



The Two Frontiers Project

Field Sampling and Processing Handbook

Sample Type(s): Water, Sediment, Biomass	Version 11.0
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Author(s): Krista A. Ryon, James R. Henriksen, Braden T. Tierney	

Purpose: This Standard Operating Procedure (SOP) has been outlined to cover the sampling and processing of general environmental samples for the study of microorganisms.

Scope: The purpose of this protocol is to collect samples for downstream metagenomic sequencing and microbial culturing. It is applicable but not limited to sediment, soil, water, biomass, and coral. The methodologies discussed here are applicable to both terrestrial environments (i.e. mountains, land, desert) and bodies of water (i.e. lakes, rivers, oceans). This applies to both flowing and standing water. However, some modifications to the protocol will be made based on the location. For the purpose of this procedure, the above is defined as such:

- **Sediment:** deposit of insoluble material, primarily rock and soil particles, transported from land areas to bodies of water.
- **Soil:** the upper layer of earth in which plants grow, a black or dark brown material typically consisting of a mixture of organic remains, clay, and rock particles.
- **Water:** water that has accumulated on the earth's surface, located on top of land or below land (i.e. groundwater).
- **Biomass:** is a multi-layered, cohesive community of microorganisms, primarily bacteria and archaea, that grow on surfaces in various environments. These mats are often structured in layers, with different microbial groups occupying distinct strata based on their metabolic functions and environmental conditions. For this context, we use this term interchangeably with microbial mat or biofilm.
- **Host samples:**
 - **Coral:** marine invertebrate living mainly in ocean environments.
 - **Plants:** Includes plant biomass (above or below ground) and soil containing roots
 - **Invertebrates:** Occasional organisms, often marine benthic



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I. About this handbook

This handbook is the official Field Sampling and Processing Guide of **The Two Frontiers Project (2FP)**. It has been developed to provide standardized, modular, and field-operational protocols for environmental microbial sampling across terrestrial and aquatic systems, including SCUBA-based marine work.

Intended Audience

This handbook is designed for:

- Field researchers (microbiologists, ecologists, biogeochemists)
- Expedition participants (including student trainees and local collaborators)
- Laboratory technicians and researchers
- Partner institutions and regulatory reviewers

Whether you are a first-time field assistant or an experienced expedition leader, this guide is intended to ensure methodological consistency, safe operations, and scientific integrity across all projects bearing the 2FP name.

Format

This document is structured into discrete modules, corresponding to stages of the sampling lifecycle:

- **Planning:** logistics, permits, team roles, buffer prep
- **Collection:** sample-type-specific protocols (water, sediment, biomass, host)
- **Processing:** homogenization, labeling, and preservation
- **Transportation:** maintaining cold chain and documentation
- **Post-field:** reset procedures and metadata archiving

Each module includes:

- Background context
- Step-by-step protocols
- Summary
- Notes on hazards, errors, and contingencies

Field Use

While this is the full reference guide, you should also carry the field-ready 2FP Sampling Slate or Laminated Quick-Protocol Cards, which summarize critical steps for each sample type. These can be customized for each expedition and sampling style (e.g., SCUBA slates, backpack-ready terrestrial kits).

Use this handbook:

- **Before** the field: for training, kit assembly, and team planning
- **During** the expedition: for verification of sampling techniques and troubleshooting
- **After** the field: for processing, preservation, shipping, and sample intake



If in doubt in the field: go back to the 2FP Field Slates or this Handbook. Follow the steps. Prioritize sterility, traceability, and safety.

General Guidelines

These guidelines underpin every step of The Two Frontiers Project's field and laboratory workflows. They are not sample-type-specific – whether sampling coral tissue at 20m depth or collecting soil in a high-altitude desert, these standards apply.

1. Sterility and Contamination Control

Protecting sample purity is essential for generating high-quality, reproducible data. Contamination at any stage – field or lab – can compromise results and waste valuable effort.

- Wear gloves at all times when handling any collection materials or biological samples. Change gloves frequently, especially between sites or sample types.
- Use pre-sterilized, single-use collection bags and tools whenever possible. If reusable tools are used, disinfect with 70–80% ethanol or flame-sterilize between uses.
- Never touch the interior of sample bags or caps of cryovials. Avoid breathing directly over open sample containers.
- Prepare buffers, aliquot, and process samples only in designated clean areas. Decontaminate work surfaces with 10% bleach followed by 70% ethanol. Let the bleach sit for 5-10 minutes before wiping. Let air dry before applying 70% ethanol.

2. Sample Integrity

Preserving the original biological and chemical state of a sample ensures it reflects the environment it came from, and ensures it can be used for downstream analysis.

- Use new collection bags and cryovials for every sample. Do not reuse Whirl-Paks or cryovials, even within the same site.
- Place samples into the appropriate preservative immediately after collection to prevent microbial community drift.
- Keep all samples upright, on ice, and protected from direct sunlight until further processing.
- Label clearly and redundantly, including: Field ID, unique sample ID (ideally generated using CUAL-ID), sample type, date, and collector initials.

3. Traceability and Metadata

Complete and accurate records make each sample scientifically valuable, enabling re-analysis, replication, and sharing within and beyond 2FP.

- Assign every sample a unique 2FP CID (Collection ID) that is traceable from field collection through to final storage.
- Record metadata at the time of collection, including: date, time, GPS coordinates (decimal degrees), collector initials, and environmental observations.
- Upload supporting materials (e.g., field photos, dive logs, handwritten notes) to the designated expedition cloud drive within 48 hours.



4. Team Safety and Role Clarity

Our safety protocols protect both people and science – a safe, well-coordinated team is essential for successful and sustainable expeditions.

- Never collect samples alone. Field teams should operate in pairs or trios with clearly assigned roles.
- For SCUBA operations, assign explicit dive tasks during the pre-dive briefing and review underwater communication signals.
- Conduct a safety briefing before each field day to review site-specific hazards, emergency protocols, and contact information.

5. Redundancy and Replication

Building in backups reduces the risk of data loss and maximizes the scientific return from every field effort.

- Where possible, collect at least two replicates per condition/sample type.
- Preserve replicates in multiple preservative types (e.g., glycerol for culturing, DNA/RNA Shield for sequencing).
- Maintain backup storage locations for key samples to safeguard against loss or spoilage.

6. Ethical Bioprospecting: Respect for Local Ecosystems and Partners

2FP's work is built on respect – for ecosystems, local communities, and shared global biodiversity. We follow not just legal requirements but ethical best practices.

- Minimize habitat disturbance and avoid sampling in protected or sacred sites without permission.
- Follow all permit and export requirements – when in doubt, assume permits are needed.
- Acknowledge the contribution of local collaborators, communities, and biodiversity stewards.
- Communicate fully to land owners and local partners the nature of your intent with a given sample taken on their land (e.g., research vs. commercial use).



II. Expedition Planning (logistics and team)

Background

Expedition planning is the foundation of every successful Two Frontiers Project sampling effort. A well-planned expedition maximizes scientific yield, minimizes environmental impact, and ensures team safety. These steps are not optional — they are the baseline for maintaining our reputation for excellence, integrity, and collaboration.

Sampling under extreme or remote conditions requires not just technical readiness, but a cohesive, role-oriented team, an ethically chosen site, and an understanding of cultural and logistical context. No matter the destination—deep-sea reef, arid soil, or coastal lagoon—each site demands its own level of preparation, precaution, and partnership.

This section outlines how to assemble an expedition team, choose your collection site, ethically engage local collaborators, and set appropriate planning timelines. These are not just organizational steps; they are an essential part of scientific stewardship.

Selecting a site

Site selection depends on scientific value, accessibility, regulatory constraints, operational feasibility, and a thorough risk assessment. This risk review should address environmental hazards (storms, wildlife, toxic gases), political or security risks, and health considerations (altitude, remoteness from medical care). Teams must also check seasonality to confirm conditions will be workable during the planned window (e.g., avoiding monsoon season, hurricanes, high-temperatures or ice cover). Pre-site reconnaissance using satellite imagery and mapping tools is strongly encouraged. Ideal sites are novel or under-characterized microbial environments, especially where geochemical or ecological gradients suggest stratified or metabolically diverse communities. Examples include shallow methane seeps, acidified coastal zones, hypersaline ponds, hydrothermal-influenced sediments, or coral–algal transition zones.

Teams must also evaluate:

- Physical access — factoring in terrain, transportation requirements, and travel time.
- Environmental conditions — such as weather patterns, sea state, depth limits, or exposure levels.
- Alignment with team capabilities — ensuring activities (e.g., SCUBA diving, mountaineering) remain within the most conservative skill and safety limits of any team member.

Backup sites should be identified in case primary locations become inaccessible. Cold chain, storage and preservation workflows must be feasible for any selected site—if they cannot be maintained, the site should not be used.

Assembling a team and assigning roles

Each 2FP expedition team should be constructed with overlapping competencies, complementary personalities, and redundant technical skills. Every high-risk expedition should include at least one member with advanced field medical capacity (e.g., Wilderness First Aid or Diver Rescue certification). All members should receive cross-training in critical contingency



tasks like cold chain repair, metadata entry, and buffer preparation, even if outside their primary role. A pre-departure communications plan must be established, assigning responsibility for satellite phone, radio protocols, and daily check-ins if operating in remote or high-risk zones. Success in the field requires more than subject-matter expertise—it requires a team capable of navigating uncertainty, adapting to variable field conditions, and operating effectively in isolated or high-stress environments.

Team members must collectively cover the following domains: scientific expertise relevant to the study objectives (e.g., microbiology, environmental chemistry), prior field experience in the terrain being sampled (such as underwater, alpine, or desert systems), leadership and decision-making capacity, and the ability to function effectively within a small, diverse team in unfamiliar environments. We also prioritize traits like tolerance for discomfort, a demonstrated capacity to manage ambiguity, and the ability to maintain productivity despite possible fatigue, logistical failure, or physical stress.

To avoid single points of failure, at least two team members must be trained in each core technical task: sample collection (e.g., diving, sediment core extraction), sample preservation (e.g., cryovial handling, buffer use, cold chain protocols), and metadata check-in (e.g., CID assignment, GPS logging, photograph management). This ensures the expedition can proceed even in the event of illness, injury, or gear failure.

All team members should be briefed on their primary and secondary roles in advance of departure. Clear role assignment avoids ambiguity and duplication during collection. Prior to fieldwork, teams should review key safety plans, sample handling protocols, emergency procedures, and data management expectations. The highest-functioning teams are those who are cross-trained, aligned on objectives, and able to rotate tasks when needed.

Ethics

All sampling must be conducted in accordance with 2FP's ethical framework, the Nagoya Protocol (where applicable), and all local regulations. Sites that fall within protected areas, sacred spaces, or community-managed ecosystems should not be sampled without explicit, documented permission. This includes marine parks, indigenous territories, archaeological sites, and other culturally or ecologically sensitive zones. In cases of uncertainty, sampling should be paused until clearance is obtained.

Teams must also evaluate the potential ecological impact of collection. If sample extraction would lead to irreversible damage to a habitat, such as destabilizing a microbial mat or breaking apart a fragile coral assemblage, an alternative sample site should be selected. In all cases, habitat disturbance should be minimized, and photographic documentation should accompany the sample to contextualize its removal. Teams must incorporate biosecurity measures to prevent cross-site contamination or the spread of invasive species, including cleaning and disinfecting gear between sampling sites. Benefit-sharing should be considered even for research-only samples, ensuring data or results are returned to local partners.

Fieldwork is not extractive research. It must be executed with transparency, humility, and respect for local communities, researchers, and stewards of the environments being studied.



Finding local collaborators

Local collaborators are essential for accessing field sites legally, working safely, and collecting ethically. In many cases, securing a collaborator is also a requirement for permitting, export, or sample use. Collaborators may be based at universities, government agencies, NGOs, field stations, or private land holdings. The goal is to identify someone who is familiar with the sampling region, understands its ecological or regulatory context, and can provide guidance or assistance with access, safety, or approvals.

