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

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RACHELE CESARONI¹, Rachele Cesaroni²

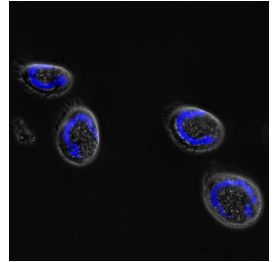
¹University of Bern, Institute of Biology; ²Universität Bern

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Protocol status: In development

We are still developing and optimizing this protocol

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Troubleshooting

- 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 μ l 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 μ g/ml FeSO_4
0.2 ml of 2 mg/ml thiamine (light sensitive; store at 4 °C)
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 μ l of medium (500 mM sorbitol, 0.5 mM Tris-HCl, pH 7.0) at room temperature and 4 μ g of DNA in MilliQ H_2O (0.2-1 μ g/ μ L) to an Eppendorf tube and vortex (after adding the DNA the final volume must be 100 μ l).
- 6 Add 6 μ l of FuGene HD Transfection Reagent directly to the medium and mix immediately.
- 7 Incubate FuGene HD Transfection Reagent and DNA mixture for 15 minutes at room temperature.
- 8 Add 10 μ l of the FuGene HD Transfection Reagent and DNA mixture to the 50 μ l of *Euplotes crassus* cells in medium (mix everything by pipetting).
- 9 One hour after addition of FuGene/DNA complexes, add 50 μ l of artificial sea water to the cells.
- 10 After another hour, add to the cells other 50 μ l 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 μ l 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.