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Minimally Invasive Coral Biopsy Technique for In-Situ Microbiome and Metabolome Sampling

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We use this protocol and it's working

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Disclaimer

This protocol was based on and modified from Roach et al., 2023.

Roach, T.N.F., Matsuda, S.B., Martin, C. *et al.* Single-polyp metabolomics reveals biochemical structuring of the coral holobiont at multiple scales. *Commun Biol* **6**, 984 (2023). <https://doi.org/10.1038/s42003-023-05342-8>

Abstract

Climate change and predicted future ocean conditions are threatening coral reefs on a global scale with compositional shifts at the macro and microscopic scale. One way to assess reef health is through studying the coral microbiome and metabolome. Prior coral microbiome and metabolome research methods included biopsying techniques that would use large quantities of tissue leaving large wounds (centimeters wide) that would induce stress, vulnerability to pathogenic disease, and increase recovery time of the scarred areas. Past research methods included using a hammer and chisel, dermal curettes, and more recently, 16-gauge blunt syringe tips for metabolomic studies (Roach et al., 2023). Our modified modern methods require less tissue quantities and allow for faster host recovery time (~2 weeks). The purpose of this protocol is to minimize the tissue needed to accurately assess the coral microbial communities and metabolomic signatures while inducing the least stress to the coral host. This technique is a less invasive approach for future coral microbiome and metabolome research.

Image Attribution

Justin Berg, Claire Moreland-Ochoa, and Rayna McClintock.



Materials

- Labels, Nitrile gloves, and materials to create sterile working space
 - Materials needed to ensure sterile environment and create appropriate labeling system for experiment.
- 25mL Luer-Lok™ Syringe
 - Size of the Luer-Lok™ Syringe can vary, however, 25mL is suggested to allow enough air for expelling coral biopsy. Larger and smaller syringes are less optimal for pressure needed.
- 16 Gauge 1/2 Inch Blunt Tipped Dispensing Needle, Luer Lock™
 - The 1/2 Inch Blunt Tip is suggested for durability in the field and to ensure uniform sample sizes. The Blunt Tip is also safer for the user compared to the beveled end tip. The specific brand and size of Blunt Tipped Dispensing Needle may vary.
- 16 Gauge 1 Inch Bevel Tipped Dispensing Needle, Luer Lock™
 - The 1 Inch Bevel Tipped is suggested for field sampling on additional coral species that may have a harder skeleton or larger polyp diameter. Caution: **increased risk to user handling the sharper bevel tipped option.**
- 5mL Luer Lock™ Syringes
 - Used for leverage, sample organization, and removing sample when using alternate bevel tipped needles
- 1.5mL conical bottom snap cap tubes
 - Used for storage of needle tips in the field to ensure no contact with foreign objects.
- Sterile 2.0mL free standing screw cap tube with cap and o-ring
 - Used to store final microbiome samples in preservative buffer. Storage of samples in this tube can be used in future high-throughput DNA Extraction steps without transferring to new tubes and risking contamination.
- Sterile 2.0mL Glass Amber Vials with a PTFE/ Silicon Septum bonded to cap
 - Used to store final metabolomic samples in preservative buffer. Samples can be stored in this vial for later metabolomic processing.
- 70% LCMS-Methanol
 - Storage buffer used for coral metabolomic samples to preserve their metabolomic state and stop enzymatic activity.
- Pipette and 200uL pipette tips
 - Used to transfer the preservative buffer for the final step of this procedure.
- Glass Pasteur Pipette
 - Used to transfer the LCMS-Methanol for preservation of coral biopsies for metabolomic sampling.



- Zymo DNA/RNA Shield Buffer

- Preservative buffer to protect and stabilize DNA and RNA at ambient temperatures during transport and storage.

Safety warnings

- ⚠ When using this technique, be aware that needle tips can break with added pressure. There are other variants of LuerLok tips, such as 1-inch blunt needle tips, or beveled needle tips, that have increased risk to user for breaking or safety risks.

