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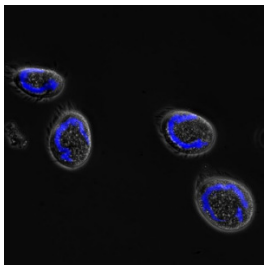
Euplotes crassus transfection using Lipofectamine 2000 as vehicle (provisional)



Forked from Euplotes crassus transfection using Lipofectamine 2000 as vehicle (provisional)

In 1 collection

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Protocol status: In development
We are still developing and optimizing this protocol

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1 Collect 4 × 10^4 well-fed Euplotes crassus cells (we used E.coli as the only food source) by centrifugation at 400 rcf for 3 minutes.

2 Wash the cells twice with artificial sea water (see attachment for the recipe) and once with 500 mM sorbitol, 0.5 mM Tris-HCl, pH 7.0 (400 rcf for 3 minutes each time). Then resuspend Euplotes crassus cells in 50 µl of the medium (500 mM sorbitol, 0.5 mM Tris-HCl, pH 7.0). To get this small volume you may require an additional minute of centrifugation.

Note

Recipe for complete sea water (1 L):

36 g Reef Crystals
1 ml Walne's solution
1 ml of 10 µg/ml $FeSO_4$
0.2 ml of 2 mg/ml thiamine (light sensîtive; store at 4 ºC)
Add distilled water up to 1 L

3 Transfer the cells into wells within a 96-well plate for transfection.

4 Dilute 2.5 µl of Lipofectamine 2000 Reagent in 25 µl of the same medium of the cells (500 mM sorbitol, 0.5 mM Tris-HCl, pH 7.0).

5 Dilute 5 µg of DNA dissolved in MilliQ H_2O (0.5-5 µg/µl) in 125 µl of the same medium of the cells (500 mM sorbitol, 0.5 mM Tris-HCl, pH 7.0).

6 Add 25 µl of the diluted DNA to 25 µl of the diluted Lipofectamine 2000 Reagent (1:1 ratio), and incubate them for 10 minutes at room temperature (before incubation mix them by pipetting up and down 5 times or vortex them for 10 seconds).

7 Add 10 µl of the transfection complexes drop-wise to the 50 µl of Euplotes crassus cells in medium (gently swirl the dish to ensure uniform distribution of the transfection complexes).

8 One hour after addition of Lipofectamine 2000 complexes, add 50 µl of artificial sea water to the cells.

9 After another hour, add other 50 µl of artificial sea water to the cells.

10 An hour later harvest the cells (400 rcf for 3 minutes), and wash them twice with artificial sea water (400 rcf for 3 min each time). Then resuspend them in 400 µl of the artificial sea water.

11 Transfer the cells into depression wells.

12 Incubate the cells at 24°C, then analyze them by fluorescence microscopy to determine gene expression.