Addendum Addendum to: Radakovits R, Jinkerson RE, Fuerstenberg SI, Tae H, Settlage RE, Boore JL, et al. Draft genome sequence and genetic transformation of the oleaginous alga Nannochloropsis gaditana. Nat Commun 2012; 3:686; PMID:22353717; http://dx.doi.org/10.1038/ncomms1688

Keywords: Nannochloropsis, algae, biofuels, genomics, lipids
Submitted: 07/12/12
Accepted: 08/18/12
http://dx.doi.org/10.4161/bioe.21880
*Correspondence to: Matthew C. Posewitz; Email: mposewit@mines.edu

Nannochloropsis species have emerged as leading phototrophic microorganisms for the production of biofuels. Several isolates produce large quantities of triacylglycerols, grow rapidly, and can be cultivated at industrial scales. Recently, the mitochondrial, plastid and nuclear genomes of Nannochloropsis gaditana were sequenced. Genomic interrogation revealed several key features that likely facilitate the oleaginous phenotype observed in Nannochloropsis, including an over-representation of genes involved in lipid biosynthesis. Here we present additional analyses on gene orientation, vitamin B12 requiring enzymes, the acetyl-CoA metabolic node, and codon usage in N. gaditana. Nuclear genome transformation methods are established with exogenous DNA integration occurring via either random incorporation or by homologous recombination, making Nannochloropsis amenable to both forward and reverse genetic engineering. Completion of a draft genomic sequence, establishment of transformation techniques, and robust outdoor growth properties have positioned Nannochloropsis as a new model alga with significant potential for further development into an integrated photons-to-fuel production platform.

Microalgae are among the most promising renewable feedstocks for the production of fuels and chemicals. They produce a variety of bioenergy carriers, including lipids and carbohydrates; however, biological productivities need to be improved before algae are leveraged in economically viable biofuel production processes. A promising strategy for increasing productivities is to “domesticate” algal strains via genetic engineering to improve lipid yields and to produce biomolecules tailored for biofuel applications. Currently, only a few algal systems with publicly available genomes are genetically tractable, including the green algae Chlamydomonas reinhardtii and Ostreococcus tauri, as well as the diatoms Phaeodactylum tricornutum and Thalassiosira pseudonana. These organisms were selected as model systems because of their phylogenetic or ecological significance and/or their ease of culturing in the laboratory. Unfortunately, these organisms do not natively exhibit the high lipid or biomass productivities required from a biofuel feedstock and would likely require extensive genetic modification to increase their bioenergy carrier yields to the necessary levels.

As an alternative to these model algal systems, we took the approach of studying an algal strain that natively produces large quantities of lipids and is cultivated at commercial scale outdoors. After evaluating a variety of organisms, Nannochloropsis gaditana CCMP526 emerged as a leading candidate that produces large quantities of lipid and sustains high biomass accumulation rates. Species of Nannochloropsis are successfully cultivated at industrial scale by several commercial interests including Aurora Algae, Solix Biofuels and Scambiotic. To begin to develop Nannochloropsis as a genetically tractable, industrially relevant alga, we
sequenced the *N. gaditana* genome and transcriptome, and developed a robust transformation protocol using endogenous promoters. These results were recently published in Radakovits et al.\(^8\) Importantly, homologous recombination was reported for *Nannochloropsis* sp. W2JB3.\(^3\) Further establishing *Nannochloropsis* as among the most promising oleaginous algae for metabolic engineering, and the recent genetic advances will enable *Nannochloropsis* to be developed into a model system for optimizing algal lipid accumulation.

**Nannochloropsis for Biofuel Production**

There are six recognized species in the *Nannochloropsis* genus that are phylogenetically divided into two clades; one consisting of *N. gaditana* and *N. salina*, and the second of *N. grana- lata*, *N. limnetica*, *N. oceanica*, and *N. oculata*. *Nannochloropsis* species in commonly accessed culture collections have been isolated from around the world primarily from the northern hemisphere (Table 1). *Nannochloropsis* is generally regarded as a marine species, but *N. limnetica* are fresh water isolates. Additionally, we have adapted *N. gaditana* CCMP526 to grow in 10% of the salinity in seawater by gradually adapting cultures to lower salt levels, which illustrates *Nannochloropsis*’ ability to thrive in a variety of culture conditions.