Below are recommended approaches for identifying and establishing contact with potential local collaborators:

1. Search the academic literature for publications involving your site or region of interest. Use Google Scholar, Web of Science, or Scopus to search for terms like the name of the lake, reef, or reserve, plus keywords like “microbial,” “sediment,” “sampling,” or “biodiversity.” Prioritize authors listed with local affiliations. Email the corresponding author directly and clearly describe your sampling plans and collaboration request.
2. Look for nearby universities or research institutes, even if they haven’t published on your specific site. Departments in marine biology, environmental science, geology, or agriculture are good starting points. Many institutions have field stations, labs, or graduate students who are actively sampling nearby. Department websites and institutional directories can help you identify staff or faculty contacts.
3. Reach out to private landowners directly if your sampling site is on private property. Use Google Maps or cadastral databases to identify ownership. Local government or town offices may be able to provide contact information. When reaching out, be clear about what the work involves, how long you’ll be there, and whether you’re requesting access, samples, or assistance. Offer to share your results and be respectful of their land use or concerns.
4. Contact dive shops, boat operators, or environmental NGOs that work in the region. These groups often have intimate knowledge of local conditions and may have assisted previous research efforts. If sampling underwater, dive operators may be able to provide both logistical support and introductions to scientific contacts.
5. Use permitting or export authorities as a point of contact. In some countries, agencies that oversee scientific permits will have approved collaborator lists or may connect you to institutions with existing sample access agreements.
6. Leverage existing 2FP or collaborator networks. Contact a 2FP program lead if you’re working in a region where we or our partners have operated in the past. Prior collaborators may be willing to assist again or can recommend others.

Whenever possible, formalize collaboration through a memorandum of understanding (MOU) or written collaboration agreement to prevent misunderstandings. Consider tapping into citizen science and conservation networks for local expertise. Once you identify a prospective collaborator, send a direct, concise inquiry. Introduce yourself, outline your project aims, state your anticipated field dates and locations, and describe what kind of collaboration you’re seeking (e.g., letters of support, permitting, joint sampling, site access, equipment sharing).



Always offer co-authorship or material/data sharing when appropriate. Be clear about what you are offering in return and never assume unpaid labor.

We recommend identifying potential collaborators at least 12 weeks out from sampling, if not more. Some permits can take up to a year to grant, so in many cases this may be far too conservative an estimate.

Permitting

Permitting is a fundamental part of expedition planning. Whether collecting from private land, public land, or international territories, failing to secure the right permissions or misrepresenting the intended use of samples can invalidate entire research programs and lead to reputational, financial, or even legal consequences. This is particularly true when it comes to commercial use designation.

Samples must be explicitly declared for commercial use at the time of collection if there is any possibility they will be used in downstream industrial, biotech, or product development applications. This is not just a legal distinction—it is an ethical one. Declaring “research only” and then pivoting to commercial exploitation violates trust, breaches permits, and can jeopardize future access for all. Always consult with permitting authorities, landowners, and legal counsel before sample acquisition.

Private lands

Building access to private land is primarily a relationship-building exercise, requiring cultural sensitivity, especially when working with indigenous territories or areas with customary ownership rules. All verbal agreements should be documented in writing promptly after meetings. Scientific merit matters but so does trust. Cold outreach often works best through local universities, conservation groups, or citizen science networks. When landowners are engaged, 2FP offers a simple, plain-language release form that clearly states what we are collecting, how samples will be used, and who retains ownership. Importantly, we allow landowners to redline this form on-site—modifying terms or restricting use as they see fit. This transparency builds credibility and usually leads to more successful collaborations.

Identifying lands of interest can be done using public data sources. For example, the USGS National Water Information System (NWIS) can help locate high-CO₂ springs or unusual water chemistry sites. Property ownership databases, county GIS maps, and water rights records can all help pinpoint access routes and decision-makers.

Public lands

Public lands are governed by a patchwork of agencies—each with its own permitting rules, timelines, and expectations. In the U.S., the key distinction is whether land is federal, state, or municipal, but even within these categories, jurisdiction can vary.

For U.S. federal lands, including Bureau of Land Management (BLM), Forest Service, and National Parks:



- Bureau of Land Management lands are often permissive. Incidental use provisions allow small-scale, non-invasive sampling—such as a few milliliters of water or grams of soil—to be conducted with only a short letter of notification or a simple form.
- National Parks are among the most restrictive, particularly regarding anything with potential commercial use. Even academic research often requires a Cooperative Research and Development Agreement (CRADA), which can take months and may limit data sharing or IP ownership.
- Partnering with an institution or PI that already holds a permit is often the fastest path to access.

For state lands, rules vary widely. Some states allow low-volume environmental sampling under educational exemptions or require only agency notification. Others demand formal scientific collection permits, often with conservation reviews or public comment periods. Always check state natural resource department websites, and start the process early—permits can take weeks to months.

In all cases, the best strategy is early outreach, clear communication of intended use (research vs. commercial), and documentation of all correspondence. Permitting should be treated as a gating item in project timelines.

Example 1 year planning timeline

Weeks Before Departure	Task	Category
52–36 weeks	Define scientific objectives, target environments, and sample types (e.g., sediment, seawater, biomass, coral). Establish hypotheses and desired downstream analyses. Assign organizational team for upfront logistics.	Science
52–36 weeks	Identify target regions and candidate sampling sites using geochemical, biological, and logistical criteria.	Science / Logistics
52–36 weeks	Research permitting requirements for each site (research-only vs. commercial use; federal, state, local, and international). Include MTA, CITES, Nagoya Protocol compliance.	Permitting
52–36 weeks	Identify and initiate contact with local collaborators, research stations, and institutional partners. Explore cost-sharing or resource-sharing opportunities.	Collaboration / Admin
36–24 weeks	Begin drafting and submitting permit applications. Include timelines for review and contingency sites if permits are delayed.	Permitting
36–24 weeks	Conduct sample use classification (research-only vs. commercial) and internal ethical review. Document any human or vertebrate exclusions.	Permitting / Ethics
36–24 weeks	Contact private landowners (if applicable) and negotiate site access agreements.	Permitting / Collaboration



36–24 weeks	Assign expedition lead, deputy lead, and begin building the initial team roster. Identify science, logistics, safety, and communications leads.	Admin / Logistics
24–12 weeks	Finalize the expedition team and assign sampling roles (collection, metadata, safety officer, sampling). Identify backups for each role.	Admin / Logistics
24–12 weeks	Draft site-specific sampling plans: sample types, target numbers, preservation methods, and cold chain strategies.	Science / Logistics
24–12 weeks	Begin detailed equipment inventory; identify gaps, expired consumables, and place early orders for long-lead-time items.	Logistics
24–12 weeks	Plan housing, transport, field base operations; identify local storage, cold chain options, and communication infrastructure.	Logistics
24–12 weeks	Initiate travel document collection (passports, visas, SCUBA certifications, vaccination records, emergency contacts).	Admin
12–8 weeks	Finalize permit submissions; follow up on pending approvals and adapt site plans as needed.	Permitting
12–8 weeks	Order remaining equipment, consumables, reagents, and microbial preservation buffers (e.g., glycerol, DMSO, DNA/RNA Shield).	Logistics / Science
12–8 weeks	Identify cold chain and preservation needs; confirm sourcing of dry ice, coolers, or portable freezers. Identify in-field freezing and refrigeration options.	Logistics
12–8 weeks	Book all transport: flights, vehicles, boats, and accommodations. Build redundancy in travel timelines.	Logistics
12–8 weeks	Draft field safety plan; identify local emergency contacts, hospitals, and evacuation routes.	Safety
8–4 weeks	Build digital and physical expedition kits using 2FP inventory template. Prepare microbiology, dive, and field sampling kits separately.	Logistics
8–4 weeks	Generate laminated quick-protocol cards, sample ID/CID labels, and waterproof metadata sheets.	Science / Logistics
8–4 weeks	Conduct team-wide review of core protocols (collection, preservation, contamination control, decontamination). Include safety and emergency procedures.	Safety / Science
8–4 weeks	Begin customs declaration and transport documentation (dry ice, MTAs, import/export permits).	Permitting / Logistics
8–4 weeks	Compile background literature, maps, and geospatial datasets for site briefings.	Science
4–2 weeks	Prepare and QC buffers for contamination checks; confirm cryovial inventory.	Science / Logistics
4–2 weeks	Conduct full team meeting with role assignments.	Admin / Logistics
4–2 weeks	Print expedition packets (maps, checklists, permits, customs letters, safety plan).	Admin
4–2 weeks	Confirm shipment logistics for gear or samples sent ahead. Identify point-of-contact for receiving shipments.	Logistics



1 week	Pack kits by functional group (e.g., dive, microbiology, sediment core, consumables). Seal and label for easy deployment.	Logistics
1 week	Assign final roles and backups for each sampling and safety task.	Admin
1 week	Reconfirm all travel, transport, accommodation, and local contact details.	Logistics
1 week	Conduct final pre-departure safety briefing and Q&A.	Safety
1 week	Upload all backup documents and trackers to expedition cloud folder and offline storage. Ensure hard copies are in field kit.	Admin
2 weeks post-expedition	Conduct expedition debrief (science outcomes, logistics review, budget reconciliation, lessons learned). Update protocols and inventory.	Admin / Science / Logistics

Resources

Resource	Type	Description	Link
2FP Expedition Template	Folder	Master planning file for objectives, CIDs, metadata, inventory, and team roles	https://github.com/two-frontiers-project/2FP-expedition-template/tree/main
BLM Permits & Land Use Authorizations	Website	U.S. Bureau of Land Management guidance on obtaining permits and authorizations for research, collecting, and other activities on BLM-managed public lands. Includes procedures for scientific research permits, filming/photography authorizations, and recreation or special use permits.	https://www.blm.gov/programs/lands-and-realty/leases-and-permits
BLM Research & Collecting on Public Land	PDF	Overview of regulations and permit requirements for conducting scientific research and collecting specimens on BLM lands, including archaeological, paleontological, and biological resources.	https://www.blm.gov/sites/default/files/documents/files/collecting_on_publiclands.pdf



Local Context	Website	Local Contexts is a global initiative that supports Indigenous communities with tools to reassert cultural authority in collections and data.	https://localcontexts.org/
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III. Sample identifiers and site metadata

Background

Sample identifiers are the backbone of traceability in 2FP's workflows, ensuring every collected specimen can be tracked from the moment of field collection to long-term storage and final data analysis. Consistent, unambiguous identifiers are essential for reproducibility, data integration, and sharing with collaborators. Complete metadata must be collected at the time of sampling, never reconstructed later, to maintain accuracy and enable replication.

Field IDs versus CUAL-IDs (CIDs)

We use two forms of identifiers for every sample:

Field ID

A short, human-readable code written directly on the collection container (e.g., Whirl-Pak, 50 mL conical). This serves as the primary reference during fieldwork. Field IDs can be simple numbers (1, 2, 3...) or can include metadata about sample type (e.g., S1, S2 for sediment; W1, W2 for water). Field IDs are assigned and labeled on containers before heading into the field, and all handwritten metadata recorded during collection should reference the Field ID. Because Field IDs are tied to a specific expedition and context, they can be reused between different sampling campaigns – but never within the same expedition.

CUAL-ID (CID)

A unique, permanent alphanumeric hash assigned during Sample Check-In back at the lab. Each CID is pre-generated, printed on cryolabel sheets prior to field deployment, and mapped to the sample's Field ID immediately upon check-in. The CID label is applied to the original collection container and later to all aliquots or preserved fractions (e.g., glycerol, DMSO, DNA Shield tubes). Once assigned, a CID is never reused under any circumstances.

Minimum metadata to collect in the field

At the time of sampling, record the following for each sample:

- GPS coordinates in decimal degrees (DD), WGS 84 datum
- Date and time of collection (local time)
- Field ID and assigned CID
- Collector initials
- Sampling method and preservation method used
- Environmental notes (e.g., turbidity, temperature, flow, substrate type)
- Site photographs and GPS screenshots

Metadata recording and handling

Metadata should be recorded both digitally and in a backup field notebook. At least one device per team must be capable of GPS logging, and all devices should be time-synced before fieldwork begins. Avoid apps or tools that auto-convert coordinate formats or datums without clear indication or transparency.



