Full Contact Microbiology (a.k.a Diatom Transformation via Bacterial Conjugation) Version 2

Jeric Harper

Abstract

Citation: Jeric Harper Full Contact Microbiology (a.k.a Diatom Transformation via Bacterial Conjugation). **protocols.io** https://www.protocols.io/view/full-contact-microbiology-a-k-a-diatom-transformat-fzrbp56
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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

Diatom cultivation (liquid)

Step 1.

Grow the diatom culture to mid-log phase (≈ $8.0E^6$ cells/ml for *Phaeodactylum tricornutum* grown on F/2 media or 5.0 E^7 cell/ml when grown on BG-11*).* We have investigated transformation efficiency throughout the growth curve and found this to be the sweetspot.

NOTES
Jeric Harper 04 Oct 2016

Cultivation Conditions

- Cultivation in 0.2 um-filtered L1 or BG-11 media prepared using 32ppt 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 bubble column bioreactors (500 - 800 ml working volume).

- 0.2 µm filtered air containing 1% CO_2 provides aeration/agitation at 0.2 vvm.

ANNOTATIONS
Jeric Harper 04 Oct 2016

- 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.

Prepare E. coli starter culture

Step 2.

Grow 1 mL of *E. coli* culture containing both the mobillity plasmid (Pta-MOB) and carrier plasmid, overnight (16-20 hrs) in LB+antibiotics, for each planned transformation. (We grow them at 37°C at 270 rpm in a shaking incubator.)

DURATION
16:00:00

ANNOTATIONS
Jernej Turnsek 19 Oct 2016

In my experience it has proven beneficial to perform conjugations with multiple donors carrying THE SAME construct. 2/3 worked beautifully, one failed completely.

Outgrow E. coli

Step 3.

On the day of transformation, use the overnight culture to inoculate 50 mL of fresh LB+antibiotic, 1:50 dilution, 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.

DURATION
03:00:00

ANNOTATIONS
Jeric Harper 06 Oct 2016

The authors of The original protocol reported that the OD_{600} range is flexible. Transformation success has been seen within OD_{600} range of 0.4 to 1.2.

P. tricornutum cell concentration

Step 4.

During the 3-4 hours the *E. coli* culture is growing, measure the *Phaeodactylum tricornutum* cell concentration with a FlowCam or haemocytometer to calculate the required volume needed to collect $2.5E^8$ cells for each transformation.

DURATION
00:05:00

Concentrate the diatom and E. coli cultures

Step 5.

For each transformation, centrifuge 50 mL of *E. coli* culture and the required *Phaeodactylum tricornutum* volume at 4000 x g for 10 minutes at 4°C.

- Resuspend *E. coli* pellet in 500 µL of SOC medium.
- Resuspend *P. tricornutum* pellet in 500 µL of L1 medium.

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

DURATION
00:10:00

NOTES
Jeric Harper 04 Oct 2016

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.

Conjugation

Step 6.

In a 1.5 mL tube mix 200 µl of *E. coli* cells with 200 µl of *Phaeodactylum tricornutum* cells.

Negative control: In a 1.5 mL tube mix 200 µl of SOC medium with 200 µl of *Phaeodactylum tricornutum* cells.

Note: Incubate and treat the negative control plates identically to conjugation plates.

ANNOTATIONS
Jeric Harper 04 Oct 2016

- 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.

- N concentration seems to be important. We have found greater transformation success in using 0.5x BG-11 medium made with seawater instead of 0.5x L1 medium.

- 5% LB is a sufficient concentration to support the E. coli culture without encouraging growth. Increasing the LB concentration results in increased algal death. We have also buffered the media but that did not increase *P. tricornutum* survivorship.

Conjugation

Step 7.

Spread the mixture (400 µL) on Conjugation Plates. (0.5x BG-11 with 5% LB and 1% agar).

NOTES

Jeric Harper 06 Oct 2016

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Conjugation

Step 8.

Incubate plates for 90 minutes at 30°C in the dark.

DURATION

01:30:00

NOTES

Jeric Harper 06 Oct 2016

P. tricornutum can survive transformation temperatures up to 32ºC. At 34ºC and above survivorship is severely hampered.

Conjugation

Step 9.

Move plates to light incubator (18°C and 100 µmol photons m^{-2} s^{-1}) for 2 days.

DURATION
48:00:00

NOTES
Jeric Harper 06 Oct 2016

- According to Diner et. al, the conjugation occurs during the 2-day incubation. Transformation efficiency increased as incubation time increased

Selection

Step 10.

