**Airbrushing Protocol**

1. Grab 1 nubbin from the -80C freezer
2. Open tin foil and allow to thaw for ~30 mins
   1. Check for small tube in the foil
   2. Check if you can read the label on the petri dish (DO NOT TOUCH THE NUBBIN) and if it matches the one on the tube; Ensure the coral ID label on the small EtOH tube is legible. If not, write a new coral ID label on the small EtOH tube. If there is no tube, please fill a new one with 100% EtOH and label clearly
   3. Make appropriate notes in your notebook of which nubbin it is and any labeling issues
3. Put on your safety gear (coat, gloves, and glasses)
4. Sample 3-4 polyps for RNA
   1. Using a STERILE razorblade, dig them out and transfer them to the microsampling tube
   2. Immediately cap and take to the -80 and place in the appropriate box
5. Before beginning, ensure that there is plenty of seawater, ethanol and DI water at your station
6. Label (sample ID, initials, and date) your other tubes
   1. 4 bead blasting tubes for the host (labeled ID-H)
   2. 4 bead blasting tubes for the symbiont (labeled ID-S)
   3. 1 1.5-ml tube for symbiont counts (labeled ID-C)
   4. 1 15-ml conical (labeled ID- extra host)
   5. Add beads to bead blaster tubes (but not the 1.5 ml tube), a bit less than up to the 0.1ml line
7. Clean the airbrush
   1. Run half a reservoir of 50% EtOH through, also rinsing the outer nozzle with EtOH
   2. Then a full reservoir of DI, and rinse the exterior of the nozzle THOROUGHLY
   3. Run half a reservoir of Instant Ocean water through
8. You may have to wait a bit longer for the nubbin to be thawed enough for airbrushing
9. When the nubbin is ready, grab a new sterile Ziploc bag
10. Fill the airbrush reservoir with seawater (use seawater only, be sure it is not EtOH)
11. Spray off all tissue into plastic bag (3-5 reservoirs, usually)
12. Seal Ziploc and cut corner off the bottom of the bag
13. Pour tissue slurry into a clean 50ml conical tube (rinsed ones will be at the sink/drying rack near the airbrushing stations) and record the volume to the nearest ml
    1. If the volume is not at least 5 ml, add seawater until it is but NOT before noting the volume
14. Homogenize immediately for 3 minutes (use the timer!)
    1. Set homogenizer to 8
    2. The lower hole must always be submerged while the upper hole must never be submerged
    3. Do not set the tube down while homogenizing
    4. Note: never lay the homogenizer flat
15. Take your symbiont count aliquot
    1. 300 µl into the 1.5 ml tube immediately after homogenizing
16. Hang up the homogenizer
17. Put the symbiont count aliquot in the fridge, just hang it in a slat of a rack
18. Pellet the symbionts
    1. Centrifuge the slurry conical at 4400 rpm for 3 minutes
    2. BALANCE THE CENTRIFUGE by having equal volume in other conicals in other buckets
19. While waiting, clean the homogenizer by running it in a conical filled with DI, bottom hole covered, top hole open
    1. Empty the conical after cleaning (every time)
    2. Wipe off with a Kimwipe and then let it air dry before replacing the cap on the bottom
20. Clean the airbrush with EtOH and then DI water. This includes wiping down the nozzle. Use just a small amount of EtOH and then run a full reservoir of DI water through; hang it back up on the stand to dry
21. After centrifuging, immediately aliquot the host fractions, being careful not to disturb the symbiont pellet
    1. 600 µl TWO TIMES into each host tube (BIG BLUE PIPETTE)
22. Pour off extra host fraction into a labeled 15 ml conical; DO NOT OVERFILL because it will expand when frozen
    1. Any excess can be poured down the drain
23. Wash the symbiont pellet GENTLY with seawater, but if it starts to be disturbed, just stop
    1. Do this by pouring water on the SIDE of the conical and swirling ever so softly and then dumping in the sink, 2-3 times if not disturbed
24. Resuspend the symbionts in 5 ml
    1. Vortex vigorously
    2. 600 µl TWO TIMES into each symbiont tube; if there is a little left that you didn’t quite take up, just take it up and split it (ish) among the tubes
25. Bead blast at 6 m/s for 2 minutes
26. Immediately freeze all tubes except symbiont count
27. Count the symbionts
28. CLEAN YOUR STATION!!!!!!!! This includes emptying all trash containers and wiping down the counter with ethanol.