Electroporation of natural communities in sea water Version 3

Andrés Ramírez, Albane Ruaud, Fernan Federici, Peter von Dassow

Abstract

This protocol has been optimized for electroporation of natural communities in coastal surface waters. As natural communities may vary in different places, we recommend 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 recommend using sea water samples kept for more than 2 days.

Protocol

Preparation of electrocompetent cells

Step 1.

Concentate your sea water sample using a Tangential Flow Filtration (TFF) system to the desired volume.

REAGENTS
- TFF concentrated sea water sample by Contributed by users

Preparation of electrocompetent cells

Step 2.

Filter the concentrate using a 1.2 µm 47 mm filter disc. When filtering, use a pressure lower than 100 mm Hg.

REAGENTS

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

Preparation of electrocompetent cells

Step 3.

Once the sea water sample is filtered, wash 5 times with 10 mL of sorbitol 800 mM solution.

AMOUNT

10 ml Additional info:

REAGENTS

- Sorbitol 800 mM Solution by Contributed by users

NOTES

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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.

Preparation of electrocompetent cells

Step 4.

Recover the filter using clean tweezers, and introduce it into a 50 mL clean conical Falcon tube. Be careful to leave the cells towards the tube's lumen.

REAGENTS

- conical tubes, 50ml by Contributed by users

Preparation of electrocompetent cells

Step 5.

Add 5 mL of sorbitol 800 mM 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.

AMOUNT

5 ml Additional info:

REAGENTS

- Sorbitol 800 mM Solution by Contributed by users
- P1000 micropipet and Tips by Contributed by users

Preparation of the electroporation reaction

Step 6.

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 7.

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

REAGENTS

Electroporation System Gene Pulser XCell by Bio-rad Laboratories

Electroporation

Step 8.

Transfer an electroporation reaction into a 0.2 cm electroporation cuvette, and run the electroporation program.

REAGENTS

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

NOTES

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

Electroporation

Step 9.

Once finished, transfer the reaction into a 12x75 mm polypropylene tube pre-filled with 3 mL of L1

algae medium.

AMOUNT
3 ml Additional info:

REAGENTS
L1 Algae Medium by Contributed by users

12x75 mm high clarity polypropylene test tubes 352063 by Corning

Electroporation

Step 10.

Once finished with the electroporation reactions, transfer the tubes to an incubator that mimics the enviromental conditions of your isolate.

Cytometric Analysis of expression

Step 11.

Check chlorophyll expression at different time points using a Flow Cytometer.

REAGENTS
Flow Cytometer by Contributed by users

NOTES
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Expression profiles may vary from experiment to experiment depending on the composition of the community on the day of sampling. Make sure to set the threshold (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). In addition, make sure to have a non electroporated control with dextran green to account for non specific binding of the dye to the community.

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Controls for expression of fluorescent protein should include a sham DNA control, ideally the same plasmid backbone, at the same concentration, but without a fluorescent protein gene (or with a non-functional one).

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.