Phaeodactylum tricornutum *transformation by means of the PDS-1000/He Microprojectile Accelerator*

1. Plate 5x10^6 cells of a P. tricornutum culture in exponential phase on 1% agar in 50% f/2-Si plates
2. Sterilize the following components and consumables by soaking for 15 min in 70% ethanol, then allowing to dry in sterile hood; alternatively, components listed in parentheses can be sterilized by autoclaving:

   Rupture disks, macroprojectiles, macrocarrier seating tool,

   (stopping screens, microcarrier launch assembly parts, macrocarrier holders, rupture disk retaining cap)
3. Prepare tungsten microcarriers as below:

- Place 60 mg of the dry tungsten particles in 1 ml of 100% ethanol in a microfuge tube. Vortex on high for 1-2 min. Repeat 3x
- Pellet particles in microfuge (1 min); wash 2x in 1 ml sterile distilled water. Suspend in 1 ml sterile water and aliquot 50 ul into sterile microfuge tubes (vortex continuously while pipetting). Store at -20 °C (to prevent oxidation of the tungsten)
- To precipitate DNA onto microprojectiles: to 50 ul of the carrier in water, add in order, while continuously vortexing, 5 ul plasmid DNA (1 ug/ul), 50 ul 2.5 M CaCl2, and 20 ul of 0.1 M spermidine (free base). Continue vortexing for 3 min, then microfuge for 10 sec at 13000 rpm and remove as much supernatant as possible. Wash with 250 ul 100% ethanol, spin again, then suspend in 60 ul of 100% ethanol. When removing aliquots to place on the macrocarrier, vortex continuously to reduce aggregation
- Leave on ice

4. Slowly open the main valve of the helium cylinder until gas pressure registers on the first gauge of the regulator. Turn the regulator adjusting screw clockwise and adjust the pressure to 1700 psi (200 psi above the burst pressure of the selected rupture disk)
5. Seat a macroprojectile into a macroprojectile holder using the seating tool. Pipette 10 ul of prepared microcarriers onto the macrocarrier; allow drying about 1 min in hood.
6. Unscrew the assembly containing the rupture disk inside and screw it back on to the acceleration tube. Gently tighten with torque wrench provided.
7. Remove the microcarrier launch assembly from the sample chamber and do the following: remove the lid, adjust the spacer rings, and put a sterile stopping screen in place, and install the macrocarrier holder (with the dried DNA-coated particles facing down). Put the lid back on and place the whole assembly in the second shelf slot in the chamber.

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Cell culture medium: f/2-Si
Reagents: $CaCl_2$, Spermidine

Apparatus: PDS-1000/He Microprojectile Accelerator (BIO-RAD)

Plasticware: cell culture dishes

Additional information

A Falciatore, R Casotti, C Leblanc, C Abrescia, C Bowler (1999) Transformation of Nonselectable Reporter genes in Marine Diatoms. Marine biotechnology New York NY 1(3): 239-251

http://www.bio-rad.com/LifeScience/pdf/Bulletin_9075.pdf

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8. Place the Petri dish containing the sample on the dish holder at the desired level. Close the bombardment chamber door.
9. With the power on, start the vacuum pump and turn the central switch to vac.
10. When the vacuum gauge shows 23 in Hg turn the switch to hold.
11. Press the fire switch continuously until the rupture disk bursts and the helium pressure gauge drops to zero.
12. Turn the central switch to vent to release the vacuum
13. Open the bombardment chamber door and remove the plate
14. Unload the macrocarrier and stopping screen from the macrocarrier launch assembly. Unload the spent rupture disk
15. Keep the cells at 18 °C in ligh-dark cycle (12h-12h)
16. After 48 h transfer the cells on plates containing phleomycin 50 ug/ml
17. The transgenic cells will be resistant to the antibiotic and will form colonies in 2 weeks