*Nannochloropsis* *gaditana* natively exhibits high photosynthetic biomass and lipid productivities. Over a period of three months we achieved an average biomass production rate of 0.65 g l\(^{-1}\) d\(^{-1}\), or ~20 g m\(^{-2}\) d\(^{-1}\) on an extrapolated aerial basis.

### Table 1. Strains of *Nannochloropsis* and status of genomic sequencing efforts

| Species               | Strain     | Year Isolated | Location Isolated | Genomic sequencing* |
|----------------------|------------|---------------|-------------------|---------------------|
| *Nannochloropsis*    | CCMP526    | 1985          | Lagune de Oualidia, Morocco | completed          |
| *gaditana*           | CCMP527    | 1952          | Great South Bay, Long Island, New York, USA | in progress        |
| *Nannochloropsis*    | CCMP532    | 1956          | Milford, Connecticut, USA | in progress        |
| *gaditana*           | CCMP536    | 1965          | Sayville, New York, USA | in progress        |
| *Nannochloropsis*    | CCMP1775   | 1995          | Cadiz Bay, Cadiz, Spain | completed          |
| *gaditana*           | CCMP1984   | 1985          | Comacchio Lagomarsi, Ferrara, Italy | completed          |
| *Nannochloropsis*    | CCMP52109  | 1958          | Continental Shelf, North Atlantic, off USA East coast | in progress        |
| *granulata*          | CCMP534    | 1986          | Bigelow Laboratory dock, West Boothbay Harbor, Maine, USA | in progress        |
| *Nannochloropsis*    | CCMP535    | 1965          | Sayville, New York, USA | in progress        |
| *granulata*          | CCMP5662   | 1993          | Skagerrak, North Atlantic | completed          |
| *Nannochloropsis*    | CCMP505    | 1971          | Morehead City, North Carolina, USA | in progress        |
| *limnetica*          | CCMP2260   | 1996          | Arrowwood Lake, North Dakota, USA | in progress        |
| *Nannochloropsis*    | CCMP2287   | 1996          | Arrowwood Lake, North Dakota, USA | in progress        |
| *limnetica*          | CCMP2271   | 1996          | Jim Lake, North Dakota, USA | in progress        |
| *Nannochloropsis*    | CCMP2272   | 1996          | Arrowwood Lake, North Dakota, USA | in progress        |
| *oculata*            | CCMP531    | 1996          | Qingdao, China | in progress        |
| *Nannochloropsis*    | CCMP1779   | 1979          | Kuwait Institute for Scientific Research, Kuwait | in progress        |
| *oceanica*           | CCMP8001   | 1996          | Gulf of Maine | in progress        |
| *Nannochloropsis*    | CCMP532    | 1996          | Great South Bay, Long Island, New York, USA | in progress        |
| *oceanica*           | CCMP1785   | 1996          | Kuwait | in progress        |
| *Nannochloropsis*    | CCMP536    | 1996          | Gulf of Maine | in progress        |
| *oceanica*           | CCMP1778   | 1996          | Gulf of Maine | in progress        |
| *Nannochloropsis*    | CCMP537    | 1996          | Gulf of Maine | in progress        |
| *salina*             | CCMP1779   | 1996          | Gulf of Maine | in progress        |
| *Nannochloropsis*    | CCMP1780   | 1996          | Gulf of Maine | in progress        |
| *salina*             | CCMP1791   | 1996          | Gulf of Maine | in progress        |
| *Nannochloropsis*    | CCMP2000   | 1996          | Gulf of Maine | in progress        |
| *salina*             | CCMP2004   | 1996          | Gulf of Maine | in progress        |

