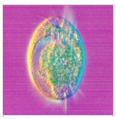

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Working

Electroporation of Euplotes crassus: conditions that allow for the uptake of DAPI.

Forked from Electroporation of Euplotes crassus: conditions that allow for the uptake of DAPI.

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

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ABSTRACT

The electroporation procedure and conditions described allow for the entry of DAPI into the cell, resulting in nuclear staining. We have not yet been successful in using these conditions for transformation of *Euplotes crassus*, but only a couple of constructs have been tried to date.

1 Grow a 600 ml culture of *Euplotes crassus* using *Dunaliella salina* grown in artificial seawater as the food source until all the algae is eaten, and then allow the *Euplotes* culture to starve an additional 24 hours.

2 Collect and concentrate cells down to ~40 ml by pouring the culture through a 20 um Nitex membrane.

Photo of 20um Nitex membrane and a frame constructed by cutting out the top and bottom of a Tupperware container.

3 Transfer the concentrated *Euplotes* cells to a 50 ml screw cap centrifuge tube and pellet cells by centrifugation at 1,000 rpm (~200 x g) for 1.5 min in a clinical centrifuge.

4 Remove all but the last 10 ml of the supernatant, resuspend cell pellet by gently flicking tube, and transfer resuspended cells to a 15 ml centrifuge tube.

5 Centrifuge cells at 200 x g for 1.5 min, remove all but the last ~100 ul of supernatant, and resuspend pelleted cells by gently agitating tube.

6 Add 1 ml of freshly prepared 0.3 M glucose to the tube of cells and gently mix.

7 Allow cells to equilibrate for 15 min at room temperature.

8 For electroporation, transfer 300 ul of cells to a 0.2 cm electroporation cuvette.

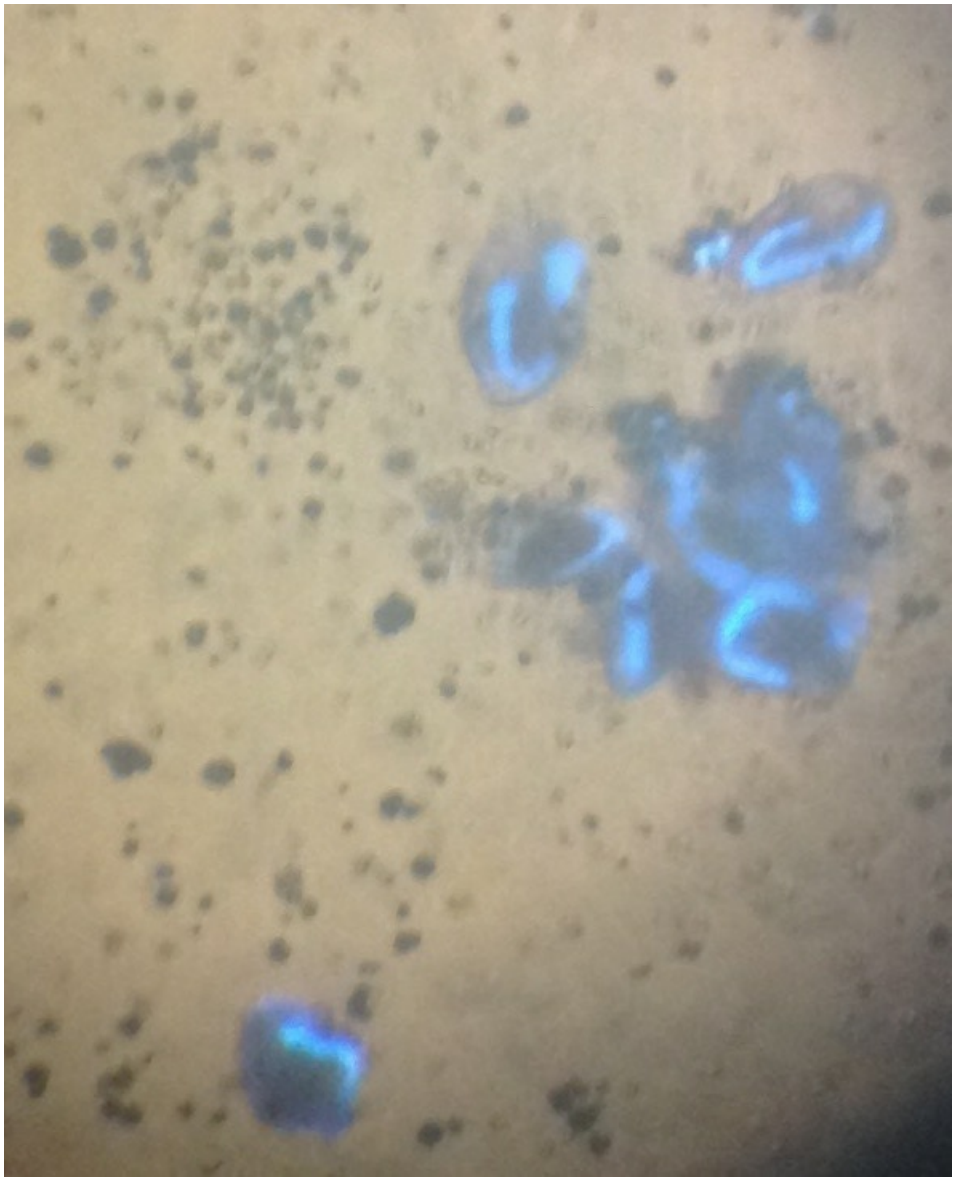
9 Add 3 ul of a 10 mg/ml solution of DAPI (or DNA) to the cells and gently agitate to mix.

10 Place cuvette in BTX ECM 630 electroporation device and electroporate using either 150 volts, 25 ohms, and 50 uF or 250 volts, 25 ohms, and

50 uF. Allow cells to recover for at least one min.

11 Transfer electroporated cells to a 15 ml screw-cap tube containing either 10 ml of a *Dunaliella* algae culture grown in artificial seawater to a low-moderate density or 9 ml of 0.3 M glucose + 1 ml of a moderate-high density algae culture.

12 Examine a sample of cells using a fluorescent microscope equipped with a DAPI filter. About 50% of cells are killed using these electroporation conditions, but a high percentage of the remaining cells should be motile and have brightly staining C-shaped macronuclei. Control, non-electroporated cells will have very dim macronculear staining, with the exception of any dead cells present in the culture.



13 NOTES: The electroporation procedure and conditions described allow for the entry of DAPI into the cell, resulting in nuclear staining. We have not yet been successful in using these conditions for transformation of *Euplotes crassus*, but only a couple of constructs have been tried to date.

In step 11, the 0.3 M glucose + algae solution should be used if subsequent selection of transformants with a drug will be attempted, as we have found that this increases the sensitivity of E. crassus to at least some selective agents (e.g., G418 or paromomycin).