**Quantifying Chl-a and Chl-c2 Concentration in Symbiodiniaceae from octocoral tissues**

Adapted from Putnam Lab Methods by T. Lindsay

**Materials**

* 100% acetone
* flammable safe fridge 4°
* quartz 96 well plate
* Microcentrifuge
* 1ml pipette and tips
* 1.5 ml microfuge tubes
* Synergy HTX Multi-Mode Microplate Reader
* Gen5 Software

**Protocol**

*This protocol uses freeze-dried and ground octocoral material. Freeze-drying these samples does not lyse the symbionts.*

**Day 1**

Sample prep (can be done in advance and stored at -80˚C)

1. Label two 1.5ml tubes with each sample name.
2. Weigh 15mg of freeze-dried material to tube 1. It cannot vary more than +/- 0.1mg. Record the exact weight.
3. Add 0.5ml DI II to tube 1 and vortex for 3+ minutes.
4. Use a pipette to transfer the supernatant from tube 1 to tube 2 to remove sclerites from the suspended material.
5. Repeat steps 3 & 4 two more times until tube 2 has 1.5 ml of liquid. The third rinse of the sclerites should be almost clear.
6. Discard tube 1 or save for sclerite morphology analysis.
7. Centrifuge tube 2 at 13,000g for 3 minutes to form a pellet of the symbionts.
8. Pipette off and discard the supernatant.
9. Proceed with the CHL extraction or keep the symbiont pellet at -80˚C until ready for extraction.

Chlorophyl extraction

1. Thaw samples
2. Add 1 mL of 100% acetone to the pellet.
3. Vortex until pellet is completely broken up and suspended in the acetone.
4. Place the tubes in a fridge in the dark at 4°C for 24 hours.

**A printer on a table

Description automatically generated with low confidenceDay 2**

Set up computer

1. Make sure the laptop is plugged in to the plate reader.
2. Turn on the computer.
3. Flip the on switch on the plate reader and wait three minutes for it to turn on. It should open and close the door a few times. It is ready when it stops with the door open.
4. Open the Gen5 software. It should give you options for which protocol to run. Open the KW\_chlorophyl protocol.

Sample Preparation

*It is best to do this part of the protocol in relative darkness or at least away from sunlight. Acetone evaporates easily, so once you’ve started to fill the wells you must work quickly.*

1. Make sure the quartz plate is clean and place it on a tray for safe transportation.
   1. It may be helpful to place a white piece of paper underneath to make it easier to see which well you’re on.
2. Take samples out of the fridge.
3. Vortex the tubes for 15 sec.
4. Centrifuge the samples at 13,000 rpm for 3 minutes to pellet any debris.
5. Pipette 200µl of acetone blank to duplicate wells.
6. Pipette 200µl of sample to duplicate wells of 96-well quartz plate.
7. Put the place on the plate reader tray and run the KW\_chlorophyl protocol.
   1. This measures absorbance at 630, 663, and 750 nm in a 96 well plate.
8. Save the output xpt file and save the data as txt file (our computer can’t do xlsx).

**Data Analysis**

1. Convert the txt file to csv.
2. Use the R code to convert raw data in chl a and chl c data.
3. Standardize for path length in 200µl of sample in 96-well quartz plate.

Explanation of analysis

Chlorophyll a and c2 concentrations are calculated from the equations in Jeffrey and Humphrey 1975 after substracting A750nm from all measurements.

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Need to correct for differences in path length of the volume in the 96 well plate compared to the 1cm path length of a cuvette.

References

Jeffrey and Humphrey 1975

Warren 2008

Synergy HTX Operating Manual

Gen5 Software Manual