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GG1 - sgRNA cloning for Phaeodactylum tricornutum

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ABSTRACT

The CRISPR-Cas9 gene mutagenesis system was adapted for the marine diatom Phaeodactylum tricornutum (CCAP-1055/1) (Figure 1). Here, Cas9 and sgRNA(s) were delivered to Phaeodactylum by bacterial-conjugation transformation on an episome, or artificial chromosome, that is stably maintained and replicated independently from and with the Phaeodactylum chromosomal DNA. Two sgRNA expression cassettes, sgRNA (1/1) and sgRNA (1/2) were cloned into the episome. The sgRNAs each contain a unique 20-nucleotide spacer sequence that , after sgRNA cassette transcription, guides the Cas9 to a nucleic acid target by complementary binding followed by Cas9 nuclease activity. Two Phaeodactylum genes, Pt_GSII (Gene ID: 51092) and Pt_cGOGAT (Gene ID: 24739), were targeted by each sgRNA, respectively. The following protocol was followed to synthesize spacer sequences and sgRNA expression cassettes

GG1_ Spacer Cloning for P.t. episome.pdf