctactggga
 cgccaagcccgagcagctggagcgctgcgcagctgtacatggacgtggaggacaagattgagggcgctcgaata
 a

CHLI1 5' - untranslated region (regulatory region) (SEQ ID NO: 130):

tcctacagagtaaaggcttaggcgatgcgcgactgaaagactgtgaatcccgccgtcgccgtggtagggatgtggg
 ccgggtgcgctgtgcgagagataaattacaggtatcaacaaggttagggcggtggaaggagcgccgttagggaa
 ctgaaatcggatctgcatcgaccctcattccgcgacttgctccttcttggcctcgccccgagctcttgagttt
 tgttcttgacctttgacacgaaccaaccgatataaaa

CHLI1 3' - untranslated region (regulatory region) (SEQ ID NO: 131):

gcggcagggccttcatggctcgtcgttggagcatttgcgaaaggctgatggcagcagatgcagccatgtcagttgt
 ggctgaagttgttggctggggcgaggcgccgagcagctgctgcgagcgccgaagcagcggtgctgcttgcgt
 atgagaggaagaccagtgccctcgaggaggcgagtgccctgtgtgagtgatcaggacgtgtgacttcggaaactgag
 ggcgggtgagtagatgtgactggggcttgcaaggaagcctactgacctatcagaaaaggtagcaggggtatatgg
 tctaggagcgttgccggagcgtggctggccagtgctagccgcgcgggctctgttgcctcgtggcgcccgccgcc
 ttcaacaacagatgcgtagaaatgcagcgatgtgacgagggcgtggcctattctgcaatgtgtgagggcgccaatgg
 cgccactgacaaatggaggagtggtcaaagcttgggtacgttttgagagctgcatcgggcagcgaggatcagtggt
 ggcgtaagaccgacggcagacggattggcaagggaataggagggaagtggtggcggtggcgcccgccgttctgtcgag
 gccgcatgagccggcgcttctagaccgtagcccattttgaaacaagcgccacgcgtgctcccgatgggggaca
 tcgatcacgggaattgattaaggggcatgtgtgggtgtgcaagtgagtgactggtaggttccgtccctgtgaggttg
 ttcgttggacgtggctgcgggttgccgcgggcttaagcgggctgaggcagagcgctggcggtgtgagccgcgag
 tatcgatctgtaacgtgc

CHLI1 Exon 1 (SEQ ID NO: 132):

atggccctgaacatgcgtgttctcctctccaaggtcgctgccaaagcagcagggccgcatctccgcgggtgccgggt
 gtgtcgagcaaggtggcctcctccgcccgcgtggcccccttccag

CHLI1 Exon 2 (SEQ ID NO: 133):

ggcgctcccgtggccgcgcagcgcgctgctctgctgg

CHLI1 Exon 3 (SEQ ID NO: 134):

tcgcgcgcgctgccgctactgaggtcaaggctgctgagggccgcactgagaaggagctgg

CHLI1 Exon 4 (SEQ ID NO: 135):

gccaggcccccccatcttccccctaccgccatcgtggccaggatgagatgaagctggcgctgattctgaacg
 tgatcgacccccagatcggtgggtgtcatgatcatggcgacgctggcactggcaagtcaccaccattcgtgcc
 tggcgatctgctgcccagatgcag

CHLI1 Exon 5 (SEQ ID NO: 136):

gtgggtgccaacgaccccccttaactcggacccccaccgacccccagctgatgagcgaggaggtgcgcaaccgcgtc
 aaggccggcgagcagctgcccgtgtcttccaagaagattcccatggtagacctgccccggcgccactgaggac
 cgcggtgcggcaccatcgacatcgagaaggcgctgaccgagg

CHLI1 Exon 6 (SEQ ID NO: 137):

gtgtcaaggcggttcgagcccgccctgctggccaaggccaaccgcggcatcctgtacgtggatgaggtcaacctgc
tggacgaccacctg

