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Transformation of *Thalassiosira pseudonana* via bacterial conjugation

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ABSTRACT

This protocol has been successfully used to express nourseothricin resistance gene, mVenus fluorescence protein and other proteins related to triterpenoids production in *Thalassiosira pseudonana* (Tp) strain [CCMP1335](#). The original protocol was published by Karas et al. (2015) where a detailed description of L1 medium and plates preparation is presented.

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Protocol status: Working
We use this protocol and it's working

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Growth and preparation of *E. coli* donor

- 1 Inoculate 5 mL LB medium (gentamicin+antibiotic 2) with bacterial colonies from the gentamicin +antibiotic 2 plates. Grow overnight.
- 2 Start a 150 mL LB subculture with the 5 mL overnight culture (recommended starting OD₆₀₀ either 0.05 or 0.1).
- 3 Grow at 37⁰C until OD₆₀₀ reaches 0.4. (A range of OD₆₀₀ from 0.4 to 0.6 has worked for me)
- 4 Centrifuge at 4000 g, 10⁰C, for 10 min.
- 5 Decant supernatant and resuspend in 800 µL SOC.

Growth and preparation of diatom cells

- 6 Measure the *Thalassiosira pseudonana* cell concentration and calculate the required volume needed to collect 2×10^8 cells. Tp cells are cultured in L1 medium.
Note: We do not know if cell density before spinning cells down matters. We have successfully tried spinning cells down at $\sim 2-4 \times 10^6$ cells/mL
- 7 Spin down 4000 g, 10oC, for 10 min
- 8 Decant supernatant and resuspend pellet in 1 mL L1 medium. Final concentration 2×10^8 cells/m

Conjugation

- 9 Mix 200 μ L diatom cells and 200 μ L E. coli cells in a 1.5 mL tube.
- 10 Pipette up and down a few times.
- 11 Plate on 1/2xL1 1% agar plates w/ 5% LB.
Note: Make sure the plates are dry.
- 12 Incubate in dark at 30⁰C for 90 minutes.
- 13 Move plates to standard diatom growth conditions. Incubate 20-24 hrs
Note: Supposed to be 18⁰C and constant light, but we just leave them at RT constant light.

Selection

- 14 Add 1 mL L1 medium and scrape.
- 15 Plate 200 μ L of the resulting suspension on pre-dried 1/2xL1 1% agar plates w/ 50 ug/ml nourseothricin.
- 16 Leave at 18oC and constant light until colonies appear in ~10-12 days.