Transformation of Skeletonema marinoi using Multipulse Electroporation

Oskar N. Johansson., Adrian K. Clarke

Abstract

The following transformation protocol is designed for the insertion of linear DNA constructs into the nuclear genome of Skeletonema marinoi by non-homologous recombination.

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Protocol

Cell preparation

Step 1.

a) Grow two 400 mL cultures in Artificial Seawater with f/2+Si supplements (Growth media) for ca. one week until dense. The standard growth conditions used in our lab are 16°C, 50-70 µmol. photons m-2 s-1, 16 h photoperiod.

b) Reduce the volume of the sedimented culture in each flask to 50 mL by suction. Resuspend cells by vigorous agitation to minimize chain length and then transfer them to a 50 mL centrifuge tube.

c) Pellet cells by centrifugation (1200 x g, 5 min, 4°C, swing-out rotor). Decant supernatant and resuspend the pellet in each tube in 10 mL of ice-cold 0.3M sorbitol.

d) Pellet cells once more by centrifugation (1200 x g, 5 min, 4°C, swing-out rotor) and resuspend in 2.5 mL of ice-cold 0.3M sorbitol. Pool both resuspensions in a single tube.

e) Store the cell suspension on ice until needed.

ANNOTATIONS
Jon Udell 17 Feb 2017

Test

ElectroporatEquipment

Step 2.

This protocol was tested using the Gene Pulser Xcell electroporator (BioRad, USA) with the recommended 2 mm cuvettes (BioRad, USA).

Combining the cell suspension with desired DNA

Step 3.

Add 3-5 µg of the linear DNA construct to a 100 µL cell suspension in a 2 mm electroporation cuvette and leave for 3-5 min at 4°C.

NB. DNA construct should include a selectable marker such as antibiotic resistance (e.g., zeocin/bleomycin)

Electroporation

Step 4.

Ensure cells are resuspended in the cuvette prior to electroporation. Electroporation is performed by the following steps:

a) Poring pulses (300 V, 6 pulses, 1 sec pulse Interval, 5 ms pulse length)

b) Transfer pulses (40 pulses [10x4], 50 ms pulse length, 0.1 s pulse interval)

c) Reverse direction of the cuvette.

d) Repeat transfer pulses (40 pulses [10x4], 50 ms pulse length, 0.1 s pulse interval)

Cell recovery

Step 5.

Transfer the electroporated cells from the cuvette using a Pasteur pipette to 30 mL liquid growth media (without selection) and leave for 48 h under standard growth conditions

Apply Selection

Step 6.

Add selection to the media and continue growth for another 48 h

Transfer to Solid media

Step 7.

a) Pellet cells in 50 mL centrifuge tubes (1200 x g, 5 min, room temperature) and resuspend in 1 mL growth medium with selection.

b) Spread 75 (for 7 cm diameter plates) or 250 µL (for 15 cm diameter plates) of the resuspension on growth media plates (f/2 + Si + selection, 0.9% Agar).

Left under standard growth conditions, colonies should appear after 2-3 weeks.