Organize Materials Prior to Coral Collection

- 1 To begin, collect: 16 Gauge 1/2 Inch Blunt Tipped Dispensing Needle - Luer LockTM (needle tip), 1.5mL conical bottom snap cap tubes, 2.0mL free standing screw cap tube with cap and o-ring, 2mL Glass Amber Vial tubes, and labels for the tubes. For microbiome sampling, organize the 1.5mL snap cap tubes and 2.0mL screw cap tubes together and label each pair of tubes with the same sample label. For metabolome sampling, organize the 1.5mL snap cap tubes and 2.0mL Glass Amber Vial Tubes together and label each pair of tubes with the same sample label. Label the 1.5mL snap cap tube with a permanent marker to ensure label will not dissolve or break down when exposed to seawater. After the tubes are labeled, place one 16-gauge needle tip in the 1.5mL snap cap tube and close the lid. Repeat this process for all samples that will be collected. For samples collected on land, place the 1.5mL snap cap tubes with needle tips into a tube rack for organization. For samples that are collected via free diving or while scuba diving, organize samples in properly labeled plastic bags for convenience while underwater. For the 2mL screw cap tubes or 2mL Glass Amber Vials, organize the samples in a labeled cryo box to be placed in a -80°C freezer at the end of this process.



16 Gauge 1/2 Inch Blunt Tipped Dispensing Needle - Luer LockTM inside of 1.5mL conical bottom snap cap tube with label on top of lid.

Biopsying the Coral

- 2 There are 3 conditions in which corals may be biopsied, ex-situ on land, free-diving/snorkeling, and while scuba diving. Each condition will have alterations to some of the methods. For each description below, the same application applies to the microbiome and metabolome sampling.
- 2.1 **Ex-situ:** Ex-situ sampling allows for increased precision of tissue samples. To begin, place on nitrile gloves to reduce contamination and safety of user. Remove coral from tank/aquaria/research area. Remove needle tip from 1.5mL snap cap tube. *(Optional: If outside work area is viable, use needle tips from original packaging, skip the 1.5mL snap cap tube step, and continue with step 3.1).* Place thumb on back of the Luer-Lok plastic

area on the needle tip while holding the coral with the other hand. Provide light support with hand behind coral specimen while inserting the needle tip into the coral tissue. The needle tip will only need to insert 2-3 millimeters. Do not apply too much pressure as corals can break. Once fully inserted, lightly push the needle tip towards one side as the biopsy breaks from remaining coral tissue and skeleton. If a denser coral skeleton, gently rock the needle back and forth. Once complete, return the coral to the tank/aquaria/research area and return the needle tip with the coral biopsy into the 1.5mL snap cap tube and close the tube. Continue this process with remaining samples.



Biopsy wound of *Montipora capitata*.



Biopsy scar after recovery ~2 weeks of *Montipora capitata*.

- 2.2 Free-Diving/Snorkeling:** Free-diving/snorkel sampling allows for field sampling in areas that may not be suitable for scuba diving. To begin, place on nitrile gloves to reduce contamination and safety of user. Find a selected area for coral collection. While on the surface above selected area, remove the needle tip from 1.5mL snap cap tube and close 1.5mL snap cap tube to remain dry inside. While free diving down, allow water to enter needle tip next to coral specimen. An air bubble in the needle tip can cause the coral biopsy to pop out. Different coral species will require different sampling techniques. Boulderizing corals, like *Porites lobata*, can be collected anywhere on the colony, but may need more pressure due to high skeletal density. Corals with small raised corallites, like *Montipora capitata*, can be collected as a whole coralite within the needle tip. For both coral species, do not apply too much pressure as this can cause more damage to the coral or break the needle tip. Once the coral biopsy is in the needle tip, lightly push the needle tip towards the side so that the skeleton can have a clean break. Once the biopsy is secured, keep thumb on back of the Luer-Lok plastic part of the needle tip while surfacing so that biopsy is not lost due to back pressure. On the surface, open the appropriate 1.5mL snap cap tube and place needle tip with the biopsy inside. Close tube and repeat for remaining samples.



Field sampling while snorkeling for *Montipora capitata*.

