Euplotes crassus transformation using FuGene HD Transfection Reagent as vehicle Version 6

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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.

Allow FuGene HD Transfection Reagent to reach room temperature and then mix by inverting or vortexing briefly (if you see a precipitate, briefly warm at 37 ºC and then let it reach room temperature).

Step 5.

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

Step 6.

Add 6 µl of FuGene HD Transfection Reagent directly to the medium and mix immediately.

Step 7.

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

Step 8.

Add 10 µl of the FuGene HD Transfection reagent and DNA mixture to one of the two wells containing 50 µl of Euplotes crassus cells in medium (mix everything by pipetting).

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

Step 10.

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

Step 11.

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 12.

Transfer the cells into glass depression wells for subsequent monitoring.

Step 13.

Incubate the cells at least for three days at 24 °C, then examine tby fluorescence microscopy to determine expression of the transformed construct.