

# Euplotes crassus transfection through microinjection into the macronucleus Version 2

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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 (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<sub>2</sub>O (for bacteria preparation see protocol 'Culturing Euplotes crassus to high densities using E. coli as the only food source').

### 📌 NOTES

**Rachele Cesaroni** 03 Mar 2017

Recipe for complete seawater (1 L):

36 g Reef Crystals

1 ml Walne's solution

1 ml of 10 µg/ml FeSO<sub>4</sub>

0.2 ml of 2 mg/ml thiamine (light sensitive; store at 4°C)

Add distilled water up to 1 L

### Step 2.

Grow cells at 24°C for 4 days with a 12h light/12h dark cycle even without aeration 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).

### 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.

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

#### **Step 5.**

Inject into the macronucleus DNA in the concentration of 3 to 5  $\mu\text{g}/\mu\text{l}$  using Eppendorf Femtotips I injection needle.

#### **Step 6.**

Recover each cell individually in 500  $\mu\text{l}$  of artificial sea water plus 0.25  $\mu\text{l}$  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').