

# Pour plating of *Thalassiosira pseudonana* (Tp)

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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 Na<sub>2</sub>SiO<sub>3</sub> x 9H<sub>2</sub>O 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.

### Step 3.

Move to 37 °C bath.

### Step 4.

Lay out plates, get cell suspensions to plate ready (have them ready in 50 mL conical tubes --> 0.5 mL total volume works fine).

### Step 5.

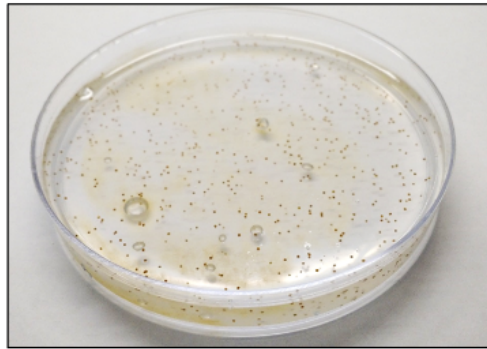
When you are ready, remove Tp L1-agar mix from 37 °C bath and allow to cool a few degrees (shake frequently and use temperature gun --> [option](#)). 32-33 °C is a sweet spot. Critical: the mixture will start to solidify at 27-28 °C.

### Step 6.

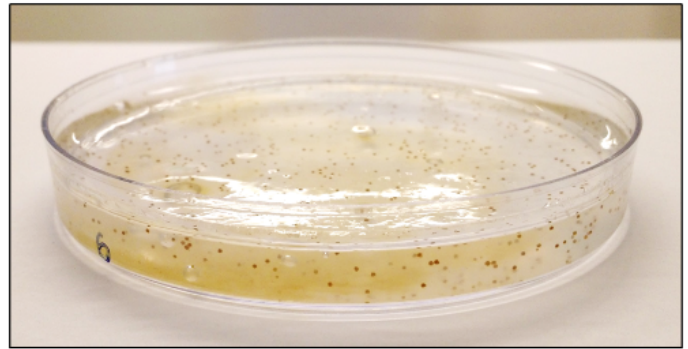
Add 40 mL of Tp L1-agar mixture to each cell suspension, then pour into plates. The extra depth from 40 mL is really useful in maintaining the plates.

### Step 7.

We find plating  $10^5$  or less cells will give clearly distinguishable colonies. Expect the outcome on the figure below after 2-3 weeks incubation at 18 °C.



$10^3$



$10^3$