Diatom Transformation via Bacterial Conjugation

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Abstract

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Before start

- Make sure you are using fresh *E. coli* cells streaked for isolation on LB + antibiotics no more than 1 week from -80°C cryostock.

Protocol

Step 1.

Diatom cultivation (liquid)

Grow the diatom culture to mid-log phase (≈ $8.0E^6$ cells/ml for *Phaeodactylum tricornutum* grown on F/2 media*).* We have investigated transformation efficiency throughout the growth curve and found this to be the sweetspot.

NOTES
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- The original protocol plated the culture (250uL of 1.0E8 cells/ml) on 1/2 strength L1, 1% agar plates for 4 days prior to transformation.

- We have been investigating liquid cultivation because many diatom species do not survive on agar.

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Cultivation Conditions

- Cultivation in 0.2 um-filtered L1 media prepared using seawater collected from the Gulf of Mexico.

- 80 - 100 µmol photons m^{-2} s^{-1} provided by Philips Daylightt Delux 40 watt T12 flourscent tubes.
- Semi-continuous operation in 1L bulbble column bioreactors (500 - 800 ml working volume).
- 0.2 µm filtered air containing 1% CO_2 provides aeration/agitation at 0.2 vvm.

Step 2.

Prepare *E. coli* starter culture

Grow 1 mL of *E. coli* culture overnight (16-20 hrs) in LB+antibiotic for each planned transformation. We grow them at 37°C at 270 rpm in a shaking incubator.

Step 3.

Outgrow *E. coli*

Dilute cells 1:50 into 50 mL of fresh LB+antibiotic for each planned transformation.

On the day of transformation, use the overnight culture to inoculate 50 mL of fresh LB+antibiotic for each planned transformation.

- Grow to an OD_{600} of 0.8 - 1.0 (37°C with 270 rpm shaking).
- This takes about 3-4 hours.

Step 4.

Concentrate the diatom and *E. coli* cultures

Centrifuge both cultures at 3000 x g for 10 minutes.

- The diatom and *E. coli* cultures should be centrifuged at around the same time to minimize the amount of time they spend concentrated.

E. coli:

- Centrifuge 50 mL of culture for each transformation and resuspend in 500 uL of SOC medium.
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Phaeodactylum tricornutum:

- Measure cell concentration with FlowCam or haemocytometer.
- Centrifuge $2.5E^8$ cells and resuspend in 500 uL of L1 medium.

NOTES

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The original protocol scraped the agar plates the culture was initiated on using 500uL F/2, then adjusted the volume to attain 5.0 E^8 cells/ml. We have found no difference so far in transformation

effficiency between liquid- and plate-initiated cultures.

Step 5.

Conjugation

- **Mix** 200 µl of *E. coli* cells with 200 µl of *Phaeodactylum tricornutum* cells.
- **Spread** the mixture on transformation plates (0.5x BG-11 with 5% LB and 1% agar).
- **Incubate** plates for 90 minutes at 30°C in the dark.
- **Move** plates to light incubator (18°C and 80 µmol photons m^{-2} s^{-1}) for 2 days.

Negative controls

- Mix 200 µl of SOC medium with 200 µl of *Phaeodactylum tricornutum* cells.
- Mix 200 µl of L1 medium with 200 µl of *E. coli* cells.
- Incubate and treat plates identically to conjugation plates.

ANNOTATIONS

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- The authors of the original protocol suggested spreading the mixture near, but not touching, the edges of the plate, as this mixture will be scraped and replated in 2 days.

- According to Diner et. al, the conjugation occurs during the 2-day incubation.

- We have found greater success in using 0.5x BG-11 medium made with seawater instead of 0.5x L1 medium.

Step 6.

Selection

- **Collect** cells by adding 1 mL of L1 medium. Use a cell scraper to concentrate cells and medium to one side of the plate. Transfer resuspended cells to a 1.5 ml microcentrifuge tube with a P1000 pipette and filter tips.
- **Plate** 200 µl of the cell suspension on selection plates.
- Incubate at 18°C and 80 µmol photons m^{-2} s^{-1} until colonies appear.

NOTES

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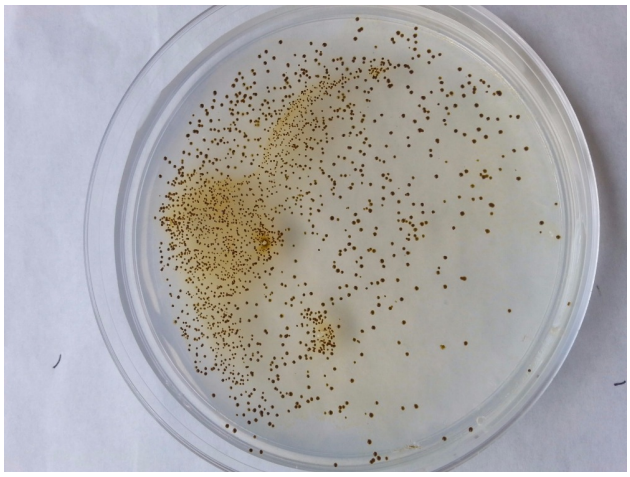
It is important to use filter tips as smalll peices of agar that are accidentaly scraped up can cause the pipette to cavitate resulting in contamination.

Step 7.

Colony identification

After a minimum of 8-12 days, untransformed *Phaeodactylum tricornutum* cells die off, and colonies of transformed cells begin to appear – in some cases, this can take 3-4 weeks.



Step 8.

This protocol was modified from the original procedure and correspondence with the authors.