

Jun 25, 2019 Version 3

Electroporation of *Heterosigma akashiwo* V.3

DOI

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

Deepak Nanjappa and Kathryn Coyne¹

¹University of Delaware



Kathryn Coyne

OPEN  ACCESS



DOI: dx.doi.org/10.17504/protocols.io.4qggvtw

Protocol Citation: Deepak Nanjappa and Kathryn Coyne 2019. Electroporation of *Heterosigma akashiwo*. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.4qggvtw>

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: June 25, 2019

Last Modified: June 25, 2019

Protocol Integer ID: 25064

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

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

- 1 Harvest exponential growth *Heterosigma akashiwo* culture ($1-2 \times 10^6$ 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 up 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 plasmid 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 $\infty\Omega$ 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.