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Protocol status: In development
 We are still developing and optimizing this protocol

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🌐 Electroporation of *Thalassiosira pseudonana*

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Protist Research to Optimize Tools in Genetics (PROT-G)

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ABSTRACT

An electroporation-mediated genetic transformation of the marine diatom *Thalassiosira pseudonana* was developed. Using a sorbitol-based buffer, *T. pseudonana* cells were successfully transformed with the *Tpfc*p/nat plasmid at an efficiency of 2820 per 10⁸ cells. This represents a six-fold improvement compared with previously published methods.

MATERIALS

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⊗ f/2 medium **NCMA Catalog #MKF250L**

375 mM sorbitol (sterile filtered)
 2 mM electroporation cuvettes (chilled on ice)
 50 mL conical centrifugation tubes
 1.5 mL centrifuge tubes
 Selection agent
 NEPC plates

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21136

Keywords: electroporation,
diatoms, Thalassiosira

- 1 Grow cultures of *T. pseudonana* on f/2 supplemented sterile seawater to a density of approximately 1.1×10^6 cells mL⁻¹

Cell collection option 1

- 2 All procedures are carried out at 4 C and cells were kept on ice.
Collect cells from 500 mL of culture (see step 1) by centrifugation for 10 min at 3000 x g in 10 x 50 mL conical centrifuge tubes. Discard supernatant and resuspend pellet in 1 mL of 375 mM sorbitol (filter sterilized)
- 3 Combine resuspended cells into one 50 mL conical centrifuge tube and centrifuge at 3000 x g for 10 min. Discard supernatant and resuspend pellet in 800 µL of 375 mM sorbitol (filter sterilized). Transfer to 1.5 mL tube and store on ice.

Cell collection option 2

- 4 All procedures are carried out at 4 C and cells were kept on ice.
Concentrate cells by vacuum filtration to approximately 300 mL. Collect cells by centrifugation in 50 mL conical centrifugation tubes at 3000 x g for 10 min. Discard supernatant and resuspend pellet in 2 mL of 375 mM sorbitol (filter sterilized).
- 5 Combine cells into new tube and centrifuge at 3000 x g for 10 min. Discard supernatant and resuspend in 1 mL of 375 mM sorbitol.

Electroporation

- 6 On ice, add linearized plasmid (in 250 mM sorbitol) to a final concentration of 0.15 µg mL⁻¹ to the concentrated cells from above.

- 7 Transfer 100 μL of cells + plasmid to a pre-chilled 2 mm gap electroporation cuvette.
- 8 Wipe cuvette dry with kimwipe and place into Biorad Gene Pulser electroporator set to 25 μF and 400 ohm resistance. In our hands, the highest transformation efficiencies were obtained using a field strength of 2.50 kV/cm.
- 9 Transfer electroporated cells to 10 mL of sterile, f/2 supplemented artificial seawater and incubate overnight at 17 °C under constant light (170 $\mu\text{E m}^{-2} \text{s}^{-1}$).
- 10 The following day, plate ca. 5×10^8 cells on selective NEPC agar plates (in our experiment we used 100 $\mu\text{g mL}^{-1}$ NAT). Controls should include no-plasmid and no electroporation treatments.
- 11 Grow plates under constant light (170 $\mu\text{E m}^{-2} \text{s}^{-1}$). Colonies appear approximately 10 days after plating.
- 12 Transfer colonies to 300 μL of f/2 supplemented artificial seawater in 96 well plates. Optical density (600 or 680 nm) can be used as a proxy for growth using a plate reader.