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Coral tissue and skeleton separation for downstream DNA and RNA extraction

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Protocol status: Working
We use this protocol and it's working

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Materials

MATERIALS

- Liquid nitrogen
- Ultrapure Distilled, Nuclease Free Water
- Tweezers
- 70% Ethanol
- RNaseZap **Ambion Catalog #**AM9780
- ELGA water
- microcentrifuge tubes
- EDTA
- 50ml Falcon tubes **Corning Catalog #**352070
- Phosphate Buffered Saline
- Kimtech Science™ Kimwipes™ Delicate Task Wipers, 1-Ply **Thermo Fisher Catalog #**06666
- Sterilin™ Weighing Boats, Square, White, small 7mL **Thermo Fisher Catalog #**WB30205
- Acrodisc® syringe filters13 mm Dia 0.2 µm Pore Size **Cole-Parmer Catalog #**4602
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- Compressed air
- 60mL sterile syringes
- Dry ice
- Autoclavable funnels
- Stainless steel chisel
- Cryogloves
- Cryolabels
- Mallot
- 1000uL sterile filter tips
- Weighing boats sterile
- Stainless Steel Spatula/Scoop

Equipment

Ultrasonic Cleaner

NAME

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BRAND

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SKU

Equipment

Acrylic box for airbrushing

NAME

NA

BRAND

NA

SKU

Acrylic box with two side holes for hands. Airbrushing inside of a box (or some similar space) helps contain the mess from airbrushing and prevent contamination.

SPECIFICATIONS

Equipment

Tissue-Tearor

NAME

Tissue homogenizer

TYPE

BioSpec

BRAND

985370EUR-14

SKU

Equipment

Airbrush

NAME

WA Platinum Airbrush

TYPE

GSI Creos

BRAND

GSI Creos Mr. Procon Boy WA Platinum Airbrush

SKU

Equipment

BioPulverizer

NAME

biospec

BRAND

59014N

SKU

59014N

SPECIFICATIONS

Preparation

1 Prepare airbrush solution of [M] 1 x PBS with [M] 10 micromolar (µM) EDTA (Hester et al., 2016; ISME J, 10, 1157–1169)

1.1 Add 5.84 mg EDTA to 2 L of [M] 1 x PBS .

1.2 Autoclave PBS/EDTA solution.

1.3 Prior to airbrushing, in a nuclease-free work space (e.g. UV hood), filter sterilize small volumes (~30-50mL) with a 0.22µm syringe filter into sterile nuclease-free falcon/corning tubes. Having small aliquots helps to avoid contamination during airbrushing.

2 Autoclave funnels (8-16 funnels recommended) and store in container cleaned with bleach, ethanol, and RNAseZap.

3 Pre-label microcentrifuge tubes with cryolabels. For extracting DNA & RNA from both tissue and skeleton, it is recommended to prepare 6 microcentrifuge tubes per coral sample.

Pre-label falcon tubes (2x per coral sample), which will be used to temporarily hold the tissue and skeleton during processing.

4 Prepare airbrushing work space.

Note: it is recommended to airbrush in laboratory space that is removed from any molecular work-DNA/RNA extractions as aerosolized particles could be a source of contamination.

4.1 Clean airbrush, tissue homogenizer, chisel, cryopulverizer with ethanol and RNaseZap. Rinse thoroughly with nuclease free water.

4.2 Connect airbrush to compressed air source. Fill with nuclease free water and purge this water out the front end of the airbrush to clean the inside of the airbrush.

4.3 Clean workspace and acrylic box with bleach, ethanol, and RNaseZap.

5 Fill one styrofoam box with liquid nitrogen (LN2) and another with dry ice. The liquid nitrogen box will be used for the cryopulverizer and the dry ice box to store samples. Liquid nitrogen can also be used to store samples.

6 Take samples you plan to process out of -80°C freezer and place on dry ice or LN2.

Note: It is good to have two people working together on this protocol: one airbrushing and one pulverizing. With two people, 16 - 20 samples can be comfortably processed in a day.

Tissue Airbrushing

7 Fill a small insulated bowl with dry ice and place a labelled falcon tube inside the bowl, such that the bottom is in contact with the dry ice and tube is upright. The dry ice will help keep the sample cold and prevent degradation while you are airbrushing.

Note: depending on the size of your tissue homogenizer, it might be easiest to use 50mL falcon/corning tubes so that the probe can fit inside the tube easily.

8 Take out an autoclaved funnel and spray with RNaseZap. Rinse with nuclease free water. Dry briefly with compressed air using airbrush.

Place the funnel inside the falcon tube (Step 6) and inside the acrylic box.

9 Take your sample out of dry ice/LN2. If needed, break the sample into a smaller piece using the sterilized chisel, and place any remaining sample back on dry ice/LN2 or back in -80°C. Place the sample inside the funnel.

