# Golden Lab - Cyano Growth Information

*Anabaena*

We grow *Anabaena* in BG-11o at a pH of: 7.5 in constant light of between 75 and 100 uE at 30°C. *Anabaena* is alive if it is a healthy green, but if it has yellowed or changed colors it is probably dead or dying.

**How we begin cultures:**

- To begin a culture you should inoculate a 100 ml flask of BG-11o (with the appropriate antibiotics added to the media) with ~10 ml of liquid culture to a final OD750 of about 0.05 for a pale mint green color.
    - A chunk of agar with culture growing on it will also work
- After inoculation keep the culture in constant light at 30°C
- You can shake the cultures at about 150 rpm, but for growth it isn't imperative that the cultures shake
    - The cells will fall to the bottom of the flask if they are not being shaken
    - Anabaena is clumpier than other cyanobacteria in N- media due to the stickiness of the heterocyst envelope.
- After 4 – 5 days the culture should be a nice forest green color and have an OD740 of about 0.2-0.4.
- We suggest that after the culture is fully grown you:
    - Transfer 10 ml of that culture to a new 100 ml flask
    - After the new flask is grown, freeze down your culture (Instructions listed below)

**Growth Media Notes:**
We don't typically add bicarbonate to cultures, as they will grow up quickly but also die quickly, but if you are bubbling in $CO_2$ however you need to buffer the cultures by adding bicarbonate.

*Anabaena* is grown in media lacking nitrogen (BG-11o) which is many times referred to as BG(N-) in our lab. If they are grown in Nitrogen+ media they can lose their ability to form heterocysts. If you need to start a culture in N+ media for a nitrogen step down, we recommend using ammonia for the nitrogen source for at least a day before nitrogen step down to fully suppress heterocyst development.

On Petri plates you should usually get colonies in 6-7 days. We add sodium thiosulfate to our plates, 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.

**pH:**
*Anabaena* is sensitive to low pH (below 7) and cultures will eventually crash if the pH goes to 11 or so.

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**Temperature:**
*Anabaena* is more sensitive to high temperatures than most cyanobacteria, but grows well at room temperature. We use 30°C as a good compromise between growth rate and plates not drying out too much, but the cells are happy between 23°C and 35°C.

**Shaking**:
In general, *Anabaena* flasks should be shaking at 150 RPM. This is to allow gas exchange and keep the surface moving. Since the cells will fall to the bottom of the flask if they are not being shaken, it is good practice to have the flasks shaking.

**Doubling Time:**
**On Agar**: Anabaena PCC 7120 will divide every 8 hours as a monolayer on a BG-11 agar plate with about 75 micro-mol photons/m2/s, but too much light is bad for the cells. Growth slows as the cells pile up in a colony.

**In liquid**: The cells divide about once a day after the culture looks green, maybe a little faster than that when the culture is very dilute, but they quickly start to self-shade. After the culture is medium green, the growth rate becomes quite slow.

Growth rates will vary widely depending on the temperature, light levels, and carbon dioxide availability. The cultures need to have good gas exchange to get rid of oxygen, which becomes a problem at higher levels, and obtain carbon dioxide.

**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 *Nostoc* and *Anabaena* strains do not tolerate light levels above 150 µE, and a light intensity of 75 µE is normally used. For reviving cultures however many times a lower light intensity is better, somewhere around 10-20 µE.

**Freezing Down Strains:**
- Grow a 100 ml liquid culture of the Cyano strain
- Get four 2 ml 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-11o media
- 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-11o plate (without antibiotics) and a BG-11o plate (with appropriate antibiotics)
- Add 500 µl of remaining volume to a 100 ml BG-11o flask (without antibiotics)

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- Add the remaining volume (~500 ul) to a 100 ml BG-11o flask (with appropriate antibiotics)
- Incubate at 30°C in **LOW** light (below 50 uE) 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 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, but if these grow and the others do not, you can spike antibiotics later.