When using environmental sensors such as pH, dissolved oxygen, or salinity probes, log calibration settings and measurement conditions alongside each reading. Capture redundant measurements and photographs whenever possible to safeguard against data loss.

Best practices

- Prepare and label all Field ID containers before field deployment.
- Carry spare pre-labeled containers for unplanned samples, but document any new IDs in the metadata sheet before collection.
- Use permanent, alcohol-resistant markers or thermal labels to prevent fading in wet or abrasive conditions.
- Apply CID labels only in a clean, organized Check-In area to avoid mislabeling.
- Immediately update the expedition metadata file with the Field ID → CID mapping and upload to the cloud folder.
- Cross-check GPS coordinates and photos before leaving a site to ensure data completeness.
For multi-day expeditions, upload digital metadata daily to the designated expedition cloud folder and verify backups are intact.

Resources

Resource	Type	Description	Link
2FP Expedition Template	Folder	Master planning file for objectives, CIDs, metadata, inventory, and team roles	https://github.com/two-frontiers-project/2FP-expedition-template/tree/main



IV. Preparation for sample collection

Background

Preparation is where 2FP's field success is decided. Every expedition begins with clear planning, structured workflows, and built-in redundancy for all critical operations. Regardless of environment – desert, reef, forest, or vent field – the same core principles apply: samples must be traceable, sterile, biologically representative, and compatible with downstream sequencing and culturing.

This section outlines how to:

- Define a sampling plan aligned with objectives and constraints
- Build and check your kit
- Prepare for safety contingencies
- Ensure metadata and cold chain coverage from the start

Defining sampling plans

Every expedition requires a clear sampling strategy that aligns with scientific goals, site constraints, and team capacity. A strong plan defines what types of samples will be collected, how many replicates are needed per site, which preservation methods will be used, and who is responsible for each step.

Sampling goals should be defined prior to departure. Specify whether the objective is metagenomic sequencing, microbial cultivation, geochemical correlation, or all of the above. Match sample types to research questions (e.g., if you are interested in complex communities, emphasize biomass; if you are interested in free-living organisms, emphasize water sampling). Ensure that sample sizes will guarantee statistical power to enable your downstream collection plans. Plan for depth of and type of sequencing for each given sample, and plan for downstream culturing activities. If no culturing is needed, certain cryopreservatives may not be required (or, for example, if phototrophs are not of interest, DMSO may not be needed). Similarly, if DNA sequencing is not needed, samples do not need to be preserved in a DNA shield.

During sample collection planning, field teams must define:

- Number of sites
- Number and type of samples per site
- Collection methods
- Preservation strategy per sample
- Which team members are responsible for collection, labeling, metadata, and preservation

Develop a field sheet or digital form to track this in real time. Do not improvise sample types in the field without confirming whether they are permitted, logistically supported, or scientifically justified.



Terrestrial Collection

Terrestrial sampling can include water from springs, rivers, and seeps, sediment, microbial mats, soil, and plant material. Site access may require hiking, climbing, or navigating through remote terrain, and often requires landowner or government approvals. Planning for terrestrial sampling must account for terrain and elevation changes, environmental conditions such as dust or dryness that can increase contamination risk, and the distance from base camp or laboratory, which directly impacts cold chain management.

Teams should also anticipate the need for alternate water sources for rinsing or mixing, and prepare for hazards such as extreme temperatures, high altitude, or wildlife encounters.

Sampling in these environments demands careful consideration of physical access routes, weight and portability of equipment, and the ability to transport samples quickly back to refrigeration or preservation stations.

Underwater Collection

Underwater sampling requires dive-qualified personnel, certified equipment, and strict adherence to local diving and sampling laws. Samples may include water, sediment, microbial mats, coral, or invertebrate surfaces. The logistical complexity of underwater work demands highly detailed dive plans, which must specify maximum and target depths for each site, the number of dives per day per diver, and post-dive no-fly times.

Roles and tasks should be clearly assigned during pre-dive briefings and reinforced through agreed underwater communication methods. Dive computers must be synced prior to sampling to ensure accurate metadata capture. All dives should be supported by the presence of an O₂ kit on board and a plan for accessing the nearest hyperbaric chamber. 2FP recommends that all divers carry DAN dive insurance. Dive plans should be reviewed daily, and all sampling gear, such as pre-assembled slates and mesh bags, should be prepared in advance to maximize underwater efficiency.

Underwater plans must include:

- Maximum and target depth for each site
- Number of dives per day per diver and proximity to flying
- Sample types to be collected per dive
- Clear leadership and task structure when diving, as well as sample-specific visual communication methods
- Adherence to all standard diving protocols (e.g., having an O₂ kit on board, knowing the location of the nearest hyperbaric chamber)
- We recommend diving with DAN dive insurance:

As with terrestrial sampling, sampling slates and mesh bags should be pre-assembled for each dive. Dive computers must be synced before collection to aid metadata matching. Dive plans should be submitted and reviewed daily.

Safety preparedness and briefing

Safety is a core pillar of every 2FP expedition. Before departure, one team member must be appointed as the Safety Officer, serving as the central resource for all safety-related information during field operations. This individual is responsible for knowing and communicating the



locations of first aid kits, nearest hospitals or clinics, emergency muster points, local hazards (including wildlife), and any other relevant safety resources. While it is ideal for the Safety Officer to be a trained first responder or medical professional, this is not a requirement; the key is that this person is proactive, detail-oriented, and able to access and relay information quickly. Any questions about safety protocols in the field should be directed to the Safety Officer. In addition to appointing this role, a safety briefing must be delivered before sampling begins. This briefing should cover general hazards, site-specific risks, and the protocols in place to address them. It may be led entirely by the Safety Officer or shared among team members with relevant expertise – for example, dive instructors may lead the diving safety component, while local collaborators may present on environmental hazards unique to the site. The goal is to ensure that every member of the team understands the risks, mitigation strategies, and emergency procedures before work starts.

Dive safety

All underwater work conducted by 2FP follows the standards of the American Academy of Underwater Sciences (AAUS). This includes comprehensive dive planning, pre-dive briefings, and adherence to operational limits based on diver training and experience. An O₂ kit must be present on the dive vessel, and the location of the nearest hyperbaric chamber must be confirmed before the first dive. Dive plans must also account for post-dive no-fly intervals and the physical limits of the least-experienced diver on the team. 2FP is in the process of obtaining full AAUS organizational membership and will update this handbook with the complete dive manual upon approval.

Pre-Dive Safety Checklist

- Confirm all divers have current medical clearance and DAN dive insurance
- Review dive plan, including max depth, bottom time, and turn pressure
- Verify dive computers are synced and functioning
- Check presence and accessibility of O₂ kit and first aid supplies
- Identify location of nearest hyperbaric chamber
- Assign underwater roles and review hand signals
- Confirm emergency recall signal for all divers
- Check weather, tides, and sea state conditions
- Ensure mesh bags, slates, and sampling gear are prepped and secured
- Verify communication plan with surface support team

Wilderness Safety

When operating in remote terrestrial or inland aquatic environments, additional hazards must be considered. These may include extreme weather, rapid changes in terrain, limited access to rescue services, and potential encounters with wildlife. Teams should carry sufficient emergency supplies – including extra food, water, shelter, and navigation tools – and should have a plan for evacuation in the event of injury or environmental hazards such as floods, landslides, or storms. The Safety Officer should be familiar with the local climate, terrain, and



wildlife risks, and all team members should know the designated muster point and contact procedures in case of separation.

Wilderness Safety Checklist

- Confirm all team members have reviewed site-specific hazards
- Identify muster points and evacuation routes
- Verify first aid kits are stocked and accessible
- Confirm at least one satellite phone or radio is functional and charged
- Ensure emergency contact numbers are stored in multiple devices
- Review expected weather and potential hazards for the day
- Verify all members have sufficient water, food, and protective clothing
- Check navigation tools (GPS units, maps, compasses) are operational
- Confirm wildlife deterrents or precautions are in place where relevant
- Review plan for communication if a member becomes separated

Other Safety Circumstances

2FP expeditions may encounter environments that present unique safety challenges outside of standard diving or remote terrestrial work. These conditions require specific preparation, equipment, and training.

Cold and Snow/Ice Regions

In cold regions — including alpine environments, polar zones, and high-latitude field sites — hazards include frostbite, hypothermia, snow blindness, and avalanche risk. Weather conditions can change rapidly, and visibility can drop to near zero. Footing may be unstable due to ice, snow bridges, or crevasses. Team members must be trained in cold-weather survival basics, avalanche awareness, and emergency shelter use.

Extreme Heat and Arid Regions

In hot or desert environments, risks include dehydration, heat exhaustion, and heatstroke. Sampling plans should avoid peak heat hours, and shade should be available during breaks.

Unstable or High-Risk Terrain

For steep slopes, scree fields, cliffs, or unstable ground, teams should move in small groups, maintain visual contact, and avoid loading unstable surfaces with excess weight. Ropes and helmets may be required.

Selecting equipment and generating inventory list/kits

Sampling equipment must be tailored to the specific requirements of the expedition, with redundancy scaled to the remoteness and risk profile of the environment. For deployments hundreds of miles from the nearest supply point, it may be necessary to bring an entire additional Pelican case dedicated to backup equipment. For operations based in or near a city, a lighter redundancy plan may be sufficient.



As a standard practice, 2FP aims to ensure that all mission-critical objectives can be achieved in at least two different ways. For example, if a powered water filtration pump fails, a manual hand pump should be available as a backup. If pre-filled DNA/RNA Shield tubes leak, a sealed set of backup DNA/RNA Shield wrapped in parafilm should be on hand. Consumables are packed at approximately 30% above projected needs to account for scope changes (e.g., unplanned sample sites), as well as for inevitable losses or breakages (e.g., dropped Whirl-Paks). Personal equipment should never be used for organizational work to ensure accountability, standardization, and compliance with 2FP's quality control standards.

The 2FP INVENTORY AND TRANSPORT spreadsheet should be used during planning to guide kit generation. While precise contents will vary by expedition, kits typically include:

1. Microbiology kit – materials for culturing and growing microbes in the field
2. Lab-in-a-box – general laboratory tools for maintaining sterile fields, pipetting, and processing
3. Consumables kit – sampling and processing consumables such as pipette tips and conical tubes
4. Dive kits – SCUBA-specific sampling gear and safety equipment
5. Terrestrial sampling kits – tools for soil, sediment, biomass, and plant collection

Many 2FP suitcases and field cases are insulated to double as coolers. When dry ice is required, we recommend using rotomolded hard coolers that are both impact resistant and have thick walls that can hold cold for extended periods of time. This can also serve as an alternative suitcase when empty, prior to loading with ice or dry ice.

For air travel, we recommend packing to minimize baggage volume rather than pre-packing by kit. Kits should be assembled upon arrival at the field site. All critical resources should be carried on the plane whenever possible (except for items that must legally be shipped, such as dry ice). Every checked bag must contain printed customs declarations, Material Transfer Agreements, and all relevant permits or letters of agreement.

