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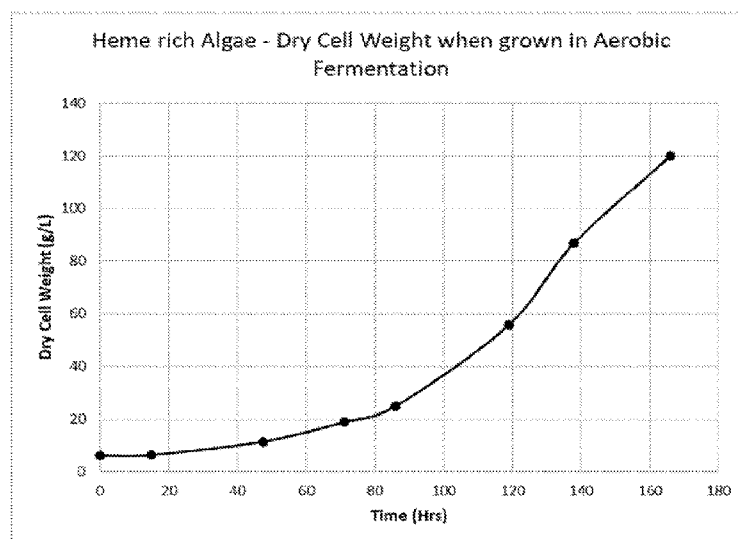


FIG. 6

(57) Abstract: Provided herein are compositions and processes for producing compositions from an algae to provide heme and a red or red-like color to edible compositions including ingredients and finished food products. Also provided are methods of growing heme-producing algae, methods of producing algae preparations therefrom and methods of making ingredients and food products with algae preparations. Also provided are compositions, including edible compositions that include heme and other nutrient components produced from algae.



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COMPOSITIONS AND METHODS FOR INCORPORATING HEME FROM ALGAE IN EDIBLE PRODUCTS

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 6, 2019, is named 20498-202379_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. Animal agriculture requires a significant amount of land use and fresh water, finite resources that are becoming increasingly difficult to access.

[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. To improve the sustainability of the food ecosystem it is imperative that products are developed that appeal to consumers who currently prefer meat.

[0005] Recent advances made 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. 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. Additionally, there is a trend to products with increased

nutrition benefit and a balance of caloric intake. A number of the current meat alternatives cannot fully satisfy these demands while maintain the taste, texture and visual appeal desired by consumers. Thus, a need exists for edible products incorporating heme-containing proteins as set forth herein.

SUMMARY OF THE INVENTION

[0006] To address both the economic and consumer concerns associated with the current approaches of incorporating heme into a product, provided herein are compositions and processes for producing such compositions that provide flavorful and nutritious alternatives to meat. In particular, provided herein are compositions and methods of producing such compositions that incorporate heme from algae, along with other nutrition components. Algae can be incorporated into finished products without the costly process of purification.

[0007] The present disclosure includes compositions of engineered algae overexpressing or accumulating heme and methods of using such engineered algae for food products. Thus, one aspect of the disclosure includes an engineered algae having a genetic modifications, where the genetic modification results in an accumulation of heme in the algae as compared to an algae lacking the genetic modification. In some embodiments, the engineered algae has reduced or absence of chlorophyll production. In some embodiments, the algae has red or red-like color. In some embodiments, the algae is capable of growth on glucose as the sole carbon source.

[0008] Preferably, the genetic modification comprises a genetic alteration to chlorophyll synthesis pathway, protoporphyrinogen IX synthesis pathway or heme synthesis pathway. In some embodiments, the genetic modification is associated with a deficiency in the expression of magnesium chelatase. Alternatively and/or additionally, the genetic modification comprises an alteration in one or more of CHLD, CHLI1, CHLI2 or CHLH1. Alternatively and/or additionally, the genetic modification comprises an alteration in an upstream regulatory region, a downstream regulatory region, an exon, an intron or any combination thereof. In some embodiments, the genetic modification comprises an insertion, a deletion, a point mutation, an inversion, a duplication, a frameshift or any combination thereof.

[0009] In some embodiments, the engineered algae has a heme content greater than the chlorophyll content. Alternatively and/or additionally, the engineered algae has a protoporphyrin IX content greater than the chlorophyll content. Alternatively and/or additionally, the engineered algae has reduced production of one or more fatty acids.

[0010] In some embodiments, the engineered algae further comprises a genetic modification that reduces or eliminates the expression of light independent protochlorophyllide oxidoreductase. In such embodiments, it is contemplated that the genetic modification comprises a mutation or deletion in one or more of ChlB, ChlL or ChlN. In some embodiments, the engineered algae has upregulated expression of ferrochelatase and/or upregulated expression of protoporphyrinogen IX oxidase. Optionally, the algae contain a recombinant or heterologous nucleic acid. In some embodiments, the engineered algae comprises a *Chlamydomonas* sp. Alternatively and/or additionally, the *Chlamydomonas* sp. is *Chlamydomonas reinhardtii*.

[0011] Another aspect of the disclosure includes an edible composition comprising an algae preparation, wherein the algae preparation comprises an engineered algae as described above or a portion thereof. In some embodiments, the edible composition comprises heme derived from the engineered algae. In some embodiments, the algae preparation comprises algae cells. In some embodiments, the algae preparation is a fractionated algae preparation. In some embodiments, the algae preparation is red or red-like in color.

[0012] In some embodiments, the edible composition has a red or red-like color derived from the algae preparation. Alternatively and/or additionally, the algae preparation confers a meat or meat-like flavor to the edible composition. Alternatively and/or additionally, the edible composition has a meat or meat-like texture derived from the algae preparation. In such embodiment, it is contemplated that the meat or meat-like texture comprises a beef or beef-like texture, a fish or fish-like texture, a chicken or chicken-like texture, a pork or pork-like texture or a texture of a meat replica.

[0013] In some embodiments, the edible composition is 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 composition is vegan, vegetarian or gluten-free. Alternatively and/or additionally, the edible composition has an appearance of blood derived from the algae preparation.

[0014] Alternatively and/or additionally, the algae preparation has a heme content greater than the chlorophyll content. Alternatively and/or additionally, the algae preparation has a protoporphyrin IX content greater than the chlorophyll content. In some embodiments, the algae 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 algae preparation provides vitamin A, beta carotene or a combination thereof to the composition. Optionally, 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. Alternatively and/or additionally, the algae 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 edible composition. Alternatively and/or additionally, the algae 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 a finished product comprising the edible composition. Alternatively and/or additionally, the algae 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 edible composition. Alternatively and/or additionally, the algae preparation has reduced fatty acid content.

[0015] In some embodiments, the edible product is combined with a protein source, a fat source, a carbohydrate, a starch, a thickener, a vitamin, a mineral, or any combination thereof. In such embodiments, it is preferred that the protein source is selected from the group consisting of textured wheat protein, textured soy protein and textured pea protein, fungal protein or algal protein. Alternatively and/or additionally, the fat source comprises at least one of refined coconut oil or sunflower oil. In some embodiments, the edible component 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.

[0016] In some embodiments, the edible product is an ingredient for a burger, a sausage, a kebab, a filet, a fish-alternative, a ground meat-like product or a meatball. In some embodiments, the burger comprises about 5% of the algae preparation, about 20% textured soy protein and about 20% refined coconut oil. Optionally, the burger further comprises about 3% sunflower oil, about 2% potato starch, about 1% methylcellulose, about 45% water and about 4-9% flavors. Alternatively and/or additionally, the burger further comprises about 0.5% Kojac gum, about 0.5% Xanthan gum, about 45% water and about 4-9% flavors. In some embodiments, fish-alternative comprises 20% textured soy protein, about 5 % of algae

preparation, about 65% water and about 10% flavors. In some embodiments, the edible composition is free of animal proteins.

[0017] In some embodiments, the algae preparation comprises an algae having an increase in protoporphyrinogen IX synthesis or accumulation. Alternatively and/or additionally, the algae preparation comprises an algae that exhibits a red or red-like color when grown in the dark conditions. In some embodiments, the algae comprised in the algae preparation are recombinant or genetically modified algae. In some embodiments, the algae preparation comprises a *Chlamydomonas* sp. Optionally, the *Chlamydomonas* sp. is *Chlamydomonas reinhardtii*.

[0018] Another aspect of the disclosure includes a method for the production of an edible composition. The method includes steps of (a) culturing an engineered algae as described above in a condition where the engineered algae exhibits a red or red-like color and wherein the engineered algae produces heme, (b) collecting the cultured engineered algae to produce an algae preparation, and (c) combining the algae preparation with at least one edible ingredient to produce an edible composition. In some embodiments, the condition comprises a fermentation condition. Alternatively and/or additionally, the condition comprises acetate as a reduced carbon source for growth of the engineered algae. Alternatively and/or additionally, the condition comprises sugar as a reduced carbon source for growth of the engineered algae. Alternatively and/or additionally, the condition comprises dark or limited light condition. Alternatively and/or additionally, the condition further comprises iron supplements.

[0019] In some embodiments, the method further comprises fractionating the cultured algae to produce the algae preparation. In some embodiments, the algae preparation has a heme content that is greater than the chlorophyll content. Alternatively and/or additionally, algae preparation has a protoporphyrin IX content that is greater than the chlorophyll content. In some embodiments, the engineered algae is a *Chlamydomonas* sp. Optionally, the engineered algae is a *Chlamydomonas reinhardtii*.

[0020] In some embodiments, the edible composition has at least one of the features selected from the group consisting of a meat or meat-like flavor, a meat or meat-like texture, a blood-like appearance and a meat or meat-like color, where the at least one of the features is derived from the algae preparation. In some embodiments, the method further comprises producing a finished product comprising the edible composition and wherein the finished

product is a beef-like food product, a fish-like product, a chicken-like product, a pork-like product or a meat replica. In some embodiments, the edible composition is free of animal proteins. In some embodiments, the algae preparation is fractionated to remove one or more of starch, protein, PPIX, fatty acids and chlorophyll.

[0021] Another aspect of the disclosure includes a method of making an engineered algae enriched in heme content. The method includes steps of (a) subjecting an algae strain to a process that produces genetic modification to create a first algae population, and (b) from the first algae population, selecting a second algae population that is enriched in heme content, and optionally, PPIX content. In some embodiments, the process comprises at least one of a random UV mutagenesis, a random chemical mutagenesis, a recombinant genetic engineering, a gene editing, or a gene silencing. In some embodiments, the method further comprises a step of culturing the first algae population in a fermentation condition. In some embodiments, the fermentation condition comprises a media having sugar as a sole carbon source. In such embodiments, it is preferred that the sugar is selected from glucose, dextrose, fructose, maltose, galactose, sucrose, and ribose. Alternatively and/or additionally, the fermentation condition comprises a brightness of less than 500 lux.

[0022] In some embodiments, the selecting the second algae population step comprises sorting or identifying algae cells having a red or red-like color. Alternatively and/or additionally, the second algae population step is performed by FACS. In some embodiments, the second algae population is selected with its capability to grow in the fermentation condition.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] **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 heme.

[0024] **FIGs. 2A** and **2B** show the composition of an exemplary algae growth media (Fig. 2A) and selection process (Fig. 2B).

[0025] **FIG. 3** is a pictorial diagram showing algae growth in complete dark condition with dextrose as the only carbon source.

[0026] **FIG. 4** is a pictorial diagram showing an exemplary fractionation of algae overexpressing heme, showing the separation into a protein and heme-enriched biomass, which is separated from the starch and carotenoid fractions.

[0027] **FIG. 5** is a pictorial diagram showing extraction process of PPIX and/or heme from the red algae.

[0028] **FIG. 6** is a graphical diagram showing an exemplary growth curve (dry cell weight) of a heme-overproducing strain when grown in aerobic fermentation conditions.

[0029] **FIG. 7** is a graphical diagram showing increased dry cell weight of *Chlamydomonas* sp. in a glucose-containing media.

[0030] **FIG. 8** is a graphical diagram showing the fractionated components of the red algae preparation before and after hexane extraction.

[0031] **FIG. 9** 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.

[0032] **FIG. 10** is a pictorial diagram showing burgers created with 0.01g, 0.1 g, 1.0g, and 5.0g of the heme enriched algae.

[0033] **FIG. 11** is a pictorial diagram showing ingredient mixes of the plant-based burger ingredients with no heme-enriched algae, with the addition of heme-enriched algae, or the ingredients with the addition of heme-enriched algae shaped into a burger before and after cooking.

[0034] **FIG. 12** is a pictorial diagram showing an example of heme-enriched meatless "tuna".

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.

[0036] 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.”

[0037] 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.

[0038] 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).

[0039] 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.

[0040] 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.

[0041] 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).

[0042] 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.

[0043] Provided herein are compositions and methods to provide heme and other nutrition components from algae. 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 heme to impart a red color and/or a meaty taste and smell.

[0044] Provided herein are strains, methods and compositions that employ algae overproducing heme. 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 heme, 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 heme, imparts a “meaty” flavor, smell and/or texture when incorporated into food and other edible products. In some embodiments, a preparation made from the algae culture overproducing heme, imparts a desired color, taste and/or smell, as well as one or more additional nutrition components such as omega-3 fatty acids, saturated fats, protein, vitamin A, beta-carotene or any combination thereof.

Algae Producing and Over-Producing Heme

[0045] Provided herein are algae strains that over-produce heme and strains that produce or accumulate heme and/or protoporphyrin IX (PPIX) content greater than chlorophyll content and that can be used to produce edible compositions and ingredients. Also provided herein are methods of making such strains and ingredients and compositions therefrom. and use with the methods herein to make such compositions. Such strains are created by modifying one or more steps in the biochemical pathways that produce heme, PPIX and chlorophyll.

[0046] 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 dehydrase. Next, porophobilinogen is converted to

hydroxymethylbilane by pophobilinogen 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.

[0047] By reducing metabolic flux towards chlorophyll, it is possible to increase metabolic flux towards heme B. In some embodiments herein, the algae strains used in the methods and compositions produced therewith are reduced in metabolic flux towards chlorophyll and increased metabolic flux towards heme B (also referred to herein as “heme”). In some embodiments, the algae strain is one where chlorophyll and carotenoid synthesis is decreased and heme synthesis or accumulation is increased. In some embodiments, the algae strain is deficient or reduced in the amount of chlorophyll. 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 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 gene products which are encoded in the chloroplast genome and are subunits of light independent protochlorophyllide oxidoreductase (LIPOR) that converts protochlorophyllide to chlorophyllide. This enzyme, when expressed, can allow algae such as *Chlamydomonas* to produce chlorophyll and remain green even when the algae is not provided with illumination. When one or more of these genes are knocked out, the algae strain has a yellow color under dark growing conditions.

[0049] In some embodiments, the algae strain is lacking or reduced in one or more of magnesium chelatase, magnesium protoporphyrinogen IX, protochlorophyllide, chlorophyllide, and chlorophyll.

[0050] 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 CHII1 and CHII2).

[0051] In some embodiments, a heme-enriched algae strain is deficient in one or more of a nuclearly encoded subunit of magnesium chelatase, for example in one or more of the subunits encoded by the genes for the subunits CHLD, CHLH and CHLI. A deficiency in one or more of these subunits reduces or eliminates chlorophyll expression. In some embodiments, the gene encoding a subunit can be modified, such as by one or more point mutations that change a codon to a stop codon, resulting in a truncated coding region. In some embodiments, the gene encoding a subunit can be modified by a deletion that removed some of or all of the gene encoding the subunit. In some embodiments, the gene encoding a subunit can be modified by a frameshift mutation, such as caused by a deletion or insertion of one or more bases into the coding region, resulting in a non-functional and/or truncated protein. In some embodiments, the gene encoding a subunit can be modified by an insertion into the coding region that creates a non-functional protein, such as by adding one or more amino acids internally or at the N or C terminus of the protein that creates a non-functional subunit or reduces the activity or stability of the subunit or enzyme.

[0052] In some embodiments, a heme-enriched algae has at least one modification in the nucleotide sequence encoding CHLD, CHLI1, CHLI2 or CHLH1 (e.g., a modification in SEQ ID NOs: 23, 25, 27, 153) including the intron, exon, regulatory regions, or full gene sequences. In some embodiments, a heme-enriched algae has at least one modification in the amino acid sequence of CHLD, CHLI1, CHLI2 or CHLH1 (e.g., a modification in SEQ ID NOs: 24, 26, 28, 151). In some embodiments, a heme-enriched algae strain contains at least one modification (point mutation, deletion, or insertion) in an exon encoding a portion of CHLD, CHLI1, CHLI2 or CHLH1. In some embodiments, a heme-enriched algae strain

contains at least one modification to a wildtype sequence of such exons, such as a modification in any of SEQ ID NOs: 47-58, 72-80, 91-102, and 132-141.

[0053] In some embodiments, a heme-enriched algae strain contains at least one modification (point mutation, deletion, or insertion) in an untranslated region of CHLD, CHLI1, CHLI2 or CHLH1, such as in the 5' untranslated region or the 3' untranslated region. In some embodiments, a heme-enriched algae strain contains at least one modification to a wildtype sequence of such untranslated regions, such as a modification in any of SEQ ID NOs: 45, 46, 70, 71, 89, 90, 130 or 131.

[0054] In some embodiments, the regulation of expression of one or more subunit of Mg-chelatase is altered to create a strain that has reduced amounts of chlorophyll. The regulatory regions of one or more of CHLD, CHLI1, CHLI2 and CHLH1 can be modified to reduce expression, such as by an insertion, deletion or one or more point mutations. Such alterations may modify, for example, transcription factor binding sites, enhancer sites, RNA polymerase interactions and transcriptional start sites in a manner the reduces or eliminates the transcription of a subunit gene.

[0055] In some embodiments, the expression of one or more subunits is altered by modifying the splicing of an intron with the gene of a subunit, such as a mutation, insertion or deletion that eliminates or alters a splicing donor or acceptor site or that otherwise alters the efficiency or accuracy of the gene splicing. In some embodiments, a heme-enriched algae strain contains at least one modification (point mutation, deletion, or insertion) in an intron of CHLD, CHLI1, CHLI2 or CHLH1. In some embodiments, a heme-enriched algae strain contains at least one modification to a wildtype sequence of such introns, such as a modification in any of SEQ ID NOs: 59-69, 81-88, 103-113, 142-150.

[0056] In some embodiments, the algae strain overexpresses one or more enzymes such that the balance of pathways favors heme production. In some embodiments, the algae strain overexpresses one or more of glutamyl-tRNA reductase, glutamyl-1-semialdehyde aminotransferase, ALA dehydrongenase, porphobilinogen deaminase, UPG III synthase, UPG III decarboxylase, CPG oxidase, PPG oxidase, and ferrochelatase. In some embodiments, the algae strain is improved for its ability to produce ALA, a rate limiting precursor of heme B synthesis. In some embodiments, the algae strain is improved 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 one or more of heme, a heme-containing protein, protoporphyrinogen IX, biliverdin IX, photochromobilin, and ferrocheletase, as compared to a wildtype strain.

[0057] 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, prolycopene and lycopene.

[0058] 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.

[0059] In some embodiments herein, algae used in the compositions and methods herein is non-GMO, does not contain heterologous nucleic acid and/or is not created using recombinant technology. In some embodiments, algae used in the compositions and methods herein is selected based on its color, heme content, rate of heme synthesis, accumulation of heme, or protoporphyrin IX content, rate of synthesis or accumulation. In some embodiments, the algae have reduced levels of chlorophyll and/or levels of chlorophyll that are less than the levels of heme and/or protoporphyrin IX. In some embodiments, algae used in the compositions and methods herein does not contain a heterologous gene for one more genes involved in heme biosynthesis or accumulation, e.g., the algae does not contain a bacterial, fungal, plant or animal-derived gene or nucleic acid that is involved in heme biosynthesis, heme accumulation, protoporphyrin IX biosynthesis, or protoporphyrin IX accumulation.

[0060] In some embodiments, algae are modified in expression of one or more genes contributing to an increase in heme synthesis or accumulation, a decrease in chlorophyll synthesis or accumulation or a combination thereof. Such modifications can be created through mutagenesis such as by exposure to UV light, radiation or chemicals.

[0061] 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 of heme. Subsequent to the creation of the modification, the algae strain can be 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 can be 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 can be used to make a modification to a component of the chlorophyll pathway such that heme expression and/or accumulation is increased. 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 CHLI1 resulting in a heme-enriched algae strain. Such modifications can be made by designing guide RNAs with modifications to one or more of SEQ ID NOs:45-113, 130-150 and/or 153 to include one or more point mutations, insertions, deletions or combinations thereof.

[0062] There are several families of engineered nucleases that can be used for gene editing described herein, 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 heme can be created by using techniques such as a CRISPR-Cas system (e.g., CRISPR-CAS9) or by the use of zinc-finger nucleases.

[0063] 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.

[0064] 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.

[0065] In some embodiments, a heme-enriched algae is created by genetically modifying a strain to modify the chlorophyll and/or heme pathways. Introduction of recombinant nucleic acids such as those that interfere with, inhibit or down-regulate expression of an endogenous gene (e.g., one or more of CHLD, CHLI1, CHLI2 or CHLH1) can alter the flux through the pathway. Such genetic modifications can include the integration of recombinant DNA in a regulatory region, exon or intron for an endogenous gene, as well as the gene silencing (e.g., introduction of antisense or siRNA for down regulating or silencing the expression of one or more endogenous genes). In some embodiments, expression of genes within the pathway can be upregulated such that the pathway produced more PPIX that can be converted to heme, or upregulates the expression or activity of ferrochelatase to produce more heme in the algae. Nucleic acids for modification of ferrochelatase can include 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. In some embodiments, a heme enriched algae may include an increased copy number of ferrocheletase or the provision of a construct to overexpress ferrocheletase (such as those provided by nucleic acid sequence SEQ ID NO: 7,

and protein sequence SEQ ID NO: 8). In some embodiments, genetic modifications include modifications to or expression of one or more genes in the chloroplast. In some embodiments, modifications are made to nuclear encoded genes or expression of such genes.

Algae Genus and Species for use in the Compositions and Methods

[0066] In the compositions and methods provided herein for producing heme and heme-containing compositions, algae strains that have a heme biosynthesis pathway are employed. In some embodiments, the algae strain for providing heme 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.

[0067] In some embodiments, the algae strain for providing heme is a single-celled algae. Illustrative and additional microalgae species of interest include without limitation, *Achnanthes orientalis*, *Agmenellum*, *Amphiprora hyaline*, *Amphora coffeiformis*, *Amphora coffeiformis lineae*, *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*, *Chlorella vulgaris* f. *tertia*, *Chlorella*
vulgaris var. *autotrophica*, *Chlorella vulgaris* var. *viridis*, *Chlorella vulgaris* var. *vulgaris*,
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 friderici*. 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, by screening, by selection or by mating with another algae strain.

[0068] In some embodiments, the algae strain for use in the methods herein and for making heme-containing compositions is selected or identified based on one or more phenotypes and/or genotypes. In some embodiments, the algae strain for overproducing heme can be created through mating processes. In some embodiments, the algae strain for overproducing heme can be created through mutagenesis, such as ultra violet mutagenesis. In some embodiments, the algae strain for overproducing heme can be generated through chemical mutagenesis with a compound that results in DNA alterations.

[0069] 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, and by genetic screening or phenotypic screening for increased expression and/or accumulation of heme, heme biosynthesis intermediates and heme biosynthesis enzymes. In some embodiments, the algae strain for use in the methods herein and for making heme-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 heme-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.

[0070] In some embodiments, algae strains are mated to combine or enhance characteristics that contribute to heme production, heme 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. 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.

[0071] 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 heme production, heme 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. In some embodiments, the strain includes further modifications, such as a modification that decreases omega oils (*e.g.*, omega-3 fatty acids) and/or a modification that allows the strain to grow on a particular carbon source such as glucose, dextrose, sucrose, etc.

[0072] In some embodiments, the algae is a *Chlamydomonas* species, such as *Chlamydomonas reinhardtii* and the strain has a visible red or reddish-brown appearance. In some embodiments, the strain also exhibits growth on glucose. In some embodiments, the strain has a genetic modification in the chlorophyll synthetic pathway, such as in a nuclearly encoded subunit of Mg-chelatase, such as in a gene encoding CHLD, CHLI1, CHLI2 or CHLH1, or in an intron or regulatory region thereof, whereby the strain overexpresses or is enriched in heme content. In some embodiments, the strain is also enriched in PPIX content. In some embodiments, the strain is capable of growing to high culture density under fermentation conditions.

Culture methods for overproducing heme strains

[0073] 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.

[0074] In some embodiments, the heme overproducing algae useful in the methods and compositions provided herein are grown in a controlled culture system, such as a small scale laboratory systems, large scale systems and closed systems and partially closed bioreactor systems. 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.

[0075] 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.

[0076] 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.

[0077] 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.

[0078] 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%, or 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.

[0079] In some embodiments, the heme-enriched strains herein are grown in dark or limited light conditions such that the pathway flux to biliverdin IX and photochromobilin are decreased, and the amount of heme in such strains is increased. In some embodiments, the heme-enriched strains herein are grown in dark or limited light condition and utilize a carbon source such as glucose.

Edible Food Products and Ingredients

[0080] Provided herein are edible products for human and animal consumption that contain heme from algae. In some embodiments, the edible product is a beef-like product, a fish-like product or a meat replica. In some embodiments, the edible product contains whole cell algae, where the algae provides heme to the composition. In some embodiments, the heme is imparted to the edible product by a whole cell algae component where the algae overproduce heme. In some embodiments, the heme is imparted to the edible product by an algae having a heme content greater than the chlorophyll content of the algae. In some embodiments, the heme is imparted to the edible product by an algae having a protoporphyrin content greater than chlorophyll content by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, or at least 50%.

[0081] In some embodiments, the edible product is a beef-like product, a fish-like product or a meat replica and the heme is provided by fractionated algae. For example, whole cell alga producing or overproducing heme can be subjected to fractionation methods to separate some or a substantial amount of biomass from the heme-containing fraction. The fractionation may remove one or more components of the algae biomass while leaving other components such as omega-3 fatty acids, fats, protein, vitamin A, beta-carotene or any combination thereof associated with the heme-containing fraction. In some embodiments, the

heme can be separated from one or more of the omega-3 fatty acids, saturated fats, protein, vitamin A, and/or beta-carotene of the algae. Extraction with solvents and buffers or a combination thereof can be used to provide a heme-enriched fraction. For example, an alga biomass or a fractions thereof can be enriched for heme through hexane extraction.

[0082] In some embodiments, the biomass is fractionated or otherwise treated to separate heme content and optionally, PPIX. 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 alga biomass herein is fractionated or otherwise treated such that the heme is separated from other components, including PPIX.

[0083] In some embodiments, the heme-containing fraction has a heme content greater than the chlorophyll content of the fraction by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, or at least 50%. In some embodiments, the heme-containing fraction has a protoporphyrin IX content greater than chlorophyll content of the fraction by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, or at least 50%. In some embodiments, the heme-containing fraction contains no chlorophyll or substantially no chlorophyll. In some embodiments, the heme-containing fraction has no chlorophyll or substantially no chlorophyll and has about 4.5% protoporphyrin IX content (on a weight per total weight basis, *e.g.*, 45 mg protoporphyrin IX in a 1 gram sample). In some embodiments, the heme-containing fraction has no chlorophyll or substantially no chlorophyll and has about 0.5% heme content (on a weight per total weight basis, *e.g.*, 5 mg heme in a 1 gram sample). In some embodiments, the heme-containing fraction has no chlorophyll or substantially no chlorophyll and has about 4.5% protoporphyrin IX content and has about 0.5% heme content (on a weight per total weight basis).

