Sample Processing & Preservation

Version 1.0
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Set-up & Pre-Lab Checks

- **Workspace & Safety**
  - Clean bench, set clean zone (trash-bag liner + trays).
  - Sanitize surfaces: 10% bleach (≥5–10 min) → wipe → 70–80% ethanol.
  - PPE: lab coat, nitrile gloves (change often), eye protection. Handle dry ice with insulated gloves.
- **Roles & Tracking**
  - Assign: processing lead, recorder, and QC.
  - Confirm TFID ↔ Field ID linkage is present on each container and in the log. Pre-stage labels for all samples from documents.
- **Tools & Reagents**
  - Forceps (2+), mortar & pestle, wide-bore tips, P1000/P200, 25/50 mL tubes.
  - Filtration kit: vacuum flask + hand pump backup; 0.22 µm filter funnels; rinse bottle (sterile water).
  - Prepare Tubes: DNA/RNA Shield; Cryo mix (50% glycerol + 10% DMSO); no-additive tubes.
  - Blue ice / fridge (4 °C) and dry ice / −80 °C access
- **General Rules**
  - Process within 24 h of collection.
  - Keep original sample cold (4 °C) between steps; cap immediately.
  - Use open flame (i.e. ethanol lamp) when possible and if allowed in area.

Processing Modules

Standardized handling to maximize microbial recovery and preserve traceability: work in a sanitized sterile field with PPE, keep samples cold, and complete processing within 24 hours using the 2FP tracking system (CIDs/TFIDs). Use the module that matches the sample type to keep records reproducible across sites.

Water

1. Mix 1 L Whirl-Pak; stand upright.
2. Assemble 0.22 µm filter; start vacuum (hand pump backup).
3. Pour slowly; keep ~3 cm headspace. Pause & remix bag if settling.
4. Forceps decon: 50% bleach ≥1 min → sterile water rinse → air-dry; tips touch only filter.
5. Remove membrane; fold (biofilm in); place in 25 mL tube with ~5 mL sample water.
6. Shake gently to wet; keep on ice.
7. Bleach/rinse funnel & flask; change gloves between samples.

Sediment/Soil/Biomass

1. Bring to bench (4 oz Whirl-Pak/tube).
2. If dry, add sterile or site water to ≤1:1 solid:liquid (target ~15 mL total).
3. Seal; invert 30 s to homogenize. Break up biomass and large pieces if possible.
4. Return to 4 °C.

Coral

1. Move fragment to mortar with 1 mL original seawater.
2. Crack skeleton (downward press) → pieces 5–10 mm; avoid powdering.
3. Add 1 mL seawater; pipette up/down to make a thick slurry.
4. Return slurry to Whirl-Pak; total liquid ~15 mL; invert 30 s.
5. Return to 4 °C.

Abort conditions: leaking container, obvious cross-contam. Pause, restart, and log incident.

Proceed to preservation after all processing for a collection event is complete.

Preservation

Proceed through all preservation types for each processed sample: DNA/RNA Shield, Cryogenic, & Original Sample. Apply the chosen method consistently across aliquots, include negative controls and planned replicates, label to maintain TFID/CIDs, and move immediately to the specified storage.

DNA/RNA Shield Aliquots (nucleic acids)

1. Goal final ratio 1:1 sample:Shield (2×)
2. Homogenize sample (invert 15 s).
3. Add 1.5 mL sample to tube pre-loaded with 1.5 mL Shield (2×).
4. Cap; invert 10×; label TFID/CID_R#_SH2x.
5. Hold at RT or 4 °C same day → −20 °C for long-term.
6. If using 1× Shield: target 1:10 sample:Shield; adjust volumes accordingly.

Cryogenic Stocks

1. Target final = 25% glycerol + 5% DMSO.
2. Use cryo mix (50% glycerol + 10% DMSO).
3. Pre-load cryovial with 0.75 mL cryo mix.
4. Add 0.75 mL sample; invert 5×; tap to clear bubbles.
5. Snap-freeze on dry ice; store at −80 °C.

Original Sample (no additive)

1. Aliquot 1.5 mL to a no-additive cryovial; keep 4 °C; ship cold.
2. Label TFID/CID_ORIG; use for chemistry/back-ups.

Controls (required)

1. Process blank: run sterile water through all steps (mortar/forceps/filter, etc.).
2. FB/FilB: collect in field; process alongside batch.
3. Assign unique TFIDs/CIDs; treat like real samples.

Post-Processing & Preservation

- **Waste & Decon**
  - Spill response: stop work, don fresh gloves/eye protection, contain with towels, neutralize bleach, bag waste, re-sanitize area, log incident.
  - Bag solids: double-bag, label as biohazard if contaminated; store in designated pickup area.
- **Cleanup**
  - Confirm TFIDs/CIDs on all tubes
  - Cap reagents & restock tips/tubes/labels.
  - Remove all waste to designated areas
  - Final decon: surfaces (bleach → ethanol), tools (bleach → sterile water), air-dry
  - Safety close-out: verify dry ice stored properly/vented; return blue ice to freezer; secure chemicals; wash hands and remove PPE.

Dry Ice Monitoring (−78.5 °C)

- Use insulated, vented coolers (never airtight); avoid confined spaces and ensure ventilation.
- Log cooler/rack temp every 60–90 min and at hand-offs.
- Replenish when dry ice volume drops ~50%.

Blue Ice Monitoring (4 °C)

- Target 2–8 °C (goal 4 °C); do not freeze samples.
- Pre-chill packs (−20 °C); line bottom/top; buffer to avoid direct contact.
- Swap when packs are flexible/mostly thawed or internal temp ≥6 °C and rising. Control moisture: wipe condensation, keep labels legible, replace wet absorbents or bag samples.