CHLI1 Exon 7 (SEQ ID NO: 138):

gtcgatgtgctgctggactcggccgcctccggctggaacaccgtggagcgcgagggatatctccatcagccacccc
gcccgccttcacccctggcggctcgg

CHLI1 Exon 8 (SEQ ID NO: 139):

gcaaccccgaggaggggtgagctgcgccccagctgctggatcgcttcggcatgcacgcccagatcggcacccgtca
aggaccccccgcctgctgtgcagatcgtgtcgcagcgtcgaccttcgacgagaacccccgcgccttcgcg

CHLI1 Exon 9 (SEQ ID NO: 140):

caaggactacgaggccggccagatggcgtgacccagcgcacatcgtggacgcgcgcaagctgctgaagcagggcga
gggtcaactacgacttcgcgctcaagatcagccagatctgctcggacctgaacgtggacggcatccgcggcgacat
cgtgaccaaccgcgcgcgccaaggccctggccgccttcgagggccgcaccgag

CHLI1 Exon 10 (SEQ ID NO: 141):

gtgacccccgaggacatctaccgtgtcattccccctgtgcctgcgccaccgcctccggaaagacccccctggctgag
atcgacgacgggtgaccgcgtgcgtgagatcttcaagcaggtgttcggcatggagtaa

CHLI1 Intron 1 (SEQ ID NO: 142):

gtgtgcagttgcatctaaagaacgtccaattcatggttactgctcgtggatctaagcgggtgggtccaccagcgtt
ccatggtccccgattcgtgcacgcag

CHLI1 Intron 2 (SEQ ID NO: 143):

gtgagaagccatgatacaaatataaggatttgaaagcggtagatctaggacccatcgaacttgagcaccgacttgc
agtcccttgccctgtccggcagactgaacttctgcgcttgctttgcag

CHLI1 Intron 3 (SEQ ID NO: 144):

gtaagtgtcgcgcaaagattttctgcgggacgggtctccctcgcaacatctgaacccatggctcggtttttttgc
cccgag

CHLI1 Intron 4 (SEQ ID NO: 145):

gtgcgcgcctcccccaacccagtttgcaaatgtgtggttaagcgtcgaaagcgtgaacagaaacaggtgttgc
gggggcccgcggaatggctgcaatgggtgctgggggcttcggagggctctgggggcgagtttggtatacacgggcg
cgacacttgaaggaaacgtcaaggacgacagcggaggcgtggagacagcgcggcccaagcagcctgtacttgt
agctgctggtcagctgaggcatcacgacttgggaccagcaccgcgcctcacggttgcaacaaggccatcacgcgc
gccaccacccacgcctcttcaaaccatgcgggcacctaccgctaccctgtgacacgctccgcacacgcgcgcgc
cgacacccccaccatgtgacag

CHLI1 Intron 5 (SEQ ID NO: 146):

gtgagagcgaggcgcggggcgtgctctgcaggctaggggaagatcaggagagccgaagcgggcccgaacagcgc
agagagagggaagacgacacccctgccgcgttttgatcacaagattcacacccttgctctccccaacgctcccg
acatag

CHLI1 Intron 6 (SEQ ID NO: 147):

gtgagcaggggcagataggcggctcgggcccgtgggcggcaggggctgtgttgctgtgttggtgtgggctgagg
ctgggtgggtgggctggcgggtggcagggatagcgggtgaggggatgggtgatgggacagaatggcgggtgggcgga
cacgtggggtcgttgagggtgtgtgggacggcaactggatgcgatatgtcggcttgccctggcggggaag
cattcgagaatggcgacgaacgagggcgggagcagcggggatgggagacgaacctgcgtgcgaagtgcg
gcgcgcgtccagttgacacgttgacgaatgtggcagtggtcgccctgagagttatgggttagaccgccagatg
agccggttaagtcgggtgtgcgggtgatcggtgtctcccttcgggtgacgcctggcaccctaaccattacc
tgtcgcgtgctgcctttgccacag