[0084] In some embodiments, a whole algae preparation used in the preparation of an edible composition has a heme content greater than the chlorophyll content of the fraction. In some embodiments, the whole algae preparation has a protoporphyrin IX content greater than chlorophyll content of the fraction. In some embodiments, the whole algae preparation contains no chlorophyll or substantially no chlorophyll. In some embodiments, the whole

algae preparation has no chlorophyll or substantially no chlorophyll and has about 4.5% protoporphyrin IX content (on a weight per total weight basis, *e.g.*, 45 mg protoporphyrin IX in a 1 gram sample). In some embodiments, the whole algae preparation has no chlorophyll or substantially no chlorophyll and has about 0.5% heme content (on a weight per total weight basis, *e.g.*, 5 mg heme in a 1 gram sample). In some embodiments, the whole algae preparation has no chlorophyll or substantially no chlorophyll and has about 4.5% protoporphyrin IX content and has about 0.5% heme content (on a weight per total weight basis).

[0085] In some embodiments, the whole algae preparation or fractionated algae preparation has no chlorophyll or substantially no chlorophyll and is made from an algae strain that does not make or accumulate chlorophyll. In some embodiments, the whole algae preparation or fractionated algae preparation has no chlorophyll or substantially no chlorophyll and is made from an algae strain that has one or more mutations in the chlorophyll synthesis pathway and/or has one or more mutations in the pathways that impact the accumulation or turnover of chlorophyll, for example, having a modification in one or more subunits of magnese chelatase such as a modification in one or more of CHLD, CHLI1, CHLI2 or CHLH1.

[0086] In some embodiments, the whole algae preparation or fractionated algae preparation contains heme at about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, 2.0%, 2.5% or more than 2.5% on a weight per total weight basis. In some embodiments, the whole algae preparation or fractionated algae preparation 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 some embodiments, the heme in the whole algae preparation or fractionated algae preparation is free heme. In some embodiments, the heme in the whole algae preparation or fractionated algae preparation is complexed with one or more proteins, for example complexed to one or more truncated hemoglobins. In some embodiments, the heme in the whole algae preparation or fractionated algae preparation is a mixture of free heme and heme complexed with protein.

[0087] In some embodiments, the whole cell or fractionated algae provides protein to the edible composition as well as providing heme. In some embodiments, the algae provides at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% of the protein to the edible

composition. In some embodiments, the algae provides greater than 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 protein in the edible product. In some embodiments, the whole cell or fractionated algae provides protein to the edible composition and the edible composition also contains protein from one or more additional sources, such as a plant-based source. In some embodiments, an alga fraction is enriched for protein as compared to the starting biomass. hexane extraction or an equivalent solvent can be used to enrich the protein content of the fraction. In some embodiments, carbohydrates and/or fatty acids are removed or reduced in amount through such extraction(s), while enriching for protein and/or enriching for heme.

[0088] In some embodiments, the whole cell or fractionated algae provides omega-3 fatty acids to the edible composition as well as providing heme. In some embodiments, the algae provides a daily recommended dosage of omega-3 fatty acids or a portion thereof to the edible product. For example, the whole cell or fractionated algae 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 edible composition.

[0089] In some embodiments, omega oils such as omega-3 fatty acids are removed from the alga biomass or a fractionated alga sample. Such oil removal can modify the aroma and taste of the alga biomass or faction, such as by decreasing or removing a “fishy” aroma or taste that can be present in an alga-derived product. In some embodiments, hexane or a similar solvent such as isohexane, heptane, butane or other alcohol, is used in the preparation of the alga biomass or fractionation to modify the aroma and taste. In some cases, hexane or similar solvent extraction removes or decreases the amount of oils, as well as enriches for heme and/or enriches for protein in the resulting product.

[0090] In some embodiments, algae biomass or fractionate algae are made using a strain deficient in one or more omega oils. Such strains can be combined with a heme-enriched strain, such as through mating to produce a heme-enriched strain that produces less omega oils.

[0091] In some embodiments, the whole cell or fractionated algae provides vitamin A to the edible composition as well as providing heme. In some embodiments, the algae provides

a daily recommended dosage of vitamin A or a portion thereof to the edible product. For example, the whole cell or fractionated algae 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, the whole cell or fractionated algae provides no more than about 2,000 µg, 2,500 µg or 3,000 µg of retinol activity equivalents (RAE) for vitamin A.

[0092] In some embodiments, the whole cell or fractionated algae provides beta-carotene to the edible composition as well as providing heme. In some embodiments, the algae provides a daily recommended dosage of beta-carotene or a portion thereof to the edible product. For example, the whole cell or fractionated algae 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. In some embodiments, the algae 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.

[0093] In some embodiments, the whole cell or fractionated algae that provides heme contains saturated fat. In some embodiments, the algae provides less than daily recommended limit for saturated fat or a portion thereof to the edible product. For example, the whole cell or fractionated algae provides 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. In some embodiments, the algae 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.

[0094] In some embodiments herein, the heme-containing whole algae or algae fraction is used to create an edible composition that is then used as an ingredient in a finished product. The ingredient may provide heme as well as omega-3 fatty acids, fats, protein, vitamin A, beta-carotene or any combination thereof to the ingredient. Such ingredient may be a colorant, texturant, binder, nutrient source, taste or flavor enhancer, or a filler.

[0095] In some embodiments, the heme-containing whole algae or algae fraction is used to create an edible composition that is a finished product. For example, the finished product may be a meat-like product such as a burger, a patty, a cake, a ground “meat,” a sausage, a kebab, a steak, cubed “meat,” a “meatball,” a filet, a drumstick, a “chicken finger,” or a “chicken nugget.” The finished product may be a meat-like product made to resemble beef, chicken, pork, wild game, turkey or other consumable meat product. The finished product may be a fish-like product made to resemble a fish filet, a fish patty or cake, a fish ball, a fish salad, ground fish, a fish nugget, a fish burger or the like, such as a tuna product, a spicy tuna product or a salmon product.

[0096] The whole algae or algae fraction may provide omega-3 fatty acids, saturated fats, protein, vitamin A, beta-carotene or any combination thereof to the finished product. In some embodiments, the whole algae or algae fraction can be reduced in omega oils and used for the finished product. Meat-like products can be made with a whole algae or algae fraction from a heme-enriched algae that is as described herein, by processing or by strain type, reduced in the amount of omega oils.

[0097] In some embodiments, the finished product comprising the whole algae or algae fraction is a cooked product. In some embodiments, the finished product comprising the whole algae or algae fraction is a uncooked product or raw product. In some embodiments, the finished product comprising the whole algae or algae fraction is a partially-cooked product.

Heme-containing preparations and products

[0098] Algae strains and cultures overproducing heme such as described herein can be used in various forms and preparations. In some embodiments, a heme-containing composition is prepared from an algae culture overproducing heme, where the composition is red or red-like in color.

[0099] In some embodiments, the heme-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 heme. In some embodiments, the fractionated or treated biomass is enriched in protein or in protein and heme. 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.

[0100] In some embodiments, the heme-containing composition is a heme-containing liquid prepared from the culture media of the cultured algae. In some embodiments, the heme-containing composition is prepared from heme found extracellularly in the algae culture. In some embodiments, the algae culture is lysed or otherwise treated to release heme from the cells. In some embodiments, the heme-containing liquid is further fractionated to remove one or more components. In some embodiments, the heme-containing liquid is fractionated to remove starch. In some embodiments, the heme-containing liquid is fractionated to remove protein. In some embodiments, the heme-containing liquid is fractionated or otherwise treated to remove carotenoids. In some embodiments, the heme-containing liquid is fractionated or otherwise treated to enrich for certain components. In some embodiments, the fractionated or treated heme-containing liquid is enriched in heme. In some embodiments, the fractionation or treatment enhances the red or red-like color of the preparation.

[0101] The heme-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 heme-containing compositions can be combined with additional components and ingredients. In some embodiments, the heme-containing composition is combined with additional ingredients to create an edible product. In some embodiments, the heme-containing composition confers a red or red-like color to the edible product. In some embodiments, the heme-containing composition confers a meat-like characteristic such as a meat-like taste, meat-like flavor aroma and/or texture to the edible product. In some embodiments, the heme-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. Alternatively, at 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.

[0102] In some embodiments, heme-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 heme-containing composition made from an over-producing algae is combined with additional ingredients to create a meat-like product whereby the addition of the heme-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 heme-containing composition are conferred to the raw or uncooked product. In some embodiments, the meat-like features conferred by the heme-containing composition is conferred to the cooked product.

[0103] In some embodiments, whole algae or fractionated algae is combined with an additional protein source in an edible composition. For example, the protein source is 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, 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 is 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 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.

[0104] 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

[0105] 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.

[0106] Embodiment 1. An engineered algae having a genetic modifications, where the genetic modification results in an accumulation of heme in the algae as compared to an algae lacking the genetic modification. 2. The engineered algae of embodiment 1, wherein the engineered algae has reduced or absence of chlorophyll production. 3. The engineered algae of embodiment 1 or embodiment 2, wherein the algae has red or red-like color. 4. The engineered algae according to any of embodiments 1-3, wherein the algae is capable of growth on glucose as the sole carbon source. 5. The engineered algae according to any of embodiments 1-4, wherein the genetic modification comprises a genetic alteration to chlorophyll synthesis pathway, protoporphyrinogen IX synthesis pathway or heme synthesis pathway. 6. The engineered algae according to any of embodiments 1-5, wherein the genetic modification is associated with a deficiency in the expression of magnesium chelatase. 7. The engineered algae according to any of embodiments 1-6, wherein the genetic modification comprises an alteration in one or more of CHLD, CHLI1, CHLI2 or CHLH1. 8. The engineered algae of embodiment 7, wherein the genetic modification comprises an alteration in an upstream regulatory region, a downstream regulatory region, an exon, an intron or any combination thereof. 9. The engineered algae according to any of embodiments 5-8, wherein the genetic modification comprises an insertion, a deletion, a point mutation, an inversion, a

duplication, a frameshift or any combination thereof. 10. The engineered algae according to any of embodiments 1-9, wherein the engineered algae has a heme content greater than the chlorophyll content. 11. The engineered algae according to any of embodiments 1-10, wherein the engineered algae has a protoporphyrin IX content greater than the chlorophyll content. 12. The engineered algae according to any of embodiments 1-11, wherein the engineered algae has reduced production of one or more fatty acids. 13. The engineered algae according to any of embodiments 1-12, wherein the engineered algae further comprises a genetic modification that reduces or eliminates the expression of light independent protochlorophyllide oxidoreductase. 14. The engineered algae of embodiment 13, wherein the genetic modification comprises a mutation or deletion in one or more of ChlB, ChlL or ChlN. 15. The engineered algae according to any of embodiments 1-14, wherein the engineered algae has upregulated expression of ferrochelatase. 16. The engineered algae according to any of embodiments 1-15, wherein the engineered algae has upregulated expression of protoporphyrinogen IX oxidase. 17. The engineered algae according to any of embodiments 1-16, wherein the algae contain a recombinant or heterologous nucleic acid. 18. The engineered algae according to any of embodiments 1-17, wherein the engineered algae comprises a *Chlamydomonas* sp. 19. The engineered algae of embodiment 18, wherein the *Chlamydomonas* sp. is *Chlamydomonas reinhardtii*.

[0107] Embodiment 20. An edible composition comprising an algae preparation, wherein the algae preparation comprises an engineered algae of any of embodiments 1-19 or a portion thereof. 21. The edible composition of embodiment 20, wherein the edible composition comprises heme derived from the engineered algae. 22. The edible composition of embodiment 20, wherein the algae preparation comprises algae cells. 23. The edible composition of embodiment 20, wherein the algae preparation is a fractionated algae preparation. 24. The edible composition according to any of embodiments 20-23, wherein the algae preparation is red or red-like in color. 25. The edible composition according to any of embodiments 20-24, wherein the edible composition has a red or red-like color derived from the algae preparation. 26. The edible composition according to any of embodiments 20-25, wherein the algae preparation confers a meat or meat-like flavor to the edible composition. 27. The edible composition according to any of embodiments 20-26, wherein the edible composition has a meat or meat-like texture derived from the algae preparation. 28. The edible composition according to embodiment 27, wherein the meat or meat-like texture comprises a beef or beef-like texture, a fish or fish-like texture, a chicken or chicken-like

texture, a pork or pork-like texture or a texture of a meat replica. 29. The edible composition according to any of embodiments 20-28, wherein the edible composition is 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. 30. The edible composition according to any of embodiments 20-29, wherein the edible composition is vegan, vegetarian or gluten-free. 31. The edible composition according to any of embodiments 20-30, wherein the edible composition has an appearance of blood derived from the algae preparation. 32. The edible composition according to any of embodiments 20-31, wherein the algae preparation has a heme content greater than the chlorophyll content. 33. The edible composition according to any of embodiments 20-32, wherein the algae preparation has a protoporphyrin IX content greater than the chlorophyll content. 34. The edible composition according to any of embodiments 20-33, wherein the algae 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. 35. The edible composition according to any of embodiments 20-34, wherein the algae preparation provides vitamin A, beta carotene or a combination thereof to the composition. 36. The edible composition of embodiment 35, 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. 37. The edible composition according to any of embodiments 20-36, wherein the algae 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 edible composition. 38. The edible composition according to any of embodiments 20-37, wherein the algae 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 a finished product comprising the edible composition. 39. The edible composition according to any of embodiments 20-38, wherein the algae 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 edible composition. 40. The edible composition according to any of embodiments 20-39, wherein the algae preparation has reduced fatty acid content. 41. The edible composition according to any of embodiments 20-40, wherein the edible product is combined with a protein source, a fat source, a carbohydrate, a starch, a thickener, a vitamin, a mineral, or any combination

thereof. 42. The edible composition of embodiment 41, wherein the protein source is selected from the group consisting of textured wheat protein, textured soy protein and textured pea protein, fungal protein or algal protein. 43. The edible composition of embodiment 41, wherein the fat source comprises at least one of refined coconut oil or sunflower oil. 44. The edible composition of any of embodiments 41-43, 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. 45. The edible composition of any of embodiments 41-44, wherein the edible product is an ingredient for a burger, a sausage, a kebab, a filet, a fish-alternative, a ground meat-like product or a meatball. 46. The edible composition of embodiment 45, wherein the burger comprises about 5% of the algae preparation, about 20% textured soy protein and about 20% refined coconut oil. 47. The edible composition of embodiment 46, further comprising about 3% sunflower oil, about 2% potato starch, about 1% methylcellulose, about 45% water and about 4-9% flavors. 48. The edible composition of embodiment 46, further comprising about 0.5% Kojac gum, about 0.5% Xanthan gum, about 45% water and about 4-9% flavors. 49. The edible composition of embodiment 45, wherein the fish-alternative comprises 20% textured soy protein, about 5 % of algae preparation, about 65% water and about 10% flavors. 50. The edible composition according to any of embodiments 20-49, wherein the edible composition is free of animal proteins. 51. The edible composition according to any of embodiments 20-50, wherein the algae preparation comprises an algae having an increase in protoporphyrinogen IX synthesis or accumulation. 52. The edible composition according to any of embodiments 20-51, wherein the algae preparation comprises an algae that exhibits a red or red-like color when grown in the dark conditions. 53. The edible composition according to any of embodiments 20-52, wherein the algae comprised in the algae preparation are recombinant or genetically modified algae. 54. The edible composition according to any of embodiments 20-53, wherein the algae preparation comprises a *Chlamydomonas* sp. 55. The edible composition of embodiment 54, wherein the *Chlamydomonas* sp. is *Chlamydomonas reinhardtii*.

[0108] Embodiment 56. A method for the production of an edible composition comprising: (a) culturing an engineered algae according to any of embodiments 1-19 in a condition where the engineered algae exhibits a red or red-like color and wherein the engineered algae produces heme; (b) collecting the cultured engineered algae to produce an algae preparation; and (c) combining the algae preparation with at least one edible ingredient to produce an edible composition. 57. The method of embodiment 56, wherein the condition

comprises a fermentation condition. 58. The method according to any of embodiments 56-57, wherein the condition comprises acetate as a reduced carbon source for growth of the engineered algae. 59. The method according to any of embodiments 56-58, wherein the condition comprises sugar as a reduced carbon source for growth of the engineered algae. 60. The method according to any of embodiments 56-59, wherein the condition comprises dark or limited light conditions. 61. The method according to any of embodiments 56-60, wherein the method further comprises fractionating the cultured algae to produce the algae preparation. 62. The method according to any of embodiments 56-61, wherein the algae preparation has a heme content that is greater than the chlorophyll content. 63. The method according to any of embodiments 56-62, wherein the algae preparation has a protoporphyrin IX content that is greater than the chlorophyll content. 64. The method according to any of embodiments 56-63, wherein the condition further comprises iron supplements. 65. The method according to any of embodiments 56-64, wherein the engineered algae is a *Chlamydomonas* sp. 66. The method of embodiment 65, wherein the engineered algae is a *Chlamydomonas reinhardtii*. 67. The method according to any of embodiments 56-66, wherein the edible composition has at least one of the features selected from the group consisting of a meat or meat-like flavor, a meat or meat-like texture, a blood-like appearance and a meat or meat-like color, where the at least one of the features is derived from the algae preparation. 68. The method according to any of embodiments 56-67, wherein the method further comprises producing a finished product comprising the edible composition and wherein the finished product is a beef-like food product, a fish-like product, a chicken-like product, a pork-like product or a meat replica. 69. The method according to any of embodiments 56-68, wherein the edible composition is free of animal proteins. 70. The method according to any of embodiments 56-69, wherein the algae preparation is fractionated to remove one or more of starch, protein, PPIX, fatty acids and chlorophyll.

[0109] Embodiment 71. A method of making an engineered algae enriched in heme content, comprising: (a) subjecting an algae strain to a process that produces genetic modification to create a first algae population; and (b) from the first algae population, selecting a second algae population that is enriched in heme content, and optionally, PPIX content. 72. The method according to embodiment 71, wherein the process comprises at least one of a random UV mutagenesis, a random chemical mutagenesis, a recombinant genetic engineering, a gene editing, or a gene silencing. 73. The method according to embodiment 71 or embodiment 72, further comprising culturing the first algae population in a

fermentation condition. 74. The method according to embodiment 73, wherein the fermentation condition comprises a media having sugar as a sole carbon source. 75. The method according to embodiment 74, wherein the sugar is selected from glucose, dextrose, fructose, maltose, galactose, sucrose, and ribose. 76. The method according to any of embodiments 73-75, wherein the fermentation condition comprises a brightness of less than 500 lux. 77. The method of any of embodiments 73-76, wherein the selecting the second algae population comprises sorting or identifying algae cells having a red or red-like color. 78. The method of any of embodiments 73-77, wherein the selecting is performed by FACS. 79. The method according to any of embodiments 73-78, the second algae population is selected with its capability to grow in the fermentation condition.

EXAMPLES

Example 1: Mutagenesis of algae and selection of strains

[0110] A wildtype strain of algae (*Chlamydomonas* sp.) was subjected to UV irradiation with an excitation wavelength of 420nm and an emission of 635nm. Strains were first selected for their ability to grow on alternatives carbon sources such as glucose. One of these selected strains was further mutagenized using similar conditions to select and/or identify for red-colored strains using fluorescence screening (e.g., Fluorescence-activated cell sorting (FACS)) or magnetic or bead-based cell sorting. These selections are illustrated in FIG. 2 and as further detailed below.

[0111] Strains of algae (*Chlamydomonas reinhardtii*) overexpressing heme 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.

[0112] To generate strains of algae overexpressing heme, green parental strains of *Chlamydomonas reinhardtii* were placed in a UV-light cross linker and exposed to 25 - 300mJ/cm² of UV-light to induce random mutations. Following the exposure to UV-light strains were recovered on agar plates and placed into the dark. Once recovered, the strains were pulled into a flask with growth media and grown placed in a shaker in the dark to limit their potential for exposure to light which could cause many of the heme rich strains to be lost. Flask for cultured for a week in the dark and then applied to a flow cytometer. Cells

were excited with a 420nm light and excitation was measured at $595\pm 15\text{nm}$ and $635\pm 15\text{nm}$. Cells that had a high excitation signal at $595\pm 15\text{nm}$ were avoided as this the fluorescent signal for Mg-protoporphyrin, a precursor to the formation of chlorophyll. Cells that had a high fluorescent excitation signal at $635\pm 15\text{nm}$ were sorted into a pulled population as this fluorescent signal is indicative of high protoporphyrin IX. Once pulled, cells were spread on a plate an individual colonies grown and their individual fluorescent characteristics determined by a 96-well plate reader. This process resulted in the identification of 50 strains that had elevated levels of protoporphyrin IX and heme.

[0113] One of these red strains was subjected to genomic sequencing at the loci involved in chlorophyll and heme biosynthesis. Sequencing indicated that the genetic modification occurred in the CHLH locus. The sequence of CHLH of the red strain is provided in SEQ ID NO: 129 (nucleotide sequence) and SEQ ID NO: 152 (amino acid sequence). 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. 9 (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.

Example 1A: Identification of heme rich *Chlamydomonas* sp. that grow on sugar as their sole reduced carbon source.

[0114] The use of sugar as a carbon source versus acetate has an economic benefit to the cost of production *Chlamydomonas* algae. To date, no strains of *Chlamydomonas reinhardtii* have been identified that grow on sugar as a carbon source. Typically, as shown in FIG. 3, *Chlamydomonas reinhardtii* requires acetate or sunlight and carbon dioxide to grow. Strains of algae from the wild or various culture collection centers were plated on agar growth media with dextrose added at 25g/L. The plates were then placed in the dark to ensure that photosynthesis could not occur. Cultures were allowed to grow for 2 weeks. At the end of two weeks cultures were studied for their ability to grow in conditions devoid of light. Strains that were capable of growing in the dark with dextrose as their primary carbon source were

then placed into shake flasks with growth medium and dextrose at 25g/L as the primary carbon source and growth for a week in the dark. Culture density and sugar concentration in the media was monitored daily to determine if dextrose was being metabolized by the strains.

[0115] Following their identification, *Chlamydomonas* sp. strains that grew on dextrose as a carbon source were mutagenized using a UV-crosslinker. Cultures were exposed to 25 - 300mJ/cm² of UV-light to induce mutations. Following the exposure to UV-light strains were recovered on agar plates and placed into the dark. Once recovered, the strains were pulled into a flask with growth media and grown placed in a shaker in the dark to limit their potential for exposure to light which could cause many of the heme rich strains to be lost. Flask for cultured for a week in the dark and then applied to a flow cytometer. Cells were excited with a 420nm light and excitation was measured at 595±15nm and 635±15nm. Cells that had a high excitation signal at 595±15nm were avoided as this the fluorescent signal for Mg-protoporphyrin, a precursor to the formation of chlorophyll. Cells that had a high fluorescent excitation signal at 635±15nm were sorted into a pulled population as this fluorescent signal is indicative of high protoporphyrin IX. Once pulled, cells were spread on a plate an individual colonies grown and their individual fluorescent characteristics determined by a 96-well plate reader. This process resulted in the identification of 20 strains that had elevated levels of protoporphyrin IX and heme and that were still able to grow on dextrose.

[0116] 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	≤ 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	≤ 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	≤ 0.01ppm	≤ 0.01ppm	ppm	MET-CH-030	Specification Met
Cadmium	≤ 0.1ppm	≤ 0.01ppm	ppm	MET-CH-030	Specification Met
Lead	≤ 0.01ppm	≤ 0.01ppm	ppm	MET-CH-030	Specification Met
Mercury	≤ 0.005ppm	≤ 0.01ppm	ppm	MET-CH-030	Specification Met
Sulfite	≤ 10 ppm	≤ 0.01ppm	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 1B: Identification of heme-overproducing algae

[0117] 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 120g/L and harvested via centrifugation. The harvested strain is red in color and can be added to compositions, such as food products, to confer a red, orange or brown color. FIG. 6 is a graph showing the cell weight of the heme overproducer strain grown in aerobic fermentation conditions.

Example 1C: High density growth of heme-overproducing algae

[0118] Strains of *Chlamydomonas* that were previously selected for their ability to overexpress heme were grown to high density. To do this, a basal media containing media components that would allow the culture to achieve 120 grams per liter was developed. The strains are fresh water algae as such media components when solubilized with water were made not to exceed 10mS/cm. Cultures were then grown using an aerobic fed-batch fermentation process. Cultures were fed with a media containing acetate as a carbon source, ammonium hydroxide as a nitrogen source, and phosphoric acid as a phosphate source. Cultures were fed using a one sided acid pH-stat to maintain the pH at 6.8. As shown in FIG. 6, cultures were allowed to grow for 7 day and titers of 120g/L of biomass were achieved. Heme and protoporphyrin IX was quantified by using a heme quantification assay (Abnova KA1617). Heme and protoporphyrin were found to be greater than 5% of the biomass by weight. Titters of greater than 1g/L of heme and protoporphyrin IX were achieved. In short, heme/protoporphyrin IX were extracted from a defined amount of algae culture by mixing the algae culture with a solution of 1.7M HCL and 80% Acetone. The mixture was allowed to sit for 30 minutes. After 30 minutes samples were centrifuge to separate the heme/protoporphyrin IX extract from the algal biomass. The soluble heme/protoporphyrin IX samples were used in the assay from Abnova and compared to a standard curve to determine the amount of heme/protoporphyrin IX in the algal biomass.

Example 2: Fractionation

[0119] Cells from a heme overproducing strain of *Chlamydomonas reinhardtii* were harvested from a fermentation culture. The harvested cells were disrupted by sonication and then the samples were separated by centrifugation at 10,000 x G. This separated the samples into a carotenoid, starch and protein/heme biomass fractions. The protein/heme biomass was then re-suspended in Phosphate buffered saline pH 7.4. Shown in FIG. 4 is the fractionation following centrifugation (left) and the resuspension of the heme-containing fraction (right). Also shown in FIG. 5 illustrates process of PPIX and heme fractionation process and/or process of generating biomass, extracts, and/or lyophilized products.

Example 3: Characterization of heme production

[0120] A number of heme assays can be used to determine the concentration of heme. In one example, the amount of heme can be quantitatively determined by mixing the algae biomass into an aqueous alkaline solution causing the heme to be converted into a uniform color. The intensity of the color can be measured by the absorbance at 400 nm which is directly proportional to the heme concentration in the sample. These measurements can then be compared to standards generated by heme at known concentrations to determine the amount of heme in algae samples.

Example 4: Preparation of a heme-enriched “meatless” burger

[0121] The heme-enriched samples can be used to prepare compositions of meat-like products produced from plant based materials and algae rich in heme. To create a heme-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 heme-enriched (“red”) algae. Shown in FIG. 10 are burgers created with 0.01g, 0.1 g, 1.0g, and 5.0g of the heme enriched algae.

