Euplotes crassus transfection through microinjection into the macronucleus Version 5

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Abstract

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Protocol

Step 1.

Dilute 1:10 Euplotes crassus cultures of two different mating types in artificial sea water (prepare 20 ml culture for each mating type), and feed them with E.coli (3 ml for each mating type). For bacteria preparation see protocol 'Culturing Euplotes crassus to high densities using E.coli as the only food source'.

NOTES
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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

Step 2.

Grow cells in 250 ml flat-bottomed flasks at 24°C for 4 days with a 12h light/12h dark cycle, and then mix the same number of cells of both mating types in a 500 ml flat-bottomed flask at room temperature (the optimal cell density for conjugation is ~1000 cells/ml). Provide no areation in both steps.

Step 3.

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

Step 4.

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

Step 5.

Inject into the macronucleus DNA (3 to 5 µg/µl) using Eppendorf Femtotips I injection needle.

Step 6.

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

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