Title: Cas9-mediated mutagenesis of GS-GOGAT genes in the diatom Phaeodactylum tricornutum

Authors: Mark Moosburner,[1,2]*, Pardis Gholami[2], James McCarthy[2], and **Andrew E. Allen**[1,2] **(aallen@jcvi.org)**

Institutions: [1]Scripps Institution of Oceanography, University of California, La Jolla, CA; [2]J. Craig Venter Institute, La Jolla, CA

Project Goals: Short statement of goals. (Limit to 1,000 characters)

Abstract text: Diatoms contribute tremendously to primary production in contemporary marine food webs partially due to their ability to quickly assimilate and out-compete other microbes for nitrogenous compounds. Nitrogen assimilation in diatoms is multifaceted in that it uniquely contains two complete glutamine synthetase - glutamate synthase (GS-GOGAT) cycles, one localized to the chloroplast and one to the mitochondria. In order to elucidate the function of genes involved in nitrogen assimilation and of entire GS-GOGAT cycles (chloroplast or mitochondria), CRISPR-Cas9 was employed in the diatom *Phaeodactylum tricornutum* to knock-out individual genes and two genes simultaneously. Episomal delivery of CRISPR-Cas9 was used to deliver an antibiotic-based selectable Cas9 and one or two guide-RNA expression cassettes via bacterial conjugation. To aid in the workflow in generating Cas9 episomes, a colorimetric cloning methodology was developed based on hierarchical Golden Gate assembly[1]. Using this Cas9 episome, mutant cell lines were generated for four genes, the chloroplastic GS (GSII) and GOGAT and the mitochondrial GS (GSIII) and GOGAT. Also, both the mitochondrial GS and GOGAT were knocked out together, which resulted in mutant cell lines with reduced growth rates. A paired knock out of the chloroplastic GS and GOGAT enzymes was not possible, potentially due to a severely decreased or abolished growth rate subsequently. The single-gene mutant cell lines for GSII and GSIII did not impair growth regardless of the nitrogenous compound supplemented for growth. Interestingly, GSII mutants appear to have elevated lipid content during early and mid-exponential phases compared to wild-type *Phaeodactylum* while growing comparably. Here, physiological growth measurements of mutant cell lines were used to hypothesize the respective functions of GSII and GSIII in the context of lipid biosynthesis.

References/Publications

1. Moosburner, M. A., Gholami, P., McCarthy, J. K., Tan, M., Bielinski, V. A., & Allen, A. E. (2020). Multiplexed knockouts in the model diatom phaeodactylum by episomal delivery of a selectable cas9. Frontiers in microbiology, 11, 5.

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