[0122] In this example, the composition of the heme-enriched algae was 4.5% protoporphyrin IX, 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 heme-enriched plant-based burger

[0123] The heme-enriched samples can be used to prepare burger compositions from plant based materials and algae rich in heme. To create a heme-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 heme-enriched (“red”) algae. Shown in FIG. 11 are the ingredient mixes of the plant-based burger ingredients with no heme-enriched algae (far left), with the addition of heme-enriched algae (second from left), the ingredients with the addition of heme-enriched algae shaped into a burger before and after cooking (thirds from left and far right photos, respectively). As shown, the addition of the heme-enriched algae confers 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.

[0124] In this example, the composition of the heme-enriched algae was 4.5% protoporphyrin IX, 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: Preparation of a heme-enriched meatless “tuna”

[0125] The heme-enriched samples can be used to prepare fish-like compositions, as shown in FIG. 12. To create a heme-enriched meatless “fish”, ingredients were mixed in the following proportions: 20% or about 20% Textured soy protein, 65% or about 65% water and 10% or about 10% Flavors and 5% or about 5% heme-enriched (“red”) algae. Shown in FIG. 12 is a square portion of the meatless “tuna.”

[0126] In this example, the composition of the heme-enriched algae was 4.5% protoporphyrin IX, 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 7: Growth of heme-enriched algae strain on glucose

[0127] A heme-enriched algae strain was grown in a media with glucose as the sole carbon source. Briefly, as shown in FIG. 2, media was prepared in water, providing per liter

of total volume 25g anhydrous glucose, 5g KNO₃, 0.5275g KH₂PO₄, 0.3925g MgSO₄*7H₂O, 0.031275g FeSO₄*7 H₂O, 0.007125g H₃BO₃, 0.002 CuSO₄, 0.002775g ZnSO₄, 0.002425g CoSO₄, 0.00325g MnCl₂*4H₂O, 0.00115g (NH₄)₆Mo₇O₂₄*4H₂O, and 0.01735g CaCl. The media was adjusted to pH 7.0, autoclaved and had a final pH between 5.5 to 6.5 The algae strain was inoculated at a density of about 0.1g/L.

[0128] The culture was placed in a dark incubator (devoid of light) and grown at 30°C on a rotating shaker platform. Culture density (measured by dry cell weight) and residual glucose concentration in the media were measured daily. FIG. 7 shows the increase in dry cell weight over time and a concomitant decrease in residual glucose in the media. Dry cell weight in this experiment reached over 25g/L dry cell weight.

Example 8: Extraction of heme fraction from whole biomass

[0129] Using the heme-enriched algae (grown similarly to Example 1), a heme-enriched fraction was prepared. Approximately 100g of algae biomass was mixed with a 1.0L of a solution containing 80% acetone and 20% 1.7M HCL for 30 minutes. The biomass was allowed to settle and then the aqueous layer was extracted (containing heme and protoporphyrin IX) away from the solid into new container. Centrifugation was applied to the extracted aqueous layer or in some experiments, the sample was filtered with a filter having a molecular cutoff of 0.4um. The resulting aqueous fraction was neutralized with 10M NaOH. Then water was added at 100 ml per 100 ml of sample. Following this mixture, the heme and protoporphyrin IX became insoluble and fell out of solution. The solution was then centrifuged to collect the solids (containing the heme and protoporphyrin IX) and dried to form a red powder. FIG. 5 shows the red-like colored fractions (containing the heme and protoporphyrin IX) collected through the steps of the procedure. From 160g of red algae biomass, 7.7g of PPIX/heme was extracted.

Example 9: Removal of fatty acids from algae biomass to enrich for heme

[0130] Dry Chlamydomonas cells were mixed together with water ethanol and hexane in a ratio of 6:77:17. Samples were allowed to separate for 4 hours. The aqueous layer containing the fatty acids was then removed. The sample was then centrifuged to full separate the solid biomass layer from any remaining fatty acids. The biomass was then dried prior to further analysis. FIG. 8 shows a biochemical analysis of the algae biomass before

and after the fatty acid extraction, demonstrating a greater than 10-fold reduction in fatty acid content after the extraction procedure.

Example 10: Targeted Modification of chlorophyll pathway to create heme-enriched strains

[0131] Guide RNAs (sgRNAs) can be designed against any of the sub-units of the magnesium chelatase gene to cause a deletion or an insertion that renders the protein complex non-functional. 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 magnesium chelatase 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 the magnesium chelatase 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 Mg-chelatase can be identified by eye as they are not green. Mutants are then sequenced to ensure that target mutation was introduced.

Example 11: Modification of chlorophyll pathway to create heme-enriched strains that are improved for different meat imitations.

[0132] Strains of algae that increase the precursors to heme such as aminolevulinic acid can be mated to strains that are overexpressing heme to further increase the amount of heme or protoporphyrin IX that are produced. 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 heme by measuring for an increase in fluorescence of the precursor protoporphyrin IX or by biochemical assay (Abnova KA1617).

[0133] Strains of algae overexpressing heme can also 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.

SEQUENCES

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

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

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

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45

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

atgcagatgcagctgaacgccaaagaccgtgcagggcgccctcaaggcgagcgccctcgctctgtccgcggcaac
 gtggcggtgcgcgagtgccgctccccctaagctggtcaccaagcgctccgaggagatcttcaaggaggtcag
 gagctgctgcccgggtggcgtgaactcgcccgctgcgcgctttccgctcggttggtggcgcccatcgctctcgac
 agggtaagggtgctactgctgggacgtcgatggcaacaagtaacatcgactacgttggtctctggggccctgcc
 atttgccggccacggcaacgacgaggtcaacaacgcctgaaggcgagatcgacaaggcgacctcgctcggtgct
 ccctgcgagctggagaacgtgctggccaagatgggtgattgaccgctgcctcggtggagatgggtgcgcttcgtg
 tcctcgggcactgaggcgctgctcggtgctgcgcgctgatgcgcgcatacaccggccgcgagaaggtgctgaag
 ttaccggctgctaccacggccacgcgcgactcctcctgggtgaaggcggtcggtggtgatcacctgggctg
 ccgactcgccgggtgtgcccagagcaccgcgcgcgcacccctgaccgccacctacaacaacctggactccgtg
 cgcgagctgttcgcgcgaacaaggcgagattgcgggtgtgatcctggagcccggtggtcggaacacgcggcttc
 attgtgccccaaggaggttcctgcagggcctgcgcgagatctgcacggctgagggcgccctgctgtgcttcgat
 gaggtcatgaccggcttcgcgattgccaaaggctgcgcgcaggagcactcggtatcacccccgacctgaccacc
 atgggcaagggtcattgggtggcgcatgctgtggggcgctacggcggaagaaggagatcatgaagatgggtgcc
 ccgcgggccccatgtaccaggccggcacccttcggggcaacccatggccatgactgccggcatcaagacgtg
 gagatcctgggcccccgccgctacgagcactggagaagggtgaccaagcgctgatcgacggcatcatggcc
 gccgccaaggagcacagccacgagatcacggcggaacatcagcgcatgtttggtctcttctctctgcaagggc
 cctgtgacctgcttcgaggacgcctggcgccgacactgccaaagttcgcgcgcttccaccggcgcatgctggag
 gagggcgctctacctggctccctcgaggttcgaggccggttcacctctctgcccactccgaggcgagcgtggat
 gccacgatcgccgcgctcgccgcgctgttcgccgcgcatctaa

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

MQMQLNAKTVQGAFAKQRPVSVRGNAVRAVAAPPKLVTKRSEEIFKEAQELLPGGVNSPVRAFRSVGGGPVFD
 RVKGAYCWDVDGNKYIDYVGSWGPALCGHGNDEVNNAKLAQIDKGTSFGAPCELENVLAKMVIDRVPSVEMVRFV
 SSGTEACLSVLRLMRAYTGREKVLKFTGCTYHGHADSFLVKAGSGVITLGLPDSGPVPKSTAAATLTATYNNLDSV
 RELFAANKGEIAGVILEPVVGNNSGFIVPTKEFLQGLREICTAEGAVLCFDEVMTGFRIAKGCAQEHFGITPDLT
 MGKVIIGGMPVGAAYGKKEIMKMWAPAGPMYQAGTSLGNPMAMTAGIKTLEILGRPGAYEHLKVTKRLIDGIMA
 AAKEHSHEITGGNISGMFGFFFCCKGPVTCFEDALAADTAKFARFHRGMLEEGVYLAPSQFEAGFTSLAHSEADVD
 ATIAAARRVFARI

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

atgcagaccactatgcagcagcgtctccagggccgtaacgtggcgggcgagcgtcgctccctcggtccctgcc
 catcgctccttccactcacaccgggctgccactcaaaccgctacgatcagcgctgctgctagctcaaccaccaag
 ctgccagcttcgcatctggagagcagcaagaaggcgctggattcgctgaagcagcaggccgtcaatcgctacgcg
 ggtgacaagaagagctccattattgccattgggtctcaccattcacaacgcaccctggagctgcgcgagaagctg
 gctgtgctgaggtgaatggcgcgctgctattgaggagctctgccagttcccgacatcgaggaggccgcggtg
 ctgtcgacgtgcaatcgcatggagctctacgttgctcggtctgtcggtggcaccgcggcggttcgcgaggtggaggag
 tggtgctctgcaccagcggcgctgctctggtgagctgcgcgcctacctgttctgctgcgcgacccgcgacgc
 acgcaccacctgatgcgcgtgctgggtggccttgactcgctgggttatggcgagggccagatctctgcccagtg
 cgccaggtctacaaggtcgcccgagaactgccccggcttcggctcgccacctgaacggcctgttcaagcaggtatc
 accgctggcaagcgctgctgctgagacctccatctccaccggctccgtctccgtctcatccgcgcgcgctcgag
 ctggcgagctcaagctccccaccacaaactggctcgacgctaaggctctgcatcatcggcgctggcaagatgtct
 acgctgctggtaagcactgcagagcaagggtgcaaggaggtgacgggtgctcaaccgctctctgcccgcgcgc
 caggcgctggcgaggaggttccctgaggtcaagttcaacatccacctgatgcccgacctgctgcagtgctggag
 gccagcgacgtcatcttcgcgcctccggctctgaggagatcctcatccacaaggagcatgtcgaggccatgtcc
 aagccatcgagcgttggtggctccaagcgccgcttcgctgacatctccgtgccccgcaacatcgcccccgccatc

aacgagctggagcacggcatcgtctacaacgtcgacgacctgaaggaggttggtggccgccaacaaggagggccgc
gcgaggccggccgagggcgcgaggtgctgatccgcgaggagcagcgcgcggttcgaggcctggcgctgactctctg
gagaccgtgcccaccatcaaggcgctgcgctccaaggccgagaccatccgcgcccgcgagtttgagaaggccgtg
tctcgctggcgaggggctatccaagaagcagctcaaggcggtggaggagctcagcaaggccatcgtcaacaag
ctgctgcacggggcccatgacggcactgcgctgcgacggcaccgatccggatgccgtgggcccagaccctcgcgaa
atggaggccctggagcgcatgtccagctctcggaggtggacgtggccgcgctggcgggcaagcagtaa

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

MQTTMQRLQGRNVAGRSVAPSVPAHRSFHSHRAATQTATISAAASSTTKLPASHLESSKKALDSLKQQAVNRYA
GDKKSSIIAIGLTIHNAPVELREKLAVPEAEWPRAIEELCQFPHEEAVALSTCNRMELYVVGLSWHRGVREVEE
WLSRTSGVPLDELRPYLFLLRDRDATHHLMRVSGGLDSLVMGEGQILAQVRQVYKVGQNCPGFGRHLNGLFKQAI
TAGKRVRAETSISTGSVSVSSAAVELAQLKLPTHNWSDAKVCIIAGKMTLLVKHLQSKGCKEVTVLNRSPLRA
QALAEFFPEVKFNIHLPDILLQCVEASDVI FAASGSEELIHKHEHVEAMSKPSDVVSGSKRRFVDISVPRNIAPAI
NELEHGIVYNVDDLKEVVAANKEGRAQAAAEVLIREEQRAFEAWRDSLETVPITIKALRSKAETIRAAEFKAV
SRLGEGLSKKQLKAVEELSKGIVNKLHGPMTALRCGTDPAVGTLANMEALERMFQLSEVDVAALAGKQ

**Light independent protochlorophyllide reductase subunit N (ch1N) nucleic acid
sequence (SEQ ID NO: 13):**

atgttatactcacaatttaaacattcggtgcctttaggccgtaagtctcccccttctttcagggggcccccttct
gggggtcgcccaacaacggctgcctcaggcctaggtcgcaacgtggccgtaagaattgggacccccgttgggcttt
gcccttcggggcccaggttaattatggcagctgcgggcaatactagcgggtgcgccgcacccccgtaggggagtcctcag
cctgcgttgtcccaggtggattctcaacttgtaattgagtggtgaacaggaaattaccatactttttgcccgaatt
agttgtgtttcttggtataccaaaaaattgaagatagttttttcttagttatttggtacaaaaacgtgtgggtat
tttttacaaaatgcttttaggggttatgattttttgcccgaacctcggtacgctatggcggaattagaagaaagcgat
atttcggcgcaattaaatgattacaaagaattaaaaacgtctatgtttacaaattaaacaagaccgtaacccaagt
gttattgtgtggattggcacatgcacaaccgaaattattaaaatggatttagaaggtatggcacccgaaactagaa
gctgaaatcggtattccaattgtggttagcacgcgcaaatggacttgattatgcttttacacaaggtgaagatact
gttttagctgcgatggtccaaaaatgcccgaattaggcgctattccagctattgtacctcagattccttctgac
tctcgtagacttagccaactatctgtageggcttcggtacccgaaaacagtgctctggggccagaaggggagcct
tcactagcccagaaggggaatggattcctaagttaacaaacaactctccatgccgagtagattctgtctcagaatct
accccgcgctttcctggacgtgctccgcacgtcgggaaaagtaactcctcaaaatttagttttatttggttcatta
cctagcacgatggcaaatcaactggagtttgtaattaaaacgccaaggtattaatgttactgggtgggttacctgcg
gctcgctattcattctttacctgcattaggtgaaaacgtgtatgtttgtgggattaatccatttttaagtcgaact
gctacttctttaatgcgtcgctcgtaaatgcaattaatctcagctcctttcccaatttggtccagatggtacaaaa
gcttgggtcgaaaaaatttgtaattgttttcggtgttacaccaactgggttagaagatcgtgaacgtcttggttg
gaaggtttaaaagattatttaaatctcgtaaaagggaaatctgttttctttatgggtgataatctgttagaaatt
tcattagcccggttttttaattcgtgtgtggtatgaccgtttatgaaatcggtattccgtacatggaccaacgattt
caagctggggaattagaattattaaaaaaaacatgcatggaaatgaacgtgccccctaccgcgtattgttgaaaaa
cctgataattactatcaaatcaacgtattaaagaattacaaccagatttagttattaccggcatggcccatgca
aaccactggaagcgcgcgccattactacgaaatggtcggttgaaatttacgtttgcgcaaatcatgggtttggc
aacgcacgtgatattctagaattagttacaaaacgttacgctgtaataaaaatctatctaaatatcaatttccg
ttagatagctgggacaagcctgcttccgtaggcgctcacaactgtcggcctaa

**Light independent protochlorophyllide reductase subunit N (ch1N) amino acid sequence
(SEQ ID NO: 14):**

MLYSQFKHSVPLGRKSPLLSGGPPSGGRPTTAASGLGRNAVRI GTPLGFALRAQVIMAAAGNTSGAPHPVGESQ
PALSQVDSQLVIECETGNYHTFCPI SCVSWLYQKIEDSFFLVIGTKTCGYFLQNALGVMIFAEPYAMAELEESD
ISAQLMYDKELKRLCLQIKQDRNP SVI VWIGTCTTEI IKMDLEGMAPKLEAEIGIPIVVARANGLDYAFTQGEDT
VLAANDYKCPGLGAI PAIVPQIPSDSRTLSQLSVAASVPENSASGPEGEPSLAQKGMD SKLTNNSPCRVDSVSES
TPAFPGRAPHVGKSTPQNLVLFGLPSTMANQLEFELKRQGINVTGWLPAARYSSLPALGENVYVCGINPFLSRT
ATSLMRRRKCKKLISAPFP IGPDGTKAWVEKICNVFGVTPTGLEDRERLVWEGLKDYLNLFVKGSVFFMGDNLLEI
SLARFLIRCGMTVYEIGI PYMDQRFQAGELELLKKTCEMNVP LPRIVEKPDNYYQIQRIKELQPDLVITGMAHA
NPLEARGITTKWSVEFTFAQIHGFGNARDILELVTKPLRRNKNLSKYQFPLDSWDKPASVGAHEL SA

Light Independent protochlorophyllide subunit B (ch1B) nucleic acid sequence (SEQ ID NO: 15):

atgaaattagcgtattggatgtatgcgggaccggctcatattggaacattacgagttgcaagctcgtttcgaaat
gtgcatgctattatgcatgctcccttaggcgatgattattttaacgtaaatgcgttcaatggttagaacgtgaacgt
gattttacgccagtgcggcaagtattggtgatcgctcatgttttagctcggttcacaagaaaaagttggtgaa
aacattcaacgaaaaagataaagaagaatgtccggatttaattttattaacaccaacatgtacctcaagatattttg
caagaagattttacaaaattttgtaaatcgcgccggcgaagtagcaaaagcgttcggatgttttattagctgacgtt
aaccattaccgagtgatgaattacaagcggctgaccgtacgttagagcaaattgtacgcttttatttagaaaaa
gaagtaataaacttcacgcggagtttaggcggccttaaaaaaccgcttcgctttgccagcgtacccaaaagccg
tctgccaatatttttaggcattgtttacactaggtttccataatcaacatgactgtcgtgaattaaaacgtttatta
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gcgtgggtttaacatcgctcccgatcgatgaagttggtttaatgacggcaatttatttagaaaaagaatttggcatg
ccttatacctcaatcagcccaatgggcattattgacacgcggcgtttattcgtgaaattgcggccattttagt
caaattagcacttcacaggcatctacaaactcaactgaaggactccagaggggagaaaaatgtcagtttaactgaa
actaattcgattatttttaataaagcaaaaatgaacaatacattaatcaacaaacgcattttgtttctcaagca
gcttggtttttcacgttctattgactgtcaaaatttaaccggtaaaaaaacgcttggtgttggtgatgcaactcac
gcggcaagtagacgaaaaattcctgtgcgcgaaatgggtattcatgttggttgcgcgggcacgtattgtaaacat
gatgcagattgggttagagagcaagtttcaggtttttgtgatcaagttttaattacagatgatcacagccaaatt
gcggaaatcattgctcaaatgaacctgcagccatttttggtacacaaatggaacgtcatgttgggaaaagggtta
gatattccttggtgggttatttctgcaccgggtacataattcaaaacttcccactaggttttagaccgtttttaggg
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attttcaacggtcatgacaataaagaagttattacagcttcgtattcctcagaaactgatttagaatggacaaaa
gaagcattagatgaactagctcgtgttcctgggtttgttcgttcaaaagttaaacgtaataactgaaaaatttgcg
cgtacaaataaaaaatcaagttattactattgaagtatgtacgcagctaagaagcgggtatcagcgtaa

Light Independent protochlorophyllide subunit B (ch1B) amino acid sequence (SEQ ID NO: 16):

MKLA YWYAGPAHIGTLRVASSFRNVHAIMHAPLGDDYFNVMSMLERERDFTPVTASIVDRHVLARGSQEKVVE
NIQRKDKEECPDLILLTPCTCTSSILQEDLQNFVNRAAEVAKRSDVLLADVNHYRVNELQAADRTLEQIVRFYLEK
EVNKLHAE LGGLKKPLRF AQRTQKPSANILGMFTLG FHNQHD CRELKRLLNDLGI EVNEVIPEG SFVHGLKNLPK
AWFNIVPYREVGLMTAIYLEKEFGMPYTSITPMGI IDTA AFIREIAAICSQISTSQASTNSTEGLQRGENVSLTE
TNSII FNKAKYEQYINQQTHFVSQA AWF SRSIDCQNL TGKKT VVFGDATHAASMTKILVREMGIHVVCAGTYCKH
DADWFREQVSGFCDQVLI TDDHSQIAEI I AQIEPAAI FGTQMERHVGKRLDIPCGVISAPVHIQNFPLGFRPFLG
YEGTNQISDLVYNSFSLGMEDHLLLEIFNGHDNKEVITRSYSSETDLEWTKEALDELARVPGFVRSKVKRNTEKFA
RTNKNQVITIEVMYAAKEAVSA

Light independent protochlorophyllide reductase subunit L (ch1L) nucleic acid sequence (SEQ ID NO: 17):

atgaaattagcagtttatggcaaggtggtattggtaaatccacaacaagttgtaacatttcaattgcattagca
aaacgtggcaaaaaagtattacaaattgggtgtgatccaaaacacgatagtagttttacattaaccgggtttttta
attccaacaattattgatactttacaaagtaagattatcattacgaagatgtttggccggaagatgttatttac
caaggctacgggagtggtgattgtgtgaagcaggtggccccgcagcggcgccgctgtggtgggtatgtgtt
ggtgaaacagttaaattattaaaagaattaaatgcattttatgaatatgatgttattctgtttgatgttttaggg
gatgttgatgtggtgggtttgctgcaccttttaattacgcgcgactattgcattattgtcacagataatggcttt
gatgcgttattttgccgcaaaccgatttgctgcttcagtgcgcgaaaaagcgcgcattcacccattacgttttagct
gggttaattgggaatcgtagccaaacgcgatttaatcgataaatacgttgaagcgtgcccgatgccagtcctta
gaggtattaccggttaattgaagacattcgtgtgtcacgcgtaaaaggtaaaacattatttgaaatggcagaacat
gattcatcattacactacatttgtgactttttatttaaatattgcggatcaattattaaactgaaccagaaggtgtt
gttcgcgcgaattagcagaccgtgaattatttactctattatcagattttctattttaaaccgtgggactcctagc
cctagtggtatctgagttcggctcaggcgcccttagcggaaacgagcggcgaaacagctcccggtaatatgggtcag
cacatgagtaacgcagtaaaaaacaaacgaacaggaaatgaatttctttcttgtgtaa

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

MKLAVYGGGIGKSTTSCNISIALAKRGKKVLQIGCDPKHDSFTTLTGFLIPTIIDTLQSKDYHYEDVWPEDVIY
QGYGSVDCVEAGGPPAGAGCGGYVVGETVKLLKELNAFYEDVILFDVLGDVVCGGFAAPLNYADYCIIVTDNGF
DALFAANRIAASVREKARIHPLRLAGLIGNRTAKRDLIDKYVEACMPVLEVLPLIEDIRVSRVKGKTLFEMAEH
DSSLHYICDFYLNIAQQLLTEPEGVVPRELADRELFTLLSDFYLNAGTPSPSGSEFGSGALSGTSGETAPGNMGQ
HMSNAVKTNEQEMNFFLV

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

atgceggattgtgctggtcagcggettccgagagctttaacgtgggcctgtacaaggatgcggcgaggctgctgaag
cgctccatgcccacgtcacactccaggtgttctccgaccgcgacctggcctccgacgccacccgctcccggctg
gaggcggtcttggggcgcgccgacatcttcttcggatcactgctgttcgactacgaccaggtggagtggtacgg
gcccggctggagcggtgctgtgcggttagtgtttgagtcggcggttgagctcatgagctgcaacaagggtggg
tcgttcacgatggcgcgcggtcccggcgcgccccggcccgcaaggcgccccggcgccccggcggtgaag
aaggttctctccatgtttggaagcggtcgcgaggaggacaagatggcgcggtcctccaatgtggtggccatgttc
agttacctggtggagacctgatggagccaacgggtgggttatctggtagttggtggttgtgttatggttgccg
tttcgggtgggtgatctgggtggtatctacaacccccctcaacctcacgcctccaggctacgtgcccgcgcct
gtggtggagactccgcactgggtgcttccacctcccgccccggcgctacttcgagtccccccgccgagtac
atgaagtggtagccaggaggggcgctgcgcgccagggcgccccgggtggttggtgctgctgtacccgaag
catgtgatcacgaccagccgtacatcccgcagctggtcagccagctggaggcgaggggctgctgcccgtgccc
atcttcacacggcggtggaggcgacacccgtggttcgcgacctgctgacctccgtgcacgagcaggatctgctt
gcacggcgagacggcgccatcagccccacctgaagcgggacgcggtcaaggaggcgcggtggtgagcacc
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cgtacgacacgcactaagcatgcctctgtttgtgagctccagccccctccccccccgtctccccctctccacctc
cctctccccctctctcccttccctctcactctccacctcttccccctccgccccaaacataacgaggcgggggtg
ctggggcgcaaggcgccctggagtacccgctgcgacctagctagctccaactccacccatcccccaatgcccgaat
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cgccacccacgcacacacacacacacacacacacacacacacacacacacacacacacacacacacacaca
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gttcgcgcagtgaggcgagtgaggcgagctgagcgtaccgcccctcaacacctcgccgcgcggcatgttc
caggagtacggggctgacgtggtcctgacttcggcatgcacggcacctggagtgggtgcttggggcgccgctg
gggaacaacggcctcagctggagcgacgtgctgctcgccgagctgcccacgtgtacgtgtacgtgccaacaac
ccctccgagctccatctggcgaaggcgcgcggtacggcacctcgtcagccacaacgtgcccgcgtacggcgcg
gccccgtctgtacaagcagcttccagacctcaaggagacgcttcaggagtaccgagggccgagggcgacgt
gcccgagcaggagccagcagcagcagcgccagtagcagcagtagcagcagtagcagcagtagcagcagcagc
agtgtggagctgcggcgcggttggcaccggtgttcgacgcctacactgaccgctgtatgctacctgacgtg
ctggagggcggtgttcagcgaggggtacacgtactgggagcgccgccccggcgccgaggtgggtgttt
cccgcagcttccaacgtacgtgtaactgcccactgcccacttctccccaaacacagagggtctcaagatc
cggaacctgctcatgcagaacacgcaggagctggacgggtgctcaaggccctgggtggggcttacgtgcttccc
gaggcgggcgggacctgctgcgggacgggtcgggcggtgctgcccacggcgccgaacatccacgcactggacccc
taccgcatgcccctcccccgccgcatggccgtggggcgggcggtggcgggcgccatctcttgagcagcaccggcg
gctaacagcgggggtggtggccgagacctgcgcctcaacctgtgggggtggactccatcaagagcaaggcgag
agtgtgggggtggtgctggcgctggtggggcggtgcccgtgcccagggtagcggcgccgctgcgcgcttccaa
ctggtgcccgtgtcagagtggggcgccgctgtggacgtgcttctgtaacatgagcggcatcttcgcgactcc
ttccagaacgtggtggagctgctcgacgacctgtttgcaaggggcgccgccccgctgacgagccagatgacatg
aacttcacgcgaacacacgccccgagccatggagaagcagggcctgtccgcccactcggccccctgttctccaa
ccggctggcgactacgggtcgatggtcaacgagcgagtgggggcaggcgagctgggccaacgagcagagctgggt
gacacgtggggcgcccgcaacgccttcagctacggcgagggaaggagcagggcacggcgcgcccgaggtgctg
caggcgctgctcaagaccacggacccggtcgtgacgagatcgacagtggtgagtagcggcctgacagacatccag
gagtagtacgccaacacggcgcccccaagagagcccgaggtggccaaaggcgacccggggccccgggtggccgg
cgcccgcgctgggtgttccattgtggaggcctttggcgggcgggcgccggcgggcgggcgccgggtggagcg
ggcggtgcccgcgctcgcgagctggaggaggtgctgcgcctggagtaccgctcgaagctgctcaacccccagtggtg
gccccggccatggcgcgagggcgagggcgccgctacgagatcagtcagcgcatgacggcggtggtgggtggtg
ggcgccaccaccgatttcaggggaggggtgggtgtgggacccaggcgccatggacacgtatgtgggcatgaggag
atggccagcaagctcaagaagaacaaccccgagggcctttgccaacgtgctgcggcgcatgctggaggcgcgggg

cgcgccatgtggagccccaacaaggaccagctggcacagctcaagtcgctgtacagcgagatggacgaccagctg
gaggggggtgacg

