88. Assembly of entire Phaeodactylum tricornutum chromosomes in yeast

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Project Goals: ***Synthetic Module Generation and Transplantation***: Using yeast as a platform (Gibson et al. 2008), we will create synthetic, extra-chromosomal elements to install new biological functions into diatoms. These functions may include traits found in related diatom species, or novel traits encoded by a heterologous pathway. As part of this phase, existing diatom promoter sequences will be characterized and evolved to create a suite of endogenous control elements needed in metabolic redesign.

Abstract:
Synthetic genomic approaches offer unique opportunities to use powerful yeast and Escherichia coli genetic systems to assemble and modify chromosome-sized molecules before returning the modified DNA to the target host. The model diatom Phaeodactylum tricornutum has an average G+C content of 48% and a 27.4 Mb genome sequence that has been assembled into chromosome-sized scaffolds making it an ideal test case for assembly and maintenance of eukaryotic chromosomes in yeast. We present a modified chromosome assembly technique in which eukaryotic chromosomes as large as ~500 kb can be assembled from cloned ~100 kb fragments. We used this technique to clone fragments spanning P. tricornutum chromosomes 25 and 26 and to assemble these fragments into single, chromosome-sized molecules. We found that addition of yeast replication origins improved the cloning, assembly, and maintenance of the large chromosomes with moderately high G+C content in yeast. Furthermore, purification of the fragments to be assembled by electroelution greatly increased assembly efficiency. These techniques offer new opportunities to design large biosynthesis pathways for expression in algae and open the door to the development of large DNA fragment replacement in algae for efficient genome editing.

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