Sep 17, 2019

Tranformation of Thalassiosira pseudonana via bacterial conjugation

DOI
dx.doi.org/10.17504/protocols.io.7fghjjw

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Protocol Citation: Ana Cristina Jaramillo Madrid, Justin Ashworth 2019. Tranformation of Thalassiosira pseudonana via bacterial conjugation. **protocols.io https://dx.doi.org/10.17504/protocols.io.7fghjjw**

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

Created: September 17, 2019

Last Modified: September 17, 2019

Protocol Integer ID: 27848

Keywords: triterpenoids production in thalassiosira pseudonana, tranformation of thalassiosira pseudonana, thalassiosira pseudonana, triterpenoids production, bacterial conjugation this protocol, bacterial conjugation, nourseothricin resistance gene, mvenus fluorescence protein, protein, other protein

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.

Troubleshooting

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 OD_{600} either 0.05 or 0.1).

3 Grow at 37^0C until OD600 reaches 0.4. (A range of OD_{600} from 0.4 to 0.6 has worked for me)

4 Centrifuge at 4000 g, 10^0C, 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 ~2-4 × 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 × 108 cells/m

Conjugation

9 Mix 200 µL diatom cells and 200 µL 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 30^0C for 90 minutes.

13 Move plates to standard diatom growth conditions. Incubate 20-24 hrs

Note: Supposed to be 18^0C and constant light, but we just leave them at RT constant light.

Selection

14 Add 1 mL L1 medium and scrape.

15 Plate 200 µL 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.