



Nuclear, Chloroplast, and Mitochondrial Genome Sequences of the Prospective Microalgal Biofuel Strain *Picochlorum soloecismus*

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ABSTRACT *Picochlorum soloecismus* is a halotolerant, fast-growing, and moderate-lipid-producing microalga that is being evaluated as a renewable feedstock for biofuel production. Herein, we report on an improved high-quality draft assembly and annotation for the nuclear, chloroplast, and mitochondrial genomes of *P. soloecismus* DOE 101.

Picochlorum soloecismus (Trebouxiophyceae, Chlorophyta) was isolated during the bioprospecting efforts of the National Alliance for Advanced Biofuels and Bioproducts (NAABB) consortium (1), after it outcompeted *Nannochloropsis salina* CCMP1776 in mixed cultures subjected to heat stress at Los Alamos National Laboratory in New Mexico. In general, *Picochlorum* strains have high growth rates, are halotolerant, can grow at temperatures ranging from 18 to 35°C, and may accumulate moderate amounts of lipids and carbohydrates (2–6). Phylogenetically, *P. soloecismus* is most closely related to the type species *Picochlorum oculatum* and *Nanochlorum eucaryotum* (99% 18S rRNA similarity). The fully sequenced genome from *Picochlorum* sp. strain SENEW3 showed compactness (genome size, 13.5 Mbp; 7,367 genes) and gene clustering (7, 8). The aforementioned characteristics make it a promising candidate for biotechnological use. Physiological characterization of *P. soloecismus* has so far demonstrated its capacity to grow under simulated outdoor pond conditions (to replicate the climate of Key West, FL) for up to 30 days (9). Others have also reported that *P. soloecismus* is amenable to genetic engineering (1).

P. soloecismus DOE 101 genomic DNA was extracted and purified using the Qiagen (Hilden, Germany) midi plant DNA kit. Rapidly growing cells from 200 ml of culture (optical density at 750 nm, 2.0) were lysed by heating to 95°C for 5 min in the lysis buffer. The supernatant containing the DNA was purified according to the manufacturer's directions for the kit. DNA was sequenced to 470× and 27× average genome coverage using Illumina (10) and 454 pyrosequencing (11), respectively. The 454 reads were assembled with Newbler version 2.3, and the resulting consensus sequences were computationally shredded into 2-kb overlapping fake reads (shreds). The 100-bp Illumina sequencing reads were assembled with Velvet version 1.0.13 (12), and the consensus sequence was computationally shredded into 1.5-kb overlapping shreds. The consensus shreds generated from Newbler and Velvet were combined into a single assembly using parallel Phrap version 1.080812 (High Performance Software LLC). Finally, the Illumina reads were used to correct potential base errors and increase consensus quality using the software Polisher (A. Lapidus, unpublished data), and misassemblies were corrected using gapResolution (C. Han, unpublished data) or Dupfinisher (13). Postassembly, the genome was annotated with Maker (14) using assembled transcripts from a nitrogen deprivation time-course study (S. N. Twary, unpublished data). Annotation generated 7,844 gene models.

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The final genome assembly contains 56 contigs, with a maximum contig size of 496 kbp, an assembly size of 15.2 Mbp, and an average GC content of 46%. The mitochondrial and chloroplast genomes are fully assembled into 38.7-kbp and 72.7-kbp circular chromosomes, respectively. This genome will be a valuable resource for phylogenetic and comparative studies and is an essential reference for future genetic engineering efforts toward the development of members of the genus *Picochlorum* for use as a biofuel and renewable chemical production platform.

Accession number(s). This genome sequence is publicly available at The Greenhouse knowledgebase at Los Alamos National Laboratory (<https://greenhouse.lanl.gov/greenhouse>). The version described in this paper is the first version and is deposited at DDBJ/ENA/GenBank under the accession number [PJAJ00000000](https://doi.org/10.1007/s11746-013-2225-0). The isolated and circular complete mitochondrial and chloroplast genome sequences are deposited under GenBank accession numbers [MG552670](https://doi.org/10.1007/s11746-013-2225-0) and [MG552671](https://doi.org/10.1007/s11746-013-2225-0), respectively.

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