- 2.3 Scuba Diving:** Scuba diving sampling allows for sampling in deeper waters. To begin, place on nitrile gloves to reduce contamination and safety of user. Find a selected area for coral collection. Descend down to the selected sampling area and remove the needle tip from 1.5mL snap cap tube (seawater will enter the tube). Different coral species will require different sampling techniques. Boulderizing corals, like *Porites lobata*, can be collected anywhere on the colony, but may need more pressure due to high skeletal density. Corals with small raised corallites, like *Montipora capitata*, can be collected as a

whole coralite within the syringe. For both coral species, do not apply too much pressure as this can cause more damage to the coral or break the needle tip. Once the coral biopsy is in the needle tip, lightly push the needle tip towards the side so that the skeleton can have a clean break. If a denser coral skeleton, gently rock the needle back and forth. Once the biopsy is secured, keep thumb on back of the Luer-Lok plastic part of the needle tip, place back inside the 1.5mL snap cap tube, and close the lid (1.5mL snap cap tube will be filled with seawater). Repeat this process for remaining samples.

- 2.4 **Alternate Bevel Tipped Technique for Freediving or SCUBA:** A Bevel Tipped Needle can be useful for alternative coral species with harder skeletons or larger polyp diameter like *Orbicella faveolata*. Bevel tip penetrate coral skeleton easily and can be used with a twisting motion to carve out a slightly larger polyp area. Prepare your 5mL syringes before fieldwork. Label each syringe. Then attach 16 Gauge 1 Inch Bevel Tipped Luer Lock Dispensing Needle. Keep the original caps on the needles but remove from outer plastic packaging. Arrange syringes for ease of use when underwater (a marker carrying case is great for this). When underwater Freediving or SCUBA diving, find target colony and select corresponding syringe. Uncap the syringe, place sharp edge of the needle in the coral polyp mouth, apply pressure and twist around polyp until sample is free, recap the needle so that the sample is still sitting in the tip of the needle but is contained in the locked cap (the cap is used instead of a 1.5mL snap cap tube to secure the sample). Repeat for remaining samples.



Labeled Syringes with 16 Gauge 1 Inch Bevel Tipped Luer Lock Needles organized in a marker carrying case ready for sampling on SCUBA (left) and an *Orbicella faveolata* colony that has been sampled sequentially using this technique.

Transferring Biopsy to Clean 2mL Screw Cap Tube (Microbiome) or 2mL Glass Amber Vial (Metabolome)

- 3 After field sampling is complete, return to a sterile environment to transfer the coral biopsy to a sterile 2mL Screw Cap Tube (Microbiome) or 2mL Glass Amber Vial (Metabolome). Organize the samples in the 1.5mL snap cap tube so that they match the order of the 2mL screw cap tubes/ Glass Amber Vials previously labeled in the cryo box.
- 3.1 **Ex-situ and Free-diving/snorkeling:** The needle tips from both the ex-situ and free-diving/snorkeling sampling method should be in dry, 1.5mL snap cap tubes. At a sterile work area, remove the needle tip from the 1.5mL snap cap tube and attach to the 25mL Luer-Lok™ Syringe (ensure there is air in the Syringe prior to attaching tip). Once the needle tip with the biopsy is attached to the 25mL Luer-Lok™ Syringe, open the 2mL screw cap tube and aim the tip inside the 2mL screw cap tube. This process remains the same for Metabolome sampling with the 2mL Glass Amber Vial replacing the 2mL screw cap tubes. Push the back of the syringe until air forces the biopsy out of the needle tip into the 2mL screw cap tube / 2mL Glass Amber Vial (*this may require increased pressure depending on the coral species*). If the biopsy does not initially come out of the needle tip, remove the needle tip from the syringe, add more air into the syringe, and re-try pushing the biopsy out of the needle tip. **Do not try and add more air into the syringe without removing the needle tip or the biopsy could be pulled back into the syringe.** Once complete, discard the used needle tip and close the 2mL screw cap tube / 2mL Glass Amber Vial. Sample will appear to be ~1mm in size at this point.



