Biolistic dye loading protocol for marine phytoplankton

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Abstract

A method to load algal cells with dextran-conjugated fluorescent dyes. This is a good way of testing and optimising conditions for biolistic DNA delivery.

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Protocol

Prepare gold partic

Step 1.
Weigh out 30 mg of 0.6 mm gold particles

Step 2.
Wash 3x with 1 ml 70% ethanol

Step 3.
Resuspend in 500 µl 70% ethanol (final concentration 60 mg/ml)

Step 4.

Mix gold thoroughly, take 60 µl into new tube

Step 5.
Spin 13000 rpm, 1 min

Step 6.
Remove supernatant

Step 7.

Wash with 200 µl deionised water - sonicate - centrifuge - remove supernatant

Step 8.

Add 8 µl of 8 mM FITC-dextran

Step 9.
Mix very thoroughly

Step 10.

Pipette 2 µl of mixture onto four carrier discs and allow to dry

Step 11.

Store at 4°C until use

Preparation of algal cells

Step 12.

Take 15 ml of exponentially growing algal culture (at approximately 5 x10^5 cells/ml). Healthy cells are essential.

Step 13.
Spin 3000 rpm, 3 min

Step 14.

Remove supernatant. Resuspend cells in 1 ml of loading buffer (10 mM HEPES pH 7.5, 1 M sorbitol, 200 mM potassium glutamate)

Step 15.
Spin 3000 rpm, 1 min

Step 16.

Remove supernatant using pipette

Step 17.

Add 50 µl of loading buffer, mix very gently

Step 18.

Pipette onto nitrocellulose filter (0.2 µm) positioned on the base of 35 mm Petri dish. Spread cells thinly using pipette tip. Allow excess liquid to soak into filter, but do not allow to dry.

Operation of biolistic device

Step 19.

Set up Bio-Rad PDS-1000 biolistic device. Insert rupture disk (1100 psi).

Step 20.
Place dish containing algal cells on second lowest shelf

Step 21.
FIRE!

Step 22.
Add 1ml of f/2 seawater media.

Step 23.
Spin 2000 rpm, 1 min

Step 24.
Remove supernatant (this is necessary to remove background dye fluorescence)

Step 25.
Resuspend cells in 1 ml of f/2 seawater media

Step 26.

Examine cells for dye fluorescence under fluorescent microscope.