Transfection of O. marina using particle bombardment

Pia Elustondo[1], Claudio Slamovits[1]

[1]Dalhousie University

dx.doi.org/10.17504/protocols.io.ui7euhn

Oct 11, 2018

In devel.

Protist Research to Optimize Tools in Genetics (PROT-G)

Pia Elustondo

PROTOCOL STATUS

In development

We are still developing and optimizing this protocol

Concentration of the O marina cultures

1

O marina are fragile and a high number of organisms die if subjected to high speed centrifugation. In contrast, since they are strong swimmers, if the speed used to pull them down is too slow, it is difficult to force them to pellet. We adapted two methods to increase the numbers and survival of organisms in the samples. (Osma et al 2016 (167): 136-147; Almeda et al 2011, (56): 403-414).
a. 4 X 50 ml tubes are incubated at 4 ºC (in the fridge) for 30 min or at 16 ºC for 1-2 h. This step slows down their movement
b. Centrifuge 2 tubes at a time for 10 min at 10 C, 500 g (while keeping the other two tubes in the fridge or in the 16 ºC incubator)
c. Discard carefully the top 40 ml and combine the pellets.
d. Check the pellets under the microscope.

This can be done the day before or the same morning of the transfection

Linearization of the DNA that will be transformed into O marina

2 Digest at least 10 ug of DNA for each transformation. Ideally cut near the 3' end of your gene of interest in the plasmid
Clean up de digested DNA. We used QiaEx II (QIAGEN), but any method is acceptable.

Preparation of the reagents for the particle bombardment

3

The particle bombardment protocol was adapted from the protocol developed by the Research team at Mara Renewables Corp and published in Biothechnol and Biofuels (Merkx-Jacques et al (2018) 11: 248) where they used this approach to transform Thraustochytrid T18.

a. Sterilize by autoclaving: the macrocarrier holders, stoppin screens , rupture disks, retaining caps and microcarrier launch assembly and 2 ml eppendorf tubes.
b.Prepare 1 % agarose in F/2 plates
c. Sterilize circular paper filter disks (4.25 cm) by placing them in Petri dishes and microwave for 3 min in intervals of 1 min each (letting them cool down between cycles)
d. Prepare sterile complete F/2 media
e. Prepare 50 % sterile glycerol
f.Prepare 70 % isopropanol
g. prepare 0.1 M spermidine (free base, tissue grade). It can be aliquoted in the -20 ºC freezer
h. Prepare 2.5 M CaCl2. It can be aliquoted in the -20 ºC freezer

Preparation of the gold particles

4 This preparation can be done in advance and stored at -20 ºC

a. Weight out 20 mg of 0.6 um gold particles ina 1.5 ml sterile Eppendorf tube
b. Add 1 ml of 70 % Ethanol
c. Vortex 5 min
d. Allow the particles to soak in the 70 % Ethanol for 15 min
e. Pellet the particles by spinning for 5-10 s at high speed
f. Discard the supernatant
g. Repeat the following wash steps three times:
    1. Add 1 ml of sterile water
    2. Vortex 1 min
    3. Allow the particles to settle for 1 min
    4. Pellet by briefly spinning
    5. Discard the fluid
h. Re-suspend the particles in 500 ul of 50 % glycerol to bring the particles to a final concentration (60 mg/ml). Store at -20

Preparation of the cultures on the (agarose) plates

5 Prepare 1 % agarose plates in F/2 media. Autoclave and pour very thin plates (0.25 -0.5 cm)
In addition , sterilize filter paper disks by heating them in the microwave in three cycles of 1 min each. I put them separately in petri dishes to make sure they are sterilized.

Righ before starting:
Apply one filter paper disk to each agarose plate and cover with 500 ul of the concentrated culture (this step is still under revisio; wer are trying to work with very concentrated cultures). The laboratory that works with Thraustochytrids uses usually a 1 OD of cells pelleted and resuspended in 100 µl. Following the application on the agar plates they let them dry. We cannot do this with O marina.

Coating the gold particles with the DNA to transfect

6
a. Transfer 25 µl of gold particle suspension (gold particle stock: 60 mg/ml) into a 2 ml Eppendorf tube and vortex. Add 2.5 - 5 µg of DNA while vortexing and continue vortexing for 3-5 min. The amount of DNA is variable and depends on the nature of the construct.
b. Carefully add to the walls of the tubes 25 µl of 2.5 M CaCl1 and 10 µl of 0.1 M spermidine, mix on the walls of the tube and then tilt back the tube up while vortexing to prevent clumping.
c. VORTEX for 3 min.
d. Incubate on ice for 1 min
e. Spin for 10 s at full speed and discard the supernatant
f. Add 70 µl of 70 % Ethanol. Re-suspend, vortex and spin down. Discard supernatant.
g. Add 70 µl of 100 % of Ethanol. Re-suspend, vortex and spin down. Discard supernatant.
h. Re-suspend in 26 µl of 100 % Ethanol by gently vortexing for 30 s
i. In the cell culture hood, spread 12 µl on the center of each macrocarrier disc and let them dry.

Bombardment of the cells

7 Biolistic PDS-1000/He Particle delivery system (Bio-Rad)

a. Test the rupture disk with the highest pressure (no cells, no gold particles)
b. Sterilize the rupture disks by soaking them in 70 % isopropanol just prior to insertion in the retaining cap. They are assembled and placed on the top of the biolistic system chamber. REMEMBER THE STOPPING SCREEN!!!
c. Sterilize the target shelf by whipping it with 70 % EtOH and drying it in the sterilie flowhood
d. Shooting parameters: for the 1100 psi rupture discs: Bring the Hg pressure to 25, press hold , fire. For the 1350 psi bring the Hg pressure to 27.5, press hold and fire. The switching should be very fast because the cells are subjected to vaccum before the rupture
e. Shoot each plate twice, rotate the plate 90 degrees between each shot.
f. After bombardment, the cells are checked under the microscope and F/2 complete media is added to the plateg.
g. Selection antibiotic (Bleomycing 15 ug/ml or Puromycing 10 ug/ml) was added two days after the transfection to allow for recovery and protein synthesis.