Cultivation and transformation of *Synechocystis* strains.

Strain	Genotype	Reference
WT Synechocystis sp. PCC 6803		

Growth media: BG-11
WT is glucose tolerant

Preparation of liquid BG-11 media [1]:
Add 1.5 g $NaNO_3$ to 1 L of ddH_2O.
Optional: Add 5.95 g/L HEPES
Add 1 ml of each filter sterilized stock solution below.
Set pH to 7.5-7.8.
Autoclave.

Stock 1 - 50 ml ddH_2O	
K_2HPO_4	1.53 g
Stock 2 - 50 ml ddH_2O	
$MgSO_4$*7 H_2O	3.75 g
Stock 3 - 50 ml ddH_2O	
$CaCl_2$*2 H_2O	1.8 g
Citric acid	0.3 g
Stock 4 - 50 ml ddH_2O	
Ferric ammonium citrate	0.3 g
EDTA (disodium magnesium salt)	0.05 g
Stock 5 - 50 ml ddH_2O	
Na_2CO_3	1 g
Stock 6 - 1000 ml ddH2O	
H_3BO_3	2.86
$MnCl_2$*4 H_2O	1.81
$ZnSO_4$*7 H_2O	0.222
Na_2MoO_4*2 H_2O	0.39
$CuSO_4$*5 H_2O	0.079
$Co(NO_3)_2$*6 H_2O	0.0494

Optional: Prepare a stock solution of 0.5 M NaHCO3 in BG-11, e.g. 100 ml:
4.2 g NaHCO3
0.595 g HEPES
100 ml ddH2O
100 ul of each of the six BG-11 stock solutions
Adjust pH to around 7.8 with NaOH*

Do NOT autoclave, filter through 0.2 um filter

*pH typically ends up at about 7.7-7.8 when adding this much NaHCO3, so further pH adjustment is usually not needed.

Then you just mix 1/10 volume of stock with 9/10 volume of your normally prepared BG-11 supplemented with HEPES. You can always double check pH with pH paper in the media you have used so far (maybe for NaHCO3 media in particular) if you think pH might have been a problem.

Optional: A buffer should be added if cultures are grown in higher CO_2 than atmospheric levels, or if grown with up to 50 mM $NaHCO_3$ supplemented. Use e.g. 25 mM HEPES (5.95 g/L) for this purpose. *Synechocystis* can also be grown on glucose (e.g. 20 mM), which is necessary at dark, anoxic conditions.

Preparation of BG-11 agar plates:

<u>For 200 ml</u>
Prepare two flasks to be autoclaved separately:

<u>1</u>

- 3 g agar (standard bacteriological agar)
- 0.3 g $NaNO_3$
- 100 ml ddH_2O

<u>2 – set pH to 7.5-7.8</u>

- 0.6 g sodium thiosulfate
- 100 ml ddH_2O
- 200 ul of each stock solution
- optional: 1.19 g HEPES

Mix the two after autoclaving and add antibiotics as appropriate at 50 °C.

Alternatively, the two flasks can be stored separately in room temp for weeks/months. When agar plates are needed, just microwave the flask with agar (the flask should preferably not be filled to more than half) until all agar has melted. Mix 1:1 with room temp liquid phase (flask 2). The mix will then be at approximately 50 °C and antibiotics can be added directly.

Use approximately 20 ml/plate (larger volumes, e.g. 30 ml, can be used for long-term storage).
Plates *can* be sealed with one layer of parafilm to keep a moister environment. This also prevents the plate from drying out too fast.

Grow plates and liquid cultures at 20-30 °C (faster growth at 28-30 °C).
Light intensities of about 50 µmol photons m^{-2} s^{-1} are commonly used, but cultures can grow well both at higher and lower intensities. Very dilute cultures or recently plated transformants may be a bit sensitive to *strong* light and can be protected by a thin layer of paper if necessary.

Doubling time depends on the growth conditions, but is typically 10-12 h at around 50 μmol photons m^{-2} s^{-1} and 1% CO_2 or in media supplemented with glucose or $NaHCO_3$.

Natural transformation

Inoculate a culture to OD_{730} 0.1 or less. Grow until approximately OD_{730} 0.7. The plasmid used should be non-replicative in *Synechocystis* and have two homologous regions (ca 1000 bp each) for integration into the genome.

Protocol:

- Centrifuge 10 mL of healthy cells at an $O.D_{730nm}$ of ~ 0.7 at 4000x*g* for 6 min
- Wash pellet twice in BG-11
- Resuspend the cell pellet in 200 µL of BG-11 in a 2 ml eppendorf tube
- Add 1 µg of target plasmid to the cells and incubate the tube in light for 5 hours at 30 °C.
- Add 4 ml BG-11 and grow in a flask 18-24 h
- Plate cells on BG-11 plates with appropriate antibiotics

References

[1]: Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Microbiology. 1979 Mar 1;111(1):1-61.