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.

ANNOTATIONS
Jeric Harper 04 Oct 2016

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.

We will often first add 500uL to collect the majority of the cells from the plate into the 1.5mL tube, then repeat with an additional 500ul to collect the remainder.

Selection

Step 11.

Spread 200 µl of the cell suspension on a Selection Plate.

NOTES
Jeric Harper 06 Oct 2016

- Dilution of the selection volume (using L1 as makeup volume) can help facilitate colony enumeration if 200µl results in too many colonies.

- To maximize the number of colonies the entire volume of resuspended cells can be plated to multiple plates.

- Collect 1 µL of the cell suspention and dilute 1:1000 for FlowCam cell count.

Selection

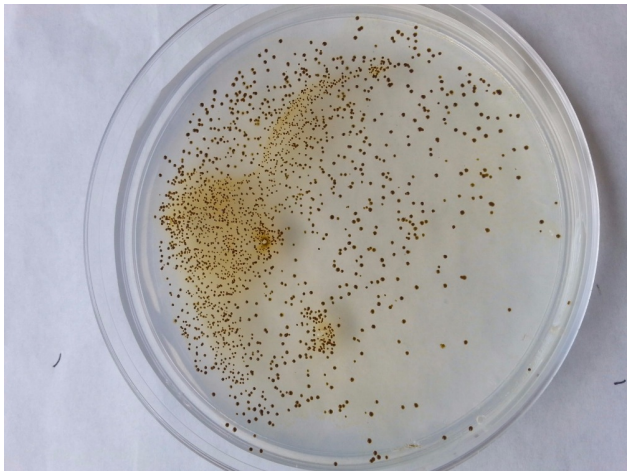
Step 12.

Incubate at 18°C and 100 µmol photons m^{-2} s^{-1} until colonies appear.

Colony identification

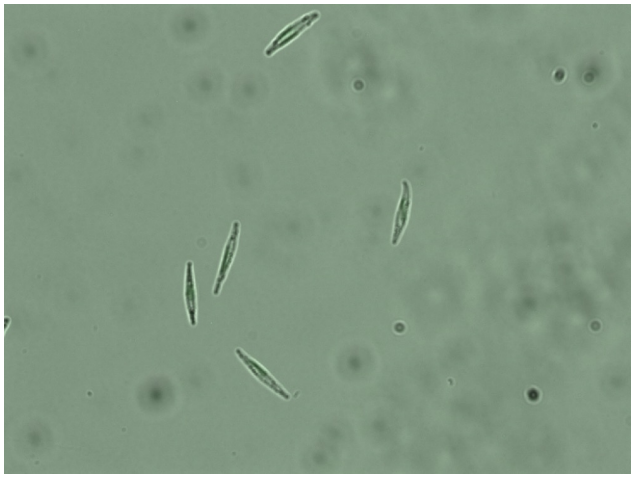
Step 13.

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.

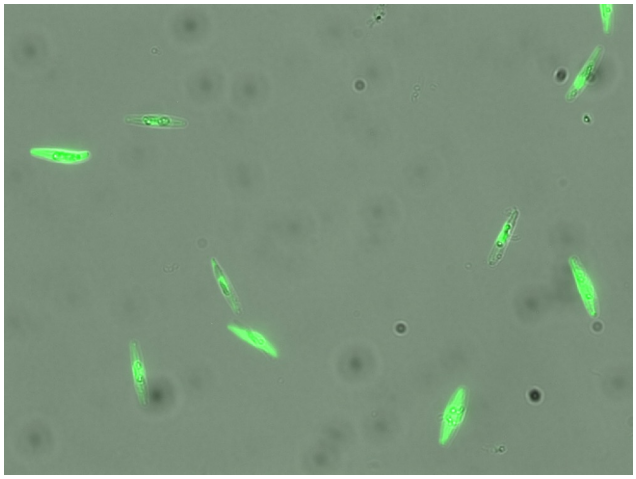


Alternatively selection can be done in liquid BG-11 Selection media using eGFP as a reporter and sorted using FACS.

un-transformed Pt



eGFP expression



ANNOTATIONS

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- For plate selection, use ImageJ protocol for colony enumeration.

- Calculate transformation efficeincy using:

Efficiency = (number of colonies on plate) ÷ (selection volume cell density (cell/mL) **x** volume (mL) put on selection plate)

Note: Be sure to include a dilution factor in the calculation if it was used in plating or counting!

Step 14.

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