Euplotes crassus transformation using Lipofectamine 2000 as vehicle Version 2

RACHELE CESARONI

Abstract

Citation: RACHELE CESARONI Euplotes crassus transformation using Lipofectamine 2000 as vehicle. **protocols.io** dx.doi.org/10.17504/protocols.io.g3xbypn
Published: 30 Jan 2017

Protocol

Step 1.

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

Step 2.

Cells were washed 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), in which they were also resuspended in 50 µl (to get this small volume an additional minute of centrifugation may be required).

NOTES
Estienne Swart 30 Jan 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 4ºC)
Add distilled water to 1 L

Step 3.

Cells were transfered into two wells within 96-well plates for transfection.

ANNOTATIONS

Rachele Cesaroni 30 Jan 2017

One of the two wells was used for the negative control.

Step 4.

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

Step 5.

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

Step 6.

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

Step 7.

10 µl of the transfection complexes were added drop-wise to the cells in the well plates (gently swirl the dish to ensure uniform distribution of the transfection complexes).

Step 8.

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

Step 9.

After another hour other 50 µl of artificial sea water were added to the cells.

Step 10.

An hour later cells were harvested (400 rcf for 3 minutes) and washed twice with artificial sea water (400 rcf for 3 minutes each time), in which they were then also resuspended in 400 µl.

Step 11.

Cells were transfered into depression wells.

Step 12.

Cells were incubated at least for three days at 24°C, then analyzed by fluorescence microscopy to determine gene expression.