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

MRIVLVSGFESFNVGLYKDAAELLKRSMNVTLQVFSDRDLASDATRSRLEAALGRADIFFGSLLFDYDQVEWLR
ARLERVPVRLVFESALELMSCNKVGSFMMGGGGPPGKAPGPPPAVKKVL SMFGSGREEDKMGGSSNVVAMF
SYLVETLMEPTGGLFGSWWLCYGWPFRLGDLGWYLQPPSTLTPPGYVPPPVPVETPALGCLHPSAPGRYFESPAEY
MKWYAREGPLRGTPAVVGVLLYRKHVITDQPYIPQLVSQLEAEGLLPVPIFINGVEAHTVVRDLLTSVHEQDLL
ARGETGAISPTLKRDAVKDVAVVSTIGFPLVGGPAGTMEGGRQAEEVAKAILGAKDVPYTVAAPLLIQDMESWSRD
GVAGLQSVVLYSLPELDGAVDTVPLGGLVGDDIYLVPERVKLAGRLKSWRTTRTKHASVCDVQPLPPSPSLSTL
PLPSSPFLSLSTLFLPLRPNI TRRGLLGASGPWSTRCDLASPTPI PQCRNSFPPEMSTHTHTHTHTHTHTHT
RHPRTHTHTHTAPPARHTPIPPHPQELLTYPADWGP AEWGPLPYLPDPDVLVRRMEAQWELRAYRGLNTSARGMF
QEYGADVVLHFGMHGTVEWLP GAPLGNGLSWSDVLLGELPNVYVYAANNPSESIVAKRRGYGTIVSHNVPPYGR
AGLYKQLSSLKETLQ EYREAAQAARARAGASSSSGSSSSSSSSSGSGSSSSSVELRAALAPVFDAYTDRLYAYLQL
LEGRLFSEGLHVLGAPPAPPQVGGFPASFQRYRKL PNCPTSPQTQEAVKIRNLLMQNTQELDGLLKGLGGRYVLP
EAGGDLRLDGSGLPTGRNIHALDPYRMPSPAAMARGAAVAAAILEQHRAANS GAWPETCAVNLWGLDSIKSKGE
SVGVVLALVGAVPVREGTGRVARFQLVPLSELGRPRVDVLCNMSGIFRDSFQNVVELLDL FARAAAAADEPDDM
NFI AKHARAMEKQGLSAT SARLFSNPAGDYGS MVNERVQGGSWANGDELGDTWAARNAFSYGRGKERGTARPEVL
QALLKTTDRI VQQIDSVEYGLTDI QEYYANTGALKRAAEVAKGDPGPGRRPRVGC SIVEAFGGAGAGAGGAGGA
GVPPPRELEEVLRL EYRSKLLNPKWARAMAAQSGSGAYEISQRMTALVGWGATTDFREGVWVDPGAMDTYVGDEE
MASKLKKNNPQAFANVLRRLMLAAGRGMWSPNKDQLAQLKSLYSEMDQLEGVT

**Magnesium Chelatase subunit 1 (CHLI1) *Chlamydomonas reinhardtii* nucleic acid
sequence (SEQ ID NO: 21):**

atggccctgaacatgcgtgtttcctcttccaaggtcgctgccaaagcagcagggccgcgcattctccgcggtgccggtt
gtgtcgagcaaggtggcctcctccgcccgcgtggccccctccagggcgctcccgtggccgcgcagcgcgtgct
ctgctggtgcgccgcgtgcccgtactgaggtcaaggtcgctgagggccgcactgagaaggagctgggcccaggcc
cgccccatcttccccctcaaccgccatcgctgggccaggatgagatgaagctggcgctgattctgaacgtgatcgac
cccaagatcggtggtgcatgatcatgggcgaccgtggcactggcaagtcaccaccattctgtgccctggcggat
ctgctgcccagatgcaggtggttgccaacgacccctttaactcggacccaccgaccccgagctgatgagcag
gaggtgcgcaaccgcgtcaaggccggcgagcagctgcccgtgtctccaagaagattcccatggtggacctgccc
ctgggcgccactgaggaccgcgtgtgcccaccatcgacatcgagaaggcgctgaccgaggggtgtcaaggcgctt
gagcccgccctgctggccaaggccaaccgcggcatcctgtacgtggatgaggtcaacctgctggacgaccacctg
gtcgatgtgctgctggactcggccgcctccggctggaacaccgtggagcgcgaggggtatctccatcagccacccc
gcccgccttcatcctggtcggtcggtcgggcaaccccgaggaggggtgagctgcgccccagctgctggatcgcttcggc
atgcacgcccagatcgccaccgtcaaggacccccgcctgctgtgagatcggtgcgcagcgcctgcaccttcgac
gagaaccccgccgcttccgcaaggactacgaggccggccagatggcgctgaccacgcgcgcctggtgacgcgcgc
aagctgctgaagcagggcgaggtcaactacgacttccgcgtcaagatcagccagatctgctcggacctgaacgtg
gacggcatccgcccgcacatcgtgaccaaccgcgcggcccaaggccctggccgccttcgagggccgcaccgaggtg
acccccgaggacatctaccgtgtcattccccctgtgcctgcccaccgcctccggaaagacccccctggctgagatc
gacgacgggtgaccgcgtgctgagatcttcaagcaggtgttcggcatggagtaa

**Magnesium Chelatase subunit 1 (CHLI1) *Chlamydomonas reinhardtii* amino acid
sequence (SEQ ID NO: 22):**

MALNMRVSSSKVAAKQQGRISAVPVVSSKVASSARVAPFQGAPVAAQRAALLVRAAAATEVKAAEGRTEKELGQA
RPIFPFTAIVGQDEMKLALILNVIDPKIGVMIMGDRGTGKSTTIRALADLLPEMQVVANDPFNSDPTDPELMSE
EVRNRVKAGEQLPVSSKKIPMVDLPLGATEDRVCGTIDIEKALTEGVKAFEPGLLAKANRGI LYVDEVNLLDDHL
VDVLLDSAASGWNTVEREGISISHPARFILVSGSNPEEGELRPQLLDRFGMHAQIGTVKDPRLRVQIVSQRSTFD
ENPAAFRKDYEAGQMALTQRIVDARKLLKQGEVNYDFRVKISQICSDLNVDGIRGDI VTNRAAKALAAFEGRTEV
TPEDIYRVIPCLRHRLRKDPLAEIDGDRVREIFKQVFGME

**Magnesium Chelatase subunit 1 (CHLI2) *Chlamydomonas reinhardtii* nucleic acid
sequence (SEQ ID NO: 23):**

Magnesium Chelatase sunubit1 (CHLI2) *Chlamydomonas reinhardtii* amino acid sequence (SEQ ID NO: 24):

Magnesium Chelatase subunit D (CHLD) *Chlamydomonas reinhardtii* nucleic acid sequence (SEQ ID NO: 25):

51

gccaaagtccaacgaggacccccgagggcgctcaagcccgacgcgccccaaagccaccgcccgcactcgctgaaggacgag
gtgcgcgacatggccaagaagggcgctccgcccgcacaaactgctgtgacacggagaacaagtctcg
agcaccgggcttgcggaggagatctccaaggcagcgagggcaagtactactacctgccccaacgccagcgacgcc
gccatcgcgggcgcccgctccggcgccatggccgcggccaagggcggtactag

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

sequence (SEQ ID NO: 26):

MKSLCHELAGPSVTGCGRRSLRKAFSGAKIAQVSRPAVLNSVQRQRLACSAVAELSAEELRAMKVSEEDSKGFD
ADVSTRRLARSYPLAAVVGQDNIKQALLGAVDTGLGGIAIAGRRGTAKSIMARGLHALLPPIEVVEGSICNADPE
DPRSWEAGLAKEYAGGPVKTKMRSAPFVQIPLGVTEEDRLVGTVDIEASMKEGKTVFQPLLAEAHRGILYVDEIN
LLDDGIANLLLSILSDGVNVVEREGISISHPCRPLLIATYNPEEGPLREHLLDRIAIGLSADVPSTSDERVAID
AAIRFQDKPQDTIDDTAELTDALRTSVILAREYLKDVITIAPEQVTYIVVEEARRGGVQGHRAELYAVKCAKACAAL
EGRERVNKDDLRLQAVQLVILPRATILDQPPPEQEQQPPPPPPPPPPPPQDQMEDEDQEEKEDEKEEEEEKENEDQD
EPEIPQEFMFSEGVIMDPSILMFAQQQQRAQGRSGRAKTLIFSDDRGRIKPMPLPKGDKVKRLAVDATLRAAAP
YQKIRRQQAISEGKVQRKVYVDKPDMSKKLARKAGALVIFVVDASGSMALNRMSAAKGACMRLLAESYTSRDQV
CLIPFYGDKAEVLLPPSKSIAMARRRLDSLPCGGGSPLAHGLSTAVRVGMQASQAGEVGRVMMVLI TDGRANVSL
AKSNEDPEALKPDAPKPTADSLKDEVDRMAKKAASAGINVLVIDTENKRVSTGFABEISKAAQGGKYYLPLNASDA
AIAAASGAMAAAKGGY

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

sequence (SEQ ID NO: 27):

atgcagacttctcgtcttcttggcgggcgacggccccaccggctgcggggcgcgacgccccaaagccgggttgcgccc
tcgccccgctggctagcaccgcccaggtcgctgcaatgtggcgactggacccccggccgcccattgaccaccttc
accgggtggcaacaagggccctgctaagcagcaggtgtcgctggatctgcgcgacgagggcgctggcatgttcacc
agcaccagcccggagatgcgcgctgtcgctccctgacgatgtgaagggctcgcttaagggtgaaggttgtgtacgtg
gtgctggaggccagtagcagtcggccatcagcgctgcggtgaagaacatcaacgccaaagaactccaaggtgtgc
ttcgaggtgggtgggtacactgctggaggagctgcgtgaccagaagaacctcgatagctcaaggaggatgtggcc
tctgccaacatcttcacggtcgctcatcttcattgaggagcttgccgagaagattgtggaggcggtgagcccc
ctgcgcgagaagctggacgcgtgacctgatcttcccgctccatgcggcggtcatgaagctgaacaagctgggcacg
ttttcgatggctcagctgggcccagtcgaagtcggtgttctcgaggttcacaaagtcgctcgcaagaacaacgac
aacttcgaggagggttgcgtgaagctgggtgcgcacctgcctaagggtgctgaagtatctgcccctcggaacaaggcg
caggacgccaaagaacttcgtgaacagcctgcagtagctgggtggcggttaactcggaacaacctggagaacctgctg
ctgaacaccgtcagcaactacgtgcccgtctgaagggcggtgacttcagcgtgggtgagccccaccgcctacccc
gatgtgggtatctggcaccctctggcctcgggcatgtacgaggacctgaaggagtacctgaactggtacgacacc
cgcaaggacatggtcttcgccaaaggacgccccctcattggcctgggtgctgcagcgctcgacactgggtgactggc
gatgagggccactacagcggtggtcgctgagctggagagccggtgctaaaggtcatccccgtcttggcgggt
ggcctggacttctccgccccgctcaagaagttcttctacgacccccctgggtcttgcccgacgcttcgtggacacc
gttgtgtcgctgacgggttcgctggtggggcgcccccgcgccaggaagcgccgaagggcattgagggcgctg
aagaacctgaacgtgcctacctgggtgctcgctgcgctgggtgttcagagacctgaggagtggtggagcagcag
ctgggctgacccccgtccaggtgggtctgacaggttgcctgcccagctggatgggtgacctggagcccatcgctg
ttcgctggcgtgactcgaaacacgggaagtcgcactcgctgcccagccgcatcgcttcgctgtgcgctcgcgcc
gtgaactgggccaacctgcgcaagaagcgcaacgcgcgagaagaagctggcgtcacctggttcagcttccccct
gacaagggcaacgtcggcactgcgcctacctgaacgtgttcggctccatctaccgctgctgaagaacctgcag
cgcgagggctacgagctggggcgccctgcgcctcggaggaggatctgatccagtcgggtgctgaccagaaggag
gccaagttcaactcgaccgacctgcacatcgctacaagatgaaggtggacgagtagcagaagctgtgccccttac
gcccaggcgctggaggagaactggggcaagcccccgccacctgaacaccaacggccaggagctgctgggtgac
ggcgccagtagggcaacgtcttcacggcgctgcagcccaccttcggctacgagggcgaccgatgcgctgctg
ttctcgaagtcggccagccccaccacggcttcgcgccctactacaccttctggagaagatctcaaggccgac
gcccgtgctgacttcggcaccacggctcgctggagttcatgcccggcaagcaggtcgccatgtcggtgtgtgc
taccctgactcgctgatcggcaccatccccaaactctactactacgcccgaacaacccgtctgaggccaccatc
gccaagcgccgctcgtacgccaacaccatttcgtacctgacgcgcctgcccagaaacggcgccctgtacaagggc
ctgaaggagctgaaggagctgatcagctcgtaccagggcatgcgtgagcttgcccgcgccgagcagatctgcgcc
accatcattgagaccgccaagctgtgcaacctggacgcgcagctgacctgcccagcgtgacgccaaggacctg
accatggacatgcgcgacagcgttgtgggcccaggtgtaccgcaagctgatggagattgagtcggcctgctgccc
tgccgctgacgctggtgggtgcccgcaccgcggaggagggcggtggccacctggtcaacatcgctgagctg
gaccgcccgaacaaccccccatcaagggcatgcccgcctcctggccgcccattggtcgcgacatcgag
tcgatttacagcggaacaacaaggcgctcctggctgacgttgaccagctgcagcgcatcaccaggacctccgc

acctgcggtgcgcgagttcgtgaaggaccgcaccggcctgaacggcgcatcggcaccaactggatcaccaacctg
 ctcaagttcacccggtctctacgtggaccctgggtgcgcggcctgcagaacggcgagttcgccagcgccaaccgc
 gaggagctgatcacctgttcaactacctggagttctgcctgaccaggtggtcaaggacaacgagctgggcgc
 ctggtagaggcgctgaacggccagtagctcgagcccgcccgccggcggtgaccccatccgcaaccccaacgtgctg
 ccacccggcaagaacatccacgccctggaccctcagtcgattcccaactcaggccgcgctgaagagcgcccgctg
 gtggtggaccgcctgctggaccgcgagcgcgacaacaacggcggaagtaccccgagaccatcgcgctgggtgctg
 tggggcactgacaacatcaagacctacggcgagtcgctggcccaggtcatgatgatgggtcggtgtcaagcccgctg
 gccgacgccccggcgcgctgaacaagctggaggtgatccctctggaggagctgggcccccccgctggacgtg
 gttgtcaactgctcggtgtgttccgcgacctgttcgtgaaccagatgctgctgctggaccgcgcatcaagctg
 gggcgcgagcaggacgagcccgatgagatgaacttcgtgcgcaagcacgccaagcagcagggcgcgagctgggc
 ctgcagagcctgcgcgacgcccaccctgtgttctccaacagctcggtctctactcgtccaacgtcaacctg
 gcggtggagaacagcagctggagcgacgagtcgagctgcaggagatgtacctgaagcgcaagtctacgcttc
 aactcggaaccgccccggcgcggtggcgagatgcagcgcgacgtgttcgagacggccatgaagacctggacgtg
 accttcagaacctggactcgtccgagatctcgtgacccgatgtgtcgcactacttcgactccgacccccaccaag
 ctggtggcgctcgtgcgcaacgacggccgcaccccccaacgcctacatcgccgacaccaccaccgccaacgcgcag
 gtccgcactctgggtgagacctgctgcctggacggccgcaccaagctgctcaaccccaagtggtaggggcatg
 cttgcctcggttacgagggcgctgcgcgagatccagaagcgcatgaccaacaccatgggctggctcgccacctcg
 ggcaggtgggacaactgggtgtacgacgagggccaactcgaccttcacgaggtgcgcccatggccgagcgctg
 atgaacaccaacccccacagcttcgcgaagctgggtggccaccttcctggaggccaacggcgcggtactgggac
 gccaaagcccgagcagctggagcgctgcgcagctgtacatggacgtggaggacaagattgagggcgctgaataa

Magnesium Chelatase subunit H (CHLH1) *Chlamydomonas reinhardtii* amino acid sequence (SEQ ID NO: 28):

MQTSSLLGRRTAHPAAGATPKPVAPSPRVASTRQVACNVATGPRPPMTTFTGGNKGPAKQQVSLDLRDEGAGMFT
 STSPEMRRVVPDDVKGRVKVKVYVLEAQYQSAISAAVKKNINAKNSKVCFEVVGYLEELRDQKNLDMLEDVA
 SANIFIGSLIFIEELAEKIVEAVSPLREKLDACLI FPSMPAVMKLNKLGTF SMAQLGQSKSVFSEFIKSARKNND
 NFEEGLKLKLVRTL PKVLKYLPSDKAQDAKNFVNSLQYWLGGNSDNLNLLNTVSNYVPALKGVD FSVAEPTAYP
 DVGIWHPLASGMYEDLKEYLNWYDTRKDMVFADAPVIGLVLRSHLVTGDEGHYSGVVAELESRGAKVIPVFAG
 GLDFSAPVKKFFYDPLGSGRTFVDTVVSLTG FALVGGPARQDAPKAIEALKNLNVPYLVSLPLVFQTTEEWLDSE
 LGVHPVQVALQVALPELDGAMEPIVFAGRDSNTGKSHSLPDRIASLCARAVNWANLRKKRNAEKKLAVTVFSFPP
 DKGNVGTAAYLNVFGSIYRVLKNLQREGYDVGALPPSEEDLIQSVLTQKEAKFNSTD LHIAYKMKVDEYQKLC PY
 AEALEENWGKPPGTLNTNGQELLVYGRQYGNVFIGVQPTFGYEGDPMRLLF SKSASPHHGFAAYYTFLEKIFKAD
 AVLHFGTHGSLEFMPGKQVGMMSGVCYPDSLIGTIPNLYYYAANNPSEATIAKRRSYANTI SYLTPPAENAGLYKG
 LKELKELISSYQGMRESGRAEQICATIIETAKLCNLDRDVTLPDADAKDLTMDMRDSVVGQVYRKLMEIESRLLP
 CGLHVVGCPPTAEAAVATLVNIAELDRPDNNPPIKMPGILARAIGRDIESIYSGNNKGVLADVDQLQRITEASR
 TCVREFVKDRITGLNGRIGTNWITNLLKFTGFYVDPWVRGLQNGEFASANREELITLFNYLEFCLTQVVKDNELGA
 LVEALNGQYVEPGPGGDP IRPNPNVLP TGKNIHALDPQSIPTQAALKSARLVDRLLDRERDNNGGKYPETIALVL
 WGTDNIKTYGESLAQVMVMVGVKPVADALGRVNKLEVI PLEELGRPRVDVVNCSGVFRDLFVNQMLLLDRAIKL
 AAQDEPDENMFVRKHAKQQAELGLQSLRDAATR VFSNSSGSYSSNVNLAVENSSWSDSQLQEMYLRKKS YAF
 NSDRPGAGGEMQRDVFETAMKTVDTVTFQNLDSSEISLTDVSHYFSDPTKLVASLRNDGRTPNAYIADTTTANAQ
 VRTLGETVRLDARTKLLNPKWYEGMLASGYEGVREIQKRMTNTMGWSATSGMVDNWVYDEANSTFIEDAAMAERL
 MNTNPNSFRKLVATFLEANGRGYWDAPKEQLERLRQLYMDVEDKIEGVE

Photochlorophyllide reductase subunit B (ch1B) nucleic acid sequence (SEQ ID NO: 29):

atgaaattagcttattggatgtacgcaggtcccgcctcatatcggtgtgttgctgttagcagctcttttaaaaaat
 gtacatgccattatgcatgctccttttaggagatgattattttaaatgtaatgcgttccatggttagaacgtgaacgt
 gattttacaccagtaacagccagtagttgtagatcgctcatgttttagcaagaggatcgcaagaaaaagtggtgaa
 aatattacgcgaaaaataaagaagaaactcctgatttaattttattaactcctactgtacgtcaagcatttta
 caagaagattttacacaattttgttgaaatcgccattagctaaaccagtagcaaatagatgaacatgcagaccataaa
 gtaactcaacaaagtgcactttcaagtgtatcccccttactaccgcttgaagaaaatacattaatagtaagtga
 ctagataagaagcttagcccgcttagcaagttgcatattaatatgcccaatatttgattcccgaaggagaagg
 gaaggggagcagactaaaaattcaattttttgttaaatctgcaactttaacaaatttgtcagaagaggaactatta
 aatcaagaacatcataccaaaacaagaaatcactctgacgttatttttagctgatgtaaacattatcgtgtaaat
 gaattacaagctgcagatcgtagcttctgaacaaattgtacgttattatatttctcaagcacaaaaacaaatgt
 ttaaacattactaaaaacagccaaacctctgtaaatattatttggtatttttactttgggttttataaatcaacat
 gattgtcgtgaattaaaaacggtttatttaattgatttaggtatttcaaatcaatgaaatcatacctgaaggcggaat

gtacacaacttaaaaaaattaccccaagcttggtttaatTTTTGTGCCctaccgtgaaattggcttaatgactgct
 atgtatttaaaatccgagtttaatatgccttacgtcgcaattactcctatgggattaattgatacggctgcttgt
 attcgttcaatttgtaaaatcattacaactcaattattaaatcagacggctacagtgcaggagccatcaaaattt
 atttaccgaaggcgacgtcatttagaacaacccaattattctcgaaacctctcaaaaagaaactattcttaagac
 aatccagatagcggaaataccctttctacaactgtagaagaaattgaaactttatttaataatatatcgatcaa
 caaactcgTTTTgtttcccaagcagcctggttttcacgttctattgactgtcaaaatttaacagggtaaaaagcc
 gtagttttcggagatgtcacacattcagctgccatgacaaaattattagcacgtgaaatgggtattaaggtttca
 tgcgctggaacttattgcaaacacgatgcggatttggtttagagagcaagttagtgggttttgtgatcaagtttta
 attaccgatgatcacacacaagtaggggatattgattgcacaattagaacctgcagccatttttgggacacaaatg
 gaacgtcacgttggtaaacgttttagatattccatgtggtgttatatctgctcctgtgcatattcaaaactttccg
 ttaggttatcgaccttttttaggttatgaaggtaacaaatcaaatagctgatttagtgataattcatttaactctt
 ggaatggaagaccatttattacaaatttttggaggtcatgattcagaaaacaattcgtcaattgcaacgcatttg
 aatacaataaacgcaataaaatttagcgccaggatatttacctgagggaaggcagtagtagaacttcaaatgta
 gtgtctacaatttctagtgaaaaaaaagccattgtatggtctccagaaggttttagcagaattaaataaagtccca
 ggatttgttcgaggaaaagttaaacgtaatacggaaaaatatgctttacaaaaaaattgttcgatgattactgta
 gaagttatgtatgcagcaaaagaagctttgtcggcttaa

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

MKLA YWYAGPAHIGVLRVSSSFKNVHAIMHAPLGDDYFNVMSMLERERDFTPVTASIVDRHVLARGSQEKVVE
 NITRKNKEETPDILILLTPTCTSSILQEDLHNFVESALAKPVQIDEHADHKVTQQSALSSVSPLLPLEENTLIVSE
 LDKKLSPPSKLHINMPNICIPEGEGEGETKNSIFVKSATLTNLSEEELNQEHTKTRNHSDVILADVNHVRVN
 ELQAADRTLEQIVRYYSQAQKQCNLNTKTAKPSVNIIGIFTLGFHNQHDRELKRLFNDLGIQINEIIEGEGN
 VHNLLKLPQAWFNFPYREI GLMTAMYLKSEFNMPYVAITPMGLIDTAACIRSI CKIITQLLNQATATVQEPSKF
 IYPKATSLEQTNILETSQKETILKDNPD SGNTLSTTVEEIIETL FNKYIDQQTRFVSQAAWFSRSIDCQNLTKKA
 VVFGDATHSAAMTKLLAREMGIKVSCAGTYCKHDADWFREQVSGFCDQVLI TDDHTQVGD MIAQLEPAAI FGTQM
 ERHV GKRLDIPCGVISAPVHIQNFPLGYRPFLGYEGTNQIADLVYNSFNLGMEDHLLQIFGGHDSENNSSIATHL
 NTNNAINLAPGYLPEGE GSSRTSNVVSTISSEKKAIVWSPEGLAELNKVPGFVRGKVKRNTEKYALQKNCSMITV
 EVMYAAKEALSA

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

31):

atgaaattagctgtttacggaaaagggtggtattggaaaatcaacgacaagttgtaatatttcgattgctttacga
 aaacgtggtaaaaaagtgttacaaattgggtgtgacctaacaatgatagtagtactttacattgacagggttttta
 attccaaccattattgatacatataagttctaaagattatcattatgaagatatttggcccgagatgttatttac
 ggaggttatgggggtgtagattgtgttgaagctggaggaccacctgccggtgcggggtgtggtggttatgttgta
 ggtgaaacggttaaaacttttaaaagagttaaatgcttttttcgaatacagatgttattttatttgatgttttaggt
 gatgttgtttgtggtggtttgctgctccattaaactacgctgattattgtattattgtaactgataatgggtttt
 gatgctttatttgcgtgcaaatcgatttgcagcttcagttcgtgaaaaagcacgtacacatccattgcgttttagcg
 ggtttaatcggaaatcgtaacataaaacgtgatttaattgataaaatattgtagaagcttgcctatgccagtatta
 gaagttttaccattaattgaagaaattcgtatttcacgtgttaaaggcaaaactttatttgaaatgtcaaataaa
 aataatatgacttcggtcatatggatggctctaaagggtgacaattctacagtaggagtgtagaactccatcg
 gaagattatatttgtaatttttatttaaatattgtgtagcaattatttaacagaaccagaaggagttattccacgt
 gaattagcagataaagaactttttactcttttatcagattttctatcttaaaatttaa

