JUN 26, 2019

OPEN ACCESS

DOI:
[dx.doi.org/10.17504/protocols.io.4abgsan](https://doi.org/10.17504/protocols.io.4abgsan)

External link:
<https://doi.org/10.3389/fmicb.2020.00005>

Protocol Citation: Mark Moosburner, Andrew E Allen 2019. GG1 - sgRNA cloning for *Phaeodactylum tricornutum*. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.4abgsan>

MANUSCRIPT CITATION:

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Jun 17, 2019

Last Modified: Jun 26, 2019

GG1 - sgRNA cloning for *Phaeodactylum tricornutum*

Frontiers in Microbiology

Mark Moosburner¹, Andrew E Allen¹

¹University of California, San Diego

A.E. Allen Lab

Mark Moosburner

Scripps Institution of Oceanography, J. Craig Venter Institu...

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

ATTACHMENTS

[GG1_ Spacer Cloning for P.t. episome.pdf](#)

PROTOCOL integer ID:

24611

Keywords: CRISPR, Cas9,
cloning, conjugation, episome,
diatom, phaeodactylum