Tetraselmis transformation by microprojectile bombardment

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Abstract

Procedure for the transformation of the nuclear genome of *Tetraselmis striata* by microprojectile bombardment.

Citation: Fulei Luan, Xiaoxue Wen, Patrick Beardslee and Heriberto Cerutti Tetraselmis transformation by microprojectile bombardment. **protocols.io**

dx.doi.org/10.17504/protocols.io.hjtb4nn

Published: 04 Apr 2017

Guidelines

The overall bombardment protocol has been modified from that used for chloroplast transformation of *Chlamydomonas reinhardtii* (Boynton and Gillham, 1993).

References

Boynton J. E. and Gillham N. W. (1993) Chloroplast transformation in *Chlamydomonas*. *Meth. Enzymol.* 217: 510-536.

Guillard R. R. L. and Ryther J. H. (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Can. J. Microbiol.* 8: 229-239.

Protocol

Step 1.

Materials

- Sterile liquid f/2 medium (permissive for the target strain, without any selective agent). [*This will be used to wash the cells by centrifugation and for cell resuspension. Note: The concentration of the original 'f medium' (Guillard and Ryther, 1962) has been reduced by half].*

- Sterile centrifugation bottles and tubes.

- Petri plates (10-cm diameter) of solid f/2 medium (1.5% agar).

- Sterile deionized water.

- Linearized plasmid DNA with an appropriate selectable marker (at 1.0 µg DNA/µL). (*Rps12PRO:Bar, conferring resistance to glufosinate, restriction enzyme digested overnight and purified by phenol/chloroform extraction)*

- 2.5 M $CaCl_2$, sterile (*Sterilize by filtration)*.

- 0.1 M Spermidine free base, sterile *(Freshly prepared and filter sterilized).*

- Macrocarriers for Helium gun, sterile. (*Sterilize by dipping in isopropanol and allowing to dry on sterile filter paper placed in a laminar flow hood*).

- Stopping screens for Helium gun, sterile. (*Sterilize by autoclaving*).

- Sterile microcentrifuge tubes and tips.

- Sterile filter papers and forceps.

- Recovery liquid growth medium, sterile. Eight-mL aliquots of f/2 medium in 50-mL capped, sterile polypropylene tubes.

Step 2.

Grow *Tetraselmis striata* (UTEX B 2565 or related strain) to a density of 5 x 10^5 cells/mL (mid-logarithmic phase) in liquid f/2 medium under continuous illumination (150 µmol m^{-2} s^{-1} photosynthetically active radiation) on an orbital shaker (190 rpm) at 23°C and ambient levels of CO_2. Approximately 1.0 L of culture at mid-logarithmic phase is needed for 24 bombardments.

Step 3.

Collect cells by centrifugation in sterile centrifugation bottles at room temperature (4,500*g* x 5 min). Discard supernatant.

Step 4.

Resuspend cells in 1/30 the initial volume of f/2 medium and pool into a single sterile centrifugation tube.

Step 5.

Collect cells by centrifugation at room temperature (4,500*g* x 5 min). Discard supernatant.

Step 6.

Resuspend cells in 6.0 mL f/2 medium (8.5 x 10^7 cells/mL). Count a 1/100 dilution with a hemacytometer under the microscope. Adjust the volume to obtain a concentration of 8.0 x 10^7 cells/mL.

Step 7.

Plate 250 µL of cell suspension evenly on a f/2-agar plate (10-cm diameter Petri plate). Allow the liquid to dry (protect from light to avoid phototactic movements of the cells). (*This procedure yields a uniform monolayer of cells on the surface of the solid f/2 medium*).

Step 8.

While plates are drying prepare the microprojectiles for bombardment.
A 20-mg aliquot of gold particles (0.6 µm in diameter, Bio-Rad) is added to a 1.5-mL microcentrifuge tube with 200 µL absolute ethanol, vortexed for 1 to 2 min and spun in a microcentrifuge for 30 sec. The pellet is then washed twice with 300 µL sterile deionized water. The pellet is finally resuspended in 250 µL sterile deionized water. If necessary, sonicate briefly to achieve uniform resuspension.

Step 9.

In a sterile 1.5-mL microcentrifuge tube, add in order:

- 50 µL gold particles resuspended in water

- 5 µL transforming DNA (1.0 µg DNA/µL)

- 50 µL $CaCl_2$ (2.5 M)

- 20 µL Spermidine free base (0.1 M)

Step 10.

Vortex for at least 3 min at room temperature and allow the tube to sit at room temperature for an additional 5 min, spin in a microcentrifuge for 10 sec and remove as much supernatant as possible.

Step 11.

Wash the pelleted particles with 250 µL of absolute ethanol, finger flick several times to resuspend, vortex briefly, and spin again.

Step 12.

Resuspend the pellet in 50 µL of absolute ethanol. Finger flick and vortex for 5 min to make sure to obtain uniform resuspension. (*These particles are enough for four bombardments).*

Step 13.

In a laminar flow hood, pipet 12 µL of the resuspended particles in the center of each macrocarrier and allow to dry at room temperature. *(Make sure that particles are well resuspended, by finger flicking or brief sonication, before dispensing on the macrocarriers)*

Step 14.

For the PDS-1000/He Particle Delivery System (Bio-Rad), dip a 1350- or 1550-psi rupture disk in isopropanol and immediately install it in the retaining cup. Place the stopping screen and the macrocarrier launch assembly in the first slot from the top of the chamber. Place the plate of target

cells (without the lid) in the third or fourth slot from the top of the chamber. Close the chamber, pull a vacuum of 20 in. Hg and bombard cells as described in the operation procedure for the PDS-1000/He system. *Negative controls are bombarded with gold particles coated with plasmid DNA lacking a selectable marker.* [*Parameters that can be optimized for different strains include: Helium pressure (rupture disks), vacuum in the chamber, and distance from the macrocarrier holder to the target plate*].

Step 15.

One to three hours after bombardment, resuspend the cells from each plate by adding 2.0 mL of f/2 medium and loosening the cell lawn by rubbing the surface of the agar with a glass spreader. Transfer the resuspended cells, under sterile conditions, to a tube containing 8 mL of f/2 medium and allow them to recover under dim lights for 18 h.

Step 16.

Add glufosinate to each recovery tube, to a final concentration of 15 µg/mL, and incubate cells for 7 days under standard culture conditions (see step 2).

Step 17.

Pellet cells by centrifugation, resuspend the pellet in fresh f/2 medium containing 30 µg/mL glufosinate and incubate cells for an additional 7 days under standard culture conditions.

Step 18.

After this selection in liquid medium, transfer cells to a sterile centrifuge tube (avoiding dead cells sticking to the walls of the tube), pellet cells by centrifugation and resuspend the pellet in 1.0 mL of f/2 medium. Spread 250 µL-aliquots of resuspended cells onto each of four selective plates (f/2 medium containing 110 µg/mL glufosinate, 1.5% agar). Seal plates with Parafilm and incubate for 4 weeks under continuous illumination (150 µmol m^{-2} s^{-1} photosynthetically active radiation).

Step 19.

Transfer colonies appearing on the selective plates (individually, with a sterile toothpick) to fresh plates with higher concentration of the herbicide (f/2 medium containing 150 µg/mL glufosinate, 1.5% agar) and incubate as before. Examine incorporation of the transforming DNA, by PCR and/or Southern hybridization, in the colonies surviving the second round of selection.