Jun 25, 2019

Electroporation of Heterosigma akashiwo V.3

Deepak Nanjappa and Kathryn Coyne[1]

[1]University of Delaware

Working dx.doi.org/10.17504/protocols.io.4qggvtw

Kathryn Coyne

ABSTRACT

This protocol was developed for transformation of *Heterosigma akashiwo* by electroporation. This species does not grow on solid medium, so transformants are grown "in bulk" and should not be considered clonal. We have had limited success with this protocol, which may need to be further optimized.

MATERIALS TEXT

Plasmid vector sequences are at DOI: 10.5281/zenodo.439653

1 Harvest exponential growth *Heterosigma akashiwo* culture (1-2 x 10^6 cells mL^{-1}) by centrifugation at 1500 rpm for 90 s in a 50 mL sterile screw-cap tube. Remove the supernatant by pipetting.

2 Resuspend the cell pellet in a small volume of MAX Efficiency® Transformation Reagent for Algae (Invitrogen, Thermo Fischer Scientific, USA) or in 384mM Sorbitol.

3 Transfer 200µl of cells into a 1.5ml Eppendorf tube and add upt to 20 µl (at least 1µg) of linearized plasmid DNA. Mix by tapping the mixture and incubate at room temperature for 10 mins. See DOI: 10.5281/zenodo.439653 for plamid sequence information.

4 Transfer the mixture to a 4 mm gap cold electroporation cuvette (BioRad). Electroporate in an electroporator, BioRad® Gene Pulser® (BioRad) at 50/75 Volts, 25 µF and ∞Ω and let it sit on bench at room temperature for 15mins. Typical electro pulse duration obtained is about 10–15 ms.

5 Transfer the electroporated sample in equal amounts to 4 wells of 12 well plate with 2 ml of fresh f/2 medium with cefotaxime (100µg/ml, to control bacterial growth).

6 Incubate in dim light for 1 day and then transfer to normal light conditions for another 2 days. After 3–4 days, add appropriate antibiotic and incubate in normal conditions.

Expected Results

7 Transformants should be visible after 20-30 days. Continue to transfer the live cells to fresh medium every 10-12 days with antibiotic and analyse transformants by PCR once sufficient culture is obtained.