CHLI1 Intron 7 (SEQ ID NO: 148):

gtgagtgcagctgccgctcgggctgctgatggtagcctgtgcgaccacggggctccgcatcttgacgaagcgt
tgtaccatagccgtcttggtccctgatttggcggcgtcctggtccgaagccttgacatctacagttcaacatggc
cgtataacgatcctgtgcccacccacacgccaccccgccag

CHLI1 Intron 8 (SEQ ID NO: 149):

gtgagcgcgcgtctctacgatacggcagacatgtacacactgcggcgactgtagagcttgcatctgcatcttcaagg
cctcgaaagagtaggggttggttctctggtggtgtcgggccacaattatgcacccgggtgtggtgcagcagct
gtgatgtcacaccttgcatcacccccctactgctgcgcctctcctctcttctgcgccgag

CHLI1 Intron 9 (SEQ ID NO: 150):

gtgagcagagcaatattgcagaggggaagggtggcggaagggtgataacggttggggatctagagggcgagatgg
atgcacacagcgcggggttggttatgcctgctgcacgcgtgcacgcacccctgatctgccggttttccaa
ctggcgatgccgtattatgacctgcagctcaccatcctcatgcttgattgcctcgtcag

CHLI1 Protein sequence (SEQ ID NO: 151):

MALNMRVSSSKVAAKQQGRI SAVPVVSSKVVASSARVAPFQGAPVAAQRAALLVRAAAATEVKAAEGRTEKELGQA
RPIFPFTAIVGQDEMKLALILNVIDPKIGGVMIMGDRGTGKSTTIRALADLLPEMQVVANDPFNSDPTDPELMSE
EVRNRVKAGEQLPVSSKKIPMVDLPLGATEDRVCCTIDIEKALTEGVKAFEPGLLAKANRGI LYVDEVNLLDDHL
VDVLLD SAASGWN TVEREGTISISHPARFILVSGSNPEEGELRPQLLDRFGMHAQIGTVKDPRLRVQIVSQRSTFD
ENPAAFRKDY EAGQMALTQRIVDARKLLKQGEVNYDFRVKISQICSDLNVDGIRGDI VTNRAAKALAAFEGRTEV
TPEDIYRVIPLCLRHLRKDPLAEIDGDRVREIFKQVFGME

Mutant protein sequence RedAlgaeCHLH (SEQ ID NO: 152):

MQTSSLLGRRTAHPAAGATPKPVAPSPRVASTRQVACNVATGPRPPMTTFTGGNKGPAKQQVSLDLRDEGAGMFT
STSPEMRRVVPDDVKGRVKVKVYVLEAQYQSAISAAVKINAKNSKVCFEVVGYLLEELRDQKNLDMLEDVA
SANIFIGSLIFIEELA EKIVEAVSPLREKLDA CLIFPSMPAVMKLNKLGTF SMAQLGQSKSVFSEFIKSARKNND
NFEGLLLKLVRTL PKVLKYLPSDKAQDAKNFVNSLQYWLGGNSDNLENLLLNTVSNYVPALKGVDFSVAEPTAYP
DVGIWHPLASGMYEDLKEYLNWYDTRKDMVFAKDAPVIGLVLQRSHLVTGDEGHYSGVVAELESRGAKVIPVFA
GLDFSAPVKKFFYDPLGSGRTFVDTVVSLTG FALVGGPARQDAPKAIEALKNLNVPYLVSLPLVFQTTEEWLDSE
LGVHPVQVALQVALPELDGAMEPIVFAGRDSNTGKSHSLPDRIASLCARAVNWANLRKKRNAEKKLA VTVFSFPP
DKGNVGTAAAYLNVFGSIYRVLKNLQREGYDVGALSALGGSDPVGADPEGGQVQLDRPAHRLQDEGGRVPEAVPL
RRGAGGELGQAPRHPEHQRPGAAGVRPPVRQLHRRAAHLRLRGRPDAPAVLEVQPPPRRLRLHLPGEDLQGR
RRAALRHRLAGVHARQAGRHVGCVL PRLADRHHPQLLLRRQQPV