*Genome status from NCBI.*
in 1 l Roux flasks sparged with air/2% CO₂ with a portion of the culture being harvested weekly. Lipid production in these conditions averaged 0.31 g l⁻¹ d⁻¹, and made up almost 50% of the algal biomass. The primary storage lipid in Nannochloropsis is triacylglycerol (TAG). The fatty acid composition of N. gaditana is relatively simple, the most abundant chain lengths being 16:0, 16:1, 18:1, 18:2, and 20:5(n-3) (EPA), with 16:0 and 16:1 typically dominating. Many algae, such as Chlamydomonas, produce little TAG during logarithmic growth and require nutrient starvation to initiate significant lipid body formation. Nannochloropsis gaditana has constitutive TAG droplets even in nutrient replete media during linear growth, and when deprived of nitrogen a single large oil droplet often encompasses the majority of the cellular volume.

Because of the exemplary lipid productivity in N. gaditana, we examined lipid metabolism genes, including those involved in fatty acid biosynthesis, TAG assembly, and lipid degradation. The number of genes involved in lipid metabolism in N. gaditana is expanded compared with the model alga C. reinhardtii, P. tricornutum and Ectocarpus siliculosus (Fig. 1). To find additional gene expansions relative to C. reinhardtii and P. tricornutum, we used Fisher’s exact test to compare the prevalence of Gene Ontology (GO) terms found in each respective genome. Several GO-terms are over-represented in N. gaditana, which may be of importance for the lipid and biomass phenotypes observed. These terms include acyl-carrier protein biosynthetic processes, auxin biosynthesis, pyruvate metabolism, carbon utilization and acetyl-CoA catalytic processes. Interestingly, GO-terms involved in the regulation of growth rate, transcription factor activity, and vitamin binding are under-represented. Many species of algae require an exogenous source of vitamin B12 for use as a cofactor in vitamin B12-dependent enzymes. This micronutrient is often acquired through symbiosis with bacteria. Nannochloropsis does not require vitamin B12 supplemented media for growth, it does contain several vitamin B12 dependent enzymes. It is estimated that about half of all algae can only produce methionine with a vitamin B12-dependent methionine synthase. Nannochloropsis contains both a vitamin B12-dependent and a vitamin B12-independent methionine synthase. Due to higher catalytic efficiency, the vitamin B12-dependent enzyme is likely preferred when vitamin B12 is available, however, methionine synthesis can occur in the absence of vitamin B12 via the vitamin B12-independent methionine synthase. Nannochloropsis also contains a vitamin B12-dependent class II ribonucleotide reductase (RNR) which catalyzes the de novo synthesis of deoxyribonucleoside triphosphates (dNTPs). This enzyme is rarely found in eukaryotes, but was described in Euglena gracilis and Dictyostelium discoideum, which suggests that the class II enzymes were present in a common, B12-dependent, eukaryotic ancestor. Eukaryotes, including Nannochloropsis, typically have class I RNRs, which are primarily active in the presence of oxygen, but the addition of a class II RNR which is oxygen-independent may facilitate deoxyribonucleotide biosynthesis during anaerobiosis in aquatic ecosystems. This suggests, along with the presence of an FeFe-hydrogenase, that Nannochloropsis experiences anaerobic conditions in its native environment and has retained the metabolic capacity to withstand anoxic challenges. A third vitamin B12-dependent enzyme, methylmalonyl coenzyme A mutase, is found in Nannochloropsis and catalyzes the isomerization of methylmalonyl-CoA to succinyl-CoA which requires a vitamin B12 derivative, adenosylcobalamin, to function. While Nannochloropsis does not require exogenous vitamin B12, the addition of this micronutrient can augment its metabolic capabilities. Further studies are required to determine if addition of this vitamin can be leveraged to stimulate growth or improve biofuel production.

