Co-Incubation protocol for transforming heterotrophic dinoflagellates (e.g. Oxyrrhis marina) Version 2

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Abstract

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Protocol

Prepare vector and transform into E. coli

Step 1.

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

Grow transformed E. coli

Step 2.

Grow *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

Step 3.

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

Mix transformed E. coli cells with prey alga

Step 4.

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

Mix transformed E. coli cells and prey alga with dinoflagella

Step 5.

Add the *coli*-prey or -seawater mix to the dinoflatellate for transformation.

Co-incubation

Step 6.

After incubation at normal culture condition for six hours, add LB with a volume equal to 1/10 the total

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volume. This step is not universal for all heterotrophic dinoflagellates so should be tested with every new species.

Add fresh medium and selection agent

Step 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.

Add fresh medium and selection agent

Step 8.

After 42 hours observe the culture under blue light.

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