10 From the PBS aliquots, pour 3-5mL of the 0.22µm filtered, autoclaved PBS solution into the airbrush. (Close this tube immediately. Do not leave open during airbrushing. Switch to a new aliquot if any contamination is suspected.)

Hold the sample using sterilized tweezers above the funnel inside the acrylic box. Remove the coral tissue and collect it in the falcon tube by airbrushing with the PBS solution.

Depending on the size of the fragment and desired volume, use more PBS solution as needed.

11 Once airbrushing is completed, put the remaining skeleton in a clean falcon tube and place the tube in LN2 or on dry ice.

12 Take falcon tube with airbrushed tissue off of dry ice and homogenize for ~5 seconds with the tissue homogenizer.

13 Using filter pipette tips, dispense this liquid into microcentrifuge tubes or directly into extraction tubes.

Note: It is a good idea to make at least 2-3 aliquots per sample. Two of 200-500uL to be used for DNA & RNA, and another aliquot can be saved as back-up sample (1-2mL).

Note: For RNA work, you can also place airbrushed tissue directly into Trizol solution and store this tissue/Trizol mixture at -80C until extraction. If you are storing in Trizol, it is important to vortex the tissue/Trizol mixture well before freezing. (However, we find the yields are higher when we freeze a tissue aliquot directly, and then add Trizol on the day of extraction--see RNA extraction protocol for more details).

14 Immediately place tissue sample aliquots on dry ice or in a dry shipper/LN2. Store at -80 °C until extraction.

Skeleton Pulverizing

15 Prepare pulverizing work space. Clean tweezers, bench top and cryopulverizer with ethanol and RNAseZap. Rinse with nuclease free water. **Thoroughly dry cryopulverizer** with kim wipes. (If any moisture remains in the pulverizer, this can freeze and seal the two pieces together.)

16 Place cryopulverizer in LN2 and allow to cool for 3 minutes.

17 Take tissue-free skeletal sample in falcon tube from Step 8 and cover skeleton in LN2 by either putting a small amount of LN2 in the falcon tube or putting skeletal fragment in a small insulated dish containing LN2.

18 Once the cryopulverizer has cooled, remove it from LN2 and place the sample in the pulverizer using tweezers. Hit the pulverizer several times with a mallet until it is a fine powder.

19 Transfer the skeletal sample into microcentrifuge tubes.

Note: It is a good idea to separate into 2-3 tubes. Two tubes with approximately 0.2 - 0.5cm^3 of skeleton can to be used for DNA & RNA extraction, and another tube can be stored as back-up sample. This will help avoid unnecessary freeze-thaw cycles.

Note: For RNA work, you can also place the skeleton directly into Trizol solution and store this skeletal/Trizol mixture at -80C until extraction. If you are storing in Trizol, it is important to vortex the tissue/Trizol mixture well before freezing. It is also recommended to add 1-5µL of 6M HCl per 1 mL of Trizol to the skeletal/Trizol mixture, as the skeleton can change the Trizol pH. (However, we find the yields are higher when we freeze the skeletal aliquot directly, and then add Trizol on the day of extraction--see RNA extraction protocol for more details).

20 Immediately place these sample tubes on dry ice or in a dry shipper/LN2. Store at -80 °C until extraction.

Cleaning

21 Clean tissue homogenizer:

After using tissue homogenizer, clean between samples by first rinsing in sequentially in 3 beakers of Elga water. Turn on briefly in the water. Unscrew the outer portion of the probe. Spray thoroughly with ethanol. Clean with RNaseZap. Rinse with nuclease-free water.

To help remove the RNaseZap from the homogenizer, fill a clean falcon tube with a few mL of nuclease-free water and turn the homogenizer on briefly. Repeat until you don't see any foaming in the water from the RNaseZap.

22 Clean cryopulverizer:

If working with replicate samples, the pulverizer can be cleaned while still frozen and dry by wiping the top and the inside of the pulverizer thoroughly with a kim wipe. A metal object (e.g. tweezers) can be used to help. Put the two pieces together and place the pulverizer back in LN2 so that it stays cold. (If it starts to warm up, humidity in the air can cause ice to form on the inside).

When switching sample type (i.e. not replicate samples), thaw the pulverizer for cleaning. The pulverizer can be placed in a drying oven (50-60C) to speed up thawing. Once completely thawed, place the pulverizer in an ultrasonicator for ~5 minutes to remove any fine skeletal powder. As specified above (Step 2), clean with ethanol, RNaseZap, and nuclease free water. Once completely dry, place in LN2 to cool down for the next sample.

23 Clean workspace and acrylic box with bleach, ethanol, and RNaseZap. (If the acrylic box is very dirty, it can be rinsed first with Elga water).

24 Clean airbrush and chisel with ethanol and RNaseZap. Rinse with nuclease free water.

Purge airbrush with nuclease free water.

25 Funnels should be washed and re-autoclaved before reuse.