**Airbrushing Protocol**

Adapted from Putnam lab protocols by T. Lindsay

Materials

* Airbrush
* Compressed air source
* Tissue homogenizer Bio-Gen Pro200 homogenizer
* 10% bleach
* DI water
* Isopropanol wipes
* Ice-cold 1x PBS (pH = 7.4)
* Forceps
* Quart size ziplock bags
* 50mL falcon tubes

Protocol

1. Remove coral frag from freezer
2. Use cleaned forceps to place frag in labeled ziplock bag
3. 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
4. Transfer slurry into sterile falcon tube and rinse sides of bag, adding the rinsate to falcon tube
5. Record total volume of tissue slurry and store on ice until homogenization
6. Homogenize slurry for 30 sec in falcon tube using tissue homogenizer.
   1. If slurry is more than 50 mL, homogenize both falcon tubes then pour back and forth between tubes to ensure even mixing
7. 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

* 1.5 mL microcentrifuge tubes
* Centrifuge
* Pipets
* Vortex

Protocol

1. Label four 1.5-mL microcentrifuge tubes for each sample
2. Add 1mL homogenized tissue blastate to two of the 1.5 mL tubes
3. Cetrifuge both 1.5 mL tubes at 13,000g for 3min
4. *Chlorophyll sample:* From one of the tubes, pipet off and discard the supernatant (~1mL). WHERE TO STORE?
5. *Coral Protein/TAC/LPO sample:* from the second tube, pipet off the supernatant and transfer into a new labeled 1.5 mL tube. Immediately place in -40˚C until ready to proceed with protein assay, total antioxidant capacity assay, or lipid peroxidation assay.
6. *Symbiont protein/TAC/LPO sample:* to the symbiont pellet from tube 2, add 1 mL 1xPBS to resuspend pellet. Vortex thoroughly and pipet up and down to fully dissolve pellet. Once dissolved, transfer 500 µL to a new labeled 1.5 mL tube. Immediately place in -40˚C until ready to proceed with assays.
7. *Symbiont density:* store the remaining 500µL of resuspended symbionts in PBS in 4˚C fridge in LTER lab until ready to count cells following the symbiont density protocol
8. *Ash-free dry weight:* Freeze the remainder of the homogenate in the falcon tube in -40˚C until use.