### Critical metabolic nodes in fatty acid biosynthesis

Critical metabolic nodes for rationally engineering improvements in fatty acid biosynthesis are at the levels of acetyl-CoA and malonyl-CoA. Factors governing the synthesis and further metabolism of these metabolites are poorly understood in algae, and our research efforts with organisms such as Chlamydomonas clearly indicate that there are significant information deficits regarding the enzymes contributing to acetyl-CoA synthesis and how these enzymes are regulated in phototrophic microorganisms. In terrestrial
plants, the acetyl-CoA used for fatty acid synthesis in the plastid is proposed to be derived primarily from the activity of a plastidic pyruvate dehydrogenase (PDH) complex, with additional potential contributions from acetyl-CoA synthetase and ATP-citrate lyase.\textsuperscript{16,17} Discrete plastidic and mitochondrial PDH complexes have been described in Arabidopsis thaliana,\textsuperscript{16,18} and genes encoding homologs for subunits corresponding to both of these distinct PDH complexes are present in \textit{N. gaditana}. The plastidic PDH complex subunits are more similar to bacterial PDH complex subunits and lack several of the well-characterized posttranslational regulatory motifs described for mitochondrial PDH complexes. Linear photosynthetic electron transport from water oxidation is optimally configured to provide the necessary ATP/NADPH ratios for fixing CO\textsubscript{2} to glyceraldehyde 3-phosphate (GAP) in the Calvin cycle. In principle, metabolism could be controlled to convert GAP, or even the primary product of the RuBisCO enzyme (3-phosphoglycerate) to acetyl-CoA, using plastidic glycolytic enzymes and in the process attaining a net production of ATP (pyruvate kinase) and reducing equivalents (NAD(P)H) via plastidic PDH and GAP dehydrogenase for subsequent use in fatty acid biosynthesis. Acetyl-CoA can also be generated by ATP citrate lyase (ACL) which catalyzes the ATP dependent cleavage of citrate into acetyl-CoA and oxaloacetate. In Arabidopsis and Chlamydomonas ACL is a cytosolic enzyme made up of two discrete subunits that produces a cytosolic pool of acetyl-CoA.\textsuperscript{19} The \textit{N. gaditana} genome encodes one monomeric ACL encoded by a single polypeptide and is similar to monomeric forms found in animals. As acetyl-CoA is at the nexus of respiratory ATP production (mitochondrion), the conversion of central metabolites to reducing equivalents, the entry point into citric acid cycle intermediates for biosynthetic precursors, and the key substrate for fatty acid biosynthesis in the plastid, control of acetyl-CoA biosynthesis is critical to this effort. By establishing that two discrete PDH complexes are present in \textit{Nannochloropsis}, efforts can now be undertaken to begin characterizing and manipulating this important metabolic node.

\textbf{Acetyl-CoA carboxylase (ACCase)} is regarded as the committed and rate limiting step in fatty acid biosynthesis, converting acetyl-CoA to malonyl-CoA. The \textit{N. gaditana} genome encodes two monomeric ACCase homologs (> 2,000 AA each), as is commonly observed in other algal strains with secondary endosymbiotic ancestry.\textsuperscript{20} In Arabidopsis, two separate ACCase enzymes have been characterized. One is encoded by a single polypeptide and is targeted to the cytosolic face of the endoplasmic reticulum where it is proposed to be involved in fatty acid elongation reactions; whereas, the second ACCase complex (4 polypeptide subunits) is targeted to the plastid and is proposed to be the primary enzyme responsible for generating malonyl-CoA for plastidic fatty-acid biosynthesis.\textsuperscript{21,22} Attempts to overexpress ACCase for improved fatty-acid synthesis have met with limited success, implying that acetyl-CoA levels are limiting or that other regulatory features need to be considered.\textsuperscript{23} However, it must be stressed that some of these efforts focused on monomeric nonplastidic forms of ACCase,\textsuperscript{23} which may not function properly in the plastid, and that posttranslational regulatory features controlling enzyme activity in algae are not well established. As in the case of the PDH complex, it is critical to develop a fundamental understanding of the physiological role, as well as the catalytic and regulatory properties of both of the \textit{Nannochloropsis} ACCase enzymes, and then leverage this understanding to improve fatty acid biosynthesis in these microalgae.
smaller bias for genes that are sequen-
tially transcribed in the same direction
(Fig. 2A). The maximum number of
consecutive genes observed with an alter-
nating orientation is 15 (two instances),
whereas the number of genes with a
continuous orientation in a single direc-
tion is 9 (one instance). This preference
is shared by other photosynthetic stra-
menopiles, but is not apparent in non-
photosynthetic stramenopiles (Fig. 2B).