CHLI1 DNA sequence (SEQ ID NO: 153):

atggccctgaacatgcgtgtttcctcttccaaaggctcgtgcccaagcagcagggccgcacatctccgcggtgccggtt
gtgtcgagcaaggtggcctcctccgcccgcgtggccccctccagggcgctcccgtggccgcgcagcgcgtgct
ctgctggtgcgcgcgcgtgccgctactgaggtcaaggctgctgagggccgcactgagaaggagctgggccaggcc
cgccccatcttccccttcacccgcatcgtgggccaggatgagatgaagctggcgctgattctgaacgtgatcgac
ccaagatcggtggtgtcatgatcatgggcgacccgtggcactggcaagtccaccaccattcgtgccctggcggt
ctgctgcccagatgcaggtggttgccaacgacccctttaactcggacccccaccgaccccgagctgatgagcgag
gaggtgcgaacccgcgtcaaggccggcgagcagctgcccggtgtctccaagaagattcccatgggtggacctgcc

ctgggcgccactgaggaccgctgtgctggcaccatcgacatcgagaaggcgctgaccgaggggtgtcaaggcgctt
gagcccgccctgctggccaaggccaaccgcggcatcctgtacgtggatgaggtcaacctgctggacgaccacctg
gtcgatgtgctgctggactcgcccgctccggctggaaacaccgtggagcgcgagggatatctccatcagccacccc
gcccgttccatcctggctcggtcggtcgggcaaccccgaggaggggtgagctgcgccccagctgctggatcgcttcggc
atgcacgcccagatcggcaccgtcaaggaccccgctgctgtgcagatcggtgcgacgctcgaccttcgac
gagaaccccgccgcttccgcaaggactacgaggccggccagatggcgctgaccagcgcatcggtggacgcgcg
aagctgctgaagcagggcgaggtcaactacgacttccgctcaagatcagccagatctgctcggacctgaacgtg
gacggcatccgcgcgacatcgtagccaaccgcgcgcgaaggccctggccgcttcgagggcgccacgaggtg
accccgaggacatctaccgtgtcatccctgtgcctgcgccaccgcctccggaagacccctggctgagatc
gacgacggtgaccgctgctgagatcttcaagcaggtgttcggcatggagtaa

[0112] Although the invention has been described with reference to the above examples, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims.

What is claimed is:

1. A composition comprising a preparation from an algae strain, wherein the algae strain overexpresses or accumulates protoporphyrin IX (PPIX).
2. The composition of claim 1, wherein the preparation is a biomass from the algae strain.
3. The composition of claim 2, wherein the preparation is a fractionated biomass from the algae strain.
4. The composition of claim 3, wherein the fractionated biomass comprises a PPIX-enriched fraction.
5. The composition of claim 4, wherein the PPIX-enriched fraction further comprises a protein-enriched fraction.
6. The composition of claim 1, wherein the preparation is an extracellular fraction of the algae culture.
7. The composition according to any of claims 1-6, wherein the preparation is red or red-like in color.
8. The composition according to any of claims 1-7, wherein the preparation contains a greater amount of PPIX than of heme.
9. The composition according to any of claims 1-8, wherein the preparation contains less than about 1%, 0.5%, 0.1%, 0.05%, 0.01%, 0.005% or 0.001% heme.
10. The composition according to any of claims 1-9, wherein the preparation contains less than about 1%, 0.5%, 0.1%, 0.05%, 0.01%, 0.005% or 0.001% heme-protein.
11. The composition according to any of claims 1-10, wherein the preparation does not contain a detectable amount of heme-protein.
12. The composition according to any of claims 1-11, wherein the preparation does not contain a detectable amount of heme.

