**Airbrushing Protocol**

Adapted from Putnam lab protocols by T. Lindsay

Materials

* Airbrush Supplies
  + Compressor
  + Hose
  + Brush
  + Bottle
* 1x PBS (pH = 7.4)
* Ice bucket & freezer beads
* Quart size ziplock bags
* 50mL falcon tubes
* Isopropanol wipes
* Forceps
* Clean Squeeze bottle
* Tissue homogenizer
* 10% bleach
* DI water
* Gloves
* Scissors

Prep

* Fill airbrush bottle and squeeze bottle with PBS
* Select samples and put them in the ice bucket
* Label falcon tube and plastic bag
* Sanitize work area and equipment with isopropanol wipes

Protocol

1. Use cleaned forceps to place coral fragment in labeled ziplock bag
2. Airbrush with 1x PBS
   1. Be sure to remove tissue from entire area including deep tissue
   2. Attempt to keep total blastate volume below 50mL to fit into one falcon tube
3. Transfer slurry into sterile falcon tube but cutting corner of bag. Rinse sides of bag using the squeeze bottle, adding the rinsate to falcon tube
4. Record total volume of tissue slurry and store in ice bucket until homogenization.
5. Homogenize slurry for 30 sec in falcon tube using tissue homogenizer at full power.
   1. If slurry is more than 50 mL, homogenize both falcon tubes then pour back and forth between tubes to ensure even mixing
6. Between each sample, clean the forceps and homogenizer in a 10% bleach solution for 30 seconds, then a first DI wash for 30 seconds, and a second DI wash for 30 seconds. Finally, wipe down with an isopropanol wipe.

**Aliquot Protocols**

Adapted from Putnam lab protocols by T. Lindsay

Materials

* 4 x 1.5 mL microcentrifuge tubes
* Centrifuge
* 1000 uL Pipet and tips
* Vortex
* 2 Disposal containers (for tips and for excess supernatant)

Protocol

1. Label four 1.5-mL microcentrifuge tubes for each sample
   1. Include project name, sample name, sample treatment, and “CHL”, “SYM-D”, “HOST-PTAC”, or “SYM-PTAC”
   2. On lid, record the sample and treatment names.
2. Pipette 1mL homogenized tissue blastate to each of the CHL and SYM-PTAC tubes.
3. Cetrifuge both 1.5 mL tubes at 13,000g for 3min.
4. From the CHL tube, pipet off and discard the supernatant (~1mL). The pellet is the chlorophyl sample.
5. From the SYM-PTAC tube, pipet off the supernatant and transfer into the HOST-PTAC tube. This is the host protein/antioxidant/lipid sample.
6. To the symbiont pellet from the SYM-PTAC tube, add 1 mL 1xPBS to resuspend pellet. Vortex for 30 seconds and pipet up and down to fully dissolve pellet. Once dissolved, transfer 500 µL to the SYM-D tube. These two tubes are the symbiont density and the symbiont protein/antioxidant/lipid samples.
7. Place the four tubes in their respective freezer boxes and record which box number they’re in.
8. Freeze the remainder of the homogenate in the falcon tube in -40˚C until use.