This could constitute a mechanism used
by *Nannochloropsis* to reduce genome
size or to allow better control of gene
transcription, as a single bi- directional
promoter controls the transcription of two
genes. The promoter for a violaxanthin/
chlorophyll a-binding protein has been
experimentally shown to be active in both
directions in *Nannochloropsis* sp W2JB3.9

Many of these gene pairs have less than
1000 bp of intergenic sequence and bidi-
rectional promoters can likely be exploited
to drive the expression of two genes of
interest, or to easily knockout two genes
with one genomic insertion.

In *N. gaditana*, we identified 9,052 gene
models using two discovery methods: (1)
gene models generated by Maker,10 which
reconciled nine lines of EST, homology
and ab initio evidence, and (2) ESTs with
homologs in other algal lineages that were
not included in the Maker gene model
set. The majority of these gene models are
supported by EST (92.3%) and homology
(69.8%) evidence. Nannochloropsis genes
on average have relatively few introns
(1.62 per gene) when compared with
model algae in the green lineage such as
*Chlamydomonas reinhardtii* (7.33 per gene)
or *C. variabilis* NC64A (6.3 per gene). Many
genes have no introns at all (more than 56% [3,102 genes]), which simplifies molecu-
lar biology and metabolic engineering
tasks.

Genes in *Nannochloropsis* have unique
orientations with respect to neighboring
gene directionality and functionality. The
majority of genes in *N. gaditana* are orien-
tated such that genes diverge from a com-
mon locus and also converge with adjacent
genes for termination, while having a
smaller bias for genes that are sequen-
tially transcribed in the same direction
(Fig. 2A). The maximum number of
consecutive genes observed with an alter-
nating orientation is 15 (two instances),
whereas the number of genes with a
continuous orientation in a single direc-
tion is 9 (one instance). This preference
is shared by other photosynthetic stra-
menopiles, but is not apparent in non-
photosynthetic stramenopiles (Fig. 2B).

This could constitute a mechanism used
by Nannochloropsis to reduce genome
size or to allow better control of gene
transcription, as a single bi- directional
promoter controls the transcription of two
genes. The promoter for a violaxanthin/
chlorophyll a-binding protein has been
experimentally shown to be active in both
directions in *Nannochloropsis* sp W2JB3.9

Many of these gene pairs have less than
1000 bp of intergenic sequence and bidi-
rectional promoters can likely be exploited
to drive the expression of two genes of
interest, or to easily knockout two genes
with one genomic insertion.

**Genome Architecture and Gene
Organization**

*Nannochloropsis gaditana* has a haploid
genome with an estimated size of 29 Mb.8
For genomic sequencing we used both 454
pyrosequencing and Illumina technolo-
gies. Sequence data were assembled into
2,087 scaffolds. Half of the genome is con-
tained on 257 scaffolds (N50 statistic) with
the 257th scaffold being 38 Kb in length
(L50 statistic). The genomic sequence
G+C content is 54.2%. All *N. gaditana*
sequences were deposited in GenBank
BioProject PRJNA73791 and have the
accession numbers JH470562-JH472444.

In *N. gaditana*, we identified 9,052 gene
models using two discovery methods: (1)
gene models generated by Maker,10 which
reconciled nine lines of EST, homology
and ab initio evidence, and (2) ESTs with
homologs in other algal lineages that were
not included in the Maker gene model
set. The majority of these gene models are
supported by EST (92.3%) and homology
(69.8%) evidence. Nannochloropsis genes
on average have relatively few introns
(1.62 per gene) when compared with
model algae in the green lineage such as
*Chlamydomonas reinhardtii* (7.33 per gene)
or *C. variabilis* NC64A (6.3 per gene). Many
genes have no introns at all (more than 56% [3,102 genes]), which simplifies molecu-
lar biology and metabolic engineering
tasks.

