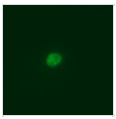

Jun 13, 2019

Electroporation of *Caecitellus sp.* with FITC-dextran

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In devel. dx.doi.org/10.17504/protocols.io.35kgq4w

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


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ABSTRACT

Caecitellus sp. is a small phagotroph belonging to the biocosoecid order of the stramenopiles. *Caecitellus* is found across global oceans but it's role in marine ecosystems, as with many heterotrophic flagellates, is unclear.

We isolated a strain of *Caecitellus* (small subunit rRNA gene was 99.53% identical to *Caecitellus parvulus* and *Caecitellus paraparvulus*) from a contaminated culture of *Oxyrrhis marina* and assessed its amenability to transformation.

Fluorescein isothiocyanate conjugated to dextran was successfully introduced into *Caecitellus* sp. using a high voltage exponential decay pulse (1000 V, 10 μF, ∞ Ω) from a BioRad GenePulser Xcell (cuvette width 0.2 cm). Cell viability was confirmed by observing motility and fluorescence 24 h after electroporation (Figure 1). Lower voltage pulses did not result in FITC uptake. Estimates of transformation effifiency were difficult to obtain due to the small size of the cells.

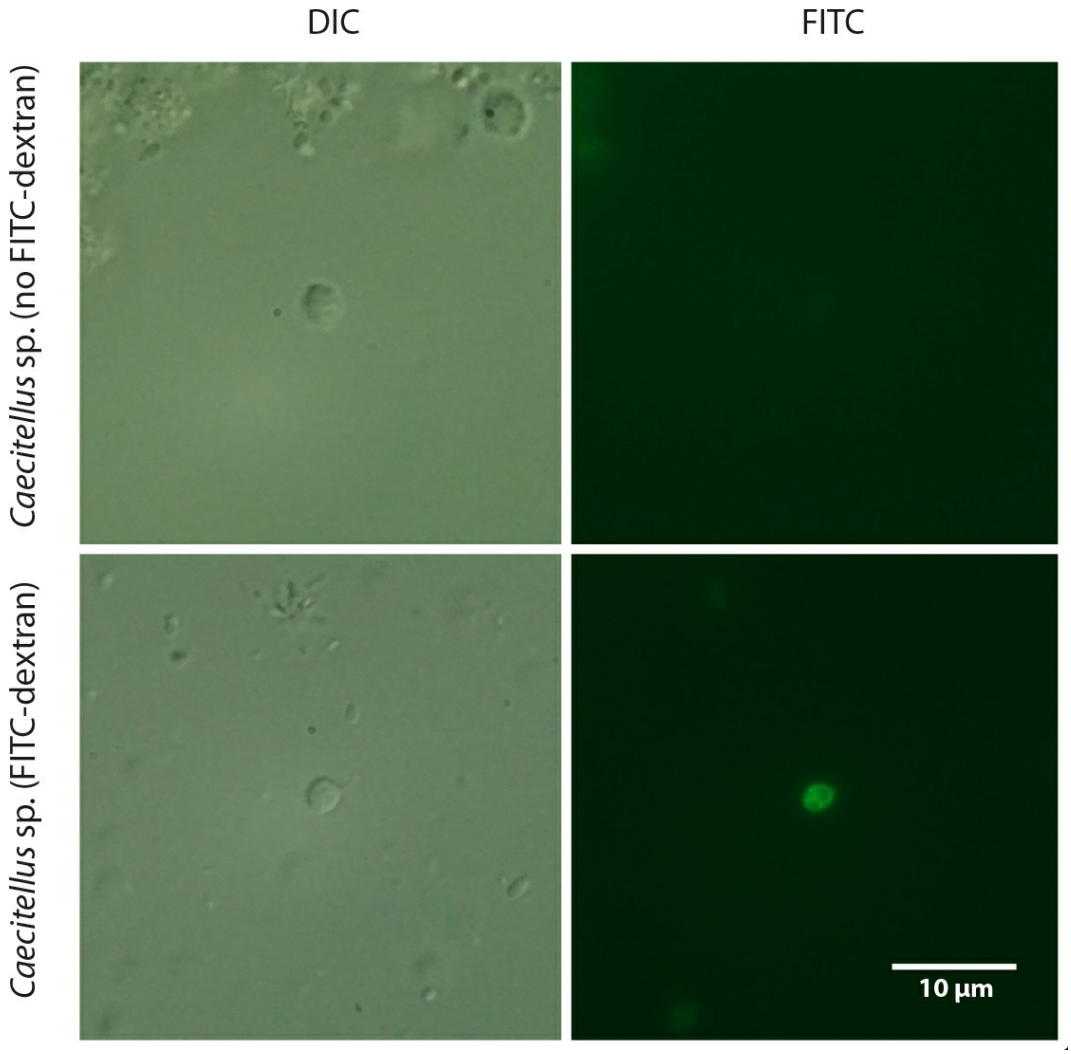

Figure 1. Electroporation of *Caecitellus* sp. with FITC-dextran.

Unfortunately, the isolated strain of *Caecitellus* perished in an incubator malfunction. However, these results may be transferable to *Caecitellus parvulus*, a model for heterotrophic flagellates.

Culturing

1 Cells were grown in f/2 media in a 20 C incubator on a 12:12 light:dark cycle.

Cells fed on natural populations of bacteria in the culture and did not need supplemental food.

Electroporation

2 Spin down cells at 3000 x g for 10 minutes.

Resuspend cells in 100 uL of BioRad Gene Pulser electroporation buffer with 2 mg/mL FITC-dextran.

Electroporate the cells using a high voltage exponential decay pulse (1000 V, 10 µF, ∞ Ω) from a BioRad GenePulser Xcell (cuvette width 0.2 cm).

After electroporation, add 900 uL of f/2 and transfer to a 1.5 mL centrifuge tube.

Wash the cells once by spinning at 7000 xg for 3 minutes, removing the supernatant, and resuspending in 1 mL fresh f/2

Place cells in a 24-well plate and allow them to recover at room temperature in the dark for 24 hours

Observations

3 Observe the cells 24 hours after electroporation.

Successful transformants will appear strongly fluorescent green and will be swimming.