

# Oyster parasite *Perkinsus marinus* transformation using Amaxa electroporator and non-proprietary electroporation buffer

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## Abstract

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## Protocol

### Cell Culture

#### Step 1.

Grow *Perkinsus marinus* at 25 °C in ATCC Media 1886 (see recipes below), until OD600 = 0.4-0.6

Harvest the equivalent of  $5-7 \times 10^7$  cells (5 ml at OD600 = 0.5) for the transformation by centrifuging the culture for 10 min at 1000 g at room temperature.

Remove supernatant completely, keep the pellet.

### DNA Mix preparation

#### Step 2.

In 1.5 ml microcentrifuge tube, add 2.5 µg of purified linearized plasmid + 2.5 µg of circular plasmid, adjust to a final volume of 60 µl using milliQ water.

Add 35 µl of 3R buffer (see recipes below), mix.

Add 10 µl of CaCl<sub>2</sub> 1.5 M (when you are ready to electroporate)

### Electroporation step

#### Step 3.

Resuspend the cells pellet with the DNA mix preparation and transfer quickly to the electroporation cuvette (2 mm)

Electroporate ASAP cells using an Amaxa electroporator, program D-023

Resuspend the cells immediately with 500 µl of fresh medium

Transfert the cells to a six well plates previously filled with 2.5 ml of fresh growth medium

Monitor transfection efficiency (by fluorescence microscopy in our case) 24-48 h post transfection.

### Buffer and Media recipe

#### Step 4.

##### 3R buffer recipe:

200 mM Na<sub>2</sub>HPO<sub>4</sub>

70 mM NaH<sub>2</sub>PO<sub>4</sub>

15 mM KCl

150 mM HEPES pH: 7.3

##### ATCC Media 1886 recipe:

1	Dulbecco's Modified Eagle's Medium Base	Sigma D5030	2.10	g
2	Nutrient Mix F-12 Ham	Sigma N6760	2.70	g
3	Instant Ocean Sea Water	18.2 g ic-salt/910mL dH <sub>2</sub> O	400.00	mL
4	L-Glutamine	200 mM	2.50	mL
5	HEPES	1.0 M	12.50	mL
6	NaHCO <sub>3</sub>	7.5% (w/v)	4.30	mL

7	Carbohydrate Solution	0.5 g Gluc + 0.1g Galc + 0.1 g Treh in 10 mL dH <sub>2</sub> O	5.00	mL
8	Lipid Concentrate	Sigma L5146	0.50	mL
9	Pluronic F68	1 g/100 mL	4.50	mL
10	Fetal Calf (Bovine) Serum		10.00	mL
11	Phenol Red	0.5% (w/v)	0.25	mL
12	Penicillin -Streptomycin Mixture	Sigma P0781	5.00	mL

- Vacuum filter through 0,2 µm filter pore and keep it on 4 °C for storage (maximum 4 weeks)
- The preference for *P. olsenii* pH is 7.6 and *P. marinus* is happy at 7.0 or a little lower (6.8)