Revisited Thalassiosira pseudonana (Tp) conjugation protocol enables delivery of a protein to Tp frustule

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Abstract

This revisited Tp conjugation protocol builds on the following two protocols: Conjugation of Thalassiosira pseudonana (DOI: dx.doi.org/10.17504/protocols.io.f55bq86) & Pour plating of Thalassiosira pseudonana (Tp) (DOI: dx.doi.org/10.17504/protocols.io.jfncjme). Reader is strongly encouraged to read both before using this one. Gene-EGFP fusion was expressed under consitutive fcp promoter and terminator.

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Protocol

Growth and preparation of E. coli (donor) cells

Step 1.

Pick bacterial colonies from your Gent20 (pTA-Mob) + Kan50 (Tp episome) plates and inoculate 3 mL LB medium. You can use pre-made glycerol stocks instead. Shake overnight at 37 °C.

Growth and preparation of E. coli (donor) cells

Step 2.

Passage 1:200 in fresh 50 mL LB. Shake at 37 °C until OD_{600} reaches 0.3–0.4.

Growth and preparation of E. coli (donor) cells

Step 3.

Centrifuge cells 10 min / 4000 rpm / 10 °C.

Growth and preparation of E. coli (donor) cells

Step 4.

Decant all supernatant and resuspend in 267 µL SOC medium.

Growth and preparation of T. pseudonana (recipient) cells

Step 5.

Determine cell density of your Tp culture. 1.3 x 10^7 cells/mL culture was used in this protocol.

NOTES

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'Tp L1' was buffered with 10 mM Tris-HCl and pH was adjusted to 8. This allows longer maintenance of Tp in culture without crashing. It also induces sexual reproduction, possibly due to excess nitrate in 'Tp L1' and extra nitrogen from Tris. Auxospores can be readily observed in this buffered 'Tp L1'. Readers are referred to this study for further information.

'Tp L1' preparation details are available in this protocol.

Growth and preparation of T. pseudonana (recipient) cells

Step 6.

Centrifuge 2 x 10^8 cells 10 min / 4000 rpm / 10 °C. Add a few mL to your calculation due to cell death and losses during centrifugation (e.g. in this protocols 20 instead of calculated 15.4 mL were spun down).

Conjugation

Step 7.

Add 200 µL Tp cells to 1.5 mL epptube.

Conjugation

Step 8.

Add 200 µL E. coli cells. Pipette up and down a few (5) times.

Conjugation

Step 9.

Plate the mix on 1/2 'Tp L1' 5% LB 1% agar plate.

NOTES

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Plates have to be pre-dried. Leave them half lid open in the hood for 90 min before plating.

Conjugation

Step 10.

Incubate plates 90 min at 30 °C in dark.

Conjugation

Step 11.

Incubate plates additional 4 hrs at 18 °C under constant light.

Conjugation

Step 12.

Scrape co-culture with 1 mL 'Tp L1' and transfer to 1.5 mL epptube. Use L spreader. Expect 600–700 µL material.

Selection

Step 13.

Plate 50 and 500 µL using the pour plating method (**crucial**). Use 100 µg/mL nourseothricin sulfate for selection in pour plates.

NOTES

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1. Regular non-superclean agar may work, but this hasn't been tested (yet).

