

# Euplotes crassus transfection using FuGene HD Transfection Reagent as vehicle (provisional) Version 9

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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.

Allow FuGene HD Transfection Reagent to reach room temperature, and then mix it by inverting or vortexing briefly (if you see a precipitate, briefly warm it at 37°C, and then let it reach room temperature).

### Step 5.

Add 90-98 µl of medium (500 mM sorbitol, 0.5 mM Tris-HCl, pH 7.0) at room temperature and 4 µg of DNA in MilliQ H<sub>2</sub>O (0.2-1 µg/µL) to an Eppendorf tube and vortex (after adding the DNA the final volume must be 100 µl).

#### **Step 6.**

Add 6 µl of FuGene HD Transfection Reagent directly to the medium and mix immediately.

#### **Step 7.**

Incubate FuGene HD Transfection Reagent and DNA mixture for 15 minutes at room temperature.

#### **Step 8.**

Add 10 µl of the FuGene HD Transfection Reagent and DNA mixture to the 50 µl of *Euplotes crassus* cells in medium (mix everything by pipetting).

#### **Step 9.**

One hour after addition of FuGene/DNA complexes, add 50 µl of artificial sea water to the cells.

#### **Step 10.**

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

#### **Step 11.**

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 12.**

Transfer the cells into glass depression wells for subsequent monitoring.

#### **Step 13.**

Incubate the cells at 24°C, then examine them by fluorescence microscopy to determine expression of the construct.