



The Two Frontiers Project (2FP) Field Sampling Protocol provides a standardized approach for collecting, documenting, and transporting environmental samples, including water, soil, and sediments, for molecular biology and microbial culturing. This protocol ensures data integrity, minimizes contamination, and supports high-quality downstream analysis for metagenomics, microscopy, and enrichment cultures.

This is designed to be paired with the 2FP Frontiers Kit and subsequent 2FP FieldLab for downstream processing. The protocol is written for field researchers, this guide outlines step-by-step procedures for pre-collection planning, metadata recording, sample acquisition, and preservation. By following these methods, teams can maintain consistency across expeditions and generate reliable scientific insights from diverse environments.

2FP is committed to responsible fieldwork that minimizes environmental impact. All sampling activities should be conducted with care to preserve natural ecosystems, avoid contamination, and leave sites as undisturbed as possible. Field teams should adhere to ethical research practices and comply with local regulations. **Do not sample on land in which you have not received express written permission with clarity regarding downstream sample usage rights.**

For any questions or additional guidance, please contact the Two Frontiers Project at twofrontiers.org or email info@twofrontiers.org.

1. Pre-Collection

1.1 Before sampling

1. Prior to sampling, ensure you acquire any requisite materials not included as consumables in the kit. In this case, if you are taking mud or sediment samples, we recommend you retrieve 1 liter of deionized water (from a local grocery store) for geochemistry measurements. We additionally recommend acquiring at least 70% rubbing alcohol/ethanol for wiping down your gloves and materials in between sampling.
2. Ensure you freeze down the ice packs provided in your kit for at least 12 hours prior to sampling and shipping samples.
3. Print out any required materials if not on hand already, including A) this protocol, B) unsigned permission forms. Ensure you bring stickers or some other material to exchange for signed permission slips.
4. We recommend pre-labeling collection materials (e.g., tubes, whirl-paks) as needed with Field IDs (see 3.4.1).

1.2 Field Notes & Metadata

- Maintain detailed field notes on a notepad or digital datasheet.
 1. Refer to your metadata sheet.
- Discuss and assess sample sites based on observations.
- Record metadata for each site:
 1. Location and site name(s) (official or descriptive)
 2. Date (YYYY-MM-DD format)
 3. Team member initials
 4. First timestamp of photo filenames
 5. GPS Coordinates
 - If available, record all digits from a lat-long display.
 - If using a mobile device, drop a pin and email or screenshot coordinates.
 - If no GPS is available, sketch a map or use your phone for later referencing.
 - Record method of securing coordinates, including device manufacturer and software.
 6. Site observations (e.g., water clarity, colors, odor, temperature, pH, unusual features).

2. Site Metadata Collection

2.1 Field Notes & 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

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. Use pH strips. For water, dip the strip in the water (either in a conical tube or in the source itself), remove, and wait 30 seconds before reading. Image the strip alongside the reference provided on the pH strip contrainter. Do not use the TDS sensor for pH, as this requires additional buffers.
- b. For mud or dirt samples that obscure the pH strip colors, place material in DI water in approximately a 1 part mud/dirt to 10 parts DI water ratio. Shake for 5 seconds and let it settle. Hold the strip in solution, wait for 30 seconds, and image as above.
- c. Write down observed pH in metadata sheet in increments of 0.5

2. Measure and record salinity

- a. Remove the cap from the salinity/total dissolved solids sensor (pictured). Power on. Ensure units are in ppm and salt is being measured. Hold in water sample (either in conical tube or directly in the water source) until values stabilize. Record in metadata sheet.
- b. If taking a sediment or other solid sample, you may dilute 1:10 as above in DI water.
- c. Wash probe tip after using

3. Measure and record temperature

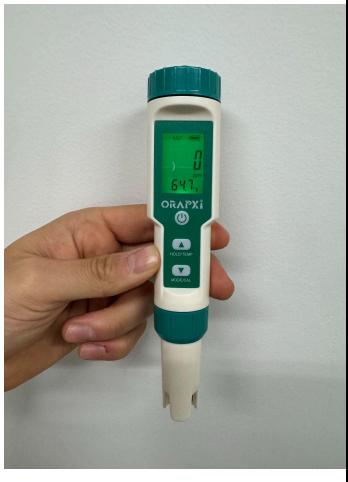
- a. Use the thermometer to measure temperature from any water sources. If none available, record ambient air temperature.

4. Measure and record additional geochemistry

- Remove one aquarium/water quality testing strips from the provided cylinder. Use the same sampling strategy as above.
- Record any additional observations on your metadata sheet (e.g., UV fluorescence, turbidity, odor, sediment type/features).

5. Record timing information

- Record the last timestamp of any photos taken during the metadata collection for this location.
- Ensure you note anything interesting about the site on the metadata sheet as well

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

3. Sample Collection

3.1 Sampling Order for a given site (assuming multiple sample types)

- 1st: Water Samples
- 2nd: Biomass Samples.
- 3rd: Sediment/soil.
- 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).

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.



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.



