Transfection by Electroporation in Euplotes crassus

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

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

1 2x104 Euplotes crassus cells were collected and resuspended in 0.3 M glucose solution (7% sea water and 93% of 0.3 M glucose solution).

2 Each round of transfection 250 µl of cells were used. 0.25 µg of Label IT® Plasmid Delivery Control Cy®3 (Mirus) were added alone or mixed with 2.5 µl of Lipofectamine® 2000 Transfection Reagent (Invitrogen) according to the supplier.

3 The sample was transferred to the 0.2 cm cuvette. Bio-Rad Gene Pulser was used. Conditions were set as follows: 0.2 kV, 25 µFD, 100 Ω. Time constant around 1.2.

4 More than 50% of cells were viable after electroporation (few cells fused together), then cells were resuspended in 3 ml of sea water.

5 The plasmid was visible in the cytoplasm immediately after the electroporation by fluorescent microscope.