Euplotes crassus transfection using Lipofectamine 2000 with repetitive exposition (provisional)

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Abstract

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Protocol

Step 1.

Collect 2 x 10^3 cells by centrifugation (3000 rpm for 3 minutes).

Step 2.

Wash the cells once with sea water and once with Opti-MEM medium (Thermo Fisher Scientific), and resuspend them in Opti-MEM medium.

Step 3.

Load 0.5 ml of cells in a 24-well plate for transfection.

Step 4.

Dilute 4 µl of Lipofectamine 2000 Reagent (invitrogen) in 200 µl of Opti-MEM medium.

Step 5.

Dissolve 5 µg of DNA (0.5-5 µg/µl resuspended in H_2O) in 250 µl of Opti-MEM medium.

Step 6.

The transfection complexes are prepared as follows: add 50 µl of diluted DNA to 50 µl of diluted Lipofectamine 2000 Reagent (1:1 ratio) and incubate for 5 min at room temperature.

Step 7.

Add 50 µl of the transfection complexes drop-wise to the cells in the wells.

Step 8.

Incubate the cells for 30 minutes at 37°C; then, 3 hours and half at 28°C.

Step 9.

After 4 hours of expositions, collect the cells from all the wells, wash them once with sea water and then resuspend them in 0.5 ml of sea water.

Step 10.

Repeat the exposition to the transfextion complexes for 3 days to improve transfection efficiency.

Step 11.

Check the cells for transfection occurrence by different methods according to the different cases.