

Sawall Lab Air Picking for Coral Tissue Analysis

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Protocols Adapted for Sawall Lab at ASU or BIOS

Materials Checklist

- ☐ Air pick and connections (small paint pistol)
 - ☐ Pressurized air cylinder or compressor
 - ☐ Ziploc bags (~20 × 10 cm, size dependent on fragment)
 - ☐ 0.45 µm filtered seawater (FSW), squishy bottle
 - ☐ 15 mL Falcon tubes
 - ☐ Electric homogenizer (Ultra-Turrax)
 - ☐ 2 mL / 1.5 mL centrifuge tubes
 - ☐ Dremel
 - ☐ Aluminum foil
 - ☐ 2 × 100–500 mL beakers with MilliQ water
 - ☐ Kimwipes
 - ☐ MilliQ water squishy bottle
 - ☐ Ice buckets (one for fragments, one for tubes)
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Protocol

1. Sample Preparation

1. Determine lipid analysis requirement

- If *not* analyzing total lipids → proceed with direct air picking
 - If analyzing total lipids:
 1. Cut a $\sim 1 \times 1$ cm fragment using a Dremel
 2. Ensure fragment fits in 20 mL glass vials
 3. Wrap in aluminum foil and store at -80°C , or place directly into vial
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2. Tube Labeling

- ☐ Coral ID
 - ☐ Time point/ Date
 - ☐ Parameter analyzed
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3. Coral Rinsing and Thawing

1. Rinse coral fragment with **filtered seawater (FSW)** to:
 - Remove debris and mucus
 - Begin defrosting
 2. **Do NOT** rinse fire corals (*Millepora alcicornis*), as tissue washes out easily.
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4. Air Picking Procedure

1. Place coral fragment inside a Ziploc bag with a small amount of FSW
 2. Hold fragment from the outside of the bag
 3. Begin air picking from one corner:
 - Use a left–right (zig-zag) motion
 - Push tissue toward the bottom of the bag
 4. Hold air pistol nozzle **1–2 mm** from tissue
 - Insert into polyps when possible (e.g., *Montastraea*)
 5. Maintain moisture:
 - Humidify with FSW every **5–10 seconds**
 - Prevent tissue drying (dry tissue is very difficult to recover)
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5. Slurry Collection and Volume Standardization

1. Cut one corner of the Ziploc bag
2. Pour slurry into a **15 mL Falcon tube**
3. Adjust volume with FSW if needed
4. Record **total slurry volume** (critical for standardization)

Tip: Standardize to **12 mL** total slurry volume for each coral.

6. Homogenization

1. Homogenize slurry using Ultra-Turrax for **~20 seconds**
 - Move probe up and down inside tube
 2. Clean homogenizer probe:
 1. Run probe in first MilliQ beaker
 2. Rinse probe (including internal hole) with MilliQ
 3. Run probe in second beaker
 4. Dry probe exterior with Kimwipe
 5. Run homogenizer for **2 seconds** outside beakers to remove residual droplets
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7. Subsampling for Downstream Analyses

Aliquot slurry as follows:

- 1 mL → Zooxanthellae count
 - 500 µL → Total proteins
 - 1 mL → Zooxanthellae identification
 - 1.5 mL → Proteomics
 - 500 µL → Chlorophyll *a*
 - 300 µL → HPLC pigment analysis (in 15 mL Falcon tube)
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8. Final Steps

1. Photograph coral skeleton with ruler for surface area analysis
 2. Place all tubes immediately at **−80 °C**
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Notes and Best Practices

- Keep slurry **on ice at all times** when possible
- For sensitive analyses (DNA, RNA, proteomics), perform air picking **on ice**