ITEM ID	Item	Process Stage	Category	Used in SCUBA?	Quantity	Packed (Y/N)	Acquire In-Field (Y/N)	Packing Container Outbound (To Expedition)	Packing Container During Expedition	Packing Container Inbound (From Expedition)
P-0001	Sharpie Markers	1, Field ID	Labeling	N	6	N	N	LIST THE SUITCASE/CONTAINER ID YOU ARE PACKING THE MATERIALS IN DURING SHIPPING	LIST THE SUITCASE/CONTAINER ID YOU ARE PACKING MATERIALS INTO UPON ARRIVAL (IF NECESSARY)	LIST THE SUITCASE/CONTAINER ID YOU ARE PACKING MATERIALS INTO UPON DEPARTURE (IF NECESSARY)
P-0002	Permaneau Marker	1, Field ID	Labeling	N	3	N	N			
P-0003	Gorilla Glue	1, Field ID	Labeling	N	2	N	N			
P-0004	Novelty Gloves (6mL), Heavy Duty (S, L, XXL)	2, Collection	Field Consumables	N	3	N	N			
P-0005	1 Liter, sterile, Whirl-pak	2, Collection	Field Consumables	N	80	N	N			
P-0006	4oz, sterile, Whirl-pak	2, Collection	Field Consumables	N	80	N	N			
P-0007	SCUBA Dive, Mesh bag	2, Collection	Field Equipment	Y	2	N	N			
P-0008	Shovel, small	2, Collection	Field Equipment	N	2	N	N			
P-0009	Clip-board, underwater	2, Collection	Field Equipment	Y	1	N	N			
P-0010	Underwater paper	2, Collection	Field Consumables	Y	50	N	N			
P-0011	Underwater pencil & eraser & sharpener	2, Collection	Field Equipment	N	3	N	N	THE VALUES POPULATED HERE ARE EXAMPLES - POPULATE THIS WITH YOUR OWN MATERIALS		
P-0012	Ice packs, blue ice	2, Collection	Field Equipment	N	10	N	N			
P-0013	Forceps, Tweezer	2, Collection	Field Equipment	N	1	N	N			
P-0014	Scopulae	2, Collection	Field Equipment	N	1	N	N			
P-0015	Small soft cooler, mini-chonzilla 1	2, Collection	Field Equipment	N	1	N	N			
P-0016	Large cooler	2, Collection	Field Equipment	N	1	N	N			
P-0017	Field Notebooks	2, Collection	Field Equipment	N	1	N	N			
P-0018	Orion™ pH 7.01 Buffer Individual Use Pouches, 25 Pack	2, Collection	Field Reagent	N	1	N	N			
P-0019	Orion™ pH 7.00 Buffer Individual Use Pouches, 25 Pack	2, Collection	Field Reagent	N	1	N	N			

Figure: Screenshot of the 2FP inventory worksheet.

Cold chain and sample storage

Once site selection and experimental design are complete, teams must finalize a cold chain plan to ensure the integrity of collected samples from the moment of collection to final storage. In



remote locations, sourcing dry ice can be challenging, yet it is essential for maintaining the viability of glycerol and DMSO stocks used in cryopreservation.

If culturing and cryopreserving samples, the cold chain plan must address:

1. Dry ice sourcing – identify nearby suppliers before deployment. Optimize for slabs over pellets, as slabs will last longer in the field.
2. Hold time – estimate how long a given quantity of dry ice will last in your cooler model (we recommend using rotomolded hard coolers that are both impact resistant and have thick walls that can hold cold for extended periods of time)
3. Refill strategies – determine how and where replenishment will occur.
4. Transit restrictions – remember that dry ice is a regulated “dangerous good” and may not be allowed on certain flights or transit routes.

When shipping with dry ice, ensure you use a carrier authorized to handle dangerous goods. In the U.S., FedEx lists these locations on its website. Always follow IATA regulations, including vented packaging and proper labeling.

In some expeditions, only DNA sequencing is planned. In these cases, cold chain demands are reduced: samples can be placed directly into an appropriate storage buffer such as Zymo Research's DNA/RNA Shield, which allows room-temperature storage for up to one month, or longer at 4°C. This approach is logically simpler but sacrifices the ability to culture microorganisms from the same samples.

For refrigeration without freezing, use blue ice packs to maintain ~4°C. These are preferred over wet ice, which can melt, leak, and increase contamination risk. Always ensure refrigeration or freezing capacity is available at the end of each day's sampling. If no blue ice is available or you need more for transport, consider freezing plastic water bottles or reusable plastic bags full of water, which can be useful when you are in places with limited resources. Hard shell blue ice packs are good for longer trips, they are very durable, produce less condensation and pack well. Soft gel packs of blue ice are flexible and lightweight, however they are less durable and don't stay cold quite as long as hard shell blue ice packs. When freezing ice packs, freeze in a standard freezer at -20°C and be mindful to not

For more details on packaging, transport regulations, and contingency planning, see **Sample Transport**.

Preparing preservation buffers

Typically, each sample is aliquoted into three different buffers to preserve different fractions of the microbial community and maintain flexibility for downstream analyses:

- **50% glycerol** is used to preserve heterotrophic microorganisms. Glycerol acts as a cryoprotectant, reducing ice crystal formation during freezing, which helps maintain cell integrity for culture recovery. It should be stored at -20°C or -80°C, and samples in glycerol must remain frozen until processing.
- **10% DMSO** is used to preserve phototrophic organisms and certain sensitive cell types. DMSO penetrates cell membranes and protects internal structures from freezing damage, but can be toxic at higher concentrations or with prolonged exposure at non-freezing temperatures. Samples in DMSO should be frozen as quickly as possible after aliquoting, ideally on dry ice, and kept at -80°C for long-term storage.



- **Zymo DNA/RNA Shield** preserves DNA and RNA by stabilizing nucleic acids and inactivating nucleases and pathogens. It allows room-temperature storage for up to one month, though refrigeration or freezing is preferred for long-term stability. This buffer is particularly useful when cold chain logistics are challenging or when only molecular analyses are planned.

The following brief protocols describe the preparation of each buffer type. All tubes should be prepared and QC'd for contamination before deployment into the field. QC typically involves plating a small aliquot of each batch on nutrient agar (TSB preferred) and confirming no microbial growth after incubation. Parafilm should be used to seal tube caps to prevent leakage during transport, especially by air. For additional protection, place tubes upright in secondary containment with absorbent material if shipping liquids, and organize by buffer type for rapid access during sampling.

A. Glycerol Cryovials (50%)

1. Preparation

1. Use ACS-certified glycerol stock to prepare a fresh 50% (v/v) solution in milliQ H₂O.
2. Mix thoroughly until homogeneous.
 1. Invert repeatedly until the solution appears uniform with no visible streaks or layering. Avoid creating bubbles, as they can interfere with accurate volume measurement and sterilization
3. Autoclave to sterilize.
 1. Dispense into a clean, autoclavable glass bottle or flask with a loose-fitting cap (do not fully tighten to avoid pressure buildup).
 2. Autoclave to sterilize: run a standard liquid cycle at 121°C for 15–20 minutes at 15 psi.
 3. After autoclaving, allow the solution to cool to room temperature before handling or aliquoting.
 4. Tighten the cap only once the bottle has cooled completely to prevent vacuum lock.
4. Calculate total volume needed (0.75 mL per cryovial plus ~10% extra to account for pipetting loss).

2. Rack Setup

1. Arrange sterile 2.0 mL cryovials in racks; loosen lids in advance.
2. Mark each completed row with one black line across the caps to track progress.

3. Aliquoting

- a. Using sterile technique and filtered pipette tips, dispense 0.75 mL per cryovial.
- b. Tighten lids progressively to maintain order and efficiency.

4. Contamination Control

- a. Plate ~0.75 mL of same solution onto a Nutrient Agar (preferred: TSB; acceptable: LB)



- b. Incubate at 30°C for 48 hours

5. Labeling & Storage

- a. Label racks: "Gly", date, initials, "**not yet cleared**"
- b. After 48 hrs, if clear: update label to "**cleared**"
- c. Store in rack or tightly re-rack into field safe boxes.

B. DMSO Cryovials (10%)

1. Preparation

- 1.1. Use molecular biology grade DMSO to prepare a 10% (v/v) solution in milliQ H₂O. Always add DMSO to water, never the reverse.
- 1.2. Filter sterilize using nylon filter bottles (250–1000 mL) using a vacuum
- 1.3. Calculate volume needed (0.75 mL per cryovial)

2. Rack Setup

- 2.1.1. Calculate required volume (0.75 mL per cryovial + ~10% extra).
- 2.1.2. Arrange sterile 2.0mL cryovials in racks; loosen lids in advance.
- 2.1.3. Mark each completed row with two black lines across the caps to track progress

3. Aliquoting

- 3.1. Using sterile technique, dispense 0.75 mL per cryovial
- 3.2. Tighten lids as you proceed

4. Contamination Control

- 4.1. Plate ~0.75 mL onto Nutrient Agar (TSB preferred)
- 4.2. Incubate at 30°C for 48 hours

5. Labeling & Storage

- 5.1. Label racks: "**DMSO**", date, initials, "**not yet cleared**"
- 5.2. After 48 hrs, if no growth: update to "**cleared**"
- 5.3. Store upright at room temperature until ready for use.

C. DNA/RNA Shield Tubes

1. Preparation

- 1.1.1. In sterile conditions, transfer required volume from the original Zymo DNA/RNA Shield bottle into a clean, labeled 50 mL conical tube. Record the date if newly opened.
- 1.1.2. This buffer does not require sterilization.

2. Aliquoting

- 2.1. Dispense 1.5 mL into a 5mL snap-top tube.

3. Labeling & Storage

- 3.1. Label tubes with preservative type, date, and initials
- 3.2. Store at room temperature or until field deployment



Resources

Resource	Type	Description	Date added	
National States Geographic Information Council	Webpage	The National States Geographic Information Council (NSGIC) is an organization in the United States of America of the states, the District of Columbia, and the territories that works to improve the use and sharing of geospatial data and GIS tools	https://nsgic.org/	2025-08-08
ISO Geospatial Metadata Standards	Webpage	ISO metadata standards, primarily the ISO 19115 series, provide a framework for describing geographic information and services	https://www.fdc.gov/metadata/iso-standards	2025-08-08
YETI cooler			<input type="button" value="Date"/>	
IATA Dry Ice Shipping Course	Website	Certification for safely shipping dry ice and temperature-sensitive cargo.	https://www.iata.org/en/training/courses/dry-ice/	2025-08-08



V. Setting up a field processing lab

Background

Prior to going out into the field, select and prepare the areas you will need for a smooth and efficient workflow. The four areas described below — prep, sample check-in, processing, and preservation — should be separated whenever possible to reduce cross-contamination and confusion. In small spaces, some functions may be combined, but sterile technique must be maintained. For example, sample processing and preservation can be done at the same table if performed by the same person in immediate sequence, within a clean working field. We recommend a closed-toe shoe-only policy in the processing and preservation areas, and lab coats in the preservation area, especially if working with an open flame.

BSL-1 Field Laboratory Practices

When operating a field lab, follow Biosafety Level 1 (BSL-1) practices unless your work or permits require higher containment. BSL-1 standards include wearing PPE (lab coat, gloves, eye protection), prohibiting food and drinks in the lab space, decontaminating work surfaces before and after use, and storing reagents and samples in designated clean areas. Most environmental microbial work falls under BSL-1, but confirm whether site-specific regulations require elevated biosafety measures.

Field Lab Stations

1. Field sampling prep area

- Prepare an area to assemble field equipment before departure to sampling sites. This space should be separate from sample processing and preservation areas and large enough to stage both incoming and outgoing gear. Maintain an inventory list here for quick restocking and replacement.

2. Sample Check-In Area

- The sample check-in area should be clean, accessible, and close to cold storage. This is where incoming samples are received, logged, and assigned Collection IDs (CIDs). It should have a computer or tablet for digital metadata entry, labeling materials, and notebooks for backup notes. Cold storage, such as coolers or portable refrigeration, should be nearby to stabilize samples immediately. The work surface should be easy to disinfect with 10% bleach followed by 70–80% ethanol before and after each use.

3. Sample Processing Area

- The sample processing area is designated for pre-processing before preservation. This may involve concentrating water samples or homogenizing sediment. All tools, racks, tubes, filters, and ethanol baths should be organized and ready before starting. Keep the area free from non-sterile gear, maintain a draft-free environment, and clean all surfaces with 10% bleach (5–10 minutes contact time) followed by 70–80% ethanol before and after use.



4. Sample Preservation Area

- The sample preservation area is used for final steps such as aliquoting into preservatives like DNA/RNA Shield, DMSO, or glycerol. This area should have prepared cryovials, reagent stocks, pipettes, and cold chain storage such as dry ice, portable -80°C freezers, or insulated coolers. If using an open flame for sterile technique, it must be secured and stable. On vessels, flame use must be approved by the captain and a fire extinguisher should be on hand. If open flames are not permitted, use a portable HEPA-filtered clean box or rely on ethanol-based surface sterilization.

