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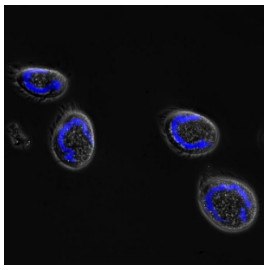
Euplotes crassus transfection using FuGene HD Transfection Reagent as vehicle (provisional)



Forked from Euplotes crassus transfection using FuGene HD Transfection Reagent 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 sensitive; 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 Allow FuGene HD Transfection Reagent to reach room temperature, and then mix it by inverting or vortexing briefly (if you see a precipitate, briefly warm it at 37°C, and then let it reach room temperature).

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).

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

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

8 Add 10 µl of the FuGene HD Transfection Reagent and DNA mixture to the 50 µl of Euplotes crassus cells in medium (mix everything by pipetting).

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

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

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.

12 Transfer the cells into glass depression wells for subsequent monitoring.

13 Incubate the cells at 24°C, then examine them by fluorescence microscopy to determine expression of the construct.