Genes in *Nannochloropsis* have unique
orientations with respect to neighboring
gene directionality and functionality. The
majority of genes in *N. gaditana* are orien-
tated such that genes diverge from a com-
mon locus and also converge with adjacent
genes for termination, while having a
smaller bias for genes that are sequen-
tially transcribed in the same direction
(Fig. 2A). The maximum number of
consecutive genes observed with an alter-
nating orientation is 15 (two instances),
whereas the number of genes with a
continuous orientation in a single direc-
tion is 9 (one instance). This preference
is shared by other photosynthetic stra-
menopiles, but is not apparent in non-
photosynthetic stramenopiles (Fig. 2B).

This could constitute a mechanism used
by Nannochloropsis to reduce genome
size or to allow better control of gene
transcription, as a single bi- directional
promoter controls the transcription of two
genes. The promoter for a violaxanthin/
chlorophyll a-binding protein has been
experimentally shown to be active in both
directions in *Nannochloropsis* sp W2JB3.9

Many of these gene pairs have less than
1000 bp of intergenic sequence and bidi-
rectional promoters can likely be exploited
to drive the expression of two genes of
interest, or to easily knockout two genes
with one genomic insertion.
More than 20 metabolic pathway gene clusters were found by analyzing the spatial distribution of GO-terms. A nitrogen assimilation gene cluster was identified that includes a nitrate reductase, nitrite reductase, and a nitrate transporter. Additionally, a hydrogenase gene cluster with a unique configuration was found that contains an FeFe-hydrogenase (HYDE, HYDF, and HYDG). This observed gene clustering can likely be used in some instances to infer the physiological function of neighboring proteins of unknown function.

**Genomic Transformation and Homologous Recombination**

For Nannochloropsis to emerge as an algal biofuel production platform, transformation of the nuclear and chloroplast genomes must be facile and routine. To date, the highest-efficiency transformations are achieved using electroporation, which includes plus and minus nitrate, and further reduce ectopic insertions, the observed high rates of homologous recombination. In an effort to increase homologous recombination efficiency, and further reduce ectopic insertions, the observed high rates of homologous recombination. In an effort to increase homologous recombination, we determined Nannochloropsis transformation efficiencies are achieved when plasmids are linearized prior to transformation. Additionally, Nannochloropsis has the ability to take up multiple pieces of exogenous DNA. Using different selectable markers, transformation frequencies of about 50% for one unselected marker and about 30% for two unselected markers were seen in Nannochloropsis sp W2JB3.9

Without using homologous flanking regions, random integration into the genome can occur and multiple integrations are possible. When using homologous flanking regions, homologous recombination can proceed with high efficiency (up to 94%) in Nannochloropsis sp W2JB3.9. The ability to create random or targeted genomic insertions in Nannochloropsis can be leveraged in forward and reverse genetic approaches. These transformation techniques complement each other and will help facilitate functional genomics studies and allow for advanced metabolic engineering strategies.

**Molecular Tool Development and Synthetic Biology in Nannochloropsis**

Chlamydomonas is the most developed model algal system and a variety of molecular tools and resources exist that expedite research. These include plastid, cosmid, and bacterial artificial chromosome libraries, methods for tagging mutant alleles, map-based cloning, RNA interference, and reporter genes. Developing a similar foundation for Nannochloropsis is a priority. One resource that may be easier to establish in Nannochloropsis relative to Chlamydomonas is a whole genome knockout library. High throughput techniques have been employed to make targeted genome scale deletion collections in organisms that have high rates of homologous recombination and a similar approach should be taken with Nannochloropsis. Autotrophic strains can also be produced rapidly with homologous recombination, which will speed the development of endogenous selection markers.

