Transformation of *Nannochloropsis oceanica* ccmp1779

Materials:

- 375 mM Sorbitol, sterile filtered and ice cold
- Recovery tubes (1 per Rx): 15 ml Falcon tubes with 5 ml f/2 (sterile and pre labeled)
- Selection Plates (1 per Rx): 150X10 mm ½ salinity f/2 agar plates containing 50 µg/ml Hygromycin B or an appropriate amount of any other Antibiotic to be used.
- 15 ml sterile Falcon Tubes
- Electroporation cuvettes with 2mm gap
- Sterile **long** 1ml filter tips
- Early log phase Nannochloropsis cells (100 Mio cells per Transformation reaction)
- Top Agar: ½ salinity f/2 culture medium with 1% Agar or 0.5% Phytoblend (Caisson)

DAY 1

1. Grow early log phase *N.oceanica* culture (to 10-30 Mio cells/ml)
2. Spin down 100 Mio cells per transformation reaction in 15 ml Falcon tubes (4000g for 15 min (4°C)).
    a. Cells can be pooled for all the reactions to save time and material
3. Wash 2x in 375mM Sorbitol (sterile, ice-cold) to remove salts.
4. Finally, resuspend pellet in 0.1 - 0.2 ml 375 mM Sorbitol per reaction.
    a. Note: the volume used per reaction depends on the volume of DNA that is added to the cells. The volume of DNA should not exceed 10% of the volume.
5. Add 3-5 µg of linearized DNA (more can be used and seems to improve transfection rate) and 30-50 µg of Salmon Sperm DNA (must be heat denatured at 95 °C for 1 min), incubate on ice for 10 min. Include no DNA control.
6. Before each Electroporation (Gene Pulser II) after setting all the parameters: Measure actual sample resistance (manual p. 12).
    a. Sample resistance> press buttons for 'actual volts' and 'time constant' at the same time
    b. Electroporation in 2 mm cuvettes:

        2.2 kV, capacitance at 50 µF, resistance at 600 Ohms

        For all electroporations record time constant (expected to be around 25ms)

        Note: actual volts, capacitance and time constant can be read *after* each electroporation, actual resistance of the sample can be read *before* the electroporation (usually 500-600 OHMS).

7. Immediately after electroporation transfer cells to culture tube with 5 mL sterile F/2 media (RT)
    a. Let the Culture recover for 48h with slow shaking and light
    b. 15 ml Falcon tubes can be placed in a Styrofoam and placed vertically in the shaker to insure mixing of the culture
    c. Culture conditions in our lab: 22°C, 100 rpm, 85 µmol quants m^{-2} s^{-2}

DAY 3

8. Melt top agar in microwave and keep at 42°C in a preheated water bath
    a. CAUTION: Be sure that the agar is not too hot, when you use it!
9. Collect cell pellet at 4500g, 10 min
10. Pipette 5 ml of top agar into each culture tube, mix well by pipetting up and down several times and pour the cells on 150 mm diameter selection plates.
11. Store on the light shelf, seal with parafilm after a few days.
12. Allow cells to grow until single colonies become visible (2-3 weeks) then pick colonies for liquid culture and screening.

SCREENING

A) Pick colonies and resuspend in 200 µl of water, spread on a selection plate to get single colony
    a. Use single colonies for analysis
B) Quick and dirty screening can be done in 96 well microtiter plates. Pick colonies to well containing 150 ul sterile f/2 with toothpick, use 48 prong plating tool to transfer the collection.
    a. Grow for a few days (depending on initial cell density 3-5 days) then screen high throughput