Biolistic Transformation of Pseudo-nitzschia multistriata and Pseudo-nitzschia arenysensis

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Abstract

This protocol allows transformation of two species of *Pseudo-nitzschia* through biolistic method.

With this method it is possible to insert one (transformation) or two (co-transformation) plasmids in the cells.

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Protocol

To be completed in advance
Step 1.

Prepare plasmid with your gene of interest at the concentration of 1 µg ml^{-1}.

To be completed in advance
Step 2.

Grow wild-type cells in f/2 medium up to mid log-phase.

To be completed in advance
Step 3.

Prepare f/2 plates (0.4% agarose in f/2 medium) and leave to dry under hood.

The day before bombarding
Step 4.

Count the cells. For each plate between 3-5x10^{7} cells are needed.

The day before bombarding
Step 5.

Centrifuge the cells at 2465 g and resuspend the pellet in a very small volume of f/2 medium.

The day before bombarding

Step 6.

Spread the cells onto agarose plates. Leave to dry under hood.

The day of the transformation

Step 7.

DNA precipitation is carried out using Tungsten M-10 microcarrier 0.7 µm particles (BioRad).

The day of the transformation

Step 8.

Dilute the tungsten particles in a final concentration of 3 mg in 50 µl of water for plate.

The day of the transformation

Step 9.

Add to particles 5 µl of plasmid (1 µg μl^{-1}) (for the transformation) or 3 µl of each plasmid (1 µg μl^{-1}) (for the co-transformation), 50 µl 2.5 M $CaCl_2$, and 20 µl of 0.1 M spermidine. Continue vortexing for 3 min, then microfuge for 10 sec and remove supernatant. Wash with 250 µl 100% ethanol, microfuge again, then resuspend in 60 µl ethanol. Keep tin ice.

The day of the transformation

Step 10.

Wash rupture disks and macrocarriers in ethanol and allow to dry.

Bombardment

Step 11.

Bombardment is carried out in a Biorad PDS-1000/He microprojectile accelerator. Shooting is carried out using 1,550 PSI rupture disks. Plates for transformation are placed in the middle slot immediately under the macrocarrier.

Bombardment

Step 12.

10µl aliquot of the prepared particles were transferred to the centre of each macrocarrier. Allow to dry.

Bombardment

Step 13.

Keep the plates in incubator overnight.

The day after the bombardment

Step 14.

Resuspend the cells (each plate) in 200 ml of f/2 medium with antibiotic. *Pseudo-nitzschia* cells grow better in liquid medium.

The day after the bombardment

Step 15.

Usually untransformed *Pseudo-nitzschia* cells are dead at 10-12 days post-application of antibiotic.