13. The composition according to any of claims 1-4, wherein the preparation does not contain a detectable amount of protein.
14. The composition according to any of claims 1-13, wherein the preparation has a protoporphyrin IX content greater than chlorophyll content.
15. The composition according to any of claims 1-4 or 6, wherein the preparation provides at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% of the total protein content to the edible composition.
16. The composition according to any of claims 1-15, wherein the preparation provides vitamin A, beta carotene or a combination thereof to the composition.
17. The composition of claim 16, wherein the vitamin A, the beta carotene or the combination thereof is at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% of the daily recommended requirement.
18. The composition according to any of claims 1-17, wherein the preparation provides less than about 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.2%, 1.5%, 2%, 5% or 10% of total saturated fat present in the composition.
19. The composition according to any of claims 1-18, wherein the preparation provides at least about 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 125 mg, 150 mg, 175 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg or 500 mg of omega-3 fatty acids to the composition.
20. The composition according to any of claims 1-19, wherein the composition has a red or red-like color derived from the preparation.
21. The composition according to any of claims 1-20, wherein the composition has a meat or meat-like flavor derived from the preparation.

22. The composition according to any of claims 1-21, wherein the composition has a meat or meat-like texture derived from the preparation.
23. The composition according to any of claims 1-22, wherein the algae is a *Chlamydomonas* sp.
24. The composition of claim 23, wherein the algae is *Chlamydomonas reinhardtii*.
25. The composition of claim 23, wherein the *Chlamydomonas* sp. is strain CC-125 deposited to the University of Minnesota *Chlamydomonas* collection center or a derivative thereof.
26. The composition of claim 23, wherein the algae has reduced or an absence of ferrochelatase activity or expression of ferrochelatase.
27. A food product comprising the composition according to any of claims 1-26.
28. The food product of claim 27, wherein the food product comprises a clean meat, a cultured meat, a synthetic meat, a plant-based meat or a non-animal cell-based meat.
29. The food product of any of claims 27-28, wherein the food product is selected from the group consisting of a beef-like food product, a fish-like product, a chicken-like product, a pork-like product and a meat replica.
30. The food product of any of claims 27-29, wherein the food product is vegan, vegetarian or gluten-free.
31. An edible ingredient comprising the composition according to any of claims 1-26.
32. The edible ingredient of claim 31, wherein the edible ingredient is part of a finished product, and wherein the finished product has a red or red-like color derived from the ingredient.
33. The edible ingredient of claim 31, wherein the edible ingredient is part of a finished product, and wherein the finished product has a meat or meat-like flavor derived from the ingredient.

34. The edible ingredient of claim 31, wherein the edible ingredient is part of a finished product, and wherein the finished product has an appearance of blood derived from the ingredient.
35. The edible ingredient according to any of claims 31-34, wherein the finished product is an ingredient for a burger, a sausage, a kebab, a filet, a ground meat-like product or a meatball.
36. The edible ingredient according to any of claims 31-34, wherein the edible composition is part of a finished product and wherein the finished product is an animal feed.
37. The edible ingredient according to any of claims 31-36, wherein the edible ingredient is combined with a protein source, a fat source, a carbohydrate, a starch, a thickener, a vitamin, a mineral, or any combination thereof.
38. The edible ingredient of claim 35, wherein the protein source is textured wheat protein, textured soy protein, fungal protein or algal protein.
39. The edible ingredient according to any of claims 37 or 38, wherein the finished product is free of animal proteins.
40. The edible composition of any of claims 37-39, wherein the fat source comprises at least one of refined coconut oil or sunflower oil.
41. The edible composition of any of claims 37-40, further comprising at least one of potato starch, methylcellulose, water, or a flavor, wherein the flavor is selected at least one of yeast extract, garlic powder, onion powder, and salt.
42. A meat substitute comprising the composition according to any of claims 1-26 or the edible ingredient according to any of claims 31-41.
43. The meat substitute of claim 42, further comprising:
 - (a) 0.01%-5% (by weight of the meat replica matrix) of a non-animal protoporphyrin IX;

- (b) a compound selected from the group consisting of glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, and galactose, and any combination thereof;
- (c) at least 1.5 mM of a compound selected from the group consisting of cysteine, cystine, thiamine, methionine, and any combination thereof; and
- (d) one or more proteins selected from the group consisting of plant proteins, fungal proteins and algal proteins,

wherein the meat substitute is a ground beef-like food product that contains no animal product, and wherein cooking the ground beef-like food product results in the production of at least two volatile compounds which have a beef-associated aroma.

