Method for electroporation of Isochrysis galbana CCMP1323

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Abstract

Citation: Glen Wheeler, Rowena Stern, Cecilia Balesteri Method for electroporation of Isochrysis galbana CCMP1323. **protocols.io**
dx.doi.org/10.17504/protocols.io.hmab42e
Published: 18 Apr 2017

Protocol

Step 1.

Prepare high concentration of plasmid DNA to allow for at least 2µg of DNA to be electroporated per sample in no more than 5 µl volume.

Step 2.

Grow I. galbana cells to 10^6 cells/ml (usually takes 1 week) in 50% salinity F/2.

Step 3.

Prepare electroporation buffer - ensure final pH is 7.2

500mM NaCl
5mM KCl
5mM CaCl2
20mM HEPES
200mM mannitol
200mM sorbitol

Filter sterilise (short term) or autoclave.

Step 4.

Remember to include a control (no DNA) sample. Add another sample if you want to calculate transformation efficiency

Step 5.

Spin down 100 ml of cells at 3000 rpm for 3 minutes, remove supernatant

Step 6.
Wash with 350µl of electroporation buffer

Step 7.
Spin down again at 3000rpm for 3 minutes, remove supernatant

Step 8.

Resuspend in 350 µl and count cells

Step 9.

Add DNA construct to cells then aliquot cells into a 4 mm wide sterile electroporation cuvette

Step 10.

Immediately take cells to the electroporator

Step 11.
Set electroporator to:a. HVb. 1800Vc. Pulse length: 50µSd. Pulses: 02e. Interval: 01 sf. Polarity: unipolar

Step 12.
Put cuvette in holder and press start

Step 13.
Record voltage and time settings afterwards

Step 14.
Immediately add 1ml of 50% salinity F/2 to cuvettes and place them in 18˚C incubator with light to allow them to recover for 2-4 hours. Take an aliquot of a control to check cell counts post electroporation.

Step 15.
Aliquot equal amount of samples into a 24 well tissue culture plate or tube and keep overnight in 18˚C incubator with light, dividing them into sufficient numbers to carry out replicates or different antibiotic concentrations. Do the same to your no DNA control. Always ensure you have a no NTC control (test sample) and an NTC control (no DNA control sample)

Step 16.
Next day, add NTC to your cultures to a final concentration of 150µg/ml. The NTC is kept at a stock concentration of 100mg/ml so approximately 3µl NTC/2ml of media.

Step 17.
Incubate at 18˚C with light and check every 4 days, taking aliquots to check for counts. It took approximately 7 weeks for substantial growth to be seen at 150µg/ml of NTC. There should be no growth in no DNA control with NTC, but growth in no DNA control grown without NTC. There should be growth in test sample without NTC and hopefully with NTC.