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

MKLAVYGGKGGIGKSTTSCNISI ALRKRGKKVLQIGCDPKHDSTFTLTGFLIPTIIDTLSSKDYHYEDIWPEDVIY
 GGYGGVDCVEAGGPPAGAGCGGYVVGETVKLLKELNAFFEYDVILFDVLGDVVC GGFAAPLNYADYCIIVTDNGF
 DALFAANRIAASVREKARTHPLRLAGLIGNRTSKRDLIDKYVEACMPVLEVLPLIEIRISRVKGKTLFEMS NK
 NNMTSAHMDGSKGDNSTVGVSETPSEDYICNFYLN IADQLLTEPEGVIPRELADKELFTLLSDFY LKI

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

33):

atgtagatggtgccacaacgatttttaaaatttaaatagtttttttgaaatgtgaaactggcaattatcatactttt
 tggccgatttagctgtgtagcttggttatatcaaaaaatcgaagatagcttttttttagtaattgggacaaaaaca

tgtgggtattttttacaaaatgcccttgaggtatgatttttgcggaacctaggtatgctatggcagaattagaa
gaaagtgatattttcagcacaattaaacgattataaagaattaaaacgtttatgtttacaaattaaacaagataga
aatcccagcggtattgtttggattggaacttgtacaactgaaattatcaaaatggatttagaagggatggctcca
cgtttagaaactgaaatcggcataccattgtttagcagcgtgctaatgggttagattatgcttttacacaagggt
gaagacacagttttatcagcaatggccttagcatccttaaaaaaagatgttccttttttagtaggtaatactggg
ttaacaaacaaccagcttctccttgaaaaatcaacttcttcagttaatgggacagacggaaaggaattacttaaa
aaatctcttgattattttgggtccgtaccaagtacagttactacacaattaactttagaattaaaaaaagaaggt
attaatgtatctggatggcttccatctgctaattataaagatttacctacttttaataaagatacacttgatgt
gggtataaatccttttttaagtgcagcagctaccacgttaatgcgtcgtagtaagtgcacattaatttgtgcaccc
tttccaataggccccgatggcacaagagtttggttgaaaaaatttgggtgcttttggcattaatcctagtctt
aatccaattactggtaataactaatttatatgatcgtgaacaaaaaattttcaacgggctagaagattatttaaaa
ttattacgtggaaaaatctgtattttttatgggtgataatttattagaaatttcttttagcacgttttttaacacgt
tgtggatgattgttttatgaaatcgggaattccatatttagataaacgatttcaagcagcagaattagctttatta
gaacaaacttgtaagaagaatgaatgtaccaatgccgcgcattgtagaaaaaccagataattattatcaaattcga
cgtatacgtgaattaaaacctgatttaacgattactggaatggcacatgcaaaccattagaagctcgaggtatt
acaacaaaatggtcagttgaatttacttttgcctcaaattcatggatttactaatacacgtgaaattttagaatta
gtaacacagcctcttagacgcaatctaattgtcaaataatctgtaaatgctattttcttaa

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

MLDGATTILNLNSFFECETGNYHTFCPI SCVAWLYQKIEDSFFLVIGTKTCGYFLQNALGVMIFAEPYAMAELE
ESDISAQLNDYKELKRLCLQIKQDRNPSVIVWIGTCTTEIIKMDLEGMAPRLETEIGIPIVVARANGLDYAFTQG
EDTVLSAMALASLKKDVPFLVNGTGLTNNQLLEKSTSSVNGTDGKELLKKSLLVFGSVPSTVTTQLTLELKKEG
INVSGLWLP SAN YKDLPTFNKDTLVCGINPFLSR TAT TLMRRSKCTLI CAPFPIGPDGTRVWIEKICGAFGINPSL
NPITGNTNLYDREQIFNGLEDYLKLLRGKSVFFMGDNLL EISLARFLTRCGMIVYEIGI PYLDRKFQAELALL
EQTKEMNVMPRI VEKPDNYYQIRRIRELKPDLTITGMAHANPLEARGITTKWSVEFTFAQIHGFTNTREILEL
VTQPLRRNLMSNQSVNAIS

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

atgcagcagtgcggttgccgctccgtccgcgctccgtccagcagggcggtcgcgcccaaggctcgctggcgctcg
gtcagccgcccgcgtgtgccgctctatgcctccgcgtgttgctaccaagacgggtgaagattggcacgcgcgggtcg
ccccggctctggcccaggcttacatgactcgcgacctgctgaagaagagcttccctgagctgagcagaggaggt
gctctggagatcgtgatcaagaccaccggtgacaaaatcctgaaccagccccggctgacatcgggtggcaag
ggctctgtttaccaaggagatcgatgatgctctgctgagcggcaagattgacatcgccgtgactccatgaaggac
gtgccacctacctgcccagggcaccatcctgcttgcacacctgccccgcgaggatgtgcgcgagtggttcac
tcgcctgtcgccaaggacctgagcagctgcccgcggcgccattgtgggtcggcctcgctgcgcgctcaggcc
cagatcctggccaagtacccccacctcaagggtggagaacttcgcgggcaacgtgcagacccgcctgcgcaagctg
aacgagggcgccctgctccgccacctgctggctctggccggctctgaagcgctggacatgactgagcacatcacc
aagaccctcagcattgacgagatgctgcccgcctgagccagggcgccattggcatgctgcccgcaccgacgac
ggcgccagccgcaacctgctggccgcccctgaaccacgaggagaccgcacatcgccgtggtgtgcgagcgcccttc
ctgaccgcccctggacggctcttgccgcacccccattgcccggctacgcgcacaagggcgccgacggcatgctgcac
ttcagcggcctggtggccaccccggaaggcagatcatgcgcgctagccgcgtggtgccccttcacggaggcg
gatgccgtcaagtgcggcgaggaggccggcaaggagctcaaggccaacggccccaaggagctgttcattgactaa

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

MQQCVGRSVRAPSSRAVAPKVAGARVSRVCRVYASAVATKTVKIGTRGSPLALAQAYMTRDLLKKSFPPELSEEG
ALEIIVIIKTTGDKILNQPLADIGGKGLFTKEIDDALLSGKIDIAVHSMKDVPTYLPEGTILPCNLPREDVRDVFI
SPVAKDLSLPAIGAIVGSASLRRQAQILAKYPHLKVENFRGNVQTRLRKLNEGACSATLLALAGLKRDLDMTEHIT
KTLSDIDMLPAVSQGAIGIACRTDDGASRNLLAALNHEETRIAVVCERAFLTALDGSCTPIAGYAHKGADGMLH
FSGLVATPDGKQIMRASRVVPFTEADAVKCGEEAGKELKANGPKELFMY

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

atgcgatcgtatctgctcaaggctcaagtggcctcatgtcagttttcgcgcacgtcgaaggctctggagactggcg
ccgggttctgacagacgacgggtgtcggggcctcactcggacaccgcactgcgcggcccccaccagcgagcccgcc
ccgccatccagcagcggaagagcgggcaacgaccactcgtgatagccacgcggccatctaagcttgcaaggag
cagacgcggcaggtgcagcagctgctgctggcgggcggcgcagctcaaggacgagcagctgcagctgagcacccctg
gaactggcgctctagggcgacacgactcagggtgtgctcgctgcgcagctcgggctcgggcgcattcaccgaggag

ctggaccaggctgtgctgtcgggcgctgcccacatgtcgggtgcacagcctgaaggactgccccgcgcgcctggcg
 cccgggctgctgtgcccgcctgctgcccggggccgacccccgggacgtcctcatcgcgccccgaggccacctcg
 ctgggcgagctggtgcccgggcagccgtgtgggcaccagcagcagccgcgcgcgcgcagatcaagcactccttc
 cccacctgcaggttgtgcagctgcgcggcaatgtggactcgcggctggggcgcatccgcagccgcgacatcggc
 gccacagtgtggcggcggcgggcctcaagcggctgggtgtgatgaactcggacgaggggtgacactaccgctacg
 ggcgcgctgggggtggtgtgcagggcagacgatgagtgggtggctggcctgctggacgccatctcgcaccgcggc
 acggccctggaggtggcggcggagcgggcgtgctggcagcgtgctgggcggcggcgcgcgtgccagcgttca
 gcgttccccgacattgcgtgggcctgccacacgcggcagccccgacagcaacacaatggacctggattgcctg
 gtggcgacctggagggcaaggagctcttcaggtacacggagtctaccggccggtcattgacgaggtggacgcg
 gtgtcgctggggtcgctgtacggcagcctgctgcgcgatgatggcgccaccaggcgcggccccctgttggcagcta
 ccttctcgcggcattag

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

MRSYLLKAQVASCQFSRTSKVWRLAPGSDRRRCRLTRTPHCAAPTSEPAPPSSSGKSGQRPLVIATRPSKLAK
 QTRQVQQLLLAAQLKDEQLQLSTLELASRGDTTQGVSLRSLGSGAFTEELDQAVLSGAADMSVHSLKDCPAALA
 PGLLLAACLPADPRDVLIAPEATSLGELVPGSRVGTSSSRRAAQIKHSFPHLQVVQLRGNVDSRLGRIRSRDIG
 ATVLAAAGLKRGLVMNSDEGDTTATGAVGVVCRADDEWVGLLDIAISHRGTALEVAEAERACLAALLGGGGACQRS
 AFPDIAWACHTRHDPDSNTMDLDCLVADLEGKELFRYTEFYRPVIDEVDVSLGSLYGSLLRMMAPPGAAPCWQL
 PSSRH

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

atgatgttgaccagactcctgggaccgccacggcttctagccggcggtcgagatccgctcggtcgccacgtc
 tcgcgaaggtcgcgctcggccacgccattctcggtcgcgagccccgcgacggctcgcgagccccgcgacgcg
 ggcggccgcgcacactccaccgcactgctgcggcgccactgggtgctcccacggcgtccggagccggcgctcgcc
 aagacgctcgacaatgtgtatgacgtgatcgtggtcggtggaggtctctcgggcctggtgacggccaggccctg
 gcggctcagcacaaaattcagaacttcttggttacggaggtcgcgagcgcgctcggcggaacattacgtccatg
 tcgggcgatggctacgtgtgggaggaggcccgaaacagcttccagcccaacgatagcatgctgcagattcggtg
 gactctggctcgagaaaggaccttgtgttcgggtgacccacgggtccccgcttcgtgtggtgggagggaagctg
 cgccccgtgccctcgggcctggacgccttcaccttcgacctcatgtccatccccggcaagatccgcgcgggctg
 ggcgccatcggcctcatcaacggagccatgccctccttcgaggagagtgtggagcagttcatccgccgaacctg
 ggcgatgaggtgttcttcgcctgatcgagcccttctgctccggcggtgtacgcgggcgacccctccaagctgtcc
 atgaaggcgcccttcaacaggatctggattctggagaagaacggcggcagcctggtgggaggtgccatcaagctg
 ttccaggaacgccagttcaacccggccccgcgcgggaccccgccctgcccgaagcccaagggccagacgggtg
 ggctcgcttcgcaaggccctgaagatgctgcggagcgccttgagcgcaacatccccgaagaatcccgctgaac
 tggagctggtgtctctgggcgcgagggcgagcggcggttacgggctggtgtacgacacggccgaggccgctgtc
 aagggtgtttgccgcgcgctggctctgaccgcgcccagctacgtggtggcggaacctggtcaaggagcaggcgccc
 gccgcgcgcgagggcctgggctccttcgactaccgcgcggtgggcgcgctgacgtgtcgtaaccgctgagcgcc
 gtgcgggaggagcgcaaggcctcgagcgggtcgtgcccgggcttcggtcagctgcacccgcgcacgcagggcatc
 accactctgggcaccatctacagctccagcctgttccccggcgcgcccgaggccacatgctgctgtcaac
 tacatcggcggcaccaccaaccgcggcatcgtcaaccagagaccacgagcagctggtggagcaggtggacaaggac
 ctgcgcaacatggtcatcaagcccgacgcgcccgaagccccgtgtggtgggcgtgcgcgtgtggcgcgcgccatc
 ccgcagttcaacctgggcaacctggagcagctggacaaggcgcgcaaggcgtggacgcggcggggctgcagggc
 gtgcacctgggggcaactacgtcagcgtgtggccctgggcaagggtggagcacggctaagagtcgcgagcc
 aacctggccaagagcgtgtccaaggccgagtcaggccctaa

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

MLLTQTPGTATASSRRSQIRSAAHVSAKVAPRPTPFVSPATAASPATAAARRTLHRTAAATGAPTASGAGVA
 KTLDNVYDVIVGGGLSGLVTGQALAAQHKIQNFLVTEARERVGGNITMSGDGYVWEEGPNFQPNDSMLQIAV
 DSGCEKDLVFGDPTAPRFVWEGKLRPVPSGLDAFTFDLMSIPGKIRAGLGAI GLINGAMPSEESVEQFIRNRL
 GDEVFFRLIEPFCSGVYAGDPSKLSMKAAFNRWIWLEKNGGSLVGGAIKLFQERQSNPAPRDRPLPPKPKGQTV
 GSFRKGLKMLPDAIERNIPDKIRVNWKLVS LGREADGRYGLVYDTPGVRVKVFARAVALTAPSYVADLVKEQAP
 AAAEALGSFDYPPVGAUTLSYPLSAVREERKASDGSVPFGQLHPRTQGITTTLGTIYSSSLFPGRAPEGHMLLLN
 YIGTTNRGIVNQTTQLVEQVDKDLNMVVKPDAPKPRVVGVRVWPRAIPQFNLGHLEQLDKARKALDAAGLQG
 VHLGGNYVSGVALGKVEHGYESAANLAKSVSKAAVKA

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

atgcagaccaaggctttcacctctgcgcgccccagcgggcccgtgcgctcaaggcgcagcgcacctcgtegggtg
 accgtgcgcgcgaccgcggccccccgcggtggcctctgccccgcgcgctcggtctctgcctctgacccccctgatg
 ctgcgcgccatccgcggcgacaagggtggagcgcgcccgcccggtgtggatgatgcgccaggccggccgctaccagaag
 gtgtaccaggacctgtgcaagaagcaccaccggttccgtgagcgctcggagcgcgtggacctggcggtggagatc
 tctctgcagccgtggcacgcgttcaagcccgacggcgctcatcctgttcagcgacattctgacccccctgcccgcc
 atgaacatcccccttcgacatggcgccccggccccatcatcatggacccccatccgcaccatggcgcaagtggagaag
 gtgacgaagctggacgctgagggccgctgcccccttcgtgggcgagtcgctgcgccagctgcgcacctacatcgcc
 aaccaggccgcggtcctgggcttcgtgggcgcccccttcacctggccacctacattgtggagggcgccagctcc
 aagaacttcgcgcacatcaagaagatggctttctccacccccgagatcctgcacgcccgtgctggacaagctggct
 gacaacgtggccgactacgtccgctaccaggccgacgcggcgccccaggtgggtgcagatcttcgactcgtgggccc
 agcgagctgcagccccaggacttcgacgtgttctccggccccctacatcaagaagtgatcgacagcgtgcgcaag
 acccaccgccagctgcccattacatcagcggtctggtggcgccctgctggagcgcagtgccctcttgctcg
 cccgacatcatctcgtggaccagtcgggtggacttcaccgacggcgctcaagcgtgcggcaccacacttcgccttc
 cagggaacatggaccccgcgctcctgttcggctccaaggacttcacgagaagcgcgtcatggacaccatcaag
 gctgcccgcgacgccgacgtgcgccacgtgatgaacctgggcccagcggtgctgcccggcaccgccgaggaccac
 gtgggcccactacttcacgtcgcccgaccgcccacgagcgcagtgtaa

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

MQTKAFTSARPPQRAAALKAQRTSSVTVRATAAPAVASAPAAASGSASDPLMLRAIRGDKVERPPVWMMRQAGRYQK
 VYQDLCKKHPTFRERSERVDLAVEISLQPWAFKPDGVILFSDILTPPLGMNIPFDMAPGPIIMDPPIRTMAQVEK
 VTKLDAEAAACPFVGESLRQLRTYIGNQAAVLGFVGAPFTLATYIVEGGSSKNFAHIKMAFSTPEILHALLDKLA
 DNVADYVRYQADAGAVVQIFDSWASELQPDQDFVSGFPYIKKVIDSVRKTHPDLPIILYISGSGGLLERMASC
 PDIIISLDQSVDFTDGVRKCGTNFAFQGNMDPGVLFGSKDFIEKRVMPTIKAARDADVRHVMNLGHGVLPGTPEDH
 VGHYFHVARTAHERM

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

atgtcgcccttgacgcgcgcgcgcacccccctacgagctagtgcggggtgtgtcctccgctctggccgccccgctg
 ttgcggcgctcccgctcacacacgtcagcctgagccctcggttcaccgtggtcagcgggcacgacgtggccggc
 accgactggcgcggttccgggggtgcccacgtggtggttctgatggcggtcgtaacctggggcagatagcc
 cggcggtctgtgcaggacgcgggggtgggcgcccgatcacctgtaagtcaacctagtggctag

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

MSALDAAAIPEYLVPGVSSALAAPLFAGVPLTHVSLSPSFTVVSGHDVAGTDWAAFRGLPTLVVLMAGRNLGQIA
 RRLVQDAGWAPDTPVSQPSG

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

ggcgccccacaaccaggacagcctacttcttgacctattaataagtcgctgctgcgactgaccattttg
 gcccgacttgctgctgtgatttgcttcgactagatccgcgggcaccaaggacgcggacagctgatagtc
 aagaactagatcctctgggagcgtctggggctgtcccgcgtgctcgccaaggaa

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

gtgccgagtgactgaggtggcaagggtgcagtgccggcgaggcaggttgctgctggggtggcaaggcgagcaggcga
 agctggtgggttgcgacgaggaggaggtgcacgtgcacgcgtaacataagaagaacagtgaggagcaggtagcg
 tgacttgactgggacgaggagcgtactgatgtgtggcgtgtgttggtatgtgagcgttacctctccctagatag
 cggcggtctccacttccaggaggatgagagccatcatgaggctttgagggggcactgggttcgtgtgtaggctgag
 gctgctgttgaagtcacaaggcagcactgcatgcgcgagtgagtggtggccggatgcatcgagttgcaggtaca
 ctgaaatgaggtgactgcggcggtatctcgtgccagtacaggttgaagcggcgggcacgggtgaatggagtaactcg
 gcctggaacgcttgcatcagatggtcgagctcaagaagatttggttgagccgttggttcgtgcgtcatattatg
 gcttgcatcttcgggagcggcaagaacggactccaatgcaggccctcgggcgagaaagattggcggtgtccgg
 ggggtgcatctcgcgcgtggggctgcatcgaatttcgcttgagtgcccttcccggggagggggggcggtagtt
 caaccccatcatcgtaggggggttgtaaatgccagcccaactaaa

CHLD Exon 1 (SEQ ID NO: 47):

atgaagtctctctgccatgagctcgctggccccagcgttactgggtgcggccggcgaagcctccggaaggctttc
agcgggtgccaagattgcgcaggtctctcgccccgctgtgcttaacagcgtgcagcgccaacagcgtctcgctgt
ctcgccgtggccgagctctcgctgctgagctgcgcg

CHLD Exon 2 (SEQ ID NO: 48):

ccatgaagggtgtctgaggaggactccaagggcttcgatgcggatgtgtcgaccgcctggcccgcctcgtaacctc
tggcgccgctggtgggccaaggacaacatcaagcaggcgtgctgctggcgccgtggacacgggctggcgga
tcgccatcgccggtcgccgcggtaccgccaagtccatcatggctcgcgccctgcacgctctgctgccgcccattg
agggtggtggagggcagcatctgcaacgcgcgacccccgaggacccccgctcctgggag

CHLD Exon 3 (SEQ ID NO: 49):

gctggcctggctgagaagtatgcggggcgccctgtgaagaccaagatgcgctcggcgcgctttgtgcagatccct
ctgggtgtgactgaggaccgcttgggtgggcactgtggacattgaggcgtccatgaag

CHLD exon 4 (SEQ ID NO: 50):

gagggcaagactgtgttcagccccggcctgctggctgaggcgacccggcgcatcctgtacgtggacgagatcaac
ctgctggatgacggcatttgccaacctgctgctgtccatcctgtcggaaggagtcaacgtggtggagcgcgagggc
atctccatcagccacc

CHLD exon 5 (SEQ ID NO: 51):

ctgcggccgctgctgattgccacctacaacccccgaggagggccctctgcgtgagcacctgctggaccgcatcg
cattggcctcagcgccgacgtccccagcaccagcgacgagcgcgtaaggc
cattgacgcagccatccgcttccaggacaagccgcag

CHLD exon 6 (SEQ ID NO: 52):

gacactattgacgacacc gcggagctcaccgacgccttgcgcacctcg

CHLD exon 7 (SEQ ID NO: 53):

gtcatcctggctcgcgagtacctgaaggacgtgaccatcgcgccggagcaggtgacctacattgtggaggaggcg
cgccgcggcgagtcagggggcacc gcgcggagctgtacgcggtcaag

CHLD exon 8 (SEQ ID NO: 54):

tgtgccaaaggcgtgtgcggtcttgaggggccgtgagcgtgtgaacaaggatgacctg
cgccaggccgtgcagctggtcatcctgccgcgcgccaccatcctggaccagccccgcccagcaggagcagccc
ccgcgcgcgcccccgccccctccccgcgcgcgcgcag

CHLD exon 9 (SEQ ID NO: 55):

gaccaaattggaggacgaggaccaggaggagaaggaggacgagaaggaggaggaggagaaggagaaacgaggaccag
gacgagcccag

CHLD exon 10 (SEQ ID NO: 56):

atccctcaggagttcatgtttgagtcaggggcgtcatcatggaccctccatcctcatgttcgcgagcagcag
cagcgcgcgaggccgctccggccgcgccaagacgtcatcttcagcgacgaccgaggccgctacatcaagccc
atgctgccaaagggtgacaagggtcaagcgctggcagtggaacggcacgcttcgcgcgcgcgcgccta
ccagaag

CHLD exon 11 (SEQ ID NO: 57):

CHLD exon 12 (SEQ ID NO: 58):

CHLD Intron 1 (SEQ ID NO: 59):

CHLD Intron 2 (SEQ ID NO: 60):

CHLD Intron 3 (SEQ ID NO: 61):

CHLD Intron 4 (SEQ ID NO: 62):

CHLD Intron 5 (SEQ ID NO: 63):

CHLD Intron 6 (SEQ ID NO: 64):

CHLD Intron 7 (SEQ ID NO: 65):

gtgaggagttggaggggaagggcgaggggatgcgacagaagcgagggcgaggggagccggggtgggttgttgc
aagtgtcgtgaattatagaatgacccccaaaagcgccggcccaacagggcctattacttgcgagtcgaatccaaccc
ctgatatagggagaatggggtagaggtcgtatcacgacagcaaggatgtacagtgggccttgggggttgggaggtg
cagggaaaaagagagagacatgggggtgggttaagcggggaataacaaatatacaccagcggttatggaagtggg
agatggaaacggggcgacgaacaggaacaggggcccggatggaggggctatgggggcatgggtgggtgggggtac
ggcgcggggagagcaggggtctgggtgaatgggcaagatgctgatgcttgggatgaagacactatgagcaaaga
aatgggtgttgacgattgccatgatcatcgagtgggggagggcggggtggcaataccggcagtcgaacagttgggg
tgcgatcaagattgattggagtaccagcagtgccgggatctggctgacgtgtctcgagcgagttgctgggggtgg
caaggagatgcaggggcagacgacgttgtgacgaccacacttacacacatttccttccccttgcgtgtgtccgtgc
gccttgcctccag

CHLD Intron 8 (SEQ ID NO: 66):

gtacgtaaacgtatattgattgctcaggtgggttagccttgggtgtggctgctgtttgacttgtgcagctgtctttgt
gtacatgttccacaaccctgtactccccatattccgcccccatccag

CHLD Intron 9 (SEQ ID NO: 67):

gtgagaggcggcgcggcggttgcgggcgaaggcgggggcgggggcgaggcaatgcggccgcgcagtgccagca
acggaagggtggctatcaacacggcgagcgacgatattcatataagagtgccatcggtgcaatgctgaatactt
gcgccaaccggatctcgctgtctccgctccaccggactgctttctcatctctccccttcacctgtgtgtatcca
cag

CHLD Intron 10 (SEQ ID NO: 68):

gtgagtccccgaggtgggtgggtgaattggggcacgaggggtatgtgggcctaagggagctgaatggggcatgt
tttcttctgagcatcacggtcagagcttgacctgtcctcccgcgtgtacccccgtgcacgggtccgacacag

CHLD Intron 11 (SEQ ID NO: 69):

gtgagtacagcgcatcccggcgcaatcattgggcctagttactgctgcaggactcgtgtgctcttaagggtggc
agctgtcagaagctctactcctcgactgaccactgtgcctttctctccttctctccctccccgcacccctc
ctcccacttctcacaacag

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

gcgacttccataaaagctcttgtaacgctgtaccaactagtaagcgggtacaattcgcttgagcccgagcaacgcg
acctttcttgctctgtggatctctgataatctaaccagacccaaaaccttttactaatctaggcaaca

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

aaaaggctgggtgtaggcctgtcgggtcgtgttaagggttgcgtgacgtgtaagtgtgacagtggtgccggta
tgtgtgtgtatacatgtgttgcggtgtgcttttgtggcggtacatggatgactgagcgggtgggacagagcac
ggttaactgacgagggcagtcctgtgcgagacggacgtttttagcggaggtgcaaggactgatgacgggctaag
ctgctggagacttggagttagagtgacaggtggatcgacgggtttctctaaggagtatgaataggcaggagggctg
gagacatttgggggtgcaaggagggcggtagtagggagatgtccatgggcggattttggcctctgtaacttcttaa
cgcca

CHLI2 Exon 1 (SEQ ID NO: 72):

atgcagagtctccagggtcagcgcgcgttactgcgggtgcgccaggggtcgggcggggtcccctgcggactcgctg
gtcgtgcgtcgtctgttgccctgccatccacgaaagccgcgaagaagccgaacttcccggtcgctcaagattcag
ggccaggaggagatgaagcttgcaactgctgctgaacgtggtcgaccccaacatcgccggagtgcttattatgggt
gaccgcggcactgccaagtccggtcgcg

CHLI2 Exon 2 (SEQ ID NO: 73):

gtccgcgccttggtggatatgcttcccgacattgacgtggttgagggcgacgccttcaacagctccccaccgac
cccaagttcatgggccccgacacctgcagcgcttccgcaacggcgagaagctgccaccgtccgcatgcggacc
ccctg

CHLI2 Exon 3 (SEQ ID NO: 74):

gtggagctgcctctgggcgccaccgaggaccgcatctgcggcaccatcgacatcgagaaggcgctgacgcagggc
atcaaggcctacgagcccgctgctg

CHLI2 Exon 4 (SEQ ID NO: 75):

gccaaaggccaaccgcggcatcctgtatgtggacgaggtgaacctgctggatgatggcctg

CHLI2 Exon 5 (SEQ ID NO: 76):

gttgatgtcgtgctggactcgtcggctagcggcctgaacactgtggagcgtgaggggtgtgtccattgtgcacct
gcccgccttcatcatgattggctcaggcaacccccag

CHLI2 Exon 6 (SEQ ID NO: 77):

gaggggtgagctgcgcccgcagctgctggatcgcttcggcatgagcgtcaacgtggccacgctgcaggacaccaag
cagcgcacgcagctggtgctggaccg