Maintaining Sterility in the Field

Maintaining sterility in the field is critical for sample quality. Gloves should be worn at all times and changed between samples or tasks. Sterile items should not be placed on unclean surfaces; use trays or racks instead. Tubes and reagent caps must remain closed unless actively in use, and all materials should be prepared in advance to minimize open handling time. When working outdoors, position yourself upwind of dust or debris, and consider using portable shelters in windy or dusty environments.

Waste Disposal

All waste must be handled as potentially contaminated. Solid waste such as gloves, tips, and used tubes should be placed in clearly labeled, leak-proof biohazard bags. Liquid waste, including rinse water and leftover buffers, should be collected in a dedicated, labeled container with a secure lid, treated with 10% bleach for at least 20 minutes before disposal. If autoclave access is available, autoclave solid waste prior to disposal. If not, double-bag and return waste to a facility with proper disposal capability. Local regulations, including those for marine or protected areas, must be followed at all times

Resources

Resource	Type	Description	Link	Date added
CDC Biosafety in Microbiological and Biomedical Laboratories	PDF	Published by the CDC and NIH, the BMBL is the primary U.S. reference for biosafety practices, facility standards, and risk assessments in microbiological work. It defines biosafety levels (BSL-1 to BSL-4), outlines safe	https://www.cdc.gov/abs/pdf/SF_19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf	2025-08-08



		<p>handling of microorganisms, and provides guidance on decontamination, PPE, and laboratory design. While most 2FP work falls under BSL-1 or BSL-2, this manual is an essential resource for aligning field and lab protocols with established safety standards.</p>		
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VI. Sample Collection

Background

This section outlines general guidelines and protocols for collecting environmental samples, including considerations for different sample types and environments, as well as key steps for ensuring sample integrity and suitability for downstream processing. All sampling must follow the relevant collection protocol for the sample type and environment, whether terrestrial or SCUBA diving.

Sampling teams and roles

We recommend working in sampling teams at all times, allocating specific tasks (e.g., metadata collection, sample tracking, sample collection) to different individuals. Teams of two or three seem to work the best. A single individual attempting to manage the entire sampling workflow, from site surveying to metadata collection, is both potentially unsafe and also quite inefficient.

We recommend assigning the following tasks across your team:

1. Sampling lead

The individual with the final say over where a sample will be collected (i.e., the person who picks a spring or a vent or other location to work at). While these decisions will usually be done by group consensus, we find, especially in situations where communication is more difficult (e.g., underwater), having a single person who can indicate “yes or no” to a given location is effective. This person also tracks timing and decides when to end sampling. This role can be separated depending on expertise (e.g., there could be a water sampling lead or a sediment sampling lead).

2. Metadata collection

An individual who is responsible for ensuring the field metadata is complete. This person should confirm that each sample is linked to a specific field ID, the field ID is readable on the collection device.

3. Sample-specific collectors

We recommend having different individuals responsible for collecting different kinds of samples. One person for water, one person for sediment/biomass, etc.

4. Sample keeper

This role is responsible for holding all collected samples and for ensuring the samples make it from the site to being checked-in in the lab. Usually appropriate to also be the metadata collector.

5. Geochemistry

This role is responsible for collecting geochemistry-specific metadata at a given site.



Additional roles include safety officer, sample photographer, and additional site-metadata collectors (e.g., the individual responsible for taking geochemistry or other data relevant to an entire site).



Sample types

Water samples

- Water is material that has accumulated on the earth's surface, located on top of land or below land (i.e. groundwater). Includes but not limited to water from rivers, lakes, ocean, ponds, streams and springs.

Biomass samples

- Biomass is a multi-layered, cohesive community of microorganisms, (e.g., bacteria, archaea and eukaryotes), that grow on surfaces in various environments. These mats are often structured in layers, with different microbial groups occupying distinct strata based on their metabolic functions and environmental conditions. You can identify biomass by:
 - Color & Texture: Look for filamentous, slimy, oily or mat-like growths on rocks, soil or surfaces of sediment. Some biofilms form a thick crust and have brittle surfaces. Common colors are green, brown, black, orange or even pink.
 - Bubble formation: A surface with bubbles, froth or foam can indicate certain types of bacteria, indicating the production of gas.
 - Smell: Some biofilms, especially in anaerobic environments (lacking oxygen/air), may produce a sulfuric or musty smell.



Minimizing Disturbance to Biofilms and Biomass

When sampling microbial mats, biofilms, or other cohesive biomass, avoid removing the entire growth from a given patch.

- Target the active surface layer (top 1–5 mm) rather than the full mat depth unless deep layers are specifically needed for the research question.

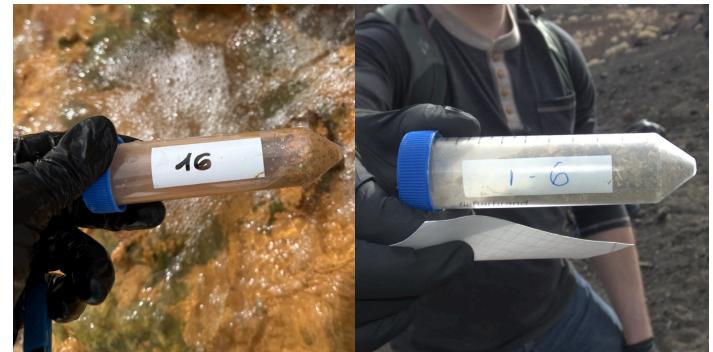


- Collect from the edges or a small subsection of the biomass, leaving most of the mat intact to continue ecological function.
- For spatially extensive mats, collect from multiple points in low-traffic areas rather than concentrating disturbance in one spot.
- Avoid dislodging or fragmenting surrounding substrate, as this can cause biomass detachment beyond the sampled area.
Record in metadata whether sampling was partial or complete, and document the approximate percent of biomass removed.

This approach preserves microbial community structure for long-term monitoring, minimizes habitat impact, and supports repeat sampling at the same location.

Sediment/Soil samples

- Sediment is defined as a deposit of insoluble material, primarily rock and soil particles, transported from land areas to bodies of water. Soil is the upper layer of earth in which plants grow, a black or dark brown material that typically consists of a mixture of organic remains, clay, and rock particles.



Sampling order

- **1st: Water Samples**
- **2nd: Sediment/soil**
- **3rd: Biomass**
- Extra samples may be collected **after planned ones**.

This order is to minimize contamination between sample types (e.g., avoiding mixing sediment into a water sample if both are collected from the same area). It is most relevant for underwater sampling.

Protocols

Pre-Collection:

- i. Pre-load sample bag with all requisite equipment, complete final gear checks.
- ii. Travel to site
- iii. Prepare a collection team by providing collection bags and assigning roles.
 - Sync dive computers and prep pH sensors.
- iv. Survey the area for immediate hazards such as:
 - Unstable terrain or rocks (terrestrial, shore-entry)
 - Slippery surfaces, sharp debris (terrestrial, shore-entry)



- Steep drop-offs or wave exposure (terrestrial, shore-entry)
- Signs of wildlife (e.g., snakes, wasps, sea urchins) (terrestrial, SCUBA)

Site Metadata

Site Documentation (To be measured once per sampling area)

1. Collect the following images of the site
 - **Overview Image:** One image of the site without people.
 - **Sampling Activity Image:** At least one image of yourself or team sampling.
 - **Sample Detail Images:** Take additional photos of sample sites with a ruler, scale card, or other measurable reference object
 - **GPS Coordinates**
 - Choose a device for GPS collection and note in the metadata (e.g., Handheld GPS, Smartphone).
 - Select scientific format to be in decimal degrees (DD) (e.g., 42.3601, -71.0589) and use World Geodetic System, 1984 (WGS 84) for recording.
 - Record horizontal accuracy (in meters) to assist with spatial analysis (plus/minus 3m)
 - For each GPS point, collect:
 - Date & Time (in time zone of site)
 - Collector Name or Initials
 - Device used
 - Coordinate format and datum
 - Environment notes (e.g., location, GPS signal quality)
 - Take a photo of the GPS point and cross-check with a second device if time permits.

Water/Site Chemistry (To be measured once per sampling area)

If using a 25ml conical for geochemistry measurements, you may reuse that tube between sites, however ensure it is rinsed with DI water between each location

- 1) Measure and record pH
 - a) For water samples: Use pH strips. Dip the strip in the water (either in a clean conical tube or directly in the source), remove, and wait 30 seconds before reading. Photograph the strip alongside the color reference on the pH strip container. Do not use the TDS sensor for pH measurements, as this requires separate calibration buffers.
 - b) For mud or sediment samples that obscure pH strip colors: Prepare a slurry by mixing approximately 1 part mud/sediment with 10 parts DI water in a clean container. Shake gently for 5 seconds, then let settle briefly. Dip the pH strip into the slurry, wait 30 seconds, and photograph as above. Note: This method provides a standardized slurry pH for comparative purposes and may differ from true in situ pH.
 - c) Record observed pH in the metadata sheet in 0.5-unit increment



2) Measure and record salinity

- a) Remove the cap from the salinity/total dissolved solids (TDS) sensor. Power on, confirm units are in ppm, and ensure the device is set to measure salt. Hold the probe in the water sample (either in a clean conical tube or directly in the source) until the reading stabilizes. Record the value in the metadata sheet.
- b) For sediment or solid samples: Prepare a slurry at approximately 1:10 ratio in DI water and measure as above.
- c) Rinse and wash the probe tip after each measurement

3) Measure and record temperature

- a) Use the thermometer to measure water temperature directly from the source. If no water is available, record ambient air temperature at the site. Record in Celsius.

4) Measure and record additional geochemistry

- a) Remove one aquarium/water quality test strip from the provided container. Follow the same sampling approach as for pH measurement.
- b) Record any other observations in the metadata sheet, including UV fluorescence, turbidity, odor, and sediment characteristics.

5) Record timing information

- a) Record the timestamp of the last photograph taken during metadata collection for this location.
- b) Note any additional relevant site observations in the metadata sheet.

TDS Sensor for Salinity	Geochemistry Strips and Reference	pH Strips and Reference	Thermometer
			



How to use a whirl-pak bag (essential for all sample types)

		
Open the Bag – Rip off the plastic seal at the top of the bag. Grasp the white pull tabs on both ends and pull outward to fully open the bag without touching the inside.	Fill the Bag – Pour or place your sample inside, staying below the fill line and avoiding contact with the interior. If collecting a water sample, fill the bag while it is under the water.	* To form a seal, top of bag MUST be folded over itself 3x times BEFORE tying twist ties*

1. Water Collection

A. Underwater (SCUBA diving)

i. Prep:

1. Put on gloves. Wear gloves while handling the sample.
2. Remove one 1 Liter Whirl-Pak bag (pre-labeled) per sample.
 1. Confirm the bag is sealed and undamaged and Field-ID label is intact.
3. Unfold the tabs of the Whirl-Pak without touching the interior or seal area.
Use a new bag for each new sample, do not reuse.

ii. Sampling:

1. At desired depth, open the bag underwater.
2. Let the water fill naturally (approximately 30 seconds)
3. Close the bag underwater using the appropriate rotating method and twist the wires at least three times.
4. Place in a mesh bag until return to the surface.



5. Once at the surface, place the sample on ice and out of direct sunlight. Inspect the bag for leaks and confirm it is sealed to avoid surface contamination. Always stand the bag upright.

B. Terrestrial

i. Prep:

1. Put on gloves. Wear gloves while handling the sample.
2. Remove one 1 Liter Whirl-Pak bag (pre-labeled) per sample.
 1. Confirm the bag is sealed and undamaged.
3. Unfold the tabs of the Whirl-Pak without touching the interior or seal area.
Use a new bag for each new sample, do not reuse.

ii. Sampling:

1. Submerge the open end of the bag into the water to the desired depth.
(Avoid submerging your ungloved arm/hand)
 1. Avoid surface scum, dense vegetation or floating debris unless part of the sample.
2. Let the water fill passively, facing upstreams (if there is a current) or away from your body.

iii. Seal

1. Once approximately 1 liter has been collected, grasp the tabs and slowly withdraw the bag from the water.
2. Expel the excess air and then twist the top at least 3 times. Remove as much air as possible, spilling some off the sides is acceptable but avoid major sample loss.
3. Bend the ends of the wire tabs together to close securely.
4. Place the sample on ice and out of direct sunlight. Inspect the bag for leaks and confirm it is sealed to avoid contamination. Always stand the bag upright.

