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Electroporation of *Oxyrrhis marina*

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ABSTRACT

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We are still developing and optimizing this protocol

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Culture condition of *O. marina*

- 1 Transfer *Oxyrrhis marina* (NIES-494) cells to 20 mL of fresh IMK medium (Nihon Pharmaceutical Co., Ltd.) in a plastic flask (IWAKI 75 cm²) at the concentration of 200 cells/mL, and add *Pyramimonas parkeae* (NIES-254) as feed at the concentration of 1×10^4 cells/mL.

Grow cells at 22°C with a light/dark cycle of 14h/8h for 14 days. After two weeks, the cell density of *O. marina* will reach approximately 1×10^4 cells/mL.

Concentration of *O. marina* cells

- 2 Collect *O. marina* and *P. parkeae* cells from 20 mL culture by centrifugation at 1,500 g for 4 min with a swing rotor.

Resuspend cells with 2 mL fresh IMK medium, and then transfer to a 12-well plastic plate. Incubate the plate in a dark condition at 22°C.

After 4 days, the cell density of *O. marina* will be increased from 1×10^5 cells/mL (0 day) to 5×10^5 cells/mL (4 days), in contrast with the drastic decrease of *P. parkeae* cells.

Electroporation with Bio-Rad Gene Pulser Xcell

- 3 Harvest *O. marina* cells at 1,500 g for 4 min with a swing rotor, and then resuspend the cell pellet by 100 µL Gene Pulser electroporation buffer (Bio-Rad #1652676) at the final concentration of 1×10^6 to 5×10^6 cells/mL.

Add DNA (5-25 μg) or RNA (5 μg) to the cell solution, and transfer it to a 0.2 cm cuvette (Bio-Rad). Immediately after electrophoresis, add 1.9 mL fresh IMK medium into the cuvette, and transfer the cells to a 12-well plastic plate.