Transformation of Phaeodactylum tricornutum by electroporation

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Abstract

Successful transformation of *Phaeodactylum tricornutum* (CMP632) by electroporation was achieved based on a modified version of Zhang & Hu (2014).

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Before start

Materials needed for transformation

- 375 mM Sorbitol, filter sterilize and store at -20°C.
- Plasmid DNA --> linearize with ScaI at 37°C (5'...AGT|ACT... 3') (3'...TCA|TGA...5')
- Salmon sperm dna--> denature the DNA by boiling for 1 minute, then place on ice
- f/2 medium + Si
- solid medium (50% ASW containing f/2 nutrients, 1.2% agar + zeocin 75 µg/ml)

Protocol

Strain and culture conditions

Step 1.

P. tricornutum (CCMP632) cells were grown axenically in f/2 + Si medium at 22°C and illuminated with 75 µmol photons $m^{-2}s^{-1}$, 12L:12D. For growth on solid medium, cultures were grown on 50% ASW containing f/2 nutrients, 1.2% agar.

Plasmid

Step 2.

pPha-T1/eGFP: carries the bleomycin-resistant gene (*sh ble*) under the *fcpB* promoter and eGFP gene (*egfp*) under the *fcpA* promoter.

NOTES

Kindly provided by Peter Kroth.

pPhaT1 GenBank accession: AF219942, [Zaslavskaia et al. (2000). Transformation of the diatom *Phaeodactylum tricornutum* (Bacillariophyceae) with a variety of selectable marker and reporter genes. *Journal of Phycology*, *36*, 379-386]

Prepare linearized plasmid DNA

Step 3.

Digest 6 µg of pPha-T1/eGFP with ScaI for 2.5 hours. Then purify linearized plasmid (i.e. Macherey-Nagel Nucleo-Spin gel and PCR clean-up kit).

Electroporation of cells

Step 4.

Grow *P. tricornutum* to a cell density of 5×10^6 cells/ml.

Electroporation of cells

Step 5.

Harvest a total of 2×10^8cells by centrifuging at 1500 *g* for 10 min at 4°C. Remove medium.

Electroporation of cells

Step 6.

Chill a 0.2 cm electroporation cuvette on ice.

Electroporation of cells

Step 7.

Wash cells three times with 1 ml 375 mM sorbitol (sterile and ice cold). If needed, spin between washes at 1500 *g* for 1-2 mins at 4°C. Before third wash, transfer cells to a 1.5 mL microcentrifuge tube, perform final sorbitol wash and then resuspend washed cells in 100 µl 375 mM sorbitol to a final density of 2×10^9cells/ml.

Electroporation of cells

Step 8.

Mix 100 µl aliquot of resuspended cells with 4 µg (0.2 µg/µl) of linearized plasmid DNA and 40 µg (10 µg/µl) of denatured salmon sperm DNA.

Electroporation of cells

Step 9.

Incubate cells/linearized plasmid/salmon sperm DNA mixture on ice for 10 min, then transfer to the 0.2 cm electroporation cuvette.

Electroporation of cells

Step 10.

Adjust Electroporation System to exponential decay and set the following parameters:

500 V field strength

25 µF capacitance

400 Ohm shunt resistance

Electroporation of cells

Step 11.

After electroporation, immediately transfer cells to 15-ml conical Falcon tubes containing 10 ml f/2 medium. Allow cells to recover by incubating in low light (30 µmol photons $m^{-2}s^{-1}$) for 24 hours before adding antibiotic selection. Incubate under normal culturing conditions for 24 hours then collect cells by centrifuging at 1500 *g* for 10 min and resuspending cells in 0.6 ml f/2 medium. Plate serial dilutions onto solid medium containing 75 µg/ml zeocin.

Electroporation of cells

Step 12.

Colonies should start appearring after about 12-14 days.