VERSION 3

SEP 25, 2019

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.7pphmmn

External link:

<http://biorxiv.org/lookup/doi/10.1101/718239>

Protocol Citation: Lu Wang, Brittany Sprecher, Huan Zhang, and Senjie Lin 2019. Co-Incubation protocol for transforming heterotrophic dinoflagellates (e.g. *Oxyrrhis marina*). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.7pphmmn>

MANUSCRIPT CITATION:

Faktorova, D. et al. "Genetic tool development in marine protists: Emerging model organisms for experimental cell biology." bioRxiv 718239 (2019).

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Co-Incubation protocol for transforming heterotrophic dinoflagellates (e.g. *Oxyrrhis marina*) V.3

Lu Wang¹, Brittany Sprecher¹, Huan Zhang¹, and Senjie Lin¹

¹University of Connecticut

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

Senjie Lin

ATTACHMENTS

[Co-Incubation Protocol for heterotrophic dinoflagellates.docx](#)

Protocol status: In development
We are still developing and optimizing this protocol

Created: Sep 25, 2019

Last Modified: Sep 25, 2019

PROTOCOL integer ID:
28111

Prepare vector and transform into *Escherichia coli*

- 1 Prepare a plasmid carrying proper promoter, selection marker, and reporter gene. Transform this plasmid into *E. coli* cells using standard heat shock protocol.

Grow transformed *E. coli*

- 2 Grow *E. coli* cells at a volume equal to 1/10 the desired volume of dinoflagellate transformation culture until the *E. coli* culture reaches an OD₆₀₀=0.7.

Harvest transformed *E. coli* cells

- 3 Centrifuge the cells at 3,000g for 5 mins to pellet the *E. coli*.

Mix transformed *E. coli* cells with prey alga

- 4 Discard the supernatant and resuspend the cells to twice the volume with either the favored prey of the species (*Dunaliella tertiolecta*) for transformation or in L1 media seawater.

Mix transformed *E. coli* cells and prey alga with dinoflagella

- 5 Add the *E. coli* prey or seawater mix to the dinoflagellate for transformation.

Co-incubation

- 6 After incubation at normal culture condition for six hours, add LB with a volume equal to 1/10 the total volume. This step is not universal for all heterotrophic dinoflagellates so should be tested with every new species.

Add fresh medium and selection agent

- 7 After 24 hours add the same volume of new media and add the selection reagent to the media. Depending on the species it may be better to add the selection reagent after 42 hours.
- 8 After 42 hours observe the culture under blue light.