Euplotes crassus transformation using Lipofectamine 2000 as vehicle Version 4

RACHELE CESARONI

Abstract

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Protocol

Step 1.

Collect 2 x 10^4 well-fed Euplotes crassus cells (we used E. coli as the only food source) by centrifugation at 400 rcf for 3 minutes.

Step 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.

NOTES
Rachele Cesaroni 06 Feb 2017

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 to 1 L

Step 3.

Transfer the cells into two wells within a 96-well plates for transfection.

NOTES
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We used one of the wells for the negative control.

Step 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).

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Add distilled water to 1 L

Step 5.

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

Step 6.

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

Step 7.

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

Step 8.

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

Step 9.

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

Step 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.

Step 11.

Transfer the cells into depression wells.

Step 12.

Incubate the cells at least for three days at 24 $^{\circ}$C, then analyzeby fluorescence microscopy to determine gene expression.