

# Euplotes crassus transfection using Lipofectamine 2000 as vehicle (provisional) Version 8

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## Abstract

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## Protocol

### Step 1.

Collect  $4 \times 10^4$  well-fed Euplotes crassus cells (we used E.coli as the only food source) by centrifugation at 400 rcf for 3 minutes.

### Step 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  $\mu$ 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.

## NOTES

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Recipe for complete sea water (1 L):

36 g Reef Crystals

1 ml Walne's solution

1 ml of 10  $\mu$ g/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 3.

Transfer the cells into wells within a 96-well plate for transfection.

#### **Step 4.**

Dilute 2.5 µl of Lipofectamine 2000 Reagent in 25 µl of the same medium of the cells (500 mM sorbitol, 0.5 mM Tris-HCl, pH 7.0).

#### **Step 5.**

Dilute 5 µg of DNA dissolved in MilliQ H<sub>2</sub>O (0.5-5 µg/µl) in 125 µl of the same medium of the cells (500 mM sorbitol, 0.5 mM Tris-HCl, pH 7.0).

#### **Step 6.**

Add 25 µl of the diluted DNA to 25 µl of the diluted Lipofectamine 2000 Reagent (1:1 ratio), and incubate them for 10 minutes at room temperature (before incubation mix them by pipetting up and down 5 times or vortex them for 10 seconds).

#### **Step 7.**

Add 10 µl of the transfection complexes drop-wise to the 50 µl of *Euplotes crassus* cells in medium (gently swirl the dish to ensure uniform distribution of the transfection complexes).

#### **Step 8.**

One hour after addition of Lipofectamine 2000 complexes, add 50 µl of artificial sea water to the cells.

#### **Step 9.**

After another hour, add other 50 µl of artificial sea water to the cells.

#### **Step 10.**

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 µl of the artificial sea water.

#### **Step 11.**

Transfer the cells into depression wells.

#### **Step 12.**

Incubate the cells at 24°C, then analyze them by fluorescence microscope to determine gene expression.