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GG2 - CRISPR-Cas9 episome cloning using red-blue screening for Phaeodactylum tricornutum

Mark Moosburner[1], Andrew Allen[1]

[1]University of California, San Diego

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

ABSTRACT

To ensure Cas9 activity upon episomal transformation, the 3' end of the Cas9 coding sequencing was transcriptionally fused to an antibiotic selectable marker, shble, by the 2A peptide. The Cas9-2A-shble construct was cloned into a Phaeodactylum episome that included a bacterial expression cassette for a red fluorescent protein (RFP). The RFP was also flanked by two BsaI restriction digest sites. The RFP vector with Cas9 was used as a cloning vector to assembly one or more sgRNA Phaeodactylum expression cassettes and a single LacZ bacterial expression cassette in place of the RFP. Upon successful assembly, the correct colonies appear blue on selective agar plates while incorrect colonies appear either red or colorless. The golden gate assembly protocol used to synthesize the episome (g51092-2, g24739-A) is as follows:

GG2_ sgRNA cloning into Cas9 episome using red-blue screening.pdf