Electroporation of Fluorescein into the coral symbiotic dinoflagellate alga Symbiodinium sp.

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Abstract

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Protocol

Harvest cells

Step 1.

Collect approximately 5 x 10^7 Symbiodinium cells by centrifugation at 1,000 x g for 3 min.

The strain we are using is SSB01 (clade B phylotype, a generous gift from Profs. John R. Pringle and Arthur R. Grossman), which is an axenic uni-algal strain closely related to the genome-sequenced strain *S. minutum* Mf1.05b (clade B).

References:

Shoguchi et al. 2013 Curr Biol, DOI:10.1016/j.cub.2013.05.062

Xiang et al. 2013 J Phycol, DOI:10.1111/jpy.12055

Washed cells with mannitol solution

Step 2.

Discard the supernatant after STEP 1, and resuspend the pellet in 1 mL of mannitol solution (0.77 M mannitol in 10% culture medium). Repeat STEP 1 and discard the supernatant.

We are using IMK medium for culturing (see the EXTERNAL LINK below).

LINK:
http://mcc.nies.go.jp/02medium-e.html#imk

Prepare for electroporation

Step 3.

Suspended the cell pellet in 0.15 mL of mannitol solution.

Add 7.5 microL of Fluorescein (Sigma #46955) stock solution (13.2 mM in EtOH) to the cell suspension and mix by pipetting.

Transfer the cell suspension to a 2-mm gap electroporation cuvette.

Step 4.

Electroporate using NEPA21 electroporator. Electroporation condition is as follows: square electric poring pulses are applied at 300 V (pulse duration, 10 ms; 4 pulses; interval 50 ms; 20% decay rate), followed by transfer pulses at 8V (pulse duration, 50 ms; 5 pulses for each direction; interval 50 ms; 40% decay rate).

Immediately after electroporation, add 1 mL of culture medium to the cuvette and transfer the cells to a 1.5-mL tube.

Collect cells by centrifugation at 2,000 x g for 3 min and wash twice with 0.5 mL of culture medium.

LINK:
http://www.nepagene.jp/e_products_nepagene_0001.html

Microscope

Step 5.

Examine electroporated cells to detect green fluorescence under the fluorescence microscope.