# **Standard protocol for coral tissue removal**

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1. Materials
   1. 2 x squirt bottles (deionized water (ddH2O), 100% ethanol (EtOH))
   2. 50 ml falcon tubes
   3. VivoHome, Professional Airbrushing kit (model (VH174)
   4. 2 ml microcentrifuge tubes, labeled (if aliquoting)—one for each aliquot
   5. Ice in a cooler
   6. Notebook
   7. Eyeglasses
   8. Nitrile gloves
   9. Plastic sandwich bags
   10. Chemwipes
   11. Drying oven
   12. Homogenizer
2. Reminder
   1. Coral samples (whole colonies or nubbins) and tissue isolates (i.e. blastates) should be kept on ice in a cooler as much as possible. This will help with mucus production, clumping, and foaming.
3. Protocol
   1. The coral tissue will be aerosolized, so use protective eyewear to avoid tissue or skeletal fragments from getting in your eye. Airbrushing should be done in the fume hood.
   2. First, label all tubes (50 ml) destined for the tissue blastate, and prepare aliquot-ready microcentrifuge tubes. Remove corals from the freezer (-20°C or -80°C) and place on ice.
   3. Isolating coral tissue:
   4. Place coral in plastic bag, seal the bag with enough room to place airbrush inside. Form a loose seal with your hand around the airbrush with one hand, holding the coral fragment (in the bag) with the other hand.
   5. Use short bursts of air + seawater to remove coral tissue. “Paint” the skeleton, moving back and form and avoid staying in one spot or holding down the “trigger” of the airbrush for too long. Pushing on the trigger gives you air > water, pulling the trigger back and down gives water > air. Use both techniques depending on your requirements (volume, colony size, complexity of skeletal architecture).
   6. Once finished, apply a few squirts of water to the coral skeleton in the bag.
   7. Remove the skeleton and place in a labeled bag or receptacle (pan, cup, new bag). Dry coral skeletons in a drying oven (60°C) thoroughly. MAKE SURE the skeleton remains with an easily identifiable label.
   8. Decant blastate into 50 ml falcon tube—carefully—and rinse bag with ~ 2ml of FSW
   9. Combine bag wash with blastate and cap the falcon tube; discard the sandwich bag.
   10. Move to the tissue homogenizer. First, rinse the probe with 100% ethanol (EtOH), then rinse with ddH2O, and wipe with chemwipes.
   11. Homogenize blastate with tissue homogenizer for 15 seconds. Once finished, rinse the homogenizer briefly, letting the wash drip into the 50 ml falcon tube.
   12. Rinse homogenizer with ddH2O into the falcon tube, and wash homogenizer with EtOH (100%). Make sure to do this after each sample.
   13. **Note blastate volume** this step is critical. You can either note volume by looking at marks on side of microcentrifuge tube (topping off blastate with ddH2O to nearest line, or desired volume). Alternatively, transfer to a graduated cylinder, measure and top off, and rinse graduated cylinder with ddH2O and 100% EtOH after each sample.
   14. Blastate is now ready for freezer or aliquoting

For our experiment:

aliquots needed per coral tissue slurry:

a. 2mL = zooxanthellae counts

b. two 1.5mL = chlorophyll a concentration

c. 0.5 mls = Total protein

1. References
   1. Wall, C. 2017. Coral tissue extraction: Airbrush method protocol. Dr. Ruth Gates’ Laboratory Hawaii Institute of Marine Biology, University of Hawaii.

LIST TO GET

Aluminium foil

Acetone

More (x2 each size) foam holders for ALL tube sizes

Waterproof notebook

2ml cryogenic vials