- 44. A method of producing a protoporphyrin IX composition, comprising:
 - (a) growing an algae population comprising an algae that is a protoporphyrin IX over-producer in a culture; and
 - (b) isolating a protoporphyrin IX composition from the culture.
- 45. The method of claim 44, wherein the step of growing comprises culturing the algae in an aerobic fermentation condition.
- 46. The method of claim 44 or claim 45, wherein the algae contains a chloroplast.
- 47. The method of claim 46, wherein biosynthesis of the protoporphyrin IX occurs in the chloroplast.
- 48. The method according to any of claims 44-47, wherein the algae is deficient for its ability to produce chlorophyll.
- 49. The method according to any of claims 44-48, wherein the algae is deficient for the ability to produce a functional Mg-chelatase enzyme.
- 50. The method according to any of claims 44-49, wherein the algae is reduced in or lacks one or more of CHLD, CHLI1, CHLI2 or CHLH1.
- 51. The method according to any of claims 44-50, wherein the algae is reduced in or lacks a functional light dependent protochlorophyllide.

52. The method according to any of claims 44-51, wherein the algae is reduced in or lacks a functional light independent protochlorophyllide.
53. The method according to any of claims 44-52, wherein the algae is reduced in or lacks ChlB, ChlL, or ChlN.
54. The method according to any of claims 44-53, wherein the algae overexpresses one or more of glutamyl-tRNA reductase, glutamate-1-semialdehyde aminotransferase, ALA dehydratase, porphobilinogen deaminase, UPG III synthase, UPG III decarboxylase, CPG oxidase, or PPG oxidase.
55. The method according to any of claims 44-54, wherein the algae is generated by mating to produce a generated strain, and wherein the generated strain is red or red-like in color.
56. The method according to any of claims 44-54, wherein the algae is generated by mutagenesis.
57. The method according to any of claims 44-56, wherein the algae is red or red-like in color.
58. The method according to any of claims 44-57, wherein the isolated protoporphyrin IX composition is an algae biomass.
59. The method of claim 58, wherein the algae biomass is fractionated.
60. The method of claim 59, wherein the algae biomass is fractionated to produce a protein-enriched fraction comprising protoporphyrin IX .
61. The method according to any of claims 44-57, wherein the isolated protoporphyrin IX composition is isolated from extracellular media of the algae culture.
62. The method according to any of claims 44-61, wherein the isolated protoporphyrin IX composition is isolated away from algae protein.
63. The method according to any of claims 44-62, wherein the algae is deficient for carotenoids.

64. The method according to any of claims 44-63, wherein the algae is a *Chlamydomonas* sp.
65. The method according to any of claims 44-64, wherein the algae is a *Chlamydomonas reinhardtii*.
66. The method of claim 64, wherein the *Chlamydomonas* sp. is strain CC-125 deposited to the University of Minnesota *Chlamydomonas* collection center or a derivative thereof.
67. The method according to any of claims 44-66, wherein progeny of a protoporphyrin IX overexpressing algae strain grows faster than its parental strain following a mating with another algae.
68. The method according to any of claims 44-66, wherein the protoporphyrin IX overexpressing algae is a strain produced by mating a strain deficient for carotenoids with a strain exhibiting a red or red-like color.
69. The method according to any of claims 44-66, wherein the protoporphyrin IX overexpressing algae is generated by mutagenesis of a first starting strain and selection of a second strain that grows faster in the dark than the first starting strain.
70. The method according to any of claims 44-66, wherein the protoporphyrin IX overexpressing algae is generated by mutagenesis of a first strain and selection of a second strain that lacks one or more carotenoids from the mutated first strain.
71. The method according to any of claims 44-70, wherein the algae lacks a functional ferrocheletase enzyme.
72. The method according to any of claims 44-70, wherein the algae is reduced in the amount of, or activity of, a ferrocheletase enzyme.
73. The method according to any of claims 44-70, wherein the algae is reduced in the amount of or lacks heme as compared to a wildtype strain.
74. A protoporphyrin IX-containing composition produced by the method according to any of claims 43-70.

