Pour plating of Thalassiosira pseudonana (Tp) Version 2

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Abstract

Here we present an efficient way to grow Tp colonies inside a 0.25% superclean agar matrix. This protocol could possibly be applicable to other marine microeukaryotes that are problematic to grow on fully solid support.

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Before start

1. Tp L1

Prepare L1 medium with Aquil salts as described by Karas et al. (2015). After sterile filtration, add 5 mL 30 g/L Na2SiO3 x 9H2O per 1L L1. More details on exact medium preparation can be found here. This medium is referred to as 'Tp L1' in the protocol.

Reference:

Karas, B.J. et al. (2015) Designer diatom episomes delivered by bacterial conjugation. Nature Communications. 6, 692-695.

2. Superclean agar

Prepare according to Waterbury & Willey (1988). Excerpt:

'100 g of agar is washed by stirring with 3 liters of double-distilled water in a 4-liter beaker. After 30 min of stirring, the agar is allowed to settle, the wash water is siphoned off, and the agar is filtered onto Whatman F4 filter paper in a Buchner funnel. This procedure is repeated once more or until the filtrate is clear. The agar is then washed with 3 liters of 95% ethanol followed by a final 3-liter wash with analytical grade acetone. The agar is then dried at 50 °C in glass baking dishes for 2-3 days and stored in a tightly covered container.'

Use bacteriological agar from Sigma --> LINK.

Reference:

Waterbury, J.B. & Willey, J.M. 1988 Isolation and Growth of Marine Planktonic Cyanobacteria. Methods in Enzymology, vol. 167, pp. 100-105.

Protocol

Step 1.

For 300 mL (= 7 plates): heat 225 mL Tp L1 up in 50 °C water bath. Autoclave 75 mL MQ water with 0.75 g superclean agar separately.

Step 2.

Cool autoclaved agar to 50 °C, mix with Tp L1, add antibiotics if necessary. Swirl/mix well.

Step 3.

Move to 37 °C bath.

Step 4.

Lay out plates and get cell suspensions at desired dilution ready (0.5 mL total volume in 50 mL conical tubes works fine).

Step 5.

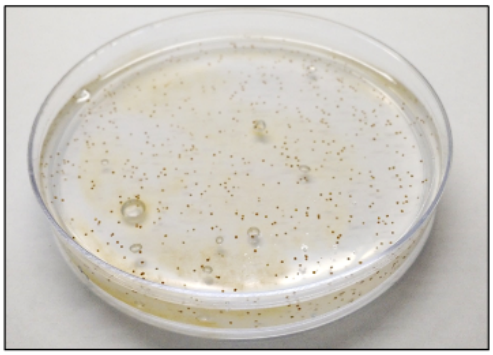
When you are ready, remove Tp L1-agar mix from 37 °C bath and allow to cool a few degrees (shake frequently and use temperature gun to monitor temperature drop --> option). 32-33 °C is a sweet spot. Critical: the mixture will start to solidify at 27-28 °C.

Step 6.

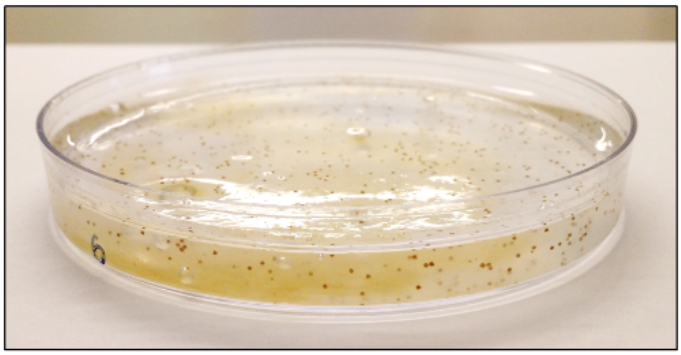
Add 40 mL of Tp L1-agar mixture to each cell suspension, then pour into plates. The extra depth from 40 mL is really useful in maintaining the plates.

Step 7.

We find plating 10^5 or less cells will give clearly distinguishable colonies. Expect the outcome on the figure below after 2 weeks incubation at 18 °C.



10^3



10^3

Step 8.

Plates behave as a liquid so you can simply pipette colonies out of matrix. Use a 20 uL pipette adjusted to 5 uL and narrow tips, carefully approach a desired colony and start pipetting a mm or so above it.

Step 9.

Dispense into 0.5 mL Tp L1 to grow an inoculum for a larger liquid culture.