Electroporation of natural communities in sea water

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Abstract

This protocol has been optimized for electroporation of natural communities in coastal surface waters. As natural communities may vary in different places we recomend to use this protocol as a starting point and re-optimize according to the results. Guidelines are provided.

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Before start

It is really important to carefully plan the sampling of sea water taking tidal and wave conditions into account. The abundance of the sea water communities may be reduced when samples are taken in periods of rough tides.

To increase the recovery of cells, make sure to use large volumes of sea water (50 liters). Samples can be stored overnight to be processed the day after, but we do not recomend to use sea water samples kept for more than 2 days.

Materials

Dextran Fluorescein 2.000.000 MW D7137 by Thermo Fisher Scientific
Sorbitol 800 mM Solution by Contributed by users
Gene Pulser Electroporation Cuvettes, 0.2 cm gap 1652086 by Bio-rad Laboratories
Mixed cellulose esters filters, 1,2 um, 47 mm RAWP04700 by Merck Millipore
conical tubes, 50ml by Contributed by users
Salmon Sperm DNA Carrier 15632011 by Thermofisher
L1 Algae Medium by Contributed by users
12x75 mm high clarity polypropylene test tubes 352063 by Corning

Electroporation System Gene Pulser XCell by Bio-rad Laboratories

- TFF concentrated cells by Contributed by users
- Vacuum pump with a support system for 47 mm filters by Contributed by users
- P1000 micropipet and Tips by Contributed by users

Protocol

Preparation of electrocompetent cells

Step 1.

Filter the TFF concentrated sample using a 1.2 um 47 mm filter disc. Use a pressure lower than 100 mm Hg. Once filtered, wash 5 times with 10 mL of Sorbitol 800 mM solution. For this step, before adding sorbitol allow the flow through to completely pass the filter, stop the pump, and then gently pour the sorbitol solution allowing it to completely cover the filter before restarting the pump to maximize the filter surface to be washed.

REAGENTS

- Sorbitol 800 mM Solution by Contributed by users

  Mixed cellulose esters filters, 1,2 um, 47 mm RAWP04700 by Merck Millipore
- Vacuum pump with a support system for 47 mm filters by Contributed by users

Step 2.

Recover the filter using clean tweezers, introduce it into a 50 mL clean conical Falcon tube. Be careful to leave the cells towards the tube's lumen. Add 5 mL of Sorbitol solution to the tube and detach the cells from the filter by pipetting sorbitol directly into the filter using a micropipette. Repeat until no more material can be detached from the filter.

REAGENTS

- Sorbitol 800 mM Solution by Contributed by users
- conical tubes, 50ml by Contributed by users
- P1000 micropipet and Tips by Contributed by users

Preparation of the electroporation reaction.

Step 3.

Prepare the electroporation mix according to the following instructions (final volume: 100 uL). Consider duplicates for each sample, and 2 different electroporation voltages.

- Dextran Green: 50 ug (0.5 mg/mL final concentration)
- Salmon Sperm DNA Carrier: 25 ug (250 ug/mL final concentration)
- Expression Vector: 2 ug
- Sorbitol 800 mM: up to 100 uL total volume

REAGENTS

Dextran Fluorescein 2.000.000 MW D7137 by Thermo Fisher Scientific

- ✓ Sorbitol 800 mM Solution by Contributed by users

Salmon Sperm DNA Carrier 15632011 by Thermofisher

- ✓ Expression Vector by Contributed by users

Electroporation

Step 4.

Setup the electroporator to "time constant" mode. Create the following programs:

1. V = 1 kV, Time constant = 20 ms
2. V = 2 kV, Time constant = 8 ms

Transfer an electroporation reaction into a 0.2 cm electroporation cuvette, run the electroporation program. Once finished, transfer the reaction into a 12x75 mm polypropylene tube pre-filled with 3 mL of L1 algae medium. Once finished with the electroporation reactions, transfer the tubes to an incubator that mimics the enviromental conditions of your isolate.

NOTE: electroporation cuvettes can be washed and re-used, however this may increase the variability in the electroporation efficiency.

REAGENTS

Gene Pulser Electroporation Cuvettes, 0.2 cm gap 1652086 by Bio-rad Laboratories

- ✓ L1 Algae Medium by Contributed by users

12x75 mm high clarity polypropylene test tubes 352063 by Corning

Electroporation System Gene Pulser XCell by Bio-rad Laboratories

Cytometric Analysis of expression

Step 5.

Check expression at different time points. Expression profiles may vary from experiment to experiment depending on the composition of the community the day of sampling. Make sure to set the treshold (trigger) of your instrument to chlorophyll in order to detect phytoplankton more efficiently and decrease noise. Electroporated cells will incorporate Dextran green and therefore will be fluorescent at 530 nm (excitation: 488 nm).
Make sure to have a non electroporated control with dextran green to account for non specific binding of the dye to the community.

REAGENTS

- ✓ Flow Cytometer by Contributed by users

Warnings

1) If expression is going to be checked using a flow cytometer, make sure that the machine's treshold (trigger) is set to chlorophyll fluorescence (Ex: 488 nm, Em: 692 nm).

2) Dextran green incorporation may vary between replicates. It is important to prepare at least duplicates for each experimental condition to maximize the likelihood of having efficiently electroporated cells.

3) Make sure the plasmidial DNA is properly desalted. To check for the presence of salt traces, a test electroporation reaction can be done using DNA diluted in molecular biology grade water. If DNA induces electrical arc, DNA has to be further desalted.