75. The composition of claim 74, wherein the composition does not contain a detectable level of heme, heme-binding protein or a combination thereof.
76. The method according to any of claims 44-73, further comprising the steps of: (a) culturing the algae strain under a dark condition, wherein the strain does not produce or is reduced in the production of chlorophyll, and (b) collecting a portion of the algae culture that is red or red-like in color to produce the protoporphyrin IX composition.
77. The method of claim 76, wherein the algae is a *Chlamydomonas* sp.
78. The method of claim 77, wherein the algae is a *Chlamydomonas reinhardtii*.
79. The method according to claim 76, wherein the algae exhibits a red or red-like color when grown in the dark condition.
80. The method of claim 76, wherein the collected portion is extracellular media from the algae culture.
81. The method of claim 76, wherein the collected portion is a biomass or fractionated biomass from the algae culture.
82. The method of claim 76, wherein the algae is grown in an aerobic fermentation condition.
83. The method of claim 76, wherein the algae is grown to a density greater than about 10g/L, 20 g/L, 30 g/L, 40g/L, 50g, L, 75 g/L, 100g/L, 125 g/L, or 150g/L.
84. The method of claim 76, wherein the algae is grown with acetate as a reduced carbon source.
85. The method of claim 76, wherein the algae is grown with sugar as a reduced carbon source.
86. The method of claim 76, wherein the algae culture is supplemented with iron during the culturing step.
87. The method of claim 76, wherein the algae culture is inoculated at a density greater than about 0.1 g/L, 1.0 g/L, 5.0 g/L, 10 g/L, 20 g/L, 50 g/L, 80 g/L, or 100 g/L.

88. The method of claim 76, further comprising fractionating the collected portion, wherein the fractionating removes from the collected portion substantially all or most of a component selected from the group consisting of carotenoids, starch, and protein.
89. The method of claim 76, further comprising fractionating the collected portion, wherein the fractionating removes from the collected portion substantially all or most of heme, heme-binding protein or a combination thereof.
90. The method of claim 76, further comprising fractionating the collected portion, wherein the fractionating produces a protein-enriched fraction.
91. The method of claim 76, wherein the algae lacks or is reduced in one or more of magnesium chelatase, magnesium protoporphyrinogen IX, protochlorophyllide, chlorophyllide, and chlorophyll.
92. The method of claim 76, wherein the algae lacks or is reduced in ferrochelatase.
93. The method according to any of claims 44-73 or 76-92, wherein the algae is not a transgenic strain.
94. A clean meat product produced by the method according to any of claims 76-93, wherein the method further comprises combining the collected portion with a clean meat manufacturing composition, wherein the collected portion provides a red or red-like color to the clean meat product.
95. The clean meat product of claim 94, wherein the collected portion is a PPIX-enriched fraction or purified PPIX.
96. An edible ingredient produced by the method according to any of claims 44-73 or 76-93, wherein the protoporphyrin IX composition confers a meat or meat-like flavor, texture, odor or any combination thereof to the edible ingredient.
97. The edible ingredient of claim 96, wherein the edible ingredient is incorporated in a finished product selected from the group consisting of a beef-like food product, a fish-like product, a chicken-like product, a pork-like product and a meat replica.
98. The edible ingredient of any of claims 96-97, wherein the edible ingredient is vegan, vegetarian or gluten-free.

99. The edible ingredient of any of claims 96-98, wherein the edible ingredient is free of animal proteins.
100. The edible ingredient of any of claims 96-99, wherein the edible ingredient does not contain any genetically modified components.