2. Sediment Collection

A. Underwater (SCUBA diving)

i. Prep:

1. Put on gloves. Wear gloves while handling the sample.
2. Remove one 4oz Whirl-Pak bag (pre-labeled) per sample.
 1. Confirm the bag is sealed and undamaged.
3. Approach the sediment site slowly to avoid disturbing the area.
4. Unfold the tabs of the Whirl-Pak without touching the interior or seal area.
Use a new bag for each new sample, do not reuse.

ii. Sampling:

1. At the site, use a clean scoop or tube to collect the surface sediment (top 1-3 centimeters).
2. Open the whirl pak, and transfer the sample into the bag.
3. Add adjacent water into the bag until the contents are approximately 75% sediment and 25% water (~15mL of liquid).
 1. Avoid vegetation, detritus or macroorganisms.



4. Carefully twist (3 times) and close the bag underwater.
5. Place in a mesh bag until return to the surface.
6. Once at the surface, place the sample on ice and out of direct sunlight. Inspect the bag for leaks and confirm it is sealed to avoid surface contamination. Always stand the bag upright.

iii. Notes:

- Do not overfill, allow space to twist the bag closed and reduce leakage risk.
- Change gloves when needed. Avoid handling samples without gloves.

B. Terrestrial

i. Prep:

1. Put on gloves. Wear gloves while handling the sample.
2. Remove one 4oz Whirl-Pak bag (pre-labeled) per sample.
 1. Confirm the bag is sealed and undamaged.
3. Identify the sampling point, and avoid areas with large detritus, roots, or debris.
4. Use a sterile scoop or tube to collect the sample.
5. Unfold the tabs of the Whirl-Pak without touching the interior or seal area.
Use a new bag for each new sample, do not reuse.

ii. Sampling:

1. Use the scoop or tube to collect the sample at the top 1-3 centimeters.
(Avoid submerging your ungloved arm/hand)
 1. Avoid surface scum, dense vegetation or floating debris unless part of the sample.
2. Transfer sediment into pre-labeled Whirl-pak bag until the contents are approximately 75% sediment and 25% water (~15mL of liquid). Add more adjacent water if needed.

iii. Seal

1. Once filled, grasp the tabs, expel the excess air and then twist the top at least 3 times. Remove as much air as possible, spilling some off the sides is acceptable but avoid major sample loss.
2. Bend the ends of the wire tabs together to close securely.
3. Place the sample on ice and out of direct sunlight. Inspect the bag for leaks and confirm it is sealed to avoid contamination. Always stand the bag upright.

3. Biomass Collection

A. Underwater (SCUBA diving)

i. Prep (before diving):

1. Put on gloves. Wear gloves while handling the sample.
2. Remove one 4oz Whirl-Pak bag (pre-labeled) per sample.
 - Confirm the bag is sealed and undamaged.
3. Approach the biomass site slowly to avoid disturbing the area. Especially if the biomass is fragile or sensitive to oxidation.



4. Unfold the tabs of the Whirl-Pak without touching the interior or seal area.
Use a new bag for each new sample, do not reuse.

ii. Sampling (during dive):

5. At the site, use a clean scoop or tweezer to collect the biomass (top 1-5mm).
6. Open the Whirl-pak, and transfer the sample into the bag.
7. Add adjacent water into the bag just enough to cover the biomass but not to dilute it (to reduce oxygen exposure and create a slurry).
 - Avoid vegetation, detritus or macroorganisms.
 - Aim for 15mL of slurry.
8. Carefully twist (3 times at least) and close the bag underwater. Squeeze out excess water if needed.
9. Place in a mesh bag until return to the surface.
10. Once at the surface, place the sample on ice and out of direct sunlight. Inspect the bag for leaks and confirm it is sealed to avoid surface contamination. Always stand the bag upright.

iii. Notes:

- Do not overfill, allow space to twist the bag closed and reduce leakage risk.
- Change gloves when needed. Avoid handling samples without gloves.

B. Terrestrial

i. Prep:

1. Put on gloves. Wear gloves while handling the sample.
2. Remove one 4oz Whirl-Pak bag (pre-labeled) per sample.
 - Confirm the bag is sealed and undamaged.
3. Identify the sampling point, and avoid areas with large detritus, roots, or debris.
4. Approach the biomass site slowly to avoid disturbing the area. Especially if the biomass is fragile or sensitive to oxidation.
5. Use a sterile scoop or tweezer.
6. Unfold the tabs of the Whirl-Pak without touching the interior or seal area.
Use a new bag for each new sample, do not reuse.

ii. Sampling:

1. Use the scoop or tube to collect the sample at the top 1-5mm active layers.
 - Avoid excess soil, surface scum, dense vegetation or floating debris unless part of the sample.
2. Open the Whirl-pak, and transfer the sample into the bag.
3. Add adjacent water into the bag just enough to cover the biomass but not to dilute it (to reduce oxygen exposure and create a slurry).
 - Avoid vegetation, detritus or macroorganisms.
 - Aim for 15mL of slurry.

iii. Seal



1. Once filled, grasp the tabs, expel the excess air and then twist the top at least 3 times. Remove as much air as possible, spilling some off the sides is acceptable but avoid major sample loss.
2. Bend the ends of the wire tabs together to close securely.
3. Place the sample on ice and out of direct sunlight. Inspect the bag for leaks and confirm it is sealed to avoid contamination. Always stand the bag upright.

4. Soil Collection

A. Terrestrial

i. Prep:

1. Put on gloves. Wear gloves while handling the sample.
2. Remove one 4oz Whirl-Pak bag (pre-labeled) per sample.
 - Confirm the bag is sealed and undamaged.
3. Identify the sampling point, and avoid areas with large detritus, roots, feces or debris.
4. Use a sterile scoop, coring device or tube to collect the sample.
5. Unfold the tabs of the Whirl-Pak without touching the interior or seal area.
Use a new bag for each new sample, do not reuse.

ii. Sampling:

1. Use the scoop or tube to collect the sample at the top 1-3 centimeters.
(Avoid submerging your ungloved arm/hand)
2. Transfer soil into a pre-labeled Whirl-pak bag until the contents are approximately 75% soil.
3. If water is nearby, add adjacent water until Whirl-pak is 75% soil and 25% water (15mL), enough to make a slurry.
 - Add more liquid to fully mix but do not over-dilute.

iii. Seal

1. Once filled, grasp the tabs, hold upright, shake and expel the excess air. Then twist the top at least 3 times. Remove as much air as possible, spilling some off the sides is acceptable but avoid major sample loss.
2. Bend the ends of the wire tabs together to close securely.
3. Place the sample on ice and out of direct sunlight. Inspect the bag for leaks and confirm it is sealed to avoid contamination. Always stand the bag upright.

5. Coral Collection

A. Underwater (SCUBA diving)

i. Prep:

1. Put on gloves. Wear gloves while handling the sample.
2. Remove one 4oz Whirl-Pak bag (pre-labeled) per sample.
 - Confirm the bag is sealed and undamaged.



3. Approach the coral but do not touch until sampling.
4. Unfold the tabs of the Whirl-Pak without touching the interior or seal area.
Use a new bag for each new sample, do not reuse.

ii. **Sampling:**

1. At the site, use a bone-cutter or coring device to collect 2-3cm of the coral tissue. If possible, use your gloved hand to snap off coral fragments.
 - Avoid areas that look diseased, bleached or stressed.
 - If possible, collect coral mucus after tissue collection.
2. Open the Whirl-pak, and transfer the sample into the bag.
3. Add adjacent seawater into the bag just enough to cover the coral but not to dilute it (to reduce oxygen exposure).
 - Aim for 15mL of slurry.
4. Carefully twist (3 times at least) and close the bag underwater. Squeeze out excess water if needed.
5. Place in a mesh bag until return to the surface.
6. Once at the surface, place the sample on ice and out of direct sunlight. Inspect the bag for leaks and confirm it is sealed to avoid surface contamination. Always stand the bag upright.

iii. **Notes:**

- Do not overfill, allow space to twist the bag closed and reduce leakage risk.
- Change gloves when needed. Avoid handling samples without gloves.

6. Plant Collection

A. Terrestrial

i. **Prep:**

1. Put on gloves. Wear gloves while handling the sample.
2. Remove one 4oz Whirl-Pak bag (pre-labeled) per sample.
 - (i) Confirm the bag is sealed and undamaged.
3. Unfold the tabs of the Whirl-Pak without touching the interior or seal area.
Use a new bag for each new sample, do not reuse.

ii. **Sampling:**

1. At the site, use a clipping or coring device to collect ~15cc of material.
2. Open the Whirl-pak, and transfer the sample into the bag.
3. Carefully twist (3 times at least) and close the bag underwater. Squeeze out excess water if needed.
4. Always stand the bag upright.

iii. **Notes:**

- Do not overfill or compress to avoid puncturing the bag.
- Change gloves when needed. Avoid handling samples without gloves.



Resources

Resource	Type	Description	Link	Date added
2FP Expedition Template	Folder	Master planning file for objectives, CIDs, metadata, inventory, and team roles	https://github.com/two-frontiers-project/2FP-expedition-template/tree/main	2025-08-08
Instructions for a Whirl-Pak® Leak-Proof Closure	Video	Manufacturer's instructions for opening, filling, sealing, and storing Whirl-Pak bags to prevent contamination.	https://www.youtube.com/watch?v=5DvyF1PfEI4	2025-08-08



VII. Sample Check-In

Background

Sample Check-In is the standardized process for receiving and registering field samples in the lab, ensuring accurate metadata capture and full traceability from collection through processing.

This step bridges field collection and laboratory processing. At Check-In, the Field ID – the temporary code written on a Whirl-Pak or other collection container – is matched to a CID (Collection ID) or other unique, pre-generated identifier. Once assigned, a CID is permanently linked to that sample and will never be reused.

The Field ID–CID mapping is recorded in the expedition metadata spreadsheet (see the Expedition Template for an example). A pre-printed cryolabel with the CID is then applied to the original sample container. Multiple cryolabels for each CID should be prepared in advance so they can also be applied to aliquots preserved in DMSO, glycerol, or DNA/RNA Shield during processing.

Assign a single individual to be responsible for Sample Check-In. This task should be completed immediately upon sample return to the field lab and ideally in a clean, organized area separate from processing or storage.

Protocol

1. Label Verification and Assignment

- A. Ensure the sample container is clearly labeled with its original Field ID.
- B. Arrange sample by date collected, site collected from and sample type.
- C. Assign a unique CID for each incoming sample.
 - o Use the CID sheet generated prior to the expedition.
 - o Apply CID label to the sample container.

2. Metadata Entry

- A. Open the expeditions metadata spreadsheet for the expedition.
- B. In the spreadsheet:
 - o Link the assigned CID to the corresponding Field ID.
 - o Record key sample metadata from the field notebook:
 - Date
 - Time
 - Collection site and GPS
 - Sample Type
 - Environmental conditions (e.g. temperature, salinity, depth)
 - Notes observed during the collection.

3. Images

- A. Confirm all sample photos have been uploaded to the appropriate location.
 - o File names must be prepared with CID (e.g. CID_012345_coral1.jpg)
- B. Upload images of all field notes (non-digital) and note each page with the date, location and page number (DD-MM-YYYY_site_pagenumber)



4. Sample Intake Area Handling

- A. Place checked in samples in the designated intake area for preparation.
 - Keep samples on ice or at 4C.
 - Group samples in racks by sample type and CID order.

Resources

Resource	Type	Description	Link	Date added
CID Guidelines	Protocol	Outline of how to generate your own unique sample identifiers.		2025-08-08



VIII. Sample Processing and Preservation

Background

Sample Processing and Preservation outlines the standardized procedures for handling environmental sample types — sediment, soil, water, coral, biomass, and plant material — collected during 2FP expeditions. The goal is to maximize microbial recovery, maintain sample integrity for both culture-dependent and culture-independent analyses, and ensure reproducibility and traceability across teams and sites.