CHLI2 Exon 7 (SEQ ID NO: 78):

gcttgcgtagcagggcggacctgacgcatttgtggactcgtgcaaggccgagcagacggcgctcacggacaagct
ggaggcgggcccgccagcgcctgcggctccgtcaagatcagcgaggagctgcag

CHLI2 Exon 8 (SEQ ID NO: 79):

atcctgatctcggacatttgcctcgccctggatgtggatggcctgcgcggtgacatttgtgatcaaccgcgcgcgc
aaggcgcttgtggccttcgagggccgcaccgaggtgaccacgaatgacgtggagcgcgtcatctcgggctgcctc
aaccaccg

CHLI2 Exon 9 (SEQ ID NO: 80):

cctgcgcaaggaccgcctggacccattgacaacggcaccaagggtggccatcctgttcaagecgatgaccgaccc
cgagatcatgaagcgcgaggaggaggccaagaagaagcgcgaggaggcgccgccaaggccaaggcggagggcaa
ggcggaccgccccacgggcgccaaggctggcgccctgggctggcttgcacctcgtcggtaa

CHLI2 Intron 1 (SEQ ID NO: 81):

gtaggtaacacaagcaattatggggcgaagatctaggctccgctgatccgggcgggcaatcggcatcgtcgggtgc
aaccgtggggcgctctgtgcacctttgctgggtgccaggttgccctgactcgctgcattcctgtaccgagccacat
tggctgctttgcagcgtgcatgggacgggtgtaggataaagcgtatgtatgcgatagcgcgggtgcaccggcttg
gcatggcaaggttgccgggtgcacatgcgtgccagcgtccctcagcatcagagtctggatctaagggtcagcg
gcttccctgcgcagtggtgctttgcgtagtgtacgaagccttataaattaaagtcacgtattgagtggtccggg
tttggggcactagtagtgccaggaggcgctgccaggttgatatgagcatatcagcaccgcgttccctgcgaaacg
cttccgttgctccttccccaccacctccccgctcataccatacatatggctatccgctcctctcattgcttg
ccctacag

CHLI2 Intron 2 (SEQ ID NO: 82):

gtgagcgggcctaccttctgaagacagtcctacgtgttgcaactgcagcgggtgttgccgacctctgcttttgctg
cgccgggaagcgcggattgcggcctcacagatcaagcccggaacgcttggtgtttccagcgggtggcacacacg
cgcgcgcgccacagtgacacctcacggccgcgctgccctgcag

CHLI2 Intron 3 (SEQ ID NO: 83):

gtgcgtagtgcatggggagaggggacgaggggagggagggcagggccaataaacggaaccccaagtcacgagaca
cagaacccgataatagctcccagatcgccaaggggtgagggcggaagccaaggatgatgcgttggccgcattgcg
tgttgacgtcaggcttacacaggggtctgactggctgtgcttgggggttggcacgcttcttgactggccccgtacg
catgctgcag

CHLI2 Intron 4 (SEQ ID NO: 84):

gtgagtggtggtggtttctgggtcagcagaggacttctgtagtaggtaaatgtgggccagggaagtgtggctaaca
tgccaaacacggggcgccaccagtgcgaagctgcattcgctgacgtgcacgggtgcaatgggtgcaaggcgaactg
caatcgcggtgcacagttgccagggctgcgtcacgcttgagtgtctgcacacgcactgcag

CHLI2 Intron 5 (SEQ ID NO: 85):

gtgcgtagcgtgcgcgcatgtacttgtctcccttgtcatgttgggaaaggctcggtccccagcctgcttgcaagat
gcggccgggtcagcagctgcggacgggtcagcacctacgtgccgaggttgtgtaacatgaatggcgttggggcgcc
gacctgccacaagctgaactgcgaccagcaaggcagctgccagcaacgcacaccgacgtgctacacgcttgtgt
tttgacctcctaaacacaccccgccgctgtctgtcacgtccacag

CHLI2 Intron 6 (SEQ ID NO: 86):

gtaagcggcgggcgcgggggacacggagggacatttcgcgagcatgggttgaggagtgggaggattcggtggc
tgcccgagtcgggagtcggagtcgcgagtcggaagtcaagcttctggcggttcgtgctgtcggggtgcgtcgc
catgatggcgctgaccggagggcgctcacgctgtgtatgtgggcgcgcag

CHLI2 Intron 7 (SEQ ID NO: 87):

gtacggggcgtagacggggcggtgcacggggccagtgaaccgacagggcagcacgcggctggcgaagagcgac
aaagtgcaggggtgaccaagaccgggtgatgccacgagagggcgcgggagccgtgcattgggtcgaggagggag
gaatgcaactttacactgatgcctctgtatacggccgccttcgagccctgcaaaccttcgctttcccccgacgc
acgcag

CHLI2 Intron 8 (SEQ ID NO: 88):

gtgagcgacgctgcgggtggatgcgggtgcgcgtgcgggttgccaacttattatgttgcactggacgcgtggctg
gcgatggcatgtcatggcggaatggatattggcggaatggataccggtaatggtagcacggggcgcgaggcct
ggcggtagtgggttgagggggcgaggactccagcgcgcatatgccatgttcagcatggccccaactgacag
cgcccgctgcctgtgcgccccgctccctccgcgcacccgctcctcctacacag

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

ctagtcagagggaactaggaggggcaacagagaa

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

gcggcctccccctcatggtagcactagttggcggttgtggttggactaggcggttagggatatatacctagtagc
ggcggtgcggagtgagggttggcgccagcgcgaggcggtggcctttcctcctggacccgagagcgctccgcg
aggagacggcgagtgagataggcagcagcgagcgagatcgatttgtgaacagtttgtggcgggatcccatagc
ggatgcagagaagaccttagagcagcttctcggtggagtgaacgagccagagcggaggggaaggcgcatgagggg
actgcagggactggaactgcgggagtgaggtccggtgctagggtccgctaaacagtgcgggtctacgcctgtgtgt
gaggtgtgcgtgtgtgtgagctgtgcggttttgttgcgcaagtaggagtgagccgagcgcgcgtactttgt
ggcgtgttggctgctggcgctgagagccaagagaggggtaaacgggttgggtattttatgggtgcgggggtgaaagc
agccctcgaggaatggagcgattctgcagcatgatgcagctgtgcctgcgcgtggatgggtggctgttgatagg
ctctgccactccggcagcaccgctacgatacctagcggtgcctggagtggtctctctgttgggtgcgtgatgtt
gggtttgccgttttgattctttgttctgtgctgaatggctgaggcggaagaccctcgtgccagtgtacagagc

ctcacggctccctcggaccccgctggggacgtccattcccgggtggcggtgtcgctcggcggtgtaaaagcaaaa
aatatattt

CHLH1 Exon 1 (SEQ ID NO: 91):

atgcagacttcctcgcttcttgccggcgacggccacccggctgcgggcgcgacgccaagccg

CHLH1 Exon 2 (SEQ ID NO: 92):

gttgcgccctcgccccgctggctagcaccgccag

CHLH1 Exon 3 (SEQ ID NO: 93):

gtcgctgcaatgtggcgactggaccccgccgcccattgaccaccttcaccgggtggcaacaagggccctgctaag
cagcaggtgtcgctggatctgcgcgacgagg

CHLH1 Exon 4 (SEQ ID NO: 94):

gcgctggcatgttcaccagcaccagcccgagatgcgcctgtcgctccctgacgatgtgaagggctcgcttaagg
tgaaggttggtgtacgtggtgctggaggccagtagcagtcggccatcagcgctgcggtgaagaacatcaacgcca
agaactccaag

CHLH1 Exon 5 (SEQ ID NO: 95):

gtgtgcttcgaggtggtgggctacctgctggaggagctgcgtgaccagaagaacctcgatatgctcaaggaggat
gtggcctctgccaacatcttcacggctcgctcatcttcattgaggagcttgccgagaag

CHLH1 Exon 6 (SEQ ID NO: 96):

attgtggaggcggtgagccccctgcgcgagaagctggacgcgtgcctgatcttcccgtccatgccggcggtcatg
aagctgaacaagctgggcacgttttcgatggctcagctgggccagtcgaagtcggtgttctcggagttcatcaag
tctgctcgcaag

CHLH1 Exon 7 (SEQ ID NO: 97):

aacaacgacaacttcgaggagggcttgctgaagctggtgcgcacccctgcctaagggtgctgaagtatctgccctcg
gacaaggcgcaggacgccaagaacttcgtgaacagcctgcagtagctggctgggcggtaactcggacaacctggag
aacctgctgctgaacaccgtcagcaactacgtgcccgctctgaagggcggtggacttcagcgctggctgagccacc
gcctaccccgatgtgggtatctggcacccctctggcctcgggcatgtacgaggacctgaaggagtacctgaactg

CHLH1 Exon 8 (SEQ ID NO: 98):

gtacgacacccgcaaggacatggtcttcgccaaaggacgcccccgctcattggcctggtgctgcagcgctcgacact
ggtgactggcgatgagggccactacagcggcggtggtcgctgagctggagagccgcggtgctaagggtcatccccgt
ctttgccg

CHLH1 Exon 9 (SEQ ID NO: 99):

gtggcctggacttctccgcccccgtaagaagttcttctacgacccccctgggctctggccgcacgttcgtggaca
ccgttggtgctgcgtgaccggcttcgcgctggtgggcggccccgcgcgagcagcgccgaaggccattgaggcgc
tgaagaacctgaacgtgccctacctggtgtcgctgcgcgctggtgttcagaccactgaggagtggtggacagcg
agctgggcgtgcaccccgctccaggtggctctgcag

CHLH1 Exon 10 (SEQ ID NO: 100):

gttgccctgcccgagctggatggtgccatggagcccatcggtgttcgctggccgtgactcgaacacccggcaagtcg
cactcgctgcccgaccgcacatcgcttcgctgtgcgctgcgcgctgaactgggccaacctgcgcaagaagcgcaac

gccgagaagaagctggccgtcaccgtgttcagcttccccctgacaagggcaacgtcggcactgccgcctacctg
aacgtgttcgggtccatctaccgcgtgctgaagaacctgcagcgcgagggctacgacgtgggcgcctgccgccc
tcggaggaggatctgatccagtcgggtgctgaccagaaggaggccaagtccaactcgaccgacctgcacatcgcc
tacaagatgaagggtggacgagtagcagaagctgtgcccttacgccgagggcgtggaggagaactggggcaagccc
cccgccacctgaacaccaacggccaggagctgctggtgtacggccgccagtagcgcaacgtcttcacggcggtg
cagcccaccttcggctacgagggcgaccgatgcgcctgctgttctcgaagtggccagccccaccagggttc
gccgcctactacaccttctggagaagatcttcaaggccgacgcgcgtgctgcacttcggcaccacaggctcgctg
gagttcatgcccggaagcaggctcggtcatgtcggtgtgtgctaccccgcactcgctgatcggcaccatccccaac
ctctactactacgccgccaacaacctgctgaggccaccatcgccaagcgcgcgtcgtagcgaacaccatttcg
tacctgacgcgcctgcccgagaacgcggcctgtacaaggccctgaaggagctgaaggagctgatcagctcgtag
cagggcatgctgagctgtggccgcgcgagcagatctgcgccaccatcattgagaccgccaagctgtgcaacctg
gaccgcgacgtgacctgcccgcgctgacgccaaggacctgaccatggacatgcgcgacagcgttgtgggccaag
gtgtaccgcaagctgatggagattgagtcgcgcctgctgccctgcggcctgcacgtggtgggtgcccgcccacc
ggcaggaggcgtggccacctggtcaacatcgctgagctggaccgcccggacaacaaccccccatcaagggc
atgcccggcatcctggccgcgcctattggtcgcgacatcgagtcgatttacagcggcaacaacaaggcgctcctg
gctgacgttgaccagctgcagcgcacaccgaggcctccgcacctgctgcgcgagttcgtgaaggaccgcacc
ggcctgaacggccgcacggcaccactggatcaccaacctgctcaagttaccggcttctacgtggacccttg
gtgcgcggcctgcagaacggcgagttcgccagcgccaaccgcgaggagctgatcacctgttcaactacctggag
ttctgcctgaccag

CHLH1 Exon 11 (SEQ ID NO: 101):

gtggtcaaggacaacgagctgggcgcctggttagaggcgtgaacggccagtagctcgagcccgcccccggcggt
gaccccatccgaacccccaaagctgctgcccaccggcaagaacatccacgccttgaccctcagtcgattcccact
cagggcgcgctgaagagcgcgcgcctggtggtggaccgcctgctggaccgcgagcgcgacacaacggcggaag
taccgagagaccatcgcgctggtgctgtggggcactgacaacatcaagacctacggcgagtcgctggcccgaggtc
atgatgatggtcggtgtcaagcccggtggccgacgcctgggcgcgctgaacaagctggaggatcctctggag
gagctgggcccgcgcctcggtggacgtggttgtcaactgctcggtgtgttcgcgcacctgttcgtgaaccagatg
ctgctgctggaccgcgcctcaagctggcgccgagcaggacgagcccgatgagatgaacttcgtgcgcaagcac
gccaagcagcaggcgcgagctgggcctgcagagcctgcgcgacgcggccaccctgtgttctccaacagctcg
ggctcctactcgtccaacgtcaacctggcggtggagaacagcagctggagcgcagagtcgcagctgcaggagatg
tacctgaagcgcaagtctgtacgccttcaactcggaccg

CHLH1 Exon 12 (SEQ ID NO: 102):

ccccggcgccggtggcgagatgcagcgcgacgtgttcgagacggccatgaagaccgtggacgtgaccttcagaa
cctggactcgtccgagatctcgtgaccgatgtgtcgcactacttcgactccgacccccaccaagctgggtggcgtc
gctgcgcaacgacggccgcacccccaaacgcctacatcgccgacaccaccacgcgcaacgcgcaggtccgcactct
gggtgagaccgtgcgcctggacgcgcgcaccaagctgctcaacccccaaagtgtacgagggcatgcttgccctcggg
ctacgagggcgtgcgcgagatccagaagcgcacgaccaacaccatgggtggtcgccacctcgggcatggtgga
caactgggtgtacgacgagggccaactcgaccttcacgaggatgcggccatggccgagcgctgatgaacaccaa
ccccaaacagcttcgcaagctggtggccaccttctggaggccaacggccgcggctactgggacgccaagcccga
gcagctggagcgcctgcgcagctgtacatggacgtggaggacaagattgagggcgtcgaataa

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):

gtgagcagcgcggaccgagcaagcgtggcgatgcagttggatttggtgttcttggtcaggcgctcgctcgatg
gccagcgcgtgtatttaatgggataaggggtgagacaaagcatctcttcgggtaaaaatcttagttttcgacagc
acgttgagaggcatgcaacttgctctttcgag

CHLH1 Intron 4 (SEQ ID NO: 106):

gtgggtaaggagttgcattatcagtggtggcatgggtgtgcgggcgtctggggcgctgcaacagcggcatcgtgcc
gaactgaccgtgccgggctacccgcgtgcag

CHLH1 Intron 5 (SEQ ID NO: 107):

gtgcgctaggggttggggctctggaggggtgtggattgcgcccagtgccctgttgcgcttggcggtcgctgtcatga
tgtgaggggtgacgtagtgcaactcaattgcctgtacgtcaccacctttgatgggctggatctgaggcaggtcagc
tcggttccctgctgcatccagtgtccctgtgcgctgcacggttgacgctgttcccccttcgcactgtctcgct
ttgcag

CHLH1 Intron 6 (SEQ ID NO: 108):

gtgtgggcacgcgctttgggaagggaggcatacatttttgggtgcggttaggctgggcgcggacttggcactcac
acggtcattgcacactcatgtctcaccttcatttacggtcccttgtgccgaactacctacag

CHLH1 Intron 7 (SEQ ID NO: 109):

gtgagcagcatcagggcagagtgcataacggattggtggcagtggggaatggaattagacggacacgtctgggc
ggcaatatgttgcgctgcagtttttgggggtgtagtgaactagaaaatagggaagagataggccacataacatccg
aaagctcatatttttgcaaccggcgacacatcacagcccacctgaagggtttttagtcaacgcgtgcaactga
ctagatgtcccccttacctgtctgatttcag

CHLH1 Intron 8 (SEQ ID NO: 110):

gtgagggcggggcggcgctgccctcggtaggggttgacagatgggtgatgggtaaccgaatgcattggccaatggggag
tgaaatcaggaaaggaggggtaacacaatgcagggcagcacctgaatcgtgaaggcggagttaggcagggatctg
tcagttcgctgtcacgtggatgggcgcagctgacctttgtgggtgttggtgtggcgacag

CHLH1 Intron 9 (SEQ ID NO: 111):

gtgagctcagctgggacatgtaggggctcggggtgcgggagcatcgatgtagaattacgggaggaggggagaggg
gagaggattgcacgaaccgagatgagggcggtggttcgggatttcggggcaaaagctcgtgagggaagcgttcagt
gactgaagagcagtggtcttcaactgccccctctgtccctcag

CHLH1 Intron 10 (SEQ ID NO: 112):

gtgcgaccgggtgccgctgcgtggccaacagcttgggtgccaccttccctgcgggtgttgatttacactgtgtgcgtgg
atgtgttggtttttcgcaacttttagtctgggctccagctctttgccttcattgatcactcgtcttacctcctgcg
ccatcatttgaatacag

CHLH1 Intron 11 (SEQ ID NO: 113):

gtgagccttaatgcaacacgtgtagccgttcgcatgggtggctgggtcatgctatgggtggatcggcgctccgcct
gcttgctactgctgttcgggtaccagcgtttactgaccccgcggtgtgccattcccaccacctaaccctcgcctt
gcag

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

gacagtgatatagcaataaccgatataataggtttggcgggcttcaccttgctctaccacagaatgtggccctgac
agtcgatttccagcccccttgccactcgtccctgattttctcaatcaactagttgggtcggttttctcgtaagg

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

ggggggcgggtggcgagtaaggcgtatggcggagcaggagatgggctgtggcgtggccgggtgttcttttgtgtga
ttggaaacatagacgggtgcccgcgcggcctgactgctgcgcggttggtgtggttgccgggggagcggggtcg
atggggcagcgcgcacgagtgggtgaaggaggaggccaggcgtgggtacacccatgggttgaggatgctag
tgagtgatgtgtgcgggggcatgggtgtgtaccattcagagtcacagcagcgggtgctggggagcgttcc
ctgctgtgcatgatgatggcgcccttcgatgaatcatctcttgaagggtccaaatgaaacgtctgaagtctgcagag
gggtgggtgctggacatgccatccaggcgggaagtgggcagctgtgtctgactacaaagtaggtcttgttttgcctgg
atagcgtttggctatgtagcgtgtattctgctcatcaatcacgccaggcgtcagggaactacccatgcaagtcggg
agcgtgggtggctctggaaaagtgttagctgctaggtggcgttggtcgggtgtcatgcatctcggcaggtaggc
ggtagcgggtggacgacctctgcagcggagcatgtgcacaagatgtgactgcgcacacccgtatatgacggcgt
tggcgtcagttgttgagagtgaacagaggagagacgagcgaagctgccatgcccttagtggcgtgggtgcgagagg
gaagaaagagagaggaaggactttgcggcagtgccccacgcgcggagtggggacacgggtcatcaacagggcggcg
gagctgggcggagtgggtgtgtgatgggacaggggtcaaggcaggttggcgaggtcggagtgggtagaccagtc
ttcagtgcaagggcattagggcctgatgtaagggtgaagcctt

Ferrochelatase Exon 1 (SEQ ID NO: 116):

atggcgtcgtttggattgatgcaaaggacgggtgcactgtccccagcttggtggaggagcgggtgttcgccggctcgt
ggcgtgctctggctcgtggcctgccagttatccagcggcaacg

Ferrochelatase Exon 2 (SEQ ID NO: 117):

gcgtggcgtgtgcagtgccaccaacgggtgtccagcagggcggtgtgctgcgcggagcggccgcttcgaccgacgt
ggctctccttcgtggacccaatgacattagaaaaccgcgacgagcagcagctggcctgcgggtggataaggctcg
cgttctgctgttaaactttggcggggccgaaaagctcgacgacgtcaagcctttcctgtataacctattcgccga
cccagaaattattcgctgccagcggcagctcagttcctgcagccgctgctcgcgacgatcatctccacgcttcg
cgccccgaagagcgcggagggtatgaggccattggcgggtggtagcccggttgctaggtattacagacgagcaggc
ggaggcgtggcgagtcctctgcgcgccaaggccaaacctgcgaacgtgtacgtggcatgcgctattggcacc
ctacacggaggaggcgtggagcacattaaggccgacggcgtcagcgcctggtcatcctccgctgtaccctca
gttctccatctctaccagcggctccagccttcgactgcttgagtcgctcttcaagagcgacatcgcgctcaagtc
gctgcggcacacgggtcatcccgctcctggtaccagcggcggggctacgtgagcgcgatggcggacctgattgtaga
g

Ferrochelatase Exon 3 (SEQ ID NO: 118):

gagctgaagaagttccgggacgtgcccagcgtggagctgtttttctccgcgcacggcgtgcccagtcctacgtg
gaggaggcgggcgacccatacaaggaggagatggaggagtgcgtgcggctcattacggacgag

Ferrochelatase Exon 4 (SEQ ID NO: 119):

gtcaagcggcgcggcttcgccaaacacgcacacgctggcctaccagagccgcgtgggccccgcgggaatggctcaa
ccgtacacggatgagtcacaa

Ferrochelatase Exon 5 (SEQ ID NO: 120):

ggagctgggcaagcgcggcgtcaagtcgctgctggcgtgcccacagctttgtcagcagcacattgagacgtt
ggaggagatcgacatggagtaccgcgagctggcggaggagagcg

Ferrochelatase Exon 6 (SEQ ID NO: 121):

gcatccgcaactggggccgcgtgccggcgtgaacaccaacgcgccttcatcgacgacctggcggacgcgggtga
tggaggcgtgcctacgtgggtgctggcggggcgacagactcgctggtgcccgtgg

Ferrochelatase Exon 7 (SEQ ID NO: 122):

gcgacctggagatgctgctgcaggcctacgaccgcgagcgcgcacgctgccgtcaccgggtgggtgatgtgggagt
ggggctggaccaagagcgcggagacgtggaacggccgcatggccatgattgccatcatcatcctggcgtgg
aggcagccagcggccagtcctcctcaaaaacctgttctggcggagtag

Ferrochelatase Intron 1 (SEQ ID NO: 123):

gtgcgataataaatttgcataccttatgaattgctcaatgactaacgagcagcgtccgcgaccacag

Ferrochelatase Intron 2 (SEQ ID NO: 124):

gtgaggggtggcattctgtaaagggagttgtggagttgggcagagcagtgagggtttgggtcgccagggcgaggatgt
tgccgggcggttggcaggaacagggctgctagggcttgctggccagcgactagggtttcgactggccagcgccg
ccggggcgcgcttgccgaagctgcacagccccaagcgcttctgtggatcaaattggaaacttggtggcagtggtat
gctagcgcttggcgcaagaccaatttttagtggtattactgttattactgtggtagcgggtgggtattcggcgcg
tggttggttggtgcagccccgtgcgactaagaccgctggcaacgacagcaagccgcccgcacccaggcatatacggc
ccaccagcaccaccgtacacaaccacgtgcctttgcactctacgcaccacagcgcgctgctgcccgtctccacctc
ccatcccaacggccctcttacccccacttcacaaccctcctctcacacgcctcctcttccccctcctcttcc
ag

Ferrochelatase Intron 3 (SEQ ID NO: 125):

gtgggcccgggcgagcgggcccgggaggggaggggaggggagggagggagggagggagggagggatgg
aaagctggcgagcggcagcgggagggacaggttagagggcgctgccccagcggcgcgaggtgggcatgggtggcg
gtaggggagcgcgtgagggactcgtcaggcatccgcatggcgggcgacttgctgctcctcaccgctgacggctgc
atctgctgtgtgcgtaacctggcctggcctggcaccgcag

Ferrochelatase Intron 4 (SEQ ID NO: 126):

gtgagggccggtgggtgggacgcggggagggagcgcggggagggggagacgcgggagcggggacaaggggtaggatac
ggggaggggaataggagaggccatggggagggatggggacacgggaggatgcacgggcctgggtggagccaggggg
aagtggacgacgagcccgcgaggagggctgggtagaaggacgcgggaggtgggtgggacaggtggacggggc
gtgtggagcatagcgcgcaagaagcgggactgagcgggttgaggggatggatgtaatacgggaagtaagaacc
cgagtggggctcagcgtgtcagcctgccttatcttgcgcgaagcgtgggggttttatttcgctgtacacacgtc
gcgcctttctgcgcgag

Ferrochelatase Intron 5 (SEQ ID NO: 127):

gtgaggaggcgccggagttttgggggaaggggtgcggcgtgaagcagagatggcaggggaggaaggaaggagcggat
gggtggctgggtgcaagcggagaggcgacagagagtggaaggttttggtggagcgggtggggagagggggcgagcag
ggatgcggccctggggatggcgggacagaagggaagcaagtttgccaagtgaaggggggggggtgctcaagaggaga
ggggcggtggaggttaagacggcctgctgggtatgctgggggttgcaaggcgcatgggcgcatggagccgggggag
tttggtgtggatgggactgcggatgggcacggcttgctactcatgtgcggtcgcgggtccgcggtgtgtcagcc
agccaggaccccatccactgggtcttctgctgctcctgggactgcttgccgccaccacccattcatcaccacca
ctgcgcagaccaccaaacaccgctgcctgaactgctctgactcttggcgctcctcag

Ferrochelatase Intron 6 (SEQ ID NO: 128):

gtgagtcgcgcctgcgcggttggttcgcggatgccggttgccggatgacgttcggcggttggcattgggtttggg
tttgaggggttggtgggtgaggtcgggattggggctcgggattgggggtcgagcgtggggctggcggtggatgatgg
cgtggctcttggaaggggcttggggaggttgccgctgtggatgcccagacagcatgggcgcgacagtgcgcattgtgc
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ctggaccgttagagaccgctacgtgtccgctagcggcctcggcctcagcctaagcgccagttagcgccagcgacac
aagcaacactgtcgctaattggcagcagcggcagcagcagcagtcacgagaatgcccgcgccggggagaaagtgtc
cctagccggggggcgccgctagctgggtttcctcagcgcggtggacgggtgggtgccttcattccgaccacccagggc
cgtccccagtcctcgtagctcgctgccttggtggccgccttgaccgccttgggcgccaccgggtgggtcgcata
acgactcgctttcgttctcgcctgacgctgtccgctgacgctctgcgcttgactctttgcgcttctcctccc
tcttccccag

