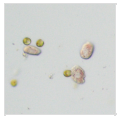

Electroporation of Oxyrrhis marina

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dx.doi.org/10.17504/protocols.io.vcne2ve

Protist Research to Optimize Tools in Genetics (PROT-G)

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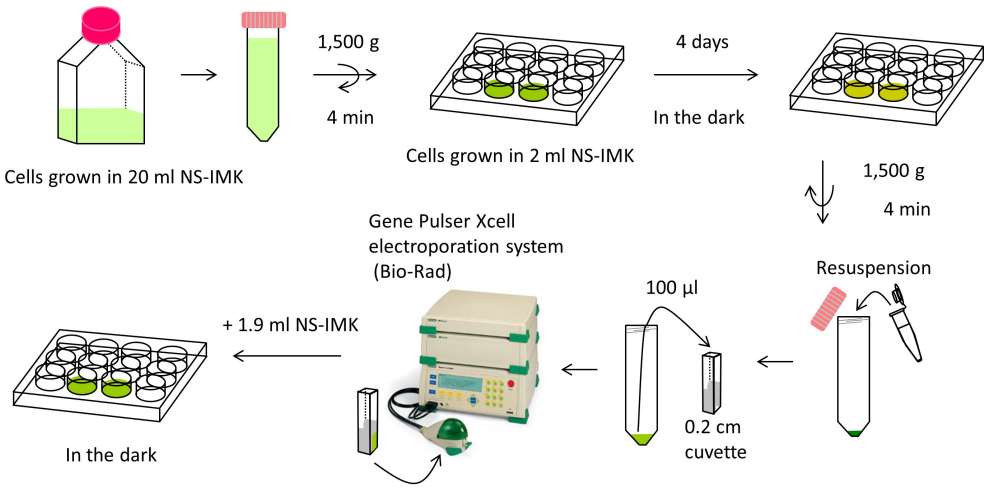
ABSTRACT



PROTOCOL STATUS

In development

We are still developing and optimizing this protocol

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 cm^2) 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℃ 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℃.

After 4 days, the cell density of *O. marina* will be increased from 1×10^5cells/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 5×10^6cells/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.