All processing should be completed within 24 hours of sample collection unless otherwise specified. Work must be conducted using sterile technique, appropriate PPE, and in full compliance with the 2FP sample tracking system. This system uses pre-generated CUAL-IDs (CIDs), unique identifiers assigned during Sample Check-In, to ensure every sample remains linked to its complete metadata record. Within 2FP, these are referred to as Two Frontiers IDs (CIDs), but for clarity they are called CIDs in this document.

Processing should be done in a clean workspace, sanitized before use with 10% bleach (5–10 minutes contact time) followed by 70–80% ethanol. Wear a lab coat, gloves, and any additional PPE appropriate for the environment. Unless conditions in the field prevent it (such as on a vessel), always process samples in a sterile field, using either a clean bench or a portable hood; if neither is available, a flame may be used for sterile work. Keep all tools, containers, and reagents organized to reduce handling time and risk of contamination.

Protocol

Sample Processing

1. Sediment

A. Initial Handling

1. Remove sediment from ice or fridge. The sediment sample will be in a 4 oz Whirl-pak.
2. Check if a CID is assigned to the sample.
3. If not already present, add ambient site water or sterile diH₂O to bring volume to ~15.0 mL, not exceeding a 1:1 sediment-to-liquid ratio.

B. Preparation

1. Check if the seal on the sample is secure.
2. Invert for 30 seconds to homogenize the sample.
3. Return the sample to 4C or on ice.

2. Biomass

A. Initial Handling

1. Remove biomass from ice or fridge. The biomass sample will be in a 4 oz Whirl-pak or, depending on available collection materials, a 25-50ml plastic conical or similar container.
2. Check if a CID is assigned to the sample.



3. If not already present, add ambient site water or sterile diH₂O to bring volume to ~15.0 mL, not exceeding a 1:1 biomass-to-liquid ratio.

B. Preparation

1. Check if the seal on the sample is secure.
2. Invert for 30 seconds to homogenize the sample.
3. Return the sample to 4C or on ice.

3. Soil

A. Initial Handling

1. Remove soil from ice or fridge. The soil sample will be in a 4 oz Whirl-pak.
2. Check if a CID is assigned to the sample.
3. If not already present, add ambient site water or sterile diH₂O to bring volume to ~15.0 mL, not exceeding a 1:1 soil-to-liquid ratio.

B. Preparation

1. Check if the seal on the sample is secure.
2. Invert for 30 seconds to homogenize the sample.
3. Return the sample to 4C or on ice.

4. Water

A. Initial Handling

1. Remove water from ice or fridge. The water sample will be in a 1 Liter Whirl-Pak.
2. Check if a CID is assigned to the sample.
3. Gently shake the bag to resuspend the sample.
4. Place the Whirl-pak in a stand to ensure it stands up-right.

B. Filtration

1. Filter Assembly

1. Wear nitrile gloves.
2. Attach a rubber stopper to the top of the vacuum flask.
3. Attach a disposable filter funnel to the rubber stopper by inserting the stem of the funnel hood into the stopper. This creates an airtight seal.

2. Water Filtration

1. Ensure the filter funnel is assembled with a 0.22 µm filter membrane in place.
2. With the Whirl-pak mixed, slowly and carefully open it.
3. Pour the water slowly into the filter funnel, pause to gently mix the water if it starts to settle in the Whirl-pak.
 - Wear clean gloves to handle any part of the filter or funnel interior (the outside of the funnel/flask can be handled with ungloved or previously used gloves, since it's downstream of the filter).
4. Stop once filled with about 3 cm of space at the top (do not overflow).
5. Apply Vacuum: Start the vacuum pump to begin filtration. During filtration make sure the pressure is sustained and no clogs occur. (Use hand pump as backup)
6. Disengage vacuum pump when adding more volume. Note the volume of water added and volume filtered.



3. Filter Membrane Removal

1. Decontaminate forceps by soaking in 50% bleach solution for at least 1 minute and then in deionized or distilled water, each stored in a 50 mL tube. Replace water frequently to ensure that forceps are free of bleach before touching the filter. After decontamination, the tips of the forceps should not come into contact with anything other than the filter or clean gloves.
2. Retrieve Filter Membrane: Now carefully remove the filter membrane from the funnel:
 - Unscrew or unclamp the filter holder. Avoid touching the top surface of the filter or inside of the funnel with fingers.
 - Using sterile forceps, gently lift the edge of the membrane.
 - Fold the membrane in half (biofilm side inwards), then in half again to quarter it. (If the filter is fragile or overloaded, you can skip folding and transfer it flat, but folding helps it fit into the vial)
 - Prepare a 25mL tube and place the folded filter into the tube. Add approximately 5mL of water from the Whirl-pak into the 15mL tube.
 - Gently shake the 15mL tube to homogenize the filter with the water in the 25mL tube.
 - Place on ice or in the fridge until processing.

4. Cleanup for Next Sample:

1. Sanitize the filter holder for reuse
2. Soak funnel parts in 10% bleach for ≥1 minute and rinse thoroughly with sterile water.
3. Empty and rinse the vacuum flask if it contains filtrate.
4. Rinse or replace tubing.
5. Decontaminate forceps again (bleach then rinse) or use a fresh pair for the next sample.
6. Change gloves before proceeding to the next sample.
7. Allow to air dry for 5 min before use again to ensure bleach is fully removed.

C. Preparation

- Return the sample to 4C or on ice.

5. Coral

A. Initial Handling

1. Remove coral from ice or fridge.
2. Check if a CID is assigned to the sample.
3. Using sterile forceps, transfer the coral fragment to a mortar.
4. Add 1.0mL of the original seawater from the Whirl-pak with a P1000 pipette, transfer pipette or pour from the Whirl-pak aseptically.



B. Preparation

1. Using the pestle, apply firm, sharp downward pressure to crack the coral skeleton.
2. Continue applying pressure to break the coral into smaller fragments (5–10 mm), exposing internal tissue—but do not grind into a powder.
 - Avoid excessive shearing that could damage microbial or DNA content.
3. Using a wide-bore pipette, add 1.0 mL of the original seawater from the sample bag/tube into the mortar.
4. Gently pipette up and down to create a homogenized suspension of coral tissue, microbes, and seawater.
 - Aim for a thick slurry rather than a liquid solution.
5. Transfer the homogenized coral from the mortar into the Whirl-pak and seal the sample bag.
 - Avoid over diluting the sample, aim for ~15mL of liquid.
6. Check if the seal on the sample is secure.
7. Invert for 30 seconds to homogenize the sample.
8. Return the sample to 4C or on ice.

6. Plants

A. Initial Handling

1. Remove samples from ice or fridge.
2. Check if a CID is assigned to the sample.
3. Using sterile forceps, transfer the plant material to a mortar.
4. Add up to ~15mL (usually ~7.5ml) of diH₂O (or the original water from the Whirl-pak for aquatic plants)

B. Preparation

1. Using the pestle, apply firm, sharp downward pressure and grinding until material is as homogenized as possible.
4. Bring the volume up to up to ~15mL using diH₂O or original seawater from the sample bag/tube.
 - a. Avoid over diluting the sample, aim for ~15mL of liquid.
9. Mix in the mortar.
10. Transfer the homogenized coral from the mortar back into the Whirl-pak (field) or 50ml tube (lab) and seal.
11. Check if the seal on the sample is secure.
12. Invert for 30 seconds to homogenize the sample.
13. Return the sample to 4C or on ice.

Sample Preservation

Proper preservation ensures the integrity of microbial communities for downstream molecular and culturing analyses. Follow the protocols below for DNA/RNA stabilization and cryogenic stock preparation for each of the sample types described above. Please see preparing preservation buffers prior to proceeding with sample preservation.



A. DNA/RNA Shield Aliquot:

- A. Homogenize the sample by shaking Whirl-pak (or tube) vigorously for 15 seconds.
- B. Use a P1000 pipette and wide-bore tip to add 1.5 mL of homogenized sample to a 5.0 mL tube containing 1.5 mL of DNA/RNA Shield.
 1. Collect up to 2 replicates.
- C. Cap tightly, invert vigorously seconds to mix.
- D. Store at room temperature or 4°C, then transfer to long-term storage at -20°C.

B. Cryogenic Stocks (DMSO & Glycerol):

- A. Homogenize the sample by shaking Whirl-pak vigorously for 15 seconds.
- B. Use a P1000 pipette and wide-bore tip to add 0.75 mL of homogenized sample into cryovials pre-loaded with:
 1. 0.75 mL of DMSO
 - Collect up to 3 replicates.
 2. 0.75 mL of Glycerol
 - Collect up to 3 replicates.
- C. Invert each tube 5 times to mix. Tap gently to ensure liquid is at the bottom of the tube before freezing.
- D. Immediately place on dry ice, then store at -80°C.

C. Original Sample Prep. Stocks:

- A. Homogenize the sample by shaking Whirl-pak vigorously for 15 seconds.
- B. Use a P1000 pipette and wide-bore tip to add 1.5 mL of homogenized sample into cryovials without ANY cryopreservant:
 1. No additive cryovials
 - Collect at least 1
- C. Immediately place on wet ice, store and transport at 4°C.

D. Controls

- A. Collect 1 batch control from the DNA/RNA shield and cryogenic stocks (DMSO & Glycerol) to serve as a control for transport and field contamination.
 1. Without banking, run these tubes through every step you would normally run a sample. For example, for a coral negative control, take sterile water, invert it, place it in a pestle, run a mortar through it, etc.
 2. **Never skip negative controls, these will be absolutely critical for bioinformatic decontamination downstream.**
- B. Assign each of these the sample CID.

Resources

Resource	Type	Description	Link	Date added
Zymo Research DNA/RNA Shield	Website	DNA/RNA Shield reagent is a DNA and RNA stabilization solution for nucleic	https://www.zymoresearch.com/products/dna-rna-shield?srsltid=AfmB0ooZJZ5gXP3Pr	2025-08-08



		acids in any biological sample	<u>6EfIXGMWHOPQJDEV</u> <u>Bw-pIGHS-3ZHd_vAn-</u> <u>TuI3N</u>	
OSHA Laboratory Safety - Cryogens and Dry Ice	PDF	This OSHA quick reference covers safe handling and storage of cryogenic liquids and dry ice in laboratory and field settings. It outlines hazards such as extreme cold burns, oxygen displacement, and pressure build-up, and provides guidance on PPE, ventilation, and container use.	https://www.osha.gov/sites/default/files/publications/OSHAquick_facts-lab-safety-cryogens-dryice.pdf	2025-08-08



IX. Sample transportation

Background

The transportation of biological and environmental samples is a critical link in the chain of custody between field collection and laboratory processing. Improper handling during transit can compromise sample integrity, lead to contamination, or result in the loss of valuable data. This protocol ensures that all 2FP samples—regardless of preservation method or destination—are transported under conditions that preserve microbial, chemical, and physical integrity for downstream applications such as sequencing, culturing, or chemical analysis. It applies to all transport methods, including hand-carry, domestic shipping, and international export. The focus is on preservative-specific temperature requirements, redundant containment to prevent leakage, and the inclusion of proper documentation for customs and regulatory clearance.

All personnel transporting samples must be trained in sample-specific handling, aware of biosafety and import/export regulations, and prepared to respond to delays, temperature excursions, or other transport-related issues. Samples must be tracked from departure to receipt, with any deviations documented immediately.

Protocols

I. General Guidelines

All samples must be clearly labeled with their CID and preservative type before leaving the field lab. A printed and digital manifest should accompany the shipment, listing each sample ID, its contents, and its destination. Secondary containment—such as sealed bags or boxes lined with absorbent material—is required for most shipments, especially those crossing national borders. Double bagging with absorbent material inside the inner bag is standard practice.

