Updated Phycocyanin Protocol

1. Filter sample using Millipore filter rig, vacuum pump, and 25 mm GF/F filter. (Don’t forget to run the pump ‘open’ for five minutes after using it.)
2. Transfer filter to either foil packet (like a stick of gum) or clear 15 ml centrifuge tube. If using centrifuge tube, wrap tubes in foil to protect from light.
3. Freeze filters. Do not extract from fresh filters- freezing and thawing is a step in the procedure.
4. Thaw filters at room temperature in the dark.
5. The rest of the steps should be completed in reduced lighting. This can be accomplished by turning off the overhead lights and turning on the light in the fume hood.
6. If filters are in foil packets, transfer to clear 15 ml centrifuge tube using forceps that are rinsed with DI water between samples.
7. Cut filters into approximately eight narrow strips (this is necessary for the tissue homogenizer to effectively grind up the filter).
8. Add 5 ml of 1.5% CaCl2 solution to each tube using pipette.
9. Use tissue homogenizer to thoroughly grind up the filter and CaCl2 solution. Each sample should take between 30 seconds and 1 minute.
10. In between samples, clean the tissue homogenizer by running it in a 50 ml centrifuge tube with fresh DI water. This helps to prevent transfer of phycocyanin between samples.
11. Using Eppendorf 5804 centrifuge in Core Molecular Lab in Sutton Hall, centrifuge tubes at 4000g (indicated by rcf\*) for 10 minutes at room temperature. The radius setting should be changed to 15.5 cm if you are using 15 ml centrifuge tubes.
12. Install phycocyanin module on the Turner Trilogy fluorometer.
13. Turn on fluorometer using switch on the back.
14. Select ‘orange’ from the GUI when prompted. Select ‘OK’ to confirm.
15. Do not calibrate- measure raw fluorescence (RFU) and convert to units using excel spreadsheet.
16. Transfer 2 ml to the Nova Biotech cuvette. You must use this cuvette with the phycocyanin module.
17. Insert cuvette into phycocyanin module and select ‘read’.
18. Rinse cuvette with DI water and dry carefully with kim-wipe.
19. Repeat process for remaining samples.
20. Clean and put away cuvette.
21. Turn off fluorometer.
22. Dilute CaCl2 waste can be disposed of down the drain. GF/F filter pieces should go in the garbage. Centrifuge tubes should be bleach- and acid-washed before reuse following the usual dishes protocol.