Needle tip attached to the LuerLock Syringe with ~10mL of air to expel the coral biopsy.

- 3.2 **Scuba Diving:** The needle tips from sampling while scuba diving will be submerged in seawater in the 1.5mL snap cap tubes. At a sterile work area, use sterile forceps to

remove the submerged needle tip from the 1.5mL snap cap tube and attach to the 25mL Luer-Lok™ Syringe (ensure there is air in the Syringe prior to attaching tip). Once the needle tip with the biopsy is attached to the 25mL Luer-Lok™ Syringe, open the 2mL screw cap tube and aim the tip inside the 2mL screw cap tube. This process remains the same for Metabolome sampling with the 2mL Glass Amber Vial replacing the 2mL screw cap tubes. Push the back of the syringe until air forces the biopsy out of the needle tip into the 2mL screw cap tube / 2mL Glass Amber Vial (*this may require increased pressure depending on the coral species and may have additional seawater*). If the biopsy does not initially come out of the needle tip, remove the needle tip from the syringe, add more air into the syringe, and re-try pushing the biopsy out of the needle tip. **Do not try and add more air into the syringe without removing the needle tip or the biopsy could be pulled back into the syringe.** Once complete, discard the used needle tip and close the 2mL screw cap tube. Sample will appear to be ~1-2 mm in size.

- 3.3 **If using the Alternate Bevel Tipped Technique:** After field sampling is complete, samples should be stored in the capped tips of 16 Gauge 1 Inch Bevel Tipped Luer Lock Dispensing Needles attached to labeled syringes. Place syringes in a cooler while returning to a sterile environment to transfer the coral biopsy to a sterile 2mL Screw Cap Tube (Microbiome) or 2mL Glass Amber Vial (Metabolome). Organize the samples in the syringes so that they match the labeled 2mL screw cap tubes/ Glass Amber Vials. Carefully remove the needle from the syringe with the cap still firmly attached, extend the syringe so it is full of 3mLs of air, place the needle back on the syringe. Then remove the cap carefully and locate the coral sample. If it is still in the needle tip, use the air in the syringe to eject the coral sample into the 2mL screw cap tube or Glass amber vial. If the sample has moved into the cap, carefully use the needle to ease it out or use sterile forceps to remove from the cap and place into 2mL screw cap tube or Glass amber vial. Discard needle into sharps container and repeat with remaining samples.

Add Preservative Buffers to Coral Biopsies for Microbiome and Metabolome Sampling

- 4 **Microbiome sampling:** After all biopsies are transferred to the 2mL Screw Cap Tubes, use a Pipette and 200uL pipette tips to add 100uL of DNA/RNA Shield to each 2mL Screw Cap Tube (**ensure the sample is submerged in the DNA/RNA Shield**). Using a 2mL Screw Tap Tube allows for storage and no additional preparations for specific high-throughput DNA Extraction Protocols. After all samples are complete, we suggest placing the samples within the cryo box in a -80°C freezer for storage until DNA extraction and Sequencing, although other temperatures may be acceptable due to the DNA/RNA Shield.



Coral biopsy in DNA/RNA Shield prior to storage in -80°C freezer.

Metabolome sampling: After all biopsies are transferred to the 2mL Glass Amber Vials, use a Glass Pasteur Pipette to add 500uL of 70% LCMS-Methanol to each 2mL Glass Amber Vial (**ensure the sample is submerged in the 70% LCMS-Methanol**). We suggest using a 2mL Glass Amber Vial as it allows for easier storage in a cryo box in a -80°C freezer for future mass spectrometry practices, although temperatures have not been fully assessed for higher temperature storage.

Protocol references

Roach, T. N. F., Matsuda, S. B., Martin, C., Huckeba, G., Huckeba, J., Kahkejian, V., Santoro, E. P., van der Geer, A., Drury, C., & Quinn, R. A. (2023). Single-polyp metabolomics reveals biochemical structuring of the coral holobiont at multiple scales. *Communications biology*, 6(1), 984. <https://doi.org/10.1038/s42003-023-05342-8>