Parachlorella strain GE-15699. The product of primers 596 and 597 is the wild type (unmodified) locus; the products of primers 405/597 and 406/597 result from targeted insertion of the bleR cassette.

[0040] **Figure 13A)** is a diagram of a donor DNA construct for promoter boosting; **B)** shows insertion sites of the donor DNA in the ACCase locus upstream of the coding region.

[0041] **Figure 14 A)** provides a schematic map of the ZnCys-2845 gene locus in Nannochloropsis with arrows depicting target sites for cas9 mediated insertion of a HygR cassette. Only locus 1 failed to result in a targeted insertion. **B)** provides the level of ZnCys-2845 gene knockdown for the various targeted insertion mutants.

[0042] **Figure 15** is a schematic diagram of the vector than included an RNAi construct for attenuation expression of the ZnCys-2845 gene. The vector included a blast gene for selection and a GFP gene for assessing penetrance of the genes of the inserted RNAi construct.

[0043] **Figure 16** provides a diagram of the 22.3 kb Donor DNA that included 6 genes, each with a separate promoter.

[0044] **Figure 17** provides photographs of PCR products diagnostic for the presence of the 22.3 kb integration fragment targeted to the acyl-CoA oxidase locus, with clones 5,6,7,8,9,20,27,38, & 31 having directed integration events.

[0045] **Figure 18** is a schematic diagram of vector pSGE-6483 that includes, in addition to a Cas9 gene, GFP gene, and HygR gene, a cre recombinase gene optimized for expression in Nannochloropsis. Each of the Cas9, GFP, HygR, and cre genes was operably linked to a separate Nannochloropsis promoter. The cre recombinase gene was operably linked to the ammonia-repressible Nitrite/Sulfite Reductase promoter.

[0046] **Figures 19A-D** show the results of flow cytometry penetrance screens of cells transformed with pSGE-6483 and the difference in peak fluorescence intensity of cells grown in ammonia versus nitrate.

[0047] **Figure 20** is photographs of gels of RT/PCR products assessing levels of the GFP transcript under different nitrogen conditions.

[0048] **Figure 21** provides a graphical representation of levels of the Cre transcript under different nitrogen conditions in transformants A11, B11, C12, D12, E12, and F12.

[0049] **Figure 22** provides a Western blot for detection of the Cre protein under different nitrogen conditions for transformants A11, B11, C12, D12, E12, and F12.

[0050] **Figure 22** provides a Western blot for detection of the Cas9 protein under different nitrogen conditions for transformants A11, B11, C12, D12, E12, and F12.

[0051] **Figure 24** is photographs of gels of PCR products of F12 and C12 cultures to determine whether the floxed GFP and BlastR gene cassettes were intact or excised by Cre-mediated recombination.

[0052] **Figure 25** is photographs of a gel of products of PCR to demonstrate in vivo excision of floxed GFP and BlastR gene cassettes.

[0053] **Figure 26** is photographs of gels of PCR products of F12 and C12 cultures to determine whether the floxed GFP and BlastR gene cassettes were intact or excised by Cre-mediated recombination.

[0054] **Figure 27** is a schematic diagram of the floxed GFP and BlastR gene cassettes.

[0055] **Figure 28** is a diagram showing cas9 mediated insertion of a floxed disruption cassette which following confirmation of the insertion, is induced for cre expression resulting in excision of the reporter (fluorescent protein) and selectable marker, allowing for recycling of these components in further engineering steps.

[0056] **Figure 29** provides a diagram of a construct for introduction into an algal cell that encodes a Type I FAS derived from an animal species. The construct also includes a gene encoding a pantetheine phosphotransferase (PPT). The genes are operably linked to algal promoters. The construct further includes a gene encoding a fluorescent protein for assessing culture-wide expression of the exogenous FAS and PPT genes.

[0057] **Figure 30** provides a diagram of a construct for introduction into an algal cell that encodes a Type I FAS derived from a labyrinthulomycete species. The gene is operably linked to an algal promoter. The construct further includes a gene encoding a fluorescent protein for assessing culture-wide expression of the exogenous FAS gene.

