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

C. Raul Gonzalez-Esquer, a Scott N. Twary, a Blake T. Hovde, a Shawn R. Starkenburga

aBioscience Division, Los Alamos National Laboratory, Los Alamos, New Mexico, USA

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 *Nannochlorum 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 470X and 27X 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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Address correspondence to Shawn R. Starkenburg, shawns@lanl.gov.
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.

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**References**

1. Unkefer CJ, Sayre RT, Magnuson JK, Anderson DB, Baxter I, Blaby IK, Brown JK, Carleton M, Cattolico RA, Dale T, Devarenne TP, Downes CM, Dutcher SK, Fox DT, Goodenough U, Jaworski J, Holladay JE, Kramer DM, Koppsch AT, Lipton MS, Marrone BL, McCormick M, Molnár I, Mott JB, Ogden KL, Panisko EA, Pellegrini M, Polle J, Richardson JW, Sabarsky M, Starkenburg SR, Stormo GD, Teshima M, Twary SN, Unkefer PJ, Yuan JS, Olivares JA. 2017. Review of the algal biology program within the National Alliance for Advanced Biofuels and Bioproducts. Algal Res 22:187–215. https://doi.org/10.1016/j.algal.2016.06.002.

2. Dahmen I, Chitourou H, Jebali A, Daassi D, Karay F, Hassairi I, Sayadi S, Abdelkaafi S, Dhouib A. 2014. Optimisation of the critical medium components for better growth of *Picochlorum* sp. and the role of stressful environments for higher lipid production. J Sci Food Agric 94: 1628–1638. https://doi.org/10.1002/jsfa.6470.

3. de la Vega M, Diaz E, Vila M, Leon R. 2011. Isolation of a new strain of *Picochlorum* sp. and characterization of its potential biotechnological applications. Biotechnol Prog 27:1535–1543. https://doi.org/10.1002/btpr.686.

4. Zhu Y, Dunford NT, Goad C. 2014. Effect of processing parameters on flocculation of *Picochlorum oklahomensis*. J Am Oil Chem Soc 91: 317–324. https://doi.org/10.1002/joca.2371-4.

5. Zhu Y, Dunford NT. 2013. Growth and biomass characteristics of *Picochlorum oklahomensis* and *Nannochloropsis oculata*. J Am Oil Chem Soc 90:841–849. https://doi.org/10.1002/joca.22250-0.

6. Wang S, Lambert W, Giang S, Goericke R, Palenik B. 2014. Microalgal assemblages in a poikilohaline pond. J Phycol 50:303–309. https://doi.org/10.1111/jpy.12158.

7. Fofonker F, Price DC, Qiu H, Palenik B, Wang S, Bhattacharya D. 2015. Genome of the halotolerant green alga *Picochlorum* sp. reveals strategies for thriving under fluctuating environmental conditions. Environ Microbiol 17:412–426. https://doi.org/10.1111/1462-2920.12541.

8. Fofonker F, Ananyev G, Qiu H, Morrison A, Palenik B, Dismukes GC, Bhattacharya D. 2016. The unexpected extremophile: tolerance to fluctuating salinity in the green alga *Picochlorum*. Algal Res 16:465–472. https://doi.org/10.1016/j.algal.2016.04.003.

9. Huesemann M, Dale T, Chavis A, Crowe B, Twary S, Barry A, Valentine D, Yoshida R, Wigmota M, Cullinan V. 2017. Simulation of outdoor pond cultures using indoor LED-lighted and temperature-controlled raceway ponds and Phenometrics photobioreactors. Algal Res 21:178–190. https://doi.org/10.1016/j.algal.2016.11.016.

10. Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, Hall KP, Evers DJ, Barnes CL, Bignell GR, Boulton JM, Bryant J, Carter RJ, Cheetham RK, Cox AJ, Ellis DJ, Flatbush MR, Gormley NA, Humphray SJ, Irving LJ, Karbelashvili MS, Kirk SM, Li H, Liu X, Maisinger KS, Murray LJ, Obradovic A, Ost T, Parkinson ML, Pratt MR, Rasolosonjatoivo IMJ, Reed MT, Rigatti R, Rodighiero C, Ross MT, Sabot A, Sankar SV, Scally A, Schroth GP, Smith ME, Smith VP, Sridhara P, Tore J, Tzenev SS, Vermaas EH, Walter K, Wu X, Zhang L, Alam MD, Anastasi C, et al. 2008. Accurate whole human genome sequencing using reversible terminator chemistry. Nature 456:53–59. https://doi.org/10.1038/nature07517.

11. Maragulies M, Egholm M, Altman WE, Attiya S, Bemben LA, Berka J, Braverman MS, Chen Y-J, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Iryk GP, Jando SC, Alenquer MLI, Jarvie TP, Jirage KB, Kim J-B, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makjihani VB, McDeke KA, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srivanasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380. https://doi.org/10.1038/nature03959.

12. Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18:821–829. https://doi.org/10.1101/gr.074492.107.

13. Han CS, Chain P. 2006. Finishing repeat regions automatically with Dupfinisher, p 141–146. In Arabnia HR, Valafar H (ed), Proceedings of the 2006 International Conference on Bioinformatics & Computational Biology. CSREA Press, Las Vegas, NV.

14. Cantarel BL, Korf I, Robb SM, Parra G, Ross E, Moore B, Holt C, Sánchez Alvarado A, Yandell M. 2008. MAKER: an easy-to-use annotation pipeline designed for emerging model organism genomes. Genome Res 18: 188–196. https://doi.org/10.1101/gr.6743907.