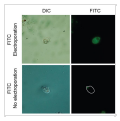

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Working

Electroporation transformation of FITC-dextran into Oxyrrhis marina - an early branching dinoflagellate.

Version 2

Nicholas Irwin[1], Elisabeth Hehenberger[1], Liz Cooney[1], Yoshihisa Hirakawa[2], Patrick Keeling[1]

[1]University of British Columbia, [2]University of Tsukuba

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Protist Research to Optimize Tools in Genetics (PROT-G)

Nick Irwin
University of British Columbia

ABSTRACT

Here we describe a protocol for transforming Oxyrrhis marina with FITC-labelled dextran (a DNA analog) by electroporation.

This protocol is reproducible, and results in a transformation efficiency of around 1%.

We have confirmed the viability of transformed cells by allowing them to recover in the dark (and thus maintain fluorescence intensity) and then by observing fluorescent cells swimming normally, days after transformation (see the video here).

NOTE ON AUTOFLUORESCENCE

Although *Oxyrrhis* is a non-photosynthetic dinoflagellate and therefore lacks plastid autofluorescence, we have observed a small amount of green autofluorescence that is typically isolated in the anterior of the cell (See top panel of figure 1).

The autofluorescence was observed in wildtype cells, cells electroporated without FITC-dextran, and cells that w

As of yet we have been unable to demonstrate the actual uptake of plasmid DNA. We have tried using rhodamine labelled plasmids to demonstrate this but the results were inconclusive due to issues with cellular autofluorescence.

We have also tried this method with a variety of expression plasmids encoding different promoters. For example, we tried the MOE promoter from *Perkinsus marinus* (a closely related species) and the generic eukaryotic CMV promoter. However no GFP fluorescence was detected in these samples.

We have also tried transforming *in vitro* synthesized GFP encoding mRNA using this protocol. As of yet, no GFP production has been detected, but this may reflect non-cannonical translational systems in dinoflagellates as the same mRNA could be translated in starfish embryos.

Although the development of expression systems in dinoflagellates remains a challenge, our results suggest that *O. marina* may be amenable to genetic transformation.

Spin down cells

1 Spin down cells at 3000xg for 10 minutes.

We tend to spin down 15-30 mL of saturated culture (cultures were fed *Dunaliella tertiolecta* and grown on a 16h/8h light/dark cycle at 20 degrees, cultures were grown in f/2 media)

If you can see a pink pellet there are likely enough cells.

The *O. marina* strain we are using is CCMP604.

Resuspend cells in electroporation buffer

2 Resuspend the *O. marina* pellet in 100 uL Biorad Gene Pulser Electroporation buffer.

Prior to resuspension we add FITC-dextran (Sigma FD70S) to 2 mg/mL in the electroporation buffer.

Electroporate the cells

3 Transfer the resuspended cells to a 0.2 cm cuvette.

Electroporate using a Biorad Gene Pulser Xcell using the following conditions:

Square wave pulse - 100 V, 3 x 5 ms pulses, 0.1 ms pause.

or for lower voltage conditions:

Square wave pulse - 75 V, 5 x 5 ms pulses, 0.1 ms pause.

We tried using the exponential protocol with short high voltage pulses (1000 V, 10 uF) and long low voltage pulses (100 V, 1000 uF) but in all instances the exponential protocol resulted in cell lysis.
We also tried raising the voltage with the square wave protocol (200V, 3 x 5 ms, 0.1 ms pause) but this also resulted in lysis.

After electroporation, add 1 mL of f/2 media to the cuvette and transfer the cells to a microcentrifuge tube.

Wash the cells and allow them to recover

4 After electroporation, spin down the cells for 3 minutes at 7000 xg and wash once with 1mL f/2 media.

We allow the cells to recover in 24-well plates for 24 hours before observing.

If you want to avoid photobleaching (ie. for FITC-dextran), you can allow the cells to recover in the dark.

A video demonstrating the results can be seen here: https://www.youtube.com/watch?v=8eHpX-GGwW0 (sorry for the low quality, the cells also seemed to be slightly squished beneath the slide)