For synthetic gene design and optimization, we determined Nannochloropsis codon usage as gene expression levels are commonly correlated with codon usage in other organisms. The most frequently used codons were identified with a relative adaptiveness analysis and are indicated in Figure 3. Nannochloropsis coding regions have a slightly higher G + C content (58.0%) than the overall genomic DNA sequence (54.2%), and have a slight preference for A or T at the second base of codons, with the average G+C content of the first, second, and third codon base being 61.0%, 45.9% and 67.0%, respectively. Sexual recombination has not been reported to date in Nannochloropsis. The absence of a tractable reproductive system in which crosses can be generated is one remaining feature that would benefit the progression of Nannochloropsis into a model alga. In the N. gaditana genome, we found a set of genes that should be sufficient to facilitate meiosis. However, transcript evidence for the expression of these genes was never observed under any of the transcriptome conditions assessed which included plus and minus nitrate, heat and cold shock, linear and stationary phase culturing, supplemental CO₂ and light/dark transitions. This suggests that (1) the correct environmental conditions to trigger meiosis were not evaluated, (2) multiple mating types may need to be present for meiosis to be initiated and/or (3) these genes are no longer transcribed because N. gaditana no longer undergoes meiosis. To determine whether Nannochloropsis can undergo a mating cycle, more environmental conditions need to be tested in addition to combing different strains that could possibly have an alternative mating type. Although difficult to establish, a tractable sexual recombination system in Nannochloropsis would complement the molecular tools already developed.

**Conclusions**

Algae can produce large quantities of lipid, have high photon to biomass conversion efficiencies, and can grow in a variety of water sources, but the lack of a genetically tractable industrially relevant alga previously limited progress. The genomic DNA sequence of Nannochloropsis gaditana, in addition to efficient transformation
protocols, will permit the rapid development of strains of Nannochloropsis for a biofuel production platform. Interrogation of the N. gaditana genome has revealed many features that contribute to our understanding of the oleaginous phenotype observed, but these findings are just a starting point for further investigations. The genomic sequence provides the basis for systems biology investigations and will serve as a platform for transferring knowledge attained from other algal systems to Nannochloropsis and other algal biofuel phenotypes.31 An informed understanding of the metabolic pathways and their regulation in Nannochloropsis will allow for metabolic engineering strategies to reroute metabolites to biofuel precursors. The tools already developed for Nannochloropsis have positioned it for rapid strain improvements and advances are likely to emerge in the near future.

References

1. Woyr K, Bush D, Darzins A, Willson B. Theoretical maximum algal oil production. Biofuel Research 2010; 3:154-161. http://dx.doi.org/10.1186/1757-1626-3-154
2. Work V11, D’Alonzo S, Radakovits R, Johnson KE, Pometz MC. Improving photosynthesis and metabolite networks for the competitive production of plant-based biofuels. Curr Opin Biotechnol 2010; 21:299-307. http://dx.doi.org/10.1016/j.copbio.2010.11.022
3. Pometz MC, Darzins A. The promise and challenges of microalgal-fossil biofuels. Biofuels, Bioproducts and Biorefining 2009; 3:63-80. http://dx.doi.org/10.1002/bbb.159
4. Radakovits R, Johnson KE, Darzins A, Pometz MC. Genetic engineering of algae for enhanced biofuel production. Eukaryot Cell 2010; 9:488-501. PMID:20183922. http://dx.doi.org/10.1128/EC.00364-09
5. Cordero S, Schwartz C, Martin JP, Diayou-Takhi B, Saux J. Metabolic and sensor-comparative functional analysis of the circadian clock in the diatom Skeletonema costatum. Plant Cell 2009; 21:3436-49. PMID:19946792. http://dx.doi.org/10.1105/tpc.111.093146
6. Ayr KE, Kettle-Panes PM, Grossman AR. Sub-cellular nuclear transformation of the diatom Thalassiosira weissflogii. Mol Cell Genet 1996; 2:573-9. PMID:8814518
7. Pisanu N, Cheek PM, Kinge N. Molecular genetic manipulation of the diatom Thalassiosira pseudonana. J Phycol 2006; 42:1059-68. PMID:17007580
8. Radakovits R, Johnson KE, Zhuangming H, Tse R, Serfling KE, Rozen J, et al. Draft genome sequence and genetic transformation of the oleaginous alga Nannochloropsis gaditana. Nat Commun 2014; 5:4017. http://dx.doi.org/10.1038/ncomms5017
9. Le K, Roquet B, Vechot KE, Kuroda T. High-efficiency homologous recombination by single-strand annealing in the chlorophyte Chlamydomonas reinhardtii. Mol Cell 2010; 39:767-77. http://dx.doi.org/10.1016/j.molcel.2010.04.037
10. Wang ZT, Ulrichs N, Jaros S, Wollmannhauser S, Goubert A. Algal lipid bodies: stress induction, purification, and biochemical characterization in the wildtype and strainulas (Chlorella vulgaris). Eukaryot Cell 2010; 9:895-60. PMID:20980796. http://dx.doi.org/10.1128/EC.00272-09
11. Work V11, Radakovits R, Johnson KE, Willson B, Effortt LG, Vrejdal D, et al. Increased lipid accumulation in the Chlamydomonas reinhardtii ec-l54 starchless strainulas imitate mutant and increased carbohydrates synthesize in complemented strains. Eukaryot Cell 2010; 9:1250-62. PMID:20542225. http://dx.doi.org/10.1128/EC.00075-09

