

Introducing dinoflagellate gene into diatom using gene gun

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Abstract

While dinoflagellate transformation tool is not yet ready, transforming dinoflagellate genes into a model alga such as a diatom is a viable option.

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Protocol

Prepare diatom culture

Step 1.

Collect *Phaeodactylum tricornutum* cells (2×10^8) from an exponentially growing liquid culture by centrifugation at 1,500x g for 10 min at 4°C, then resuspend in 200 µL 50% seawater to a final density of 10^9 cells ml⁻¹.

Prepare diatom cells on agar plate

Step 2.

Spread the cell suspension on 9 cm 1.5% agar plates containing 50% seawater supplemented with f/2 nutrients.

Bombardment

Step 3.

Carry out transformation using the Biolistic PDS-1000/He Particle Delivery System (Bio-Rad). M17 tungsten particles (Bio-Rad) are coated with DNA using 1.25M CaCl₂ and 20mM spermidine. Use a burst pressure of 1550 psi and a vacuum of 28 Hg, and 3 µg of plasmid carrying a dinoflagellate rhodopsin gene.

Recovery at low light

Step 4.

The bombarded cells are incubated on the plate under low light (50 µmol photons m⁻²s⁻¹) at 20°C for a day.

Plate the transformed cells

Step 5.

Resuspend the cells in 600 µL sterile 50% seawater, and 200 µL of this suspension is plated onto solid

medium containing $50 \mu\text{g ml}^{-1}$ Zeocin and KAS compound antibiotics, incubate under a 14 h light/10 h dark cycle ($100 \mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$) at 20°C .

Replate the transformed cells

Step 6.

After 3 weeks, re-streak colonies on fresh 9 cm 1% agar plates containing $75 \mu\text{g ml}^{-1}$ Zeocin.