Simple & rapid genotyping of marine microeukaryotes

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Abstract

This is an effective protocol for genotyping diatom exconjugants which we believe is readily applicable to any other genetically manipulated marine microeukaryote.

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Guidelines

1. We find Phire Plant Direct PCR Master Mix to be a top choice for direct genotyping of diatom exconjugants.

2. Review protocol details here.

3. Note that we regularly run 10 µL reactions as opposed to 20 µL which is the minumum volume recommended by the manufacturer.

Before start

1. Your exconjugant colonies should be growing as small - ~300 µL - liquid cultures in the presence of appropriate antibiotics. A 48-well plate works great for this purpose.

2. Adjust primer Tm using this Tm Calculator.

Protocol

Prepare PCR master mix.

Step 1.

--> volumes are per reaction

--> x (number of strains + 2)

AMOUNT
4 µl Additional info: MQ
AMOUNT
0.5 µl Additional info: 10 µM forward primer
AMOUNT
0.5 µl Additional info: 10 µM reverse primer
AMOUNT
5 µl Additional info: Phire Plant Direct PCR Master Mix

Aliquot into PCR tubes and add 0.5 µL of strains you wish to genotype.

Step 2.

--> Include appropriate positive (if available) and negative controls.

--> Include WT or knockout strain you used for transformation as a template in one of the reactions.

Passage genotype+ strains.

Step 3.

--> 1:100 or 1:50 dilution into 10 mL fresh medium is a good place to start.

Proceed with phenotypic characterization.

Step 4.