Euplotes transfection using Lipofectamine 3000 (provisional) Version 2

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Abstract

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Protocol

Step 1.

Collect 2x10^{3} cells in starvation by centrifugation (3000 rpm for 3 minutes).

Step 2.

Wash the cells once with sea water and once with 500 mM sorbitol, 0.5 mM Tris-HCl pH 7.0, (3000 rpm for 3 minutes). Then resuspend *Euplotes* cells in the same medium and aliquot them in 20 µl drops for transfection.

Step 3.

Dilute 3 µl of Lipofectamine 3000 Reagent in 100 µl of the same medium of the cells (500 mM sorbitol, 0.5 mM Tris-HCl pH 7.0).

Step 4.

Dilute 2 µg of DNA (0.5-5 µg/µl which is resuspended in TE buffer pH 7.0 to 8.0) in 100 µl of the same medium of the cells (500 mM sorbitol, 0.5 mM Tris-HCl pH 7.0) and add 4 µl of P3000 Regent (2 µl/µg DNA).

Step 5.

The transfection complexes are prepared as follows: add 5 µl of diluted DNA to 5 µl of diluted Lipofectamine 3000 Reagent (1:1 ratio) and incubate for 10-15 minutes at room temperature.

Step 6.

10 µl of the transfection complexes were added, drop-wise, to the cells in the drops and they are incubated at their optimal growth temperature.

Step 7.

One hour after addition of Lipofectamine 3000 complexes, dilute *Euplotes* cells were with 20 µl of sea water.

Step 8.

Observe the cells After 2 - 4 days under the fluorescent microscope.