

# Freezing and Reviving Cyano Strains

## Freezing Cyano Strains

- Grow a 100 ml liquid culture of the Cyano strain.
- Go to the freezer vial drawer and take **four** sterile plastic freezer vials containing DMSO (80 µl filter-sterilized).
- Spin down the whole culture and resuspend it in 4 ml fresh medium.
- Place 1 ml of cells into each of the vials (4) with 80 µl of filtered DMSO, and invert several times to mix.
- Freeze IMMEDIATELY by placing the plastic vials in the appropriate box of the appropriate -80°C freezer. **(DMSO is lethal to the Cyano cells at room temperature)**

## Reviving Cyano Strains

- Thaw the vial in your hands quickly.
- Streak a loopful of cells onto a BG(N+) plate (without antibiotics) and a BG(N+) plate (with appropriate antibiotics).
  - a. PCC 7942 may not come up well from a loop streak. Drop 10-50 µl onto the surface of the plates as well for this strain
- Add 500 µl of remaining volume to 100 ml BG(N+) in a flask (without antibiotics).
- Add the remaining volume (~500 µl) to 100 ml BG(N+) in a flask (with appropriate antibiotics).
- Incubate at 30°C in **LOW** light and do **NOT** shake the flasks yet. (You could also wrap the plates and the flasks in one layer of cheesecloth.)
- When the plates and the flasks green up a bit (1-3 days) move the flasks to a shaker until they are ready.
- Use the plate and flask that had the appropriate antibiotics added. (The plate and flask without antibiotics were only for back up purposes.)
- Check plates and flasks for signs of contamination, and double-check with microscope and/or by streaking a modified-Omni medium plate if contamination is suspected.