# Euplotes crassus transformation through microinjection into the macronucleus

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## Abstract

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## Protocol

### Step 1.

Euplotes crassus cells of two different mating types were diluted 1:10 in artificial sea water and were fed with E.coli (1:1000).

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 to 1 L

### Step 2.

Cells of both mating types were grown at 24ºC for 4 days with a 12h light/12h dark cycle and then mixed at 24ºC (after few hours they started to form pairs).

ANNOTATIONS
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Areation was provided while cells were growing, but not while cells were mating.

### Step 3.

50h after formation of the pairs (donut cells) cells were isolated and put into 2% BSA diluted in artificial sea water in order to prepare drops for microinjection (ideally one cell each drop).

NOTES

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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.

**Step 4.**

When drops were ready a layer of Mineral Oil was put above to not let the drops evaporate.

NOTES

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**Step 5.**

Phenol/chloroform extracted, precipitated and resuspended in injection buffer (114 mM KCl, 20 mM NaCl, 3 mM sodium phosphate buffer) DNA (artificial nanochromosome with telomeres, but without 3' overhangs) 3-5 µg/µl was injected into the macronucleus using Eppendorf Femtotips II injection needle.

**Step 6.**

Cells were then recovered in 400 µl of sea water plus E.coli (0.4 µl) at put at 24ºC.