Each shipment must include official documentation: a cover letter on organizational letterhead describing the samples, any required Material Transfer Agreements (MTAs), and all relevant permits. Waterproof contact information should be affixed to both the primary and secondary containers. Even when not legally required, we recommend declaring samples at customs to avoid seizure.

When shipping with dry ice, use only carriers certified to handle “dangerous goods” and never seal dry ice in an airtight container. If driving or traveling with dry ice, ensure proper ventilation, use a CO₂ monitor, and avoid sealed compartments. Always include a temperature logger placed near the samples, and use trackers such as AirTags to monitor location during transit.

Packing Checklist:

- Printed and digital manifest.
- Samples double bagged with absorbent material inside inner bag.
- Waterproof labels with CID, preservative, and contact details.
- Official letter, MTAs, and permits in shipment.
- Temperature logger and shipment tracker.
- Verified carrier compliance for dry ice or dangerous goods.



II. Temperature Requirements

Room Temperature Samples (e.g., DNA/RNA Shield):

Samples preserved at room temperature should be transported in insulated containers or padded boxes. Double bagging with absorbent material is required for most international shipments. Avoid prolonged exposure to heat or direct sunlight; use blue ice when ambient temperatures exceed 21 °C. If using data loggers, set them to record every 5–10 minutes, and limit exposure to ambient air during transfers between storage and transport containers.

Frozen Samples (e.g., DMSO/glycerol cryovials):

Ship on dry ice for short-term transport. If dry ice is not available in the field, keep on blue/wet ice or at 4C until samples can be frozen down further. Do not allow samples' temperatures to rise past the minimum temperature you have preserved them at beforehand for any extended period of time (e.g., do not transfer from 4C to room temp or -80 to 4C). Double bag the samples with an absorbent material in the inner bag (required for most international transit) and clearly label the contents. Ensure sufficient dry ice is packed for the full transport duration (typically ≥5 kg per day).

III. Documentation & Compliance

The movement of biological and environmental samples across state or national borders is regulated to protect agriculture, public health, and biodiversity. Many environmental sample types require specific permits, and requirements vary depending on the origin, destination, and intended use of the material. It is the responsibility of the transporting party to verify and comply with all applicable rules before departure. Failure to comply can result in confiscation, destruction, or legal penalties, and may jeopardize future research access.

Permit requirements by sample type:

- **Soils** – Transporting soil, particularly agricultural soil, often requires permits from agencies such as the USDA Animal and Plant Health Inspection Service (APHIS) in the United States. International imports/exports may require phytosanitary certificates, even for small volumes. Some countries prohibit soil import entirely without sterilization or fumigation. When in doubt, request a formal determination from the relevant authority before travel.
- **Marine sediment/seawater** – Typically exempt from permitting if free from large invertebrates and clearly designated as research samples. However, certain countries treat all marine sediments as regulated materials if collected within marine protected areas. Confirm local collection and export rules, especially for sediment containing visible fauna or collected from culturally or ecologically sensitive sites.



- **Corals** – All stony corals, whether alive, dead, or in fragment form, are regulated under the Convention on International Trade in Endangered Species (CITES). Even small pieces or skeletons often require both export and import CITES permits. This applies regardless of whether the coral is preserved in ethanol, dried, or fixed in resin. Allow for extended permitting timelines (often several months).
- **Other sensitive materials** – Samples containing plant matter, freshwater organisms, endangered species, or microbiological cultures may trigger additional regulations. This includes the Nagoya Protocol in countries with access and benefit-sharing frameworks.

Descriptive cover letter requirements:

Every shipment—whether hand-carried or couriered—must include a descriptive letter on official organizational letterhead that:

1. Lists sample types, quantities (e.g., mL or g), and preservation methods (e.g., ethanol, DNA/RNA Shield, glycerol).
2. States explicitly that samples are non-pathogenic, non-living, and non-host origin.
3. Explains the intended use (e.g., “for scientific research only, with no commercial value”).
4. Confirms that packaging meets IATA Dangerous Goods Regulations and airline/courier guidelines for leak-proof, insulated, and labeled transport.
5. Includes full sender and recipient contact information.
6. References any relevant permits, MTAs, or collaborating institutions.

Below is an example letter declaring samples that do not require additional permitting/clearance describing the work. This is modeled after a letter we use when flying internationally with samples. Note that we indicate the non-host, non-pathogenic nature of the samples.

“I am writing on behalf of The Two Frontiers Project (a 501(c)(3) non-profit research organization) to declare the contents of a research sample shipment being transported by hand from XXX to the United States. The shipment consists of non-hazardous, non-living environmental research samples, approximately 200 mL of seawater and 112.5 grams of marine sediment collected from the XXX. These samples do not contain any animal or human material, are not infectious, and are being imported for scientific research purposes only. They are stored in a deactivating solution, on ice.

The samples are being hand-carried on a commercial flight from XXX to XXX under a valid Material Transfer Agreement (MTA) between the University of Palermo and The Two Frontiers Project. A copy of the MTA and all relevant supporting documents is attached for your reference. The research involves the hand collection of surface marine sediment by SCUBA diving, in sterile



containers, and sterile collection of seawater. No commercial value is attached to these samples.

Regulatory Compliance: We affirm that these materials are non-pathogenic and pose no known risk to agriculture or public health. The marine water and sediment have been screened to ensure they contain no visible invasive organisms. We are not transporting any organisms derived from soil or from agriculture. The samples are packaged according to IATA and airline regulations: they are sealed in leak-proof containers and packed on gel ice/blue ice in an insulated cooler to preserve low temperature. Secondary samples are kept at room temperature."

Best practices for documentation:

- Carry at least two spare printed copies of all permits and cover letters in separate locations (e.g., one with the shipment, one with the traveler, and one stored in cloud storage).
- Keep digital copies of all documentation accessible offline on a mobile device or USB drive.
- If a shipment passes through multiple carriers or customs points, label each container with a "Documentation Inside" note in waterproof ink.
- Where possible, secure permits that cover multiple shipments or multiple sample types to reduce administrative burden.
- For international shipments, confirm if the country of transit (not just origin/destination) has any biological material restrictions.

Proactive compliance measures:

- Initiate permitting processes early—CITES and APHIS approvals can take months.
- Maintain a master log of permits, expiration dates, and conditions of use for expeditions.
- When working with international collaborators, determine if permits must be obtained by both the sending and receiving institutions.
- Keep a copy of the expedition's Material Transfer Agreement (MTA) in the shipment and in the traveler's carry-on.

IV. Emergency

- Include contact information on the outside of the package in case of shipping delay or damage to the box/container.
- Store replicates separately, so if there are issues, backups may be available.
- Include a brief SOP or quick-reference sheet with emergency procedures in case of customs seizure, temperature excursion, or container rupture.
- Provide an emergency contact number with 24/7 coverage (e.g., field coordinator or lab PI) on both the exterior and interior of the cooler.



- Carry additional blank customs declaration letters and copies of permits in case originals are lost or damaged.

Resources

Resource	Type	Description	Link	Date added
NIH Material Transfer Agreement (MTA) Guidelines	Website	Overview of MTA purpose, negotiation, and compliance	https://www.nih.gov/research/research-conducted-at-nimh/collaborations-and-partnerships/material-transfer-agreements/material-transfer-agreements	2025-08-08
World Courier	Website	Specializing in global biopharma logistics	https://www.worldcourier.com/	2025-08-08
IATA Dry Ice Shipping Course	Website	Certification for safely shipping dry ice and temperature-sensitive cargo.	https://www.iata.org/en/training/courses/dry-ice/	2025-08-08
U.S. APHIS Soil Permit Guidance	Website	Requirements for importing or transporting soil and related materials.	https://www.aphis.usda.gov/aphis/resources/permits/plant-pests/soil	2025-08-08
CITES Permitting Process	Website	Guidance on obtaining CITES permits for international transport of regulated biological materials.	https://cites.org/eng/prog/Permit_system	2025-08-08



X. Post-sampling reset and team debrief

Background

The conclusion of a Two Frontiers Project (2FP) sampling event marks a critical transition—from field operations to laboratory processing, data integration, and scientific analysis. Just as rigorous planning precedes deployment, structured debriefing and systematic equipment reset are essential to close the operational loop. These procedures ensure consumables are tracked, equipment is maintained, and lessons are documented for future expeditions.

Among these steps, the formal team debrief is especially important. It is often the only dedicated time for participants to collectively reflect, synthesize observations, and capture lessons while they are fresh. Expeditions are high-tempo and resource-constrained, and without a deliberate process, failures, workarounds, and best practices can be lost to memory or siloed with individuals. A structured debrief transforms those experiences into shared institutional knowledge, feeding directly into checklists, workflows, and protocols.

Debriefs also have a cultural function: they validate the intense labor of fieldwork, acknowledge challenges faced, and reinforce communication norms within the team. Without them, mistakes recur, resources are wasted, and progress slows.

Protocols

Equipment Reset and Consumable Replenishment

After every expedition, all field and laboratory gear should be cleaned, decontaminated, dried, and repacked according to the master inventory list. Any broken, missing, or malfunctioning items must be recorded in the equipment damage/loss tracker.

Consumables—including cryovials, Whirl-Paks, labels, ethanol, PPE, and reagents—must be restocked or added to an immediate purchase order. A full usage log for all major equipment and consumables should be completed to:

- Identify overstocked items to reduce packing volume next time.
- Flag understocked or quickly consumed items to avoid shortages.
- Improve future budget accuracy based on real usage.

The sample manifest and cold chain logs must be finalized and archived. Data from all loggers, temperature sensors, and GPS devices should be downloaded, synced, and stored securely.

The expedition lead should circulate a post-expedition email summarizing:

- Sample status and storage location.
- Any logistical notes relevant for follow-up.
- Next steps for sample processing, analysis, and reporting.
- Acknowledgments for team contributions and outstanding needs.



Mandatory Team Debrief

- A formal debrief must be held at the conclusion of every major sampling event.
- Attendance is mandatory for all expedition participants, regardless of role.
- The debrief should be scheduled for no later than two weeks after the final sampling operation, ideally before full team dispersal.
- The Debrief Form must be filled out continuously ***during the expedition***. All team members must have access so they can add comments to it real-time. It is a living document that captures:
 - Equipment failures or successes.
 - Logistical challenges (e.g., customs issues, transport delays, housing failures).
 - Site-specific access considerations.
 - Personnel and communication breakdowns or improvements.
- The form we use is the one pictured below and linked in the Resources section:
 - Each row represents an incident or lesson learned.
 - The status is marked as “Pending,” “Resolved,” “Unresolved,” or “No longer relevant.”
 - Each entry must include a description, the person who entered it, the date, and any comments or suggested solutions.

A	B	C	D	E	F
#	description	status	entered_by	date	comments
		Pending			
		Resolved			
		Pending			
		No longer relevant			
		Unresolved			
		Pending			
		Pending			

Figure: Debrief form template.

- During the debrief meeting, the team must:
 - Go through each row in the Debrief Form.
 - Confirm status updates in real time.
 - Discuss any unresolved issues as a group.
 - Assign follow-up actions where needed.
- The final version of the Debrief Form must be:
 - Archived in the expedition records.
 - Reviewed during planning for subsequent expeditions.
 - Used to refine checklists, workflows, and training materials.
- This is not a bureaucratic exercise. The debrief process is mission-critical for avoiding repeat failures and evolving our standards.
- If it's not written down and reviewed, it will be forgotten.
- Lessons learned are not “nice to have”—they are the foundation of safer, leaner, and more effective expeditions.



Maintaining gear and decontamination

All equipment used in the field must undergo full cleaning and decontamination according to standard protocols before being returned to storage or redeployed. Follow relevant regulatory and environmental guidelines, such as the **EPA Field Equipment Decontamination Procedures**.

Resources

Resource	Type	Description	Link	Date added
EPA Field Equipment Decontamination Procedures	Website	SOP for cleaning and resetting gear for future expeditions.	https://www.epa.gov/quality/field-equipment-cleaning-and-decontamination	2025-08-08
2FP Expedition Template	Folder	Master planning file for objectives, CIDs, metadata, inventory, and team roles	https://github.com/two-frontiers-project/2FP-expedition-template/tree/main	2025-08-08