[0058] **Figures 31A-B** provide flow cytometry traces (histograms) in which the flow cytometry profile of a *Nannochloropsis* tranformant that includes a *Danio rerio* Type I FAS gene is overlaid with the flow cytometry profile of a wild type (non-transformed) algal cell culture. The figure also provides Western blots showing levels of FAS protein expression in the profiled transformed lines. Line 6201-38 (rightmost flow cytometry trace in **Figure 31B**) shows no difference in its fluorescence profile relative to non-transformed cells and shows no detectable FAS protein in the Western blot (third lane from the right). Other transformed lines show fully penetrant expression with fluorescence peaks that are distinct from the wild type peak. These strains also have detectable FAS protein as evidenced by the Western blots. WE3730 is the wild type strain which does not include a Type 1 FAS protein.

[0059] **Figures 32A-B** provide flow cytometry traces (histograms) of *Nannochloropsis* transformants in which the flow cytometry profile of a tranformant that includes a *Danio*

rerio Type I FAS gene is overlaid with the flow cytometry profile of a wild type (non-transformed) algal cell culture. The figure also provides a Western blot comparing levels of FAS protein expression in the profiled transformed lines. WE3730 is the wild type strain which does not include a Type 1 FAS protein.

[0060] **Figure 33** provides flow cytometry traces (histograms) of *Nannochloropsis* transformants in which the flow cytometry profile of a tranformant that includes a labyrinthulomycete Type I FAS gene is overlaid with the flow cytometry profile of a wild type (non-transformed) algal cell culture. The figure also provides a Western blot comparing levels of FAS protein expression in the profiled transformed lines. WE3730 is the wild type strain which does not include a Type 1 FAS protein.

[0061] **Figure 34** provides a graph of FAS activity as assayed from cell extracts of transformants. Algal transformants having the labyrinthulomycete Type I FAS gene have the highest activity.

[0062] **Figures 35A-B** provide a graph of *in vivo* FAS rate determination using isotope tracer (^{13}C) incorporation for ChytFAS transgenic lines grown under phototrophic conditions (A) and mixotrophic conditions (B). ChytFAS strain 6167-B outperformed the wild type strain under photoautotrophic conditions (A). Strain 6167-A was also tested under mixotrophic conditions, where it outperformed wild type in FAME production (B).

[0063] **Figure 36** provides a graph of *in vivo* FAS rate determination using isotope tracer incorporation for DrFAS over-expression strains grown under photoautotrophic (A, labeled with ^{13}C bicarbonate) and acetate-boosted mixotrophic (B, labeled with ^{13}C acetate) conditions. All DrFAS transformants were able to outperform the wild type strain under mixotrophic conditions.

DETAILED DESCRIPTION

[0064] Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. Many of the techniques and procedures described or referenced herein are well understood and commonly employed using conventional methodology by those skilled in the art.

[0065] The singular form "a", "an", and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes one or more cells,

CRE expression cassette containing the Cre recombinase from P1 Bacteriophage codon optimized for *Nannochloropsis gaditana*, which contains the same N-terminal NLS used for the Cas9 construct and also includes an *N. gaditana* intron inserted into the Cre coding region (engineered Cre gene provided as (SEQ ID NO:111). The *Nannochloropsis*-engineered Cre gene was operably linked to the "Ammonia repressible Nitrite/Sulfite Reductase" promoter (SEQ ID NO:112) at the 5' end of the Cre gene and the "Nitrite/Sulfite Reductase" terminator (SEQ ID NO:113) at the 3' end of the Cre gene. The BlastR selectable marker and GFP reporter cassettes are arranged in tandem in the construct, and together they are flanked by identical lox sites in the same orientation. Features that are flanked by loxP sites are commonly referred to as "floxed". An ammonia-repressible promoter was to repress expression of the Cre gene as much as possible until after generating antibiotic resistant colonies and establishing full phenotypic penetrance of GFP. Additionally, cloning Cre into a vector that contains lox sites proved to be problematic, as even basal levels of Cre expression in *E. coli* looped out the floxed BlastR and GFP once Cre was cloned in. To get around this hurdle, an intron was inserted into the Cre gene disrupting the catalytic and nucleophilic domains. This resulted in the final stable vector pSGE-6483 (**Figure 18**) which doesn't self-excise its floxed markers in *E.coli.*