3.2 Required Notes Per Sample

- **Field ID** (Unique sample name to be used only in the field, e.g., YYYY-MM-DD_#1).
- **Collected by** (Name/Initials).
- **Date and Time**.
- **Sample Type** (Water, sediment, biomass, etc.).
- **Sample-specific metadata** (color, smell, etc)

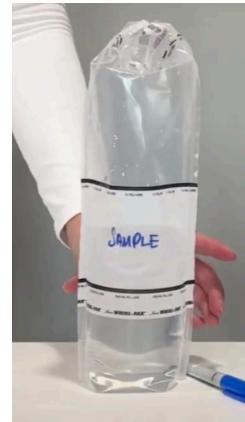
3.3 Equipment Preparation

- **Label all tubes and bags** with a **unique field sample name**.
- **Ensure sterility:**
 - **50mL tubes & Whirl-Pak bags:** Pre-sterilized.
 - **Shovel, bucket, and tools:** Rinse with local water; optionally wash with **70% ethanol**.
 - **Gloves:** Wear **ethanol-washed gloves** to reduce contamination.

3.4 Collection Methods

1. **Methods:**
 - **BEFORE ANYTHING ELSE: Select collection material (tube or whirl-pak) from kit, write a field identifier on it (this could be anything, but we recommend a site identifier as well as a numeric identifier, like S1_1). Record this field ID on the sample-specific metadata sheet.**
 - Do not touch any part of the sample or interior of the tubes/whirl-paks with an ungloved hand to avoid contamination. Try to not even breath on any of the samples you're collecting!
 - Direct collection with a **50mL tube or coring device**.
 - **50mL tube**
 1. Wear gloves.
 2. Prepare 50mL tube. Ensure the tube is properly labeled.
 3. Once site and sample type is determined, either use a device (i.e. scoopula, coring device) or the tube itself to collect.
 4. Fill the tube.
 - Avoid abundant vegetation or plant material.
 - Don't collect soils, collect sediment. If collecting sediment from an area with water, fill to 50% with sediment and the rest with water from the sample source.
 5. Cap the tube tightly and IMMEDIATELY place on ice.
 - Use a **Whirl-Pak bag** for larger sample volumes.

How to use a whirl-pak bag

		
<p>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.</p>	<p>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.</p>	<p>Seal the Bag – Close opening by pulling wire ends horizontally out to the side and folding top over itself at least four times to the closure line. Then twist tie the wire ends shut to form a loop.</p>

If collecting in deep water or at a distance, you may use the telescoping sampling pole – affix a 25ml



tube in the clamp, open it, and collect with a scraping motion from the site of interest. Close the tube on the sampling pole and then remove the sample.

- Fill tubes as much as possible, leaving **minimal headspace**.
- If collecting underwater with a conical tube, **submerge the tube before capping**.
- Avoid abundant vegetation or plant material.

2. Record Visual Observations in Sample Metadata Sheet:

- For example, sample color (e.g., green, purple, yellow, white, UV fluorescence, etc.).

3.5 Sample Storage

- Place all samples in a **cooler with ice packs immediately following collection**.
- Place in an external sampling bag first if damp/wet.
- Dispose of used **gloves** and sanitize hands if necessary.

4. Cleanup & Transport

4.1 Field Cleanup

- **Pack up all equipment and leave no trace** at the site.
- Ensure sample **labels are clear and readable**.

4.2 Sample Transport to Lab

- **Transport within 48 hours to a field lab or lab facility for processing**.

- Maintain refrigerated temperature (**do not freeze samples**).
- Use **ice packs in hot or ambient temperatures** to maintain a sample stabilizing temperature of 39.2°F / 4°C. Use a **heating pack** if transporting in freezing temperatures, below 20°F / -7°C for over 30 min.

4.2 Shipping

- Before shipping, twist caps on all samples as tightly as possible to avoid leakage. Confirm whirl-paks are additionally tightly sealed.
- Standard practice for shipping samples is to double bag all materials in case of leakage. We ask that you double bag whirl-paks individually. Tubes can be bagged together if necessary.
- We have also provided you with a remote thermometer so we can monitor sample temperature during transit (pictured to the right). Please ensure this is in the cooler, on top of the samples, prior to sealing the kit.
- Place the double bagged samples – with the ice you froze before collecting – in the provided cooler, and return the cooler to the plastic case.
- If you have a letter describing your efforts or collaboration with 2FP, please place it in the case on top (or in any fedex packaging you use on the outside of the kit/shipping container). Ensure that this letter – and anywhere else you mention the samples – describes the samples precisely as "**Exempt Environmental Samples and Unregulated Biological Material**".
- Use the zip ties provided to seal the plastic case.
- Please overnight FedEx the case (even better if you can put it in a cardboard shipping box, but this isn't necessary for domestic shipments) to the following address:



Attn: James Henriksen
 1499 Campus Delivery, NESB, CSU
 Fort Collins, CO 80523-1499
 Phone: (706) 540-5440
 Email: info@twofrontiers.org

- Please send all shipping information to info@twofrontiers.org with "Sample Shipment" followed by the FedEx tracking number in the subject line. If possible, avoid shipping on Fridays.