Golden Lab - Cyano Growth Information

Synechococcus elongatus

We grow *Synechococcus elongatus* in BG-11 at a pH of 7.5 in constant light at 30°C.

How we begin cultures:

- To begin a subculture you should inoculate a 100 ml flask of BG-11 (with the appropriate antibiotics added to the media) with ~5-10 ml of existing liquid culture
    - A chunk of agar with culture growing on it will also work, but wait until the medium turns green to start shaking the culture
- After inoculation keep the culture in constant light at 30°C
- You can shake the cultures, but for growth it isn't imperative that the cultures shake
    - If you do shake the cultures you usually will see faster growth
    - If you are just maintaining stocks, put them at low light without shaking
- After 2 – 4 days the culture should be a nice forest green color
- We suggest that after the culture is fully grown you:
    - Transfer 5 ml of that culture to a new 100 ml flask
    - After the new flask is grown, freeze down your culture (Instructions listed below)
    - Don't keep shaking a fully grown flask—there is no point; move it to a shelf in low light to store it

Growth Media Notes:
We don't typically add bicarbonate to cultures, as they will grow up more quickly but also die more quickly if it is added. However, if you are bubbling in CO_2 you need to buffer the cultures by adding bicarbonate because acidification will kill the culture.

On Petri plates you should usually get colonies in 5-7 days. We add sodium thiosulfate (pentahydrate) to our plates (final conc of: 0.24819 g/L), but it is not used in our liquid cultures. The purpose of the sodium thiosulfate is to increase the efficiency of plated cells by scavenging the free radicals caused by autoclaving.

Condensation can sometimes occur on plates. You should be monitoring your plates to avoid this, as you don't want puddles of water on your plates, and flipping a plate usually fixes this problem. Which way you need to flip depends on the plate's position in a particular chamber, so just try one orientation and, if you see condensation forming, invert the plate.

pH:
Synechococcus elongatus tolerates a high pH (8-9), and as a culture grows the medium will become more alkaline. Below a pH of 7, however, the cells don't grow well and they are killed quickly below about pH 6.

Temperature:
We use 30°C as a good compromise between growth rate and plates not drying out too much, but the cells are happy up to 40°C (optimal around 38°C) and grow faster when warmer than 30°C.

Shaking:
In general *Synechococcus elongatus* flasks should be shaking at 150 RPM. This is to allow gas exchange and keep the surface moving, but for a general subculture of a flask you don't have to have it shaking to get growth (but you usually will see faster growth).

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Doubling Rate:
The doubling rate is directly related to light intensity as *Synechococcus elongatus* eats light and is tolerant to relatively high light. As a culture grows the doubling rate will continuously decrease as the cells shade each other and there is less light penetration, so each doubling is slower than the previous one. If a steady rate of growth is required, use a turbidostat to control culture density.

Light:
We grow all our cultures in constant light, so you do not need light/dark cycles to get growth. In the lab we have a variety of lighting conditions that are used, but between 80 µE (means $\mu E\ m^{-2}.s^{-1}$) and 500 µE is a ballpark range. In general we try to keep the light levels above 100 µE and it only is a problem if you get over 500 µE. For reviving cultures however many times a lower light intensity is better, somewhere around 10-20 µE.

Appearance:
Healthy *Synechococcus elongatus* should be medium green to blue green; if it has yellowed or changed colors it is probably dead, dying, or limited for a nutrient. Depending on the nutrient, it may or may not be healthy when yellow-green; *e.g.*, the wild-type strain deals very well with low iron conditions, but lack of combined nitrogen is not survivable for long. Growth of resistant strains in some antibiotics will cause the culture to be more green or blue, and this color change may not reflect how well the culture is growing. Most people equate bluer with healthier and denser, but this correlation is not valid.

Freezing Down Strains:
- Grow a 100 ml liquid culture of the Cyano strain
- Get four 2 ml sterile plastic sterile plastic freezer vials / screw cap tubes
- Label the tubes and add 80 µl of filter-sterilized DMSO to the tubes
- Spin down the whole 100 ml culture and resuspend it in 4 ml fresh BG-11 medium
- Place 1 ml of cells into each of the vials (4) and invert several times to mix
- Freeze IMMEDIATELY by placing the plastic vials in a -80°C freezer
    - **(DMSO is lethal to the Cyano cells at room temperature)**

Reviving Frozen Strains:
- Thaw the vial in your hands quickly
- Streak a loopful of cells onto a BG-11 plate (without antibiotics) and a BG-11 plate (with appropriate antibiotics) or plop a drop from a pipette onto the surface of the plate
- Add 500 µl of remaining volume to a 100 ml BG-11 flask (without antibiotics)
- Add the remaining volume (~500 ml) to a 100 ml BG-11 flask (with appropriate antibiotics)
- Incubate at 30°C in **LOW** light and do **NOT** shake the flasks yet
- When the plates and the flasks green up a bit (1-3 days) move the flasks to a shaker until they are fully grown and ready to be used
- Use the plate and flask that had the appropriate antibiotics added
    - The plate and flask without antibiotics were only for back up purposes
- Refreeze more samples when you thaw out the last of the 4 vials.