Medina Lab
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Coral sample prep for molecular work – Coral Grinding Protocol

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Samples must be on ice at all times, especially if intended for RNA work.

Materials needed:

1. Styrofoam boxes to work in, 2 or 3
2. Dry ice (enough to keep samples, work area, and tools cold)
3. Ethanol (to clean, 70-90% is ok)
4. RNAse Away (or equivalent)
5. Kimwipes
6. Frozen chisel and mortars (at least one of each per sample)
7. Aluminum foil
8. Scoops and forceps (at least 2 per sample, clean often)
9. Hammer
10. Chisel
11. Liquid nitrogen (optional)
12. Ladle (if you chose to use liquid nitrogen, depending on LN storage).
13. Pre-labeled cryovials (label the lid too!)
14. Your sample!

Directions:

1. Clean the work area, scoops, forceps, and chisel with ethanol and RNAse Away. Keep clean scoops and forceps cold by placing on dry ice covered by a kimwipe.
2. Place the cold mortar on the dry ice and take the aluminum foil off it. Unwrap the sample and place it in the mortar. Work quickly (transfer as little body heat as possible).
3. If there is a lot of skeleton (looks white) you want to remove as much as possible. Use the chisel and the hammer to break the fragment. You can also use the pestle to give the sample a couple of smashes. Be careful that your sample does not fly away. Remove skeleton with forceps and toss it. Alternatively, overtime, condensation will build up inside the mortar unless you are frequently adding liquid nitrogen. So, you may just transfer your sample (normally, the colored (coral) tissue) to a new clean mortar. If the sample is too big and you can save some, wrap it in aluminum foil or place it in a new cryovial. Don't forget to label it!
4. Use the pestle to grind the coral sample until it looks like powder.
5. Transfer the sample to an extraction or storage tube.
6. Proceed with steps 2-6 for further samples. Clean scoops and forceps with ethanol and RNAse Away between each sample.

7. Wash the mortar and pestles with distilled water, then MilliQ water. Let air dry. Wipe with ethanol, then RNAse Away, then cover with aluminum foil. Freeze in the -80 º C.
8. Rinse scoops and forceps with distilled water. Air dry and put them away in drawer. Put all materials back in place and the samples in the -80.