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| **1. Aim/Rationale of Protocol** |

* The purpose of this protocol is to extract and purify genomic DNA from coral samples that is suitable for PCR, qPCR, sequencing, and other downstream applications. DNA is recovered from the coral host, *Symbiodinium*, and other members of the holobiont.

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| **2. Reagents needed** |

* **DNAB** (DNA Buffer; store at 4°C)

50 mL 4M NaCl

50 mL of 0.5M EDTA

Make up to 500 mL with ddH2O

* **CTAB mix** (store at -20°C)

0.75g CTAB

add ~20 mL H2O to dissolve CTAB

12.5 mL of 4M NaCl

75 µL E. coli tRNA (at 20 mg/mL stock)

Make up to 50 mL with ddH2O

* **3M Sodium Acetate** (store at room temperature)

12.3g Na Acetate anhydrous

Dissolve in ~40mL ddH2O

Adjust pH to 5.2 with Glacial Acetic Acid

Make up to 50 mL with ddH2O

Dilute an aliquot to 0.3M for use in protocol

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| **3. Workflow** |

### DNA Extraction/Isolation:

* These steps should be undertaken in batches no larger than 24, which is the maximum capacity of the centrifuges.
* DNA can be extracted from any quantity of sample archive. The protocol below extracts from 100 µL, but volumes can be proportionally adjusted up to the second ethanol precipitation if desired (e.g., to increase absolute DNA yields).

*Proteinase K digest:*

1. Defrost Proteinase K and vortex well to ensure it is fully mixed. Prepare a set of new 1.5 mL microcentrifuge tubes for the samples you intend to process and add 5 µL of Proteinase K (10 mg/mL, stored in freezer) to the bottom of each tube (you can use the same pipette tip).
2. Add 150 µL of sample archive to each of your new tubes. Use a new pipette tip for each sample. Vortex samples well. These tubes now contain 150 µL of sample and 5 µL of Proteinase K (155 µL total). Return the SDS samples to the archives.
3. Incubate for 2-3h at 55°C.

*Organic Extraction:*

1. Defrost CTAB mix (stored at -20°C). You can defrost CTAB by microwaving for 5 seconds on “high”, or by heating in water bath.
2. Add twice volume (310 µL) of CTAB mix, vortex, and incubate at 65°C for 30-60 minutes.
3. Allow samples to cool. In fume hood, add equal volume (465 µL) of chloroform. Be sure to ‘charge’ (i.e., fill and empty pipette tip with chloroform 2 to 3 times) the pipette tip before first use, or your tip will leak chloroform. Vortex sample and invert several times, but be careful that caps are tight – leaking chloroform will erase your sample labels! Put in rack on rotating platform (speed 500) for 2-3 hours.

*First ethanol Precipitation:*

1. Centrifuge at 10,000g (RCF) for 10 minutes. Align tubes in centrifuge so that hinges are on the outside. While spinning, prepare a new set of labeled 1.5 mL tubes. Remove samples from centrifuge and very carefully pipette off top in two rounds of ~185 µL (using 200 µL pipette) into new tube. Dispose the rest of the contents into appropriate waste container.
2. Add twice volume (750 µL) of 100% (200-proof) ethanol (EtOH). Ensure caps are shut tightly and invert samples in their rack several times, together with a few brief shakes to make sure samples are well mixed.
3. Put samples in freezer for at least 2 hours to promote DNA precipitation. If the EtOH is pre-chilled, you can leave it in the -20°C freezer for only a 1/2 hour.

**- Potential Stopping Point -**

Keep in freezer to allow DNA to precipitate

*Second Ethanol Precipitation:*

1. Put samples in centrifuge (ensuring that the hinges of the tubes are on the outside) and spin for 10 minutes at 10,000g (RCF).
2. Remove samples from centrifuge and look for a white DNA pellet at the bottom of the tube on the same side as the hinge. Carefully decant off ethanol from all the tubes into a waste container. The pellet should remain stuck to the inside of the tube.
3. Put tubes, with their caps open, in the Vacufuge/Speedvac. Be careful when putting the tubes in and don’t touch the inside of the caps. Speedvac at 45°C for 40 minutes.
4. Remove samples from centrifuge and add 100 µL of 0.3 M NaOAc **(DO NOT USE THE STOCK 3M!)**. Vortex sample well to dissolve pellet. When the pellet is dissolved the sample will appear “syrupy” and will not bounce around as droplets inside the tube.
5. Once the pellet is dissolved, add 200uL of 100% Ethanol, vortex and invert several times and put in freezer for at least 2hrs.

**- Potential Stopping Point -**

Keep in freezer to allow DNA to precipitate

*Ethanol Wash and Resuspension of Purified DNA:*

1. Remove samples from freezer, and centrifuge for 10 minutes at 10,000g (RCF). Decant supernatant into appropriate waste container.
2. Add 100 µL of 70% Ethanol, and vortex thoroughly (this is the “Ethanol Wash” step). Centrifuge for 10 minutes at 10,000g (RCF), and again decant supernatant into appropriate waste container.
3. Put samples in Vacufuge with the caps open, and speedvac at 45°C for 60 minutes to thoroughly dry the pellet.
4. Take samples out of centrifuge and add 100 µL TE buffer (10 mM Tris, 1 mM EDTA). Vortex briefly to mix and store at -20°C in freezer. Sample is now ready for PCR.