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Biolistic transformation of *Pseudo-nitzschia multistriata*

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Pseudo-nitzschia multistriata, biolistic transformation

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- absolute EtOH
- *Pseudo-nitschia multistriata* cell culture
- F/2 medium (Guillard, 1975)
- agarose
- 100x20 mm Petri dishes
- Gold microcarriers 0.6 µm diameter (BIORAD)
- $CaCl_2$ 2.5M
- spermidine 0.1M
- DNA plasmids
- 1550 PSI rupture disks (BIORAD)
- PDS-1000/He stopping screens (BIORAD)
- PDS-1000/He macrocarriers (BIORAD)
- PDS-1000/He macrocarrier holders
- zeocin (InvivoGen)

Before starting

1 Sterilize stopping screens and macrocarrier holders by autoclaving.

2 Prepare Gold microcarriers as below:

2.1 Place 60 mg of dry gold microcarriers in 1 ml of 100% EtOH in a microcentrifuge tube.
Vortex on high speed for 1-2 minutes.
Repeat 3X.

2.2 Pellet microcarriers in microcentrifuge (1 minute at max speed).
Wash 2X in 1 ml of sterile distilled water.
Suspend in 1 ml of sterile water and aliquot 50 ul into sterile microcentrifuge tubes (vortex continuously while pipetting).
Store at -20°C.

First day: Cells plating

3 Plate 5 x 10^6 cells of *P. multistriata* culture in exponential phase (1.5-2 x 10^5 cells/ml) on 0.4% agarose in F/2 plates. Make a circular cell mat of diameter 4 cm.

Microcarriers prepartion

4 To 50 ul of the microcarriers in water, add in order, while continuously vortexing:
- 3 ul target plasmid (1ug/ul) + 3 ul selection plasmid (1ug/ul)

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- 50 ul of $CaCl_2$ 2.5M
- 20 ul of spermidine 0.1M

5 Continue vortexing for 3 min.

6 Centrifuge for 10 sec at 13000 rpm.
Remove as much supernatant as possible.

7 Wash with 250 ul EtOH 100%.
Spin again.
Centrifuge for 10 sec at max speed.
Remove supernatant.
Resuspend in 60 ul EtOH 100%.
Leave on ice.

PDS-1000/He Microcarrier accelerator preparation

8 Sterilize rapture disks and macrocarriers by soaking for 15 min in 70% EtOH.
Allow them to dry in sterile hood.

9 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 to pressure of 1800 psi (200 psi above the burst pressure of the selected rapture disks).

Biolistic shot

10 Seat macrocarriers into macrocarrier holders.

11 Pipette 10 ul of the prepared microcarriers onto the macrocarrier, while contonuously vortexing.
Allow drying about 1 minute in hood.

12 Unscrew the assembly containing the rapture disk inside and screw it back on to the acceleration tube.
Gently tighten with torque wrench.

13 Remove the microcarrier launch assembly from the sample chamber and do the following:
- remove the lid,

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- adjust the spacer rings,
- put a sterile stopping screen in place,
- install the macrocarrier holder (with dried DNA-coated particles facing down),
- put the lid back on and place the whole assembly in the second shelf slot in the chamber,
- place the Petri dish containing the sample on the third shelf slot,
- close the bombardment chamber door.

14 With the power on, start the vacuum pump and turn the central switch to hold.
Press the fire switch continuously until the rapture disk bursts and the helium pressure gauge drops to zero.
Turn the central gauge to vent to release the vacuum.

15 Open the bombardment chamber door and remove the plate.
Unload the macrocarrier and stopping screen from the macrocarrier launch assembly.
Unload the spent rapture disk.
After all shots, close the main valve and shoot until bottle pressure goes to zero.

Cells recovery

16 Keep the cells at 18°C in light-dark cycle (12h-12h).

17 After almost 4/5 hours, detach cells from the solid medium by adding 5 ml of F/2 liquid medium without selection and gently pipetting.
Transfer on flasks in a total volum of 200 ml of medium without selection.

Second day: Cells selection

18 After 24 h add zeocin 1ug/ml to flasks.
The transgenic cells will be resistant to antibiotic and will be visible in 2 or 3 weeks.

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