Introducing dinoflagellate gene into diatom using gene gun

Version 2

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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 cultu

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℃ , 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 $m^{-2}s^{-1}$) at 20℃ 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 µg ml^{-1} Zeocin and KAS compound antibiotics, incubate under a 14 h light/10 h dark cycle (100 µmol photons $m^{-2} \cdot s^{-1}$) at 20°C.

Replate the transformed cells

Step 6.

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