Euplotes crassus transformation using FuGene HD Transfection Reagent as vehicle Version 3

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Abstract

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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
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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 a 96-well plates for transfection.

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

Step 4.

FuGene HD Transfection Reagent was allowed to reach room temperature and mixed by inverting or vortexing briefly (if a precipitate is visible, briefly warm at 37ºC and then let it reach room temperature).

Step 5.

90-98 µl of medium at room temperature (500 mM sorbitol, 0.5 mM Tris-HCl pH 7.0) and 4 µg of DNA in MilliQ H_2O (0.2-1 µg/µL) were added to an Eppendorf tube and vortexed (after adding the DNA the final volume must be 100 µl).

Step 6.

6 µl of FuGene HD Transfection Reagent were added directly to the medium and mixed immediately.

Step 7.

FuGene HD Transfection Reagent and DNA mixture were incubated for 15 minutes at room temperature.

Step 8.

10 µl of the FuGene HD Transfection reagent and DNA mixture were added to the wells containing 50 µl of cells in medium (everything was mixed by pipetting).

Step 9.
One hour after addition of FuGene/DNA complexes, 50 µl of artificial sea water were added to the cells.

Step 10.

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

Step 11.

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

Step 12.

Cells were transfered into glass depression wells for subsequent monitoring.

Step 13.

Cells were incubated at least for three days at 24°C, then examined by fluorescence microscopy to determine expression of the transformed construct.