Synechocystis sp. PCC 6803 Transformation Protocol

Extracted from: Protocol based on protocol from the University of Turku received from Dr. Julie Zedler. Version: September 2017

Materials:

- *Synechocystis* target strain culture (healthy, logarithmic phase)
- 5 µg of purified plasmid per transformation
- fresh BG-11 medium
- BG-11 (NO antibiotics) plates
- BG-11 + 20% Antibiotics plates (depend on what the plasmid is you are using)

Protocol:

1. Day 1
   a. Pellet 5 – 10 mL of a healthy target strain (logarithmic phase, OD_{750} around 1-3) per transformation (4000 rpm, 10 min, RT in falcon tubes) (don't forget a negative control!!!).
   b. Discard supernatant and resuspend cells in 100 µL fresh BG-11 per 5-10 mL culture used.
   c. Transfer 100 µL of cells into clean eppy, add approximately 5 µL (1 µg) of plasmid DNA. Mix the suspension by pipetting carefully.
   d. Incubate the mixture shaking O/N at 30°C.
   e. Remark: I usually put the eppys in a brown 5 mL falcon tube and put that in the shaking incubator.
2. Day 2
   a. Plate the cell/DNA suspension straight on BG-11 plates (no antibiotics!).
   b. Spread cells and leave plates to dry properly in the sterile hood.
   c. Incubate O/N in the 30°C incubator with light protection (cover with some tissue).
3. Day 3
   a. Remove light protection and incubate in full light until the next day.
4. Day 4
   a. Wash cells carefully off the plate with approx. 400 µL fresh BG-11.
   b. Remark: I find it best to hold the plate vertical with one hand and repeatedly flush cells off to the bottom by pipetting.
   c. Transfer suspension on BG-11 plates with 20% antibiotics.

d. Spread cells and leave plates to dry properly in the sterile hood.
e. Incubate in full light at 30°C for approx. 7 to 14 days.

5. After colonies are visible….
   a. Re-streak single colonies on full concentration antibiotics BG-11 plates.
   b. Check for example by PCR. (Full segregation can take quite a while, keep on antibiotics).