[00255] pSGE-6483 was transformed into *Nannochloropsis gaditana* and plated onto PM128 agar media that contains ammonia but not nitrate, where the medium contained 100 mg/L of blasticidin. Colonies were re-patched onto the same selective PM128 media for analysis and archiving, and screened for full phenotypic penetrance of GFP by flow cytometry as described in Example 3. Six lines were carried forward for parallel serial culturing in either media containing ammonium as the sole nitrogen source (PM128) or media containing sodium nitrate as the sole nitrogen source (PM129), with no blasticidin selection in either medium. After 2 weeks of serial culturing, the strains were examined for loss of GFP signal by flow cytometry, excision of the floxed GFP/BlastR cassettes by diagnostic PCR, Cre expression by Western Blot and qRT-PCR, and Cas9 expression by Western Blot.

[00256] GFP histograms revealed mixed results for the different strains. Strain 6483-F12 was the only strain which showed an obvious GFP signal switch between NH_4 and NO_3 cultures (**Figure 19D**). Strains B11 and C12 appeared to have lost GFP signal in both NH_4 and NO_3 (**Figure 19B**), while strains A11, D12, and E12 appeared to have maintained GFP signal in both NH_4 and NO_3 (**Figures 19A and C**).

chytrid FAS for the ChytFAS transformants. **Figures 31A** and **31B** show the flow cytometry traces of 6 DrFAS transformants that were found to have complete penetrance, as the transformants displayed a single fluorescence peak that was shifted with respect to the wild type fluorescence peak. In **Figures 31A** and **31B**, Western blots are shown in which it can be seen that each fully penetrant clone also demonstrated protein expression. Unlabeled lanes on the gel show protein reactivity of clones that were not determined to be fully penetrant (i.e., they displayed more than one peak, one of which coincided with wild-type, or background, fluorescence, or they displayed a single peak that was coincident with the wild type or background peak). Thus, screening for protein level alone does not result in the identification of fully penetrant lines (expression throughout the culture). **Figures 32A** and **32B** provide the flow cytometry traces of 6 DrFAS lines that demonstrated complete penetrance and the Western blots of these lines with anti-animal FAS antibody. Interestingly, for these fully penetrant lines, protein level as assessed by Western signal intensity does correspond to the degree of separation of the transformant peak from the background (wild type) peak; for example, strains 6200-33 and 6200-37 have the most intense Western bands and the greatest separation of their flow cytometry fluorescence peaks from the wild type fluorescence peak, demonstrating that the of GFP gene expression is reflected in the degree of expression of the linked gene.

[00269] Two lines having fully penetrant ChytFAS expression were also assessed by Western for FAS protein expression (**Figure 33**). Although 6167-B had a GFP fluorescence peak shifted farther to the right (at a higher fluorescence value) than the 6167-A GFP fluorescence peak was shifted (Figure 37A), this difference was not reflected in the protein abundance as detected by Western blot. Interestingly though, strain 6167 demonstrated higher FAS activity in assays than did strain 6167A, as described below.

[00270] To analyze FAS activity in selected transformants, cell extracts of lines 6167-A and 6167-B expressing Chytrid FAS, and strains 6200-33, 6200-38, 6200-43, 6201-43, and 6201-48 expressing DrFAS, all selected as demonstrating complete penetrance (Figure 33 and Figure 34), were assayed. Malonyl-CoA dependent NADPH oxidation measured at ABS 340 nm was determined on clarified, desalted extracts in triplicate. Aliquots of cell cultures were pelleted and the pellets (approximately 200-400 µl packed volume) were resuspended in 2 ml of ice cold extraction buffer (50 mM HEPES pH 7.0 (or Tris pH 8.0), 100 mM KCl, 2 mM DTT (from fresh 1 M stock), 1 protease inhibitor cocktail from Roche at right concentration (*e.g.* 1 tablet for 10 ml). A similarly sized yeast pellet was treated the same way as a positive control extract.