Mutant sequenced RedAlgae CHLH DNA (SEQ ID NO: 129):

atgcagacttctcgttcttggccggcgacggccacccggctgcgggcgcgacgcccgaagcgggttgccgcc
tcgccccgcgtggctagcaccgcaggtcgcggtgcaatgtggcgactggacccggcgcccatgaccacctc
accgggtggcaacaaggccctgctaagcagcaggtgtcgctggatctgcgcgacgagggcgctggcatgttcacc

agcaccagcccggagatgcgcggtgtcgctccctgacgatgtgaagggtcgcgtaagggtgaagggtgtgtacgtg
gtgctggaggcccagtagcagctcgcccatcagcgtgcggtgaagaacatcaacgccaagaactccaagggtgtgc
ttcgagggtgggtgggtacctgctggaggagctgcgtgaccagaagaacctcgatatgctcaaggaggatgtggcc
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aacttcgaggagggtgtgtgaagctgggtgcgcaccctgcctaagggtgctgaagtatctgcccctcggacaaggcg
caggacgccaagaacttcgtgaacagcctgcagtagctgggtgggtaactcggacaacctggagaacctgctg
ctgaacaccgtcagcaactacgtgcccgtctgaagggtggtgacttcagcgtggctgagccccaccgcctacccc
gatgtgggtatctggcaccctctggcctcgggcatgtacgaggacctgaaggagtacctgaactggtacgacacc
cgcaaggacatgggtcttcgccaaggacgccccgtcattggcctgggtgctgcagcgtcgcacctgggtgactggc
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gttgtgtcgctgacgcgttctcgctgggtgggcccgcgcgcaggacgcgcggaaggccattgaggcgctg
aagaacctgaacgtgcctacctgggtgtcgctgcccgtgggtgttccagaccactgaggagtgggtggacagcgag
ctgggctgacccccgtccagggtgggtctgacaggttgcctgcccagctggatgggtgccaatggagcccactcgtg
ttcgctggccgtgactcgaacaccggcaagtcgcactcgctgcccagaccgcatcgcttcgctgtgcgctcgcgc
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gacaaggggcaacgtcggcactgcgcctacctgaacgtgttcgggtccatctaccgctgctgaagaacctgacg
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cgccaggcctgaggaagaaactggggcaagccccgcgcacctgaacaccaacggccaggagctgtgggtga
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caccatcattgagaccgccaagctgtgcaacctggaccgcgacgtgacctgcccgcgctgacgccaaggacct
gacctggacatcgccgacagcgttgtgggcccaggtgtaccgcaagctgatggagattgagtcggcctgctgccc
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ggaccgcccggacaacaaccccccatcaagggtatgcccggcatcctggcccgcccatgggtcgcgacatcga
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cgaggagctgatcacctgttcaactacctggagttctgacctgacccaggtgggtcaaggacaacgagctgggccc
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gcccaccgggaagaacatccacgcccctggacctcagtcgattcccactcaggccgcgctgaagagcgcccgcct
gggtggtagaccgctgtgtagccgagcgcgacaacaacggcggaagtaacccgagacctcgcgctgggtgct
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ggccgacgcccgtgggcccgtgaacaagctggaggtgatccctctggaggagctgggcccggccccgcgtggagct
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ggcgccgagcaggacgagccgatgagatgaacttcgtgcgcaagcagccaagcagcaggcgccggagctggg
cctgcagagcctgcccagcgcggccaccctgtgttctccaacagctcggtcctactcgtccaacgtcaacct
ggcggtggagaacagcagctggagcgacgagtcgcagctgcaggagatgtacctgaagcgcaagtcgtacgcctt
caactcggaccgccccggcgccgggtggcgagatgcagcgcgacgtgttcgagacggccatgaagaccgtggagct
gaccttcagaacctggactcgtccgagatctcgctgaccgatgtgtcgactacttcgactccgacccccaccaa
gggtgggtggcgtcgctgcgaacgacggccgcacccccaaacgcctacatcgccgacaccaccacgccaacgca
ggtccgcactctgggtgagacgtgcgcctggacgcccgcaccaagctgctcaacccccaaagtggtacgagggtat
gcttgccctcggttacgaggcgctgcgcgagatccagaagcgcatgaccaacaccatgggtgggtcgccacctc
gggcatggtggacaactgggtgtacgacgaggccaactcgaccttcatcgaggatgcggccatggccgagcgct
gatgaacaccaacccccaaacagcttccgcaagctgggtggccaccttctggaggccaacggccggcgtactggga
cgccaagcccagcagctggagcgccctgcgcagctgtacatggacgtggaggacaagattgaggggcgtcgaata
a

CHL1 5' - untranslated region (regulatory region) (SEQ ID NO: 130):

tctacagagtaaaggctctaggcgatgcgcgactgaaagactgtgaatcccggcgctcgccgtgggtgggtatgtggg
ccggtgcgctgtcgagaggataaattacaggtatcaaaaggttagggcgctggaaaggagcggcgctagggaa
ctgaaatcggatctgcatcggaacctcattccgcgactgtccttcttttgcctcgccccgcagctcttgagttt
tgttcttgacctttgacacgaaccaaccgatataaaa

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

gcggcaggccttcatggtcgtcgttggagcatttgcggaaaggctgatggcagcagatgcagccatgtcagttgt
 ggctgaagttgttggctggggcgggagcgggcagcagctgctgcgagcggccgaagcagcgggtgctgcttgcgt
 atgagaggaagaccagtgcctcgaggaggcagtgctgtgtgagtgtaggacgtgtgacttcggaaactgag
 ggcgggtgagttagatgtgactggggcttgcaggaagcctactgacctatcagaaaaaggtagcaggggtatatgg
 tctaggagcgttgcggagcgtggctggccagtgtagccgcgcgggctctgttgcctcgtggcgccgcgcgc
 ttcacaacagatgccgtagaaatgcagcagtgtagcagggcgtggcctattctgcaatgtgtgaggcgccaatgg
 cggcactgacaaatggaggagtgggtcaaagcttgggtacgttttgagagctgcatcgggcagcagggatcagtg
 gcggtaagaccgacggcagacggattggcaagggaataggagggacgtgggcgtgggcgcccgcgcttgcgcag
 gccgcagtagccggcgcgttctagaccgtagcccatttgaacaagcggccacgcgtgctcccgatgggggaca
 tcgatcacgggaattgattaaggggcagtggtgtgtgcaagtgagtgactgggtgggtcccgccctgtgaggttg
 tttcgttggacgtggctgccgggttgcgcgcgggctaagcgggcctgaggcagagcgtggcggtgtagccgcgag
 tatcgatctgtaacgtgc

CHLI1 Exon 1 (SEQ ID NO: 132):

atggccctgaacatgcgtgtttcctcttccaaggtcgctgccaaagcagcagggccgcacatctccgcgggtgccggtt
 gtgtcgagcaaggtggcctcctccgccgcgtggcccccttccag

CHLI1 Exon 2 (SEQ ID NO: 133):

ggcgctcccgtagggcgagcgcgcgtgctctgctgg

CHLI1 Exon 3 (SEQ ID NO: 134):

tgcgcgcgcgtgccgctactgaggtcaaggctgctgagggccgcactgagaaggagctgg

CHLI1 Exon 4 (SEQ ID NO: 135):

gccaggcccgccccatcttcccttcaccgccatcgtgggccaggatgagatgaagctggcgctgattctgaacg
 tgatcgaccccaagatcggtgggtgtcatgatcatgggcgacccgtggcactggcaagtccaccaccattcgtgcc
 tggcggtatctgctgcccgagatgcag

CHLI1 Exon 5 (SEQ ID NO: 136):

gtggttgccaacgacccctttaactcggacccaccgaccccgagctgatgagcagaggaggtgcgcaaccgcgtc
 aaggccggcgagcagctgcccgtgtcttccaagaagattcccatgggtggacctgcccctgggcgccactgaggac
 cgcgtgtgcggcaccatcgacatcgagaaggcgtgaccgagg

CHLI1 Exon 6 (SEQ ID NO: 137):

gtgtcaaggcgttcgagcccgccctgctggccaaggccaaccgcggcatcctgtacgtggatgaggtcaacctgc
 tggacgaccacctg

CHLI1 Exon 7 (SEQ ID NO: 138):

gtcgatgtgctgctggactcggccgcctccggctggaacaccgtggagcgcgagggatatctccatcagccacccc
 gcccgccttcacccctggtcggctcgg

CHLI1 Exon 8 (SEQ ID NO: 139):

gcaaccccgaggagggtagctgcgccccagctgctggatcgcttcggcatgcacgcccagatcggcaccgtca
 aggaccccgccctgctgtgtagatcgtgtcgagcgtcgaccttcgacgagaaccccgccgccttccg

CHLI1 Exon 9 (SEQ ID NO: 140):

caaggactacgaggccggccagatggcgctgaccagcgcatcgaggacgcgcgcaagctgctgaagcagggcga
gggtcaactacgacttccgcgtcaagatcagccagatctgctcggacctgaacgtggacggcatccgcccgcacat
cgtgaccaaccgcgcgcgaaggccctggccgcttcgagggccgcaccgag

CHLI1 Exon 10 (SEQ ID NO: 141):

gtgacccccgaggacatctaccgtgtcattccctgtgcctgcgccaccgcctccggaaagacccccctggctgag
atcgacgacggtgaccgcgtgcgtgagatcttcaagcagggtgttcggcatggagtaa

CHLI1 Intron 1 (SEQ ID NO: 142):

gtgtgcagttgcatctaaagaacgtccaattcatggttactgctcgtggatctaagcggttggctcaccagcgtt
ccatgggtccccgattcgtgcacgcag

CHLI1 Intron 2 (SEQ ID NO: 143):

gtgagaagccatgatatacaaatataaggatttgaagcggtagatctaggacccatcgaacttgagcaccgacttgc
agtcccttgccctgtccggcgactgaacttctgcgcttgctttgcag

CHLI1 Intron 3 (SEQ ID NO: 144):

gtaagtgtcgcgcaaagattttctgccgggacgggtctccctcgcaacatctgaacccatggctcggtttttttgc
cccgag

CHLI1 Intron 4 (SEQ ID NO: 145):

gtgcgcgcctcccccaacccagtttggcaaatgtgtggttaagcgtcgaaagcgtgaacagaaacaggtgttgc
ggggggcgcggaatggctgcaatgggtgctgggggcttcggagggtctgggggcgagtttgggtatacacgggcg
cgcacacttgaaggaaacgtcaaggacgacagcggaggcgtggagacagcgcggcccaagcagcctgtacttgt
agctgctgggtcagctgaggcatcacgacttgggaccagcaccggcctcacggttgcacaaggccatcacgcgc
gccaccaccacgcctcttcaaaccatgccggcacctaccgctacccctgtgacacgctccgcacacgcgcgcc
cgcacacccccaccatgtgacag

CHLI1 Intron 5 (SEQ ID NO: 146):

gtgagagcgaggcgcggggcgtgctctgcaggctaggggtgaagatcaggagagccgaagcggggccgaacagcgc
agagagaggcaagacgacacccctgccgcgttttgatcacaagattcacacccttgctctccccaacgctcccg
acatag

CHLI1 Intron 6 (SEQ ID NO: 147):

gtgagcaggggcagataggcggctcgggcggctgggcggcaggggctgtgttggtgtgttgggtgtgggctgagg
ctgggtgggtgggctggcgggtggcaggatagcggtaggggatgggtgatggggcagaatgggcgggtgggcgga
cacgtggggctcgttgaagggtgtgtggggacggcaactggatgcgatatgtcggcttggccctggcggggaaag
cattcgcagaatggcgacgaacgaggccggggagcgagcggggatgggagacgcaacctgcgctgcgaagtgcg
gcgcgcgtccagttgacacgttgacgaatgtggccagtgttcgcctgagagtatatgggttagaccgccagatg
agccgggttaagctgggtggtcgcgggttgatcggctgcttcccttcgggtgcacgcctggcaccctaacattacc
tgtccgctgctgccctttgccacag

CHLI1 Intron 7 (SEQ ID NO: 148):

gtgagtgcagctgccgctgcggctgctgatgggtgacctgtgcgaccacggggctccgcatttctggacgaagcgt
tgtaccatagccgtcttggtccctgatttgggcggctctggtccgaagccttgacatctacagttcaacatggc
cgtataacgatcctgtgccaccacacgcgcaccgccag

CHLI1 Intron 8 (SEQ ID NO: 149):

gtgagcgcgcgctctacgatacggcagacatgtacacactgccccgactgtagagcttgcaattgcatttcaagg
cctcgaaagagtaggggtggtcggttctctggtggtgtccggccacaattatgcaccccggtgttggtgcagcagct
gtgatgtcacaccttgcatcacccccctactgctgcccgcctctctctctctctcgccgcag

CHLI1 Intron 9 (SEQ ID NO: 150):

gtgagcagagcaatatgtcagaggggaaggggtggcggaaggggtgataacgggtggggatctagaggggagagatgg
atgcacacagcgcggggttggttatgcatgcctgcatggagcgctgcacgcacccctgatctgccggttttccaa
ctggcgatgccgtattatgacctgcagctcaccatcctcatgcttgatttgctcgctcag

CHLI1 Protein sequence (SEQ ID NO: 151):

MALNMRVSSSKVAAKQQGRI SAVPVVSSKVAASSARVAPFQGAPVAAQRAALLVRAAAATEVKAAEGRTEKELGQA
RPIFPFTAIVGQDEMKLALILNVIDPKIGGVMIMGDRGTGKSTTIRALADLLPEMQVVANDPFNSDPTDPELMSE
EVRNRVKAGEQLPVSSKKIPMVDLPLGATEDRVCGTIDIEKALTEGVKAFEPGLLAKANRGILYVDEVNLLDDHL
VDVLLDSAASGWNTVEREGISISHPARFILVSGSNPEEGELRPQLLDRFGMHAQIGTVKDPRLRVQIVSQRSTFD
ENPAAFRKDYEQMALTRIVDARKLLKQGEVNYDFRVKISQICSDLNVDGIRGDIVTNRAAKALAAFEGRTEV
TPEDIYRVIPLCLRHLRLKDP LAEIDDGDRVREIFKQVFGME

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

MQTSSLLGRRTAHPAAGATPKPVAPSPRVASTRQVACNVATGPRPPMTTFTGGNKGPAKQQVSLDLRDEGAGMFT
STSPERMRRVPPDDVKGRVKVKKVVVLEAQYQSAISAAVKINAKNSKVCFEVVGYLEELRDQKNLDMLKEDVA
SANIFIGSLIFIEELAEKIVEAVSPLREKLDACLI FPSMPAVMKLNKLGTF SMAQLGQSKSVFSEFIKSARKNND
NFE EGLLKLVRTLPKVLKYLPSDKAQDAKNFVNSLQYWLGGNSDNLENLLNTVSNVYPALKGVD FSVAEPTAYP
DVGIWHPLASGMYEDLKEYLNNWYDTRKDMVF AKDAPVIGLVLRSHLVTGDEGHYSGVVAELESRGAKVI PVFAG
GLDFSAPVKKFFYDPLGSGRTFVDTVVSLTG FALVGGPARQDAPKAIEALKNLNVPYLVSLPLVFQTTEEWLDSE
LGVHPVQVALQVALPELDGAMEPIVFAGRDSNTGKSHSLPDRIASLCARAVNWANLRKKRNAEKKLAVTVFSFPP
DKGNVGTAAAYLNVFGSIYRVLKNLQREGYDVGALSALGGGSDPVGADPEGGQVQLDRPAHRLQDEGGRVPEAVPL
RRGAGGELGQAPRHPHQRP GAAGVRPPVRQRLHRRAAHLRLRGRPDAPAVLEVGQPPPRRLRRLHLPGEDLQGR
RRAALRHPRLAGVHARQAGRHVGCVL PRLADRHHPQPLLLRRQQPV

CHLI1 DNA sequence (SEQ ID NO: 153):

atggccctgaacatgcgtgtttctcttccaaggtcgctgccaaagcagcagggccgcacatctccgcggtgccggtt
gtgtcgagcaaggtggcctcctccgcccgcgtggccccctccagggcgctcccggtggccgcgcagcgcgtgct
ctgctggtgcgcgcgcgtgccgctactgaggtcaaggctgctgagggccgcactgagaaggagctgggcccaggcc
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gtcgatgtgctgctggactcggccgcctccggctggaacaccgtggagcgcgaggggtatctccatcagccacccc
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gacgacgggtgaccgcgtgcgtgagatcttcaagcaggtgttcggcatggagtaa

[0134] Although the invention has been described with reference to the above example, 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. An engineered algae having a genetic modification, where the genetic modification results in an accumulation of heme in the algae as compared to an algae lacking the genetic modification.
2. The engineered algae of claim 1, wherein the engineered algae has reduced or absence of chlorophyll production.
3. The engineered algae of claim 1 or claim 2, wherein the algae has red or red-like color.
4. The engineered algae according to any of claims 1-3, wherein the algae is capable of growth on glucose as the sole carbon source.
5. The engineered algae according to any of claims 1-4, wherein the genetic modification comprises a genetic alteration to a chlorophyll synthesis pathway, protoporphyrinogen IX synthesis pathway or heme synthesis pathway.
6. The engineered algae according to any of claims 1-5, wherein the genetic modification is associated with a deficiency in the expression of magnesium chelatase.
7. The engineered algae according to any of claims 1-6, wherein the genetic modification comprises an alteration in one or more of CHLD, CHLI1, CHLI2 or CHLH1.
8. The engineered algae of claim 7, wherein the genetic modification comprises an alteration in an upstream regulatory region, a downstream regulatory region, an exon, an intron or any combination thereof.
9. The engineered algae according to any of claims 5-8, wherein the genetic modification comprises an insertion, a deletion, a point mutation, an inversion, a duplication, a frameshift or any combination thereof.
10. The engineered algae according to any of claims 1-9, wherein the engineered algae has a heme content greater than chlorophyll content.

11. The engineered algae according to any of claims 1-9, wherein the engineered algae has a protoporphyrin IX content greater than chlorophyll content.
12. The engineered algae according to any of claims 1-11, wherein the engineered algae has reduced production of one or more fatty acids.
13. The engineered algae according to any of claims 1-12, wherein the engineered algae further comprises a genetic modification that reduces or eliminates expression of light independent protochlorophyllide oxidoreductase.
14. The engineered algae of claim 13, wherein the genetic modification comprises a mutation or deletion in one or more of ChlB, ChlL or ChlN.
15. The engineered algae according to any of claims 1-14, wherein the engineered algae has upregulated expression of ferrochelatase.
16. The engineered algae according to any of claims 1-15, wherein the engineered algae has upregulated expression of protoporphyrinogen IX oxidase.
17. The engineered algae according to any of claims 1-16, wherein the engineered algae contains a recombinant or heterologous nucleic acid.
18. The engineered algae according to any of claims 1-17, wherein the engineered algae is a *Chlamydomonas* sp.
19. The engineered algae of claim 18, wherein the *Chlamydomonas* sp. is *Chlamydomonas reinhardtii*.
20. An edible composition comprising an algae preparation, wherein the algae preparation comprises an engineered algae of any of claims 1-19 or a portion thereof.
21. The edible composition of claim 20, wherein the edible composition comprises heme derived from the engineered algae.
22. The edible composition of claim 20, wherein the algae preparation comprises algae cells.
23. The edible composition of claim 20, wherein the algae preparation is a fractionated algae preparation.

24. The edible composition according to any of claims 20-23, wherein the algae preparation is red or red-like in color.
25. The edible composition according to any of claims 20-24, wherein the edible composition has a red or red-like color derived from the algae preparation.
26. The edible composition according to any of claims 20-25, wherein the algae preparation confers a meat or meat-like flavor to the edible composition.
27. The edible composition according to any of claims 20-26, wherein the edible composition has a meat or meat-like texture derived from the algae preparation.
28. The edible composition according to claim 27, wherein the meat or meat-like texture is a beef or beef-like texture, a fish or fish-like texture, a chicken or chicken-like texture, a pork or pork-like texture or a texture of a meat replica.
29. The edible composition according to any of claims 20-28, wherein the edible composition is 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.
30. The edible composition according to any of claims 20-29, wherein the edible composition is vegan, vegetarian or gluten-free.
31. The edible composition according to any of claims 20-30, wherein the edible composition has an appearance of blood derived from the algae preparation.
32. The edible composition according to any of claims 20-31, wherein the algae preparation has a heme content greater than chlorophyll content.
33. The edible composition according to any of claims 20-32, wherein the algae preparation has a protoporphyrin IX content greater than chlorophyll content.
34. The edible composition according to any of claims 20-33, wherein the algae 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.

35. The edible composition according to any of claims 20-34, wherein the algae preparation provides vitamin A, beta carotene or a combination thereof to the composition.
36. The edible composition of claim 35, 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.
37. The edible composition according to any of claims 20-36, wherein the algae 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 edible composition.
38. The edible composition according to any of claims 20-37, wherein the algae 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 a finished product comprising the edible composition.
39. The edible composition according to any of claims 20-38, wherein the algae 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 edible composition.
40. The edible composition according to any of claims 20-39, wherein the algae preparation has reduced fatty acid content.
41. The edible composition according to any of claims 20-40, wherein the edible product is combined with a protein source, a fat source, a carbohydrate, a starch, a thickener, a vitamin, a mineral, or any combination thereof.
42. The edible composition of claim 41, wherein the protein source is selected from the group consisting of textured wheat protein, textured soy protein and textured pea protein, fungal protein or algal protein.

43. The edible composition of claim 41, wherein the fat source comprises at least one of refined coconut oil or sunflower oil.
44. The edible composition of any of claims 41-43, further comprising at least one of potato starch, methylcellulose, water, and a flavor, wherein the flavor is selected from the group consisting of yeast extract, garlic powder, onion powder, salt, and any combination thereof.
45. The edible composition of any of claims 41-44, wherein the edible product is an ingredient for a burger, a sausage, a kebab, a filet, a fish-alternative, a ground meat-like product or a meatball.
46. The edible composition of claim 45, wherein the burger comprises about 5% of the algae preparation, about 20% textured soy protein and about 20% refined coconut oil.
47. The edible composition of claim 46, further comprising about 3% sunflower oil, about 2% potato starch, about 1% methylcellulose, about 45% water and about 4-9% flavors.
48. The edible composition of claim 46, further comprising about 0.5% Kojac gum, about 0.5% Xanthan gum, about 45% water and about 4-9% flavors.
49. The edible composition of claim 45, wherein the fish-alternative comprises 20% textured soy protein, about 5 % of algae preparation, about 65% water and about 10% flavors.
50. The edible composition according to any of claims 20-49, wherein the edible composition is free of animal proteins.
51. The edible composition according to any of claims 20-50, wherein the algae preparation comprises an algae having an increase in protoporphyrinogen IX synthesis or accumulation.
52. The edible composition according to any of claims 20-51, wherein the algae preparation comprises an algae that exhibits a red or red-like color when grown in the dark conditions.

53. The edible composition according to any of claims 20-52, wherein the algae in the algae preparation are recombinant or genetically modified algae.
54. The edible composition according to any of claims 20-53, wherein the algae preparation comprises a *Chlamydomonas* sp.
55. The edible composition of claim 54, wherein the *Chlamydomonas* sp. is *Chlamydomonas reinhardtii*.
56. A method for the production of an edible composition comprising:
- (a) culturing an engineered algae according to any of claims 1-19 in a condition where the engineered algae exhibits a red or red-like color and wherein the engineered algae produces heme;
 - (b) collecting the cultured engineered algae to produce an algae preparation; and
 - (c) combining the algae preparation with at least one edible ingredient to produce an edible composition.
57. The method of claim 56, wherein the condition comprises a fermentation condition.
58. The method according to any of claims 56-57, wherein the condition comprises acetate as a reduced carbon source for growth of the engineered algae.
59. The method according to any of claims 56-58, wherein the condition comprises sugar as a reduced carbon source for growth of the engineered algae.
60. The method according to any of claims 56-59, wherein the condition comprises dark or limited light conditions.
61. The method according to any of claims 56-60, wherein the method further comprises fractionating the cultured algae to produce the algae preparation.
62. The method according to any of claims 56-61, wherein the algae preparation has a heme content that is greater than chlorophyll content.
63. The method according to any of claims 56-62, wherein the algae preparation has a protoporphyrin IX content that is greater than chlorophyll content.

64. The method according to any of claims 56-63, wherein the condition further comprises iron supplements.
65. The method according to any of claims 56-64, wherein the engineered algae is a *Chlamydomonas* sp.
66. The method of claim 65, wherein the engineered algae is a *Chlamydomonas reinhardtii*.
67. The method according to any of claims 56-66, wherein the edible composition has at least one feature selected from the group consisting of a meat or meat-like flavor, a meat or meat-like texture, a blood-like appearance and a meat or meat-like color, wherein the at least one feature is derived from the algae preparation.
68. The method according to any of claims 56-67, wherein the method further comprises producing a finished product comprising the edible composition and wherein the finished product is a beef-like food product, a fish-like product, a chicken-like product, a pork-like product or a meat replica.
69. The method according to any of claims 56-68, wherein the edible composition is free of animal proteins.
70. The method according to any of claims 56-69, wherein the algae preparation is fractionated to remove one or more of starch, protein, PPIX, fatty acids and chlorophyll.
71. A method of making an engineered algae enriched in heme content, comprising:
(a) subjecting an algae strain to a process that produces genetic modification to create a first algae population; and
(b) from the first algae population, selecting a second algae population that is enriched in heme content, and optionally, PPIX content.
72. The method according to claim 71, wherein the process comprises at least one of a random UV mutagenesis, a random chemical mutagenesis, a recombinant genetic engineering, a gene editing, or a gene silencing.

73. The method according to claim 71 or claim 72, further comprising culturing the first algae population in a fermentation condition.
74. The method according to claim 73, wherein the fermentation condition comprises a media having sugar as a sole carbon source.
75. The method according to claim 74, wherein the sugar is selected from the group consisting of glucose, dextrose, fructose, maltose, galactose, sucrose, and ribose.
76. The method according to any of claims 73-75, wherein the fermentation condition comprises a brightness of less than 500 lux.
77. The method of any of claims 73-76, wherein the step of selecting the second algae population comprises sorting or identifying algae cells having a red or red-like color.
78. The method of any of claims 73-77, wherein the selecting is performed by FACS.
79. The method according to any of claims 73-78, wherein the second algae population is selected with its capability to grow in the fermentation condition.
80. The edible composition according to any of claims 20-59, wherein the algae preparation comprises an algae having an increase in protoporphyrinogen IX synthesis or accumulation.
81. The edible composition according to any of claims 20-59, wherein the algae preparation comprises an algae that exhibits a red or red-like color when grown in dark conditions.

