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Euplotes crassus transfection through microinjection into the macronucleus V.2

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1 Dilute 1:10 Euplotes crassus cultures of two different mating types in artificial sea water (20 ml tot volume for each mating type) and feed them with E.coli (3 ml for each mating type). Before to add E.coli to the Euplotes crassus cells, pellet them and wash them once with ddH_2O (for bacteria preparation see protocol 'Culturing Euplotes crassus to high densities using E. coli as the only food source').

Note

Recipe for complete seawater (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 up to 1 L

2 Grow cells at 24ºC for 4 days with a 12h light/12h dark cycle even without areation and then mix the two mating types at room temperature (the optimal cell density for conjugation is ~1000 cells/ml and the volume <2.5 cm in high).

3 Isolate single Euplotes crassus cells with a donut shape after 2 days into artificial sea water with2% BSA in order to prepare drops for microinjection (ideally one cell each drop).

Note

Cells have a donut shape (anlagen) and macronucleus is easier to inject.
50h after formation of the pairs a round of amplification of the genome occurs.

4 When drops are ready cover them with a layer of Mineral Oil to not let them evaporate.

5 Inject into the macronucleus DNA in the concentration of 3 to 5 µg/µl using Eppendorf Femtotips I injection needle.

6 Recover each cell individually in 500 µl of artificial sea water plus 0.25 µl of E.coli at 24ºC (for bacteria preparation see protocol 'Culturing Euplotes crassus to high densities using E. coli as the only food source').