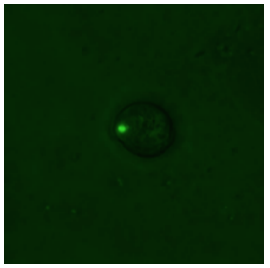
Sep 25, 2019 Version 3

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



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

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Attachments

DOCX

Co-Incubation Protoc...

17KB

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 OD_{600}=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 dinoflatellate 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.