Modified Bacterial Conjugation Protocol For Pseudo-nitzschia multiseries

April Woods, G Jason Smith

Abstract

This is a modified version of 'Conjugation of Thalassiosira pseudonana', publishedby J. Turnsek dx.doi.org/10.17504/protocols.io.f55bq86 .

The protocol was modified to enhance viability in the diatom *Pseudo-nitzschia multiseries.*

This version was used to transform *Pseudo-nitzschia multiseries* isolate 15091C3 with an episomal plasmid derived from pPtPUC3 to express a egfp gene under control of the *P. multiseries* actin promoter and termination domains.

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Protocol

Growth and preparation of E. coli pTA-MOB host and episomal plasmid donor

Step 1.

Innoculate G418+Kan plates with the appropriate host strain and grow overnight (use within 7 days). Pick isolated colonies and and inoculate into 10 mL LB medium. Grow overnight at 37°C, 200 rpm.

Preparation of E. coli donor (con

Step 2.

Measure OD600 and start a 150 mL LB subculture (recommended starting OD600 either 0.05 or 0.1).

Preparation of E. coli donor (cont)

Step 3.

Grow at 37°C, 200 rpm until OD_{600} reaches 0.3-0.4

Preparation of E. coli donor (cont)

Step 4.

Centrifuge at 4,000 rpm, 10°C, for 10 min.

Decant supernatant and resuspend bacterial cell pellet in 100 µL SOC*

ANNOTATIONS

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*This is the first modification to the conjugation protocol. By resuspending the E. coli into a highly concentrated aliquot, I can add less volume of SOC duing the conjugation and avoid osmotic stress to the diatom cells. It is necessary to pipette up and down quite a bit to get all the cells into the reduced volume solution.

Growth and preparation of Pseudo-nitzschia cells

Step 5.

P. multiseries stock cultures are maintained in filtered (0.2µ) autoclaved seawater (FASW) + Guillard's Marine Enrichment f/2 medium.

Subcultures are maintained in active mid-log phase growth, by serial batch culture in FASW+f/2

Typical cell numbers at log phase harvest are in the 10-30 *10^3 cells/mL range. Centrifugal concentration of 100mL of culture yields around 2*10^6 cells for conjugation.

Harvest Pseudo-nitzschia cells

Step 6.

Spin down cells at 4000 rpm, 10°C, for 20 min in 50mL falcon tubes. Remove supernatant with vacuum pipette.

Add additional 50mL culture and repeat centrifugation. Pellet will contain cells from a total of 100mL culture.

Re-suspend cell pellet in 1 mL of seawater enrichement media. Allow suspension to come to room temperature.

Conjugation

Step 7.

Mix 450 µL *Pseudo-nitzschia* cells and 50 µL *E. coli* cells in a 1.5 mL tube.

Mix fully by gentle pipeting.

Incubate mixture at 30 °C for 5 min.

NOTES

April Woods 07 Apr 2017

This is the major modification for*Pseudo-nitzschia* conjugation. A final concentration below 10% SOC greatly enhances *Pseudo-nitzschia* viability.

Conjugation

Step 8.

Gently spread mixture on pre-warmed 1% agar plates containing 5% (v/v) LB and 50% (v/v) filter sterilized seawater - L1 media.

NOTES

April Woods 29 Nov 2016

Plates were incubated at 37° for an hour before plating cells.

Conjugation

Step 9.

Incubate in dark at 30°C for 90 minutes.

DURATION

01:30:00

Conjugation

Step 10.

Move plates to standard *Pseudo-nitzschia* growth conditions - in my case 15°C and constant light - for 4 hours.

Conjugation

Step 11.

Add 1 mL reduced Nitrogen L1 medium*.

Gently scrape agar surface with a cell scraper or L spreader to resuspend bacterial and diatom cells.

Expect to recover 500 µL co-culture suspension after scraping.

NOTES
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At tis point, we use L1 media enrichment and adjust the amount of Nitrogen so that Silica is not a limiting nutrient. Nitrogen:Silica ration is 1:3.

Prepare for Solid Phase Growth in 1% (w/v) LGTA

Step 12.

LGTA has been prepared
following https://www.protocols.io/view/low-gel-temperature-agarose-lgta-media-gsibwce

LGTA has been maintained at 20 °C. LGTA can also be microwaved to reliquify from solid storage, be sure to cool to 20 °C.

- Aliquot 4 mL, molten LGTA (20 °C) into sterile culture tubes (e.g. Falcon 2059).
- Add as much cell suspension as you are able to scrape off the conjugation plate (ca. 500uL) ***into*** LGTA.
- Gently vortex to mix.

NOTES
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The LGTA contains the same adjusted 1:3 NO3:Si ratio.

ANNOTATIONS
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Alternatively, recovery and growouts can be done in liquid culture with FASW+L1media. Add conjugation suspension to FASW+L1 to ≤10% (v/v) and aliquot samples into culture plates or tissue culture flasks.

PLATING CULTURES:

Step 13.

Transfer 1 mL to well of Costar 12-well culture plate avoiding introducing bubbles.

Repeat for 4 wells.

For selection experiments may reduce volume to 500uL per well and include selection-free matched control wells.

(LGTA will solidify during subsequent incubation at 15 °C.)

ANNOTATIONS

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For long term solid phase culture, LGTA plugs can be overlaid with 1-2 mL FASW+L1 and selection agent as needed.

Screening

Step 14.

Observe plates under fluorescence microscopy for viability and growth (chlorophyll fluorescence).

Screen for expression of egfp under fluorescence microscopy.

As cells grow, some may emerge from the agarose and into the liquid overlay. These are available for isolate pics.

Gel plugs may be removed and resuspended into liquid growth.

Isolate cultures may be screened for presence of plasmid by PCR.

DNA extraction of isolate cultures may be used to transform E. coli and selected for Kanamycin resistance.