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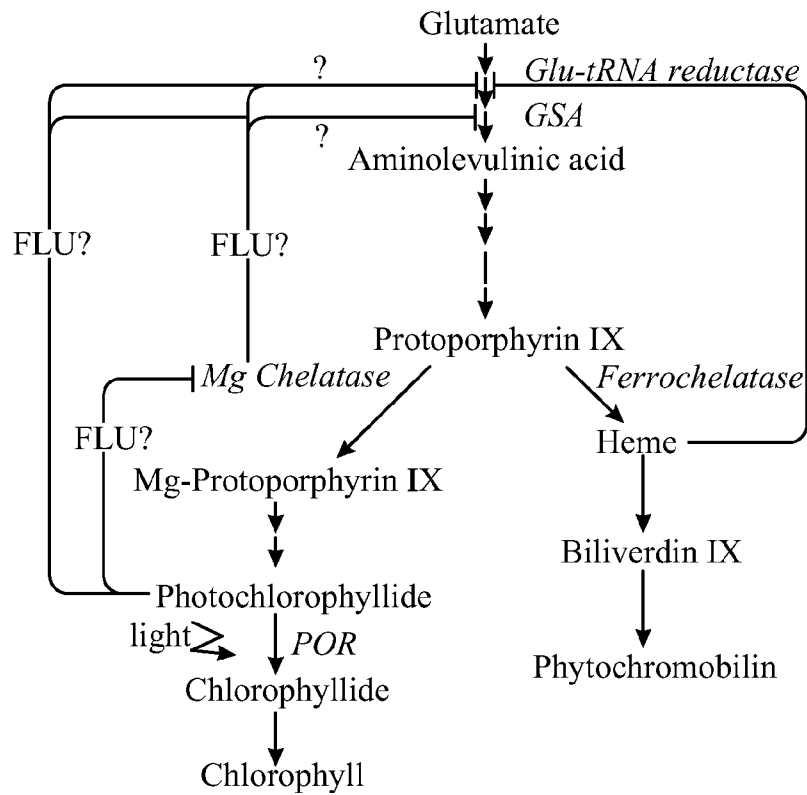
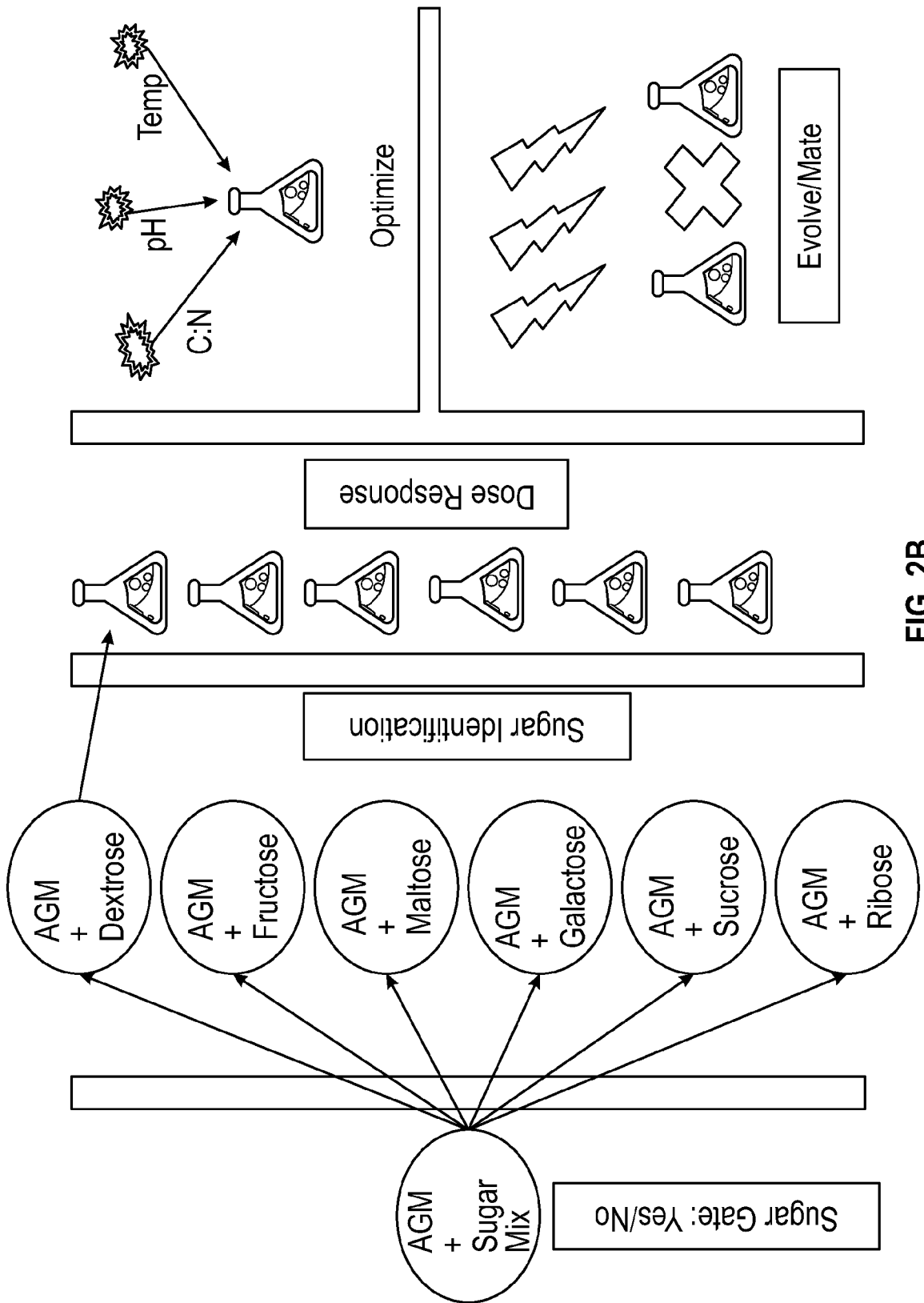


FIG. 1

Algae Growth Media (AGM)		
	Substance	g/L
1	KH ₂ PO ₄	0.175
2	CaCl ₂ *2H ₂ O	.025
3	MgSO ₄ *7H ₂ O	.075
4	NaNO ₃	2.00
5	K ₂ HPO ₄	.075
6	NaCl	.025
7	NaEDTA*2H ₂ O	.01
8	KOH	.0062
9	FeSO ₄ *7H ₂ O	.00498
10	H ₂ SO ₄	1.84
11	H ₃ BO ₃	.00805
12	F/2 Vitamin Solution	1mL
13	Trace Elements	1mL

FIG. 2A

SUBSTITUTE SHEET (RULE 26)



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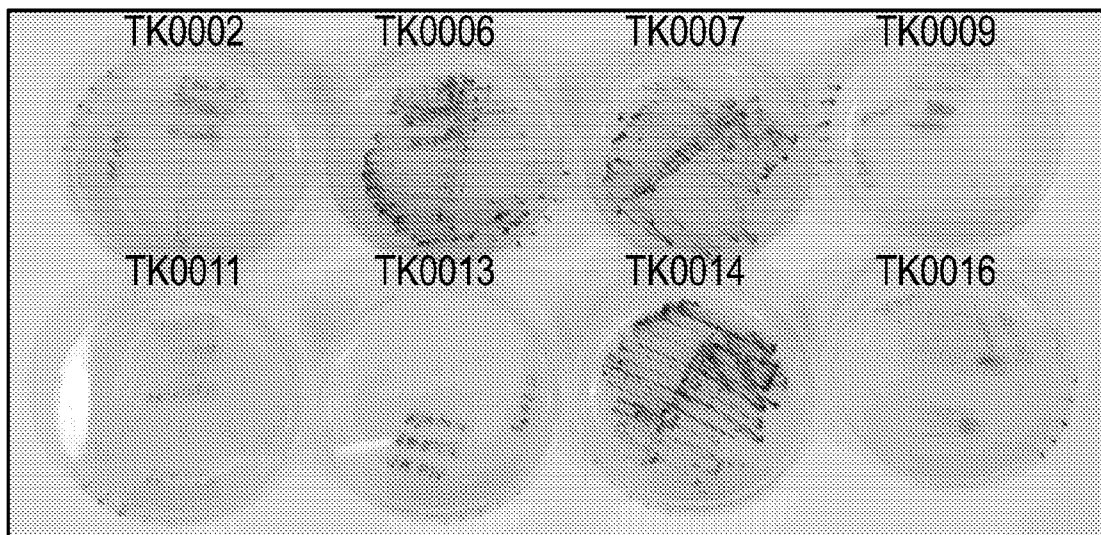


FIG. 3

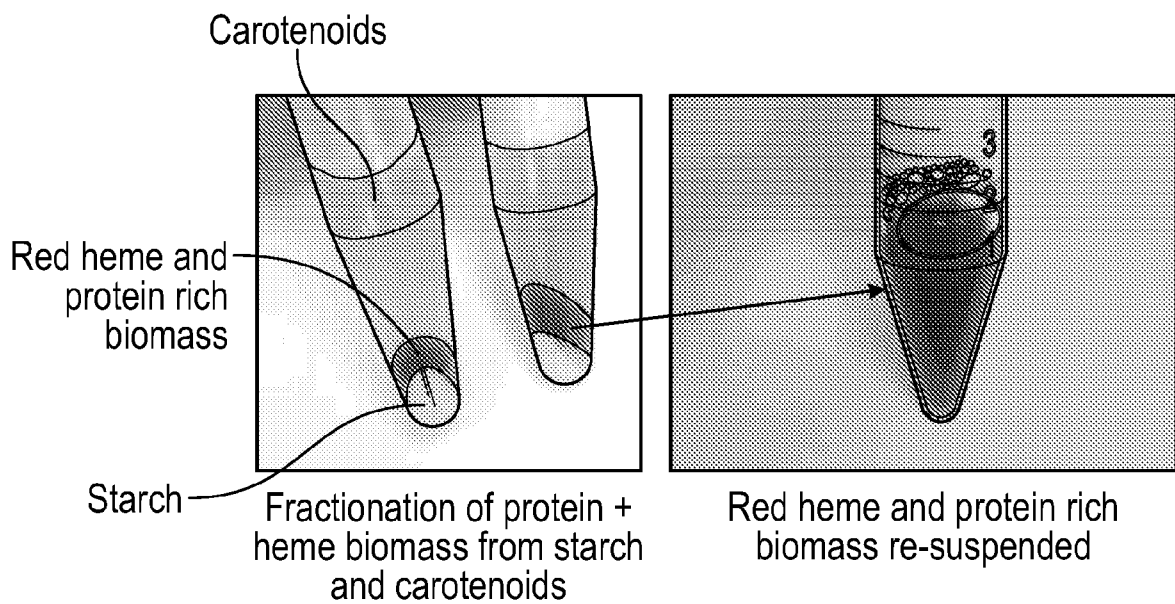


FIG. 4

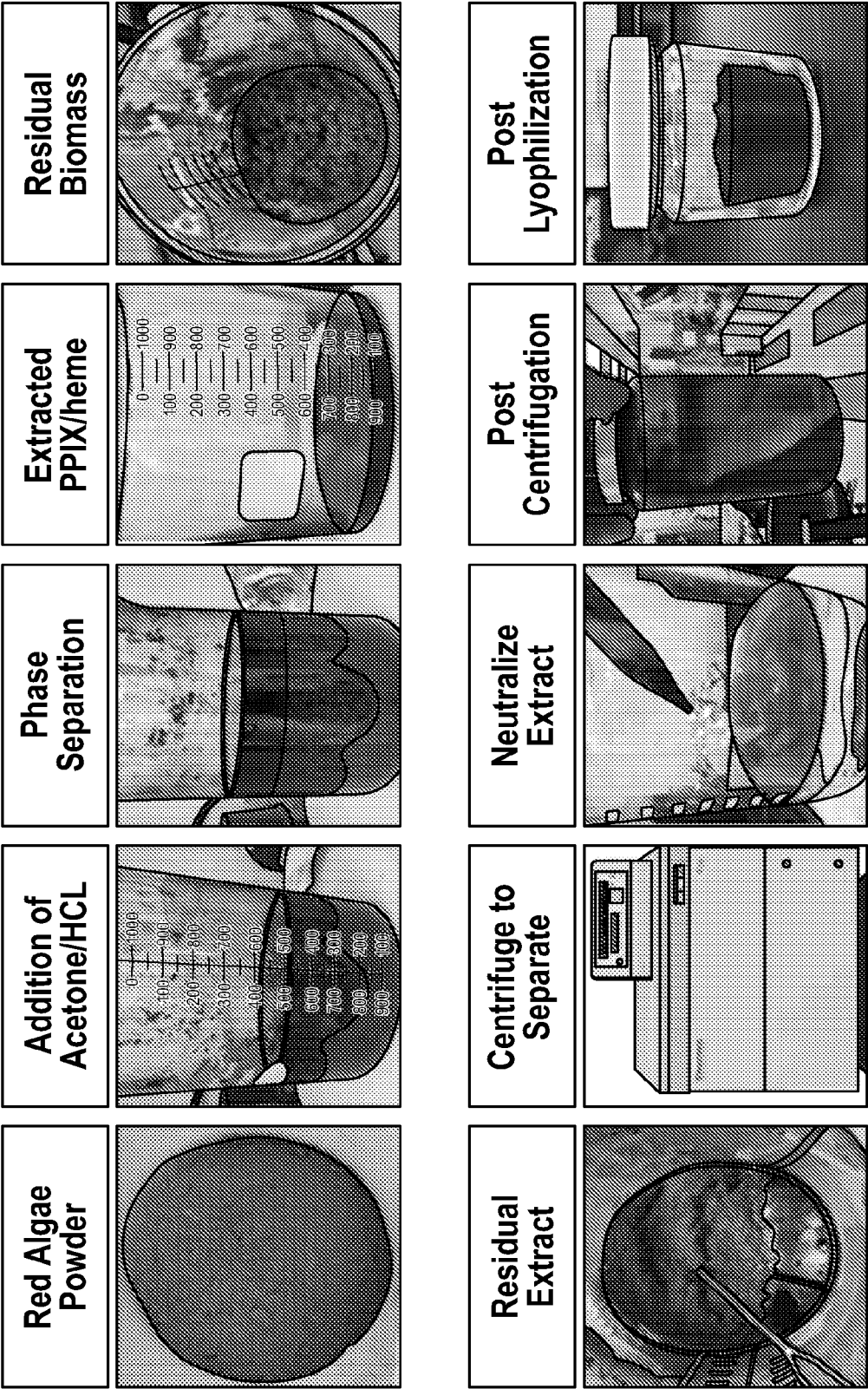


FIG. 5

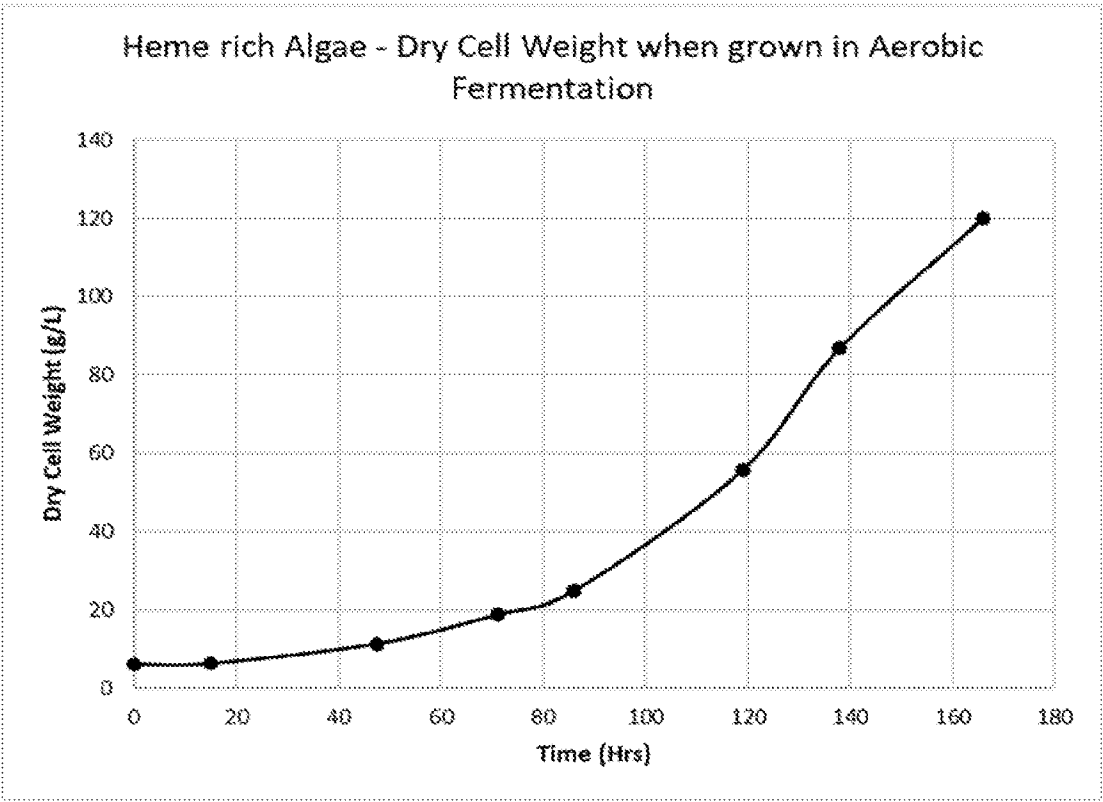


FIG. 6

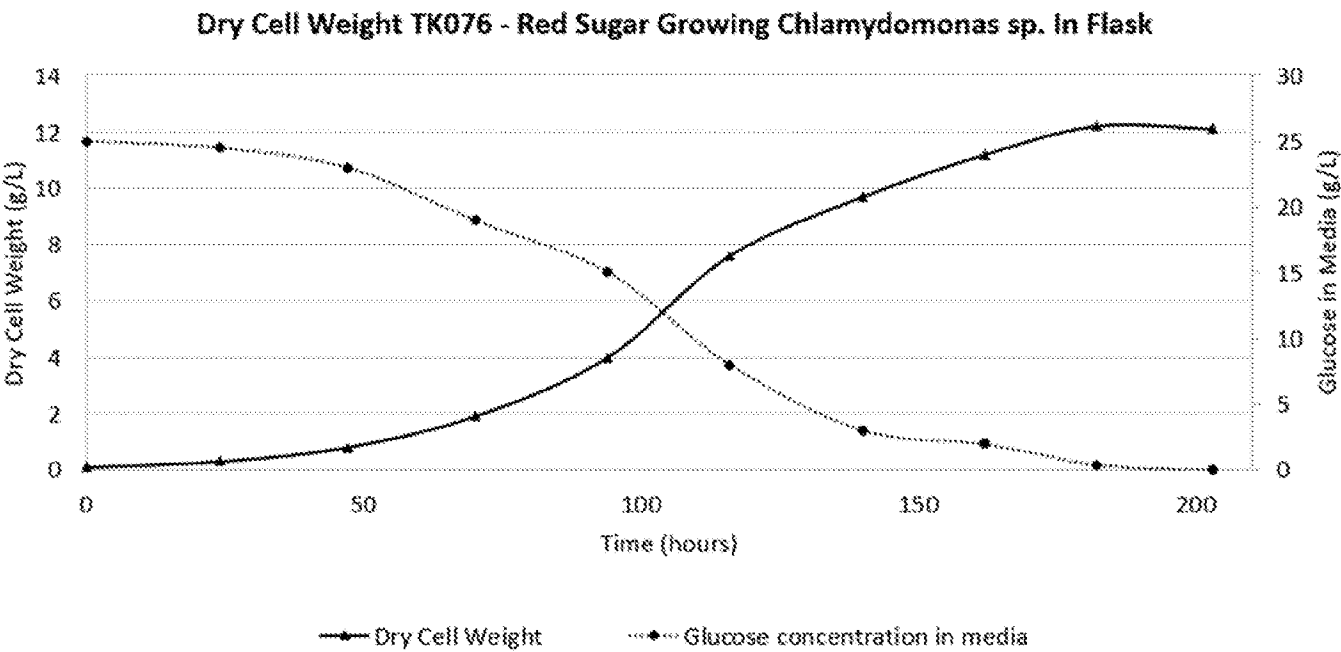


FIG. 7

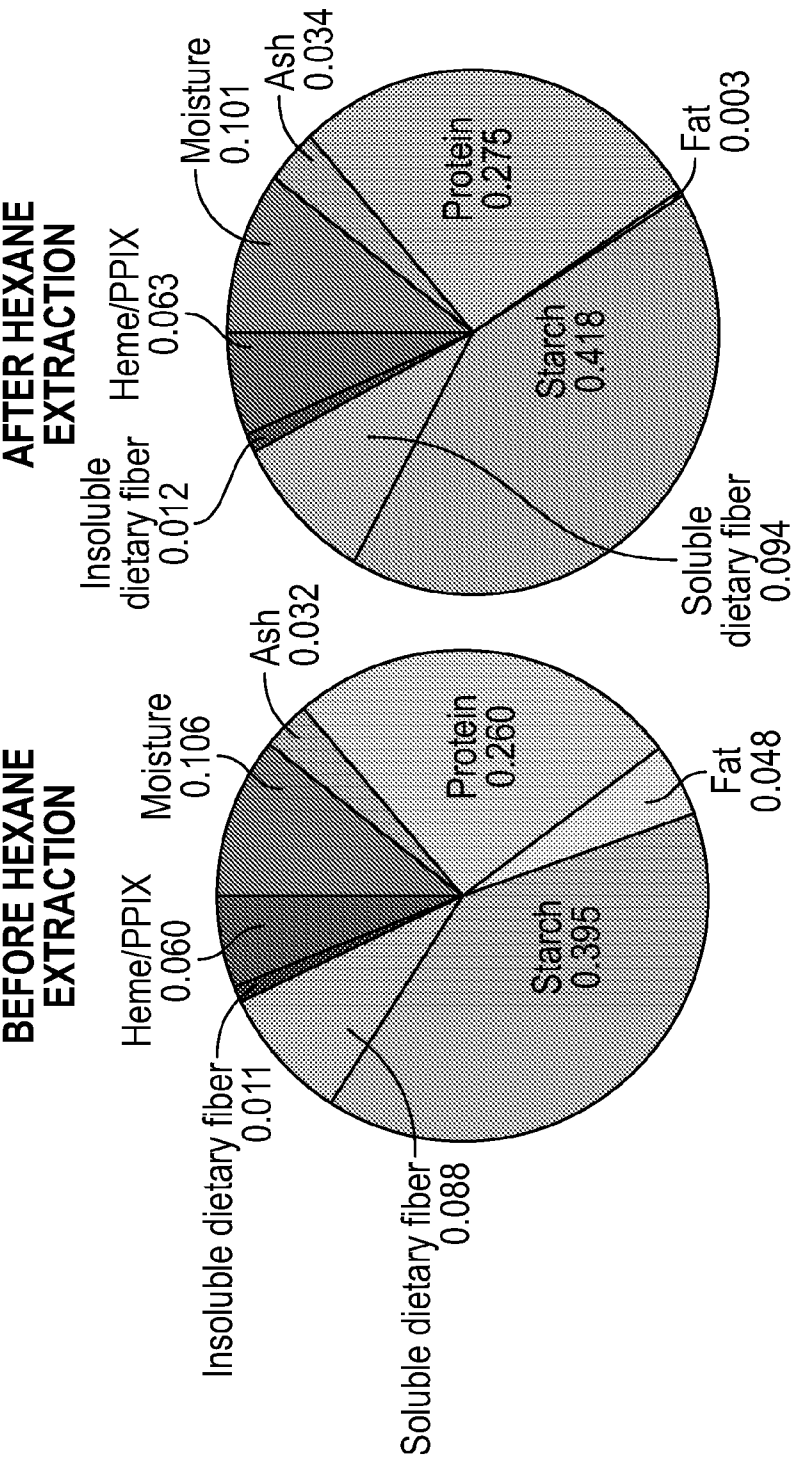


FIG. 8

Seq_1 1621 S I Y R V L K N L Q R E G Y D V G A L P 1679
TCCATCTACCGGTGCTGAAGAACCCTGCAGCGCGAGGGCTACGACGTGGGCGCCCTG-CC
|||||
Seq_2 1621 TCCATCTACCGGTGCTGAAGAACCCTGCAGCGCGAGGGCTACGACGTGGGCGCCCTGTCC 1680
S I Y R V L K N L Q R E G Y D V G A L S

FIG. 9

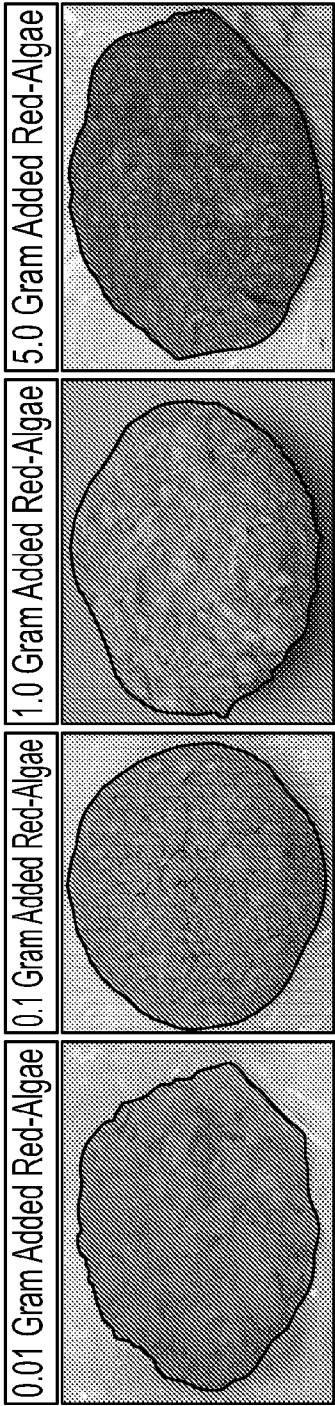


FIG. 10

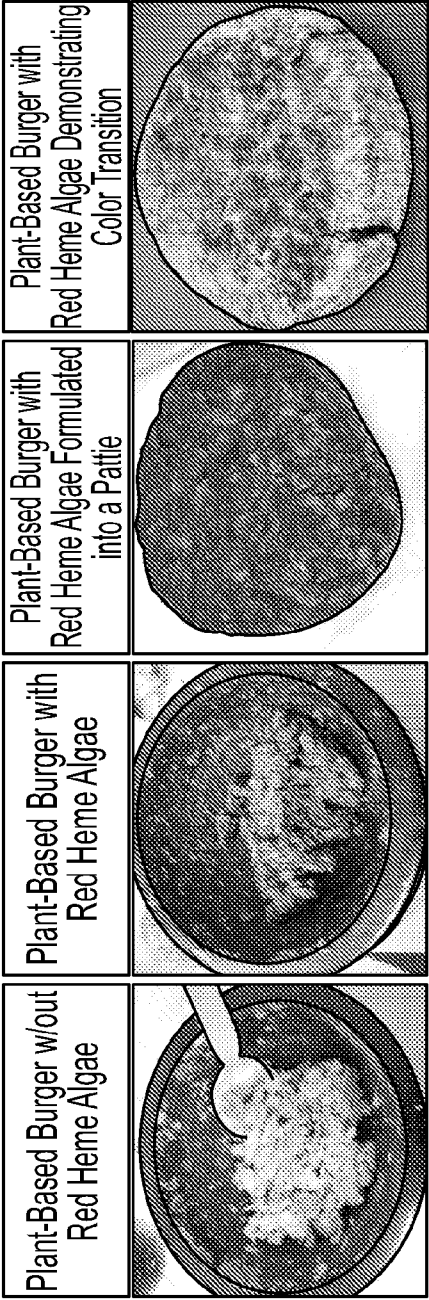


FIG. 11

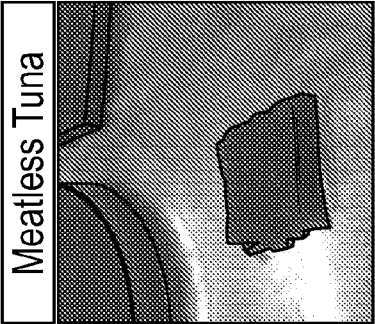


FIG. 12