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
R.E.J. was supported by a Graduate Research Fellowship from the National Science Foundation. R.R. and this research were supported with funding provided by Conoco-Phillips through a grant to the Colorado Center for Biofuels and Biorefining (C2B2).

12. Croft MT, Lawrence DA, Bauer-Dreyer E, Watson MJ, Smith AG. Algae: a viable source of B12 through a symbiotic relationship with bacteria. Nature 2001; 410:90-3. PMID:11259754. http://dx.doi.org/10.1038/nature00916
13. González JC, Barromeo RV, Huang S, Somero GN. Matriono M. Composition of cellulosome-dependent cellulases and cellulases linked to two solutions in the same chemical potential. Biochemistry 1992; 31:605- 61. PMID:13392886. http://dx.doi.org/10.1021/bi00461a013
14. Tovar B, Tovar S, Roza C, Holman U, Martin W, Rockad P. Efiiciency a class III enzyme and the phosibiotic enzyme of sub-tropics B12 deponen. J Bioe Chem 2001; 276:5404-11. PMID:11040894. http://dx.doi.org/10.1074/jbc.M108022200
15. Gavrilovski C, Dubini A, Subramanian V, Wang Y, Magnieru J, Mesi R, et al. Alternative fermentation metabolism in Chlorophycea: ruobiotic microorganisms lacking pyruvate dehynogen and both pyruvate formate lyase and alcohol dehydrogenase. Plant Cell 2012; 24:392-7. PMID:22595317. http://dx.doi.org/10.1105/tpc.2011.11.095466
16. Ro, Reh B, Rek S, Nikul B, Wardke ES. The role of pyruvate dehydrogenase and the pyruvate dehydrogenase complexes in biofuel and carbonate metabolism in developing Arabidopsis. Plant Physiol 2012; 160:57-68. PMID:22690030. http://dx.doi.org/10.1104/pp.111.195472
17. Olse D, Nikula C, Wardke ES, Aozol-Coh Livs- et the metabolic stress. Plant Physiol 2014; 159:685-97. PMID:25020865. http://dx.doi.org/10.1104/pp.14.00281
18. Tessa Minka A, Moneck J, Randall DD. Regiolistics of pyruvate dehydrogenase complex activity in plant cells. Eur J Biochem 2004; 271:1624-31. PMID:15096285. http://dx.doi.org/10.1111/j.1432-1033.2004.03840.x
19. Flisud LM, Nikula C, Petriks J. Robin-coenzyme a characteristis of the pyruvate dehydrogenase generation by ATP synthase in Arabidopsis. Plant Cell 2005; 17:182-203. PMID:15883388. http://dx.doi.org/10.1105/tpc.104.033888
20. Hoofnagle L, Thomas EM. Comprehensive guide to pyruvate carbonic acid: C1-cell Biotechnology 2012; 32:pms. PMID:22524446. http://dx.doi.org/10.1007/s00253-012-0607-1
21. Rasheda S, Carrot flux and fatty acid synthe- identification of plant. J Appl Physiol 2012; 112:39-62. PMID:22217563. http://dx.doi.org/10.1152/japplphysiol.00233.2011

www.landesbioscience.com

Bioengineered 43