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Electroporation of *Thalassiosira pseudonana*

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ABSTRACT

An electroporation-mediated genetic transformation or the marine diatom *Thalassiosira pseudonana* was developed. Using a sorbitol-based buffer, *T. pseudonana* cells were successfully transformed with the *Tp*fcp/nat plasmid at an efficiency of 2820 per 10^8 cells. This represents a six-fold improvement compared with previously published methods.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Bugge, J. 2015. Electroporation-mediated Transformation and post-transcriptional gene regulation of nitrate reductase in the marine diatom Thalassiosira pseudonana. M.S., Clark University, 89 pp.

MATERIALS

NAME	CATALOG #	VENDOR
f/2 medium	MKF250L	NCMA

MATERIALS TEXT

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

1 Grow cultures of *T. psuedonana* on f/2 supplemented sterile seawater to a density of approximately 1.1 x 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 m in 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 uL of 375 mM sorbitol (filter sterilized). Transfer to 1.5 mL tube and store on ice.

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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 supernatnat and resuspend pellet in 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 mM 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} tothe 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 o 2.50 kv/cm.

9 Transfer electroporated cells to 10 mL of sterile, f/2 supplemented artiicial seawater and incubate overnight at 17 C under constant light (170 µE m^{-2} s^{-1}).

10 The following day, plate ca. 5 x 10^8 cells on selective NEPC agar plates (in our experiment we used 100 µg mL^{-1} NAT). Controls should include no-plasmid and no electroporation treatments.

11 Grow plates under constant light (170 µE m^{-2} 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 denisty (600 or 680 nm) can be used as a proxy for growth using a plate reader.

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