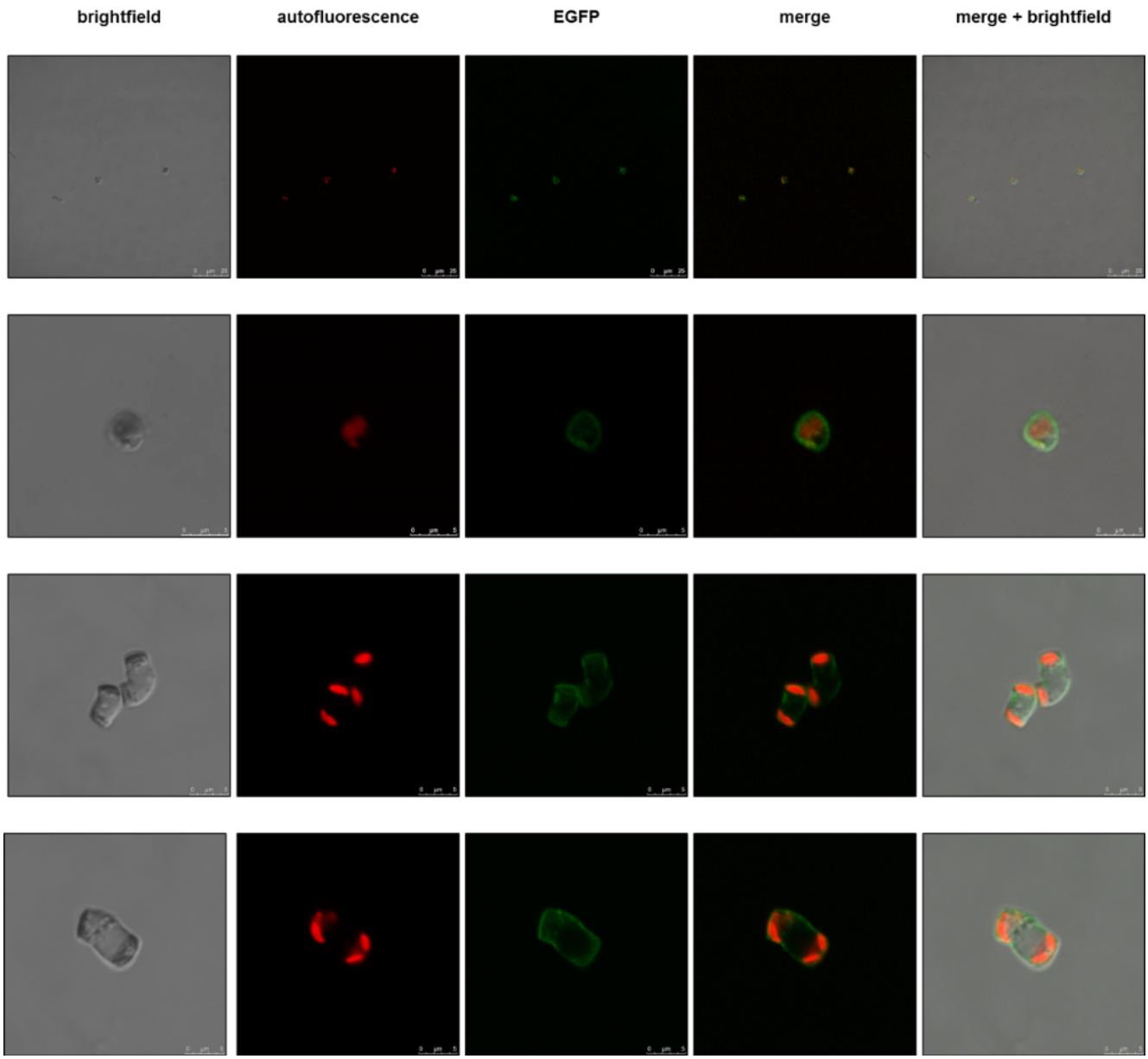
2. Some WT Tp colonies were observed in/on control plates which could be due to antibiotic depletion (2 x 10^7 cells were plated). Control plates contained 100 µg/mL nourseothricin sulfate.

Brief summary of results

Step 14.

Dozens of colonies emerged in pour plates after 2 weeks and 300 µL minicultures were started from single colonies. 200 µg/mL nourseothricin sulfate was used for selection in these minicultures. Leica TCS SP5 was used to screen minicultures for EGFP signal. Scale bars below: 25 µm (top row), 5 µm.

EXPECTED RESULTS



Brief summary of results

Step 15.