Glass beads-based transformation protocol for Perkinsus marinus

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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 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 and resuspend cells in 330 µl of fresh medium.

DNA/Cells mixture

Step 2.

In 1.5 ml microcentrifuge tube, add 2.5 µg of purified linearized plasmid + 2.5 µg of circular plasmid with the equivalent of 300 µl of glass beads (Sigma G-8772).

330 µl of the concentrated cell suspension is then added to the tube.

Tubes are then vortexed for 30 seconds at max speed.

A further 500 µl ATCC 1886 medium is added to each tube, mixed and the contents are transfered to 6-well plates in a final volume of 3 ml.

Cells are grown at 25 ºC and screened periodically for transformation (in our experience > 1 week).

ATCC Media 1886 recipe:

Step 3.

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_2O	400.00	mL
4	L-Glutamine	200 mM	2.50	mL
5	HEPES	1.0 M	12.50	mL
6	$NaHCO_3$	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_2O	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. olseni pH is 7.6 and P. marinus is happy at 7.0 or a little lower (6.8)