



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-F701

Soil Microbial Field Sampling for Molecular Analysis

Standard Operating Procedure

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1. Purpose

This SOP describes field procedures for collecting soil samples intended for molecular analysis, including DNA extraction, PCR/ddPCR, amplicon sequencing, and metagenomics. Soil microbial communities are sensitive to temperature, moisture, and oxygen exposure, and nucleic acids degrade rapidly in the absence of proper preservation. The procedures in this SOP are designed to minimize DNA and RNA degradation from the moment of collection through transport to the laboratory,

ensuring that molecular results accurately reflect the in situ microbial community (Frosteg aard et al., 1999; Knight et al., 2018).

This SOP supplements the general soil sampling procedures in [SOP-F400](#) with additional precautions specific to molecular work.

2. Scope

- **Applies to:** Field collection of soil and rhizosphere samples for DNA extraction ([SOP-L700](#)), ddPCR ([SOP-L701](#)), amplicon sequencing, metagenomics, and other molecular analyses
- **Does not apply to:** Soil sampling for chemical or physical analysis only (see [SOP-F400](#)); water eDNA sampling (see [SOP-F700](#)); laboratory-side DNA extraction and processing
- **Location:** All EA Program field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve sampling design; specify target depth and replication
Trained Personnel	Collect and preserve samples per this SOP
New Students	Complete training (including contamination awareness) before participating in molecular sampling

4. Definitions

- **Contamination (molecular soil sampling):** Introduction of exogenous DNA from other samples, the environment, or the researcher's own microbiome into a soil sample; prevented by sterile tool sterilization between samples, frequent glove changes, and use of field blanks (Frosteg aard et al., 1999).
- **Cryogenic freezing (flash-freezing):** Rapid cooling of a soil sample in liquid nitrogen (-196 °C) or on dry ice (-78 °C) immediately after collection; arrests microbial metabolism and nuclease activity; required for RNA work and recommended for optimal DNA preservation (Knight et al., 2018).
- **Dry ice (solid CO₂):** Used for field flash-freezing at -78 °C; causes frostbite on direct skin contact; must be handled with insulated cryogenic gloves and transported in a vented or insulated container.
- **Field blank:** An empty sterile tube or bag opened and closed at the field site during sampling; processed through the full extraction and PCR workflow in the lab; detects airborne or handling contamination; any positive amplification in a field blank invalidates the associated samples unless concentrations fall below a pre-established threshold (Frosteg aard et al., 1999).
- **Flash-freezing:** See cryogenic freezing.

- **LifeGuard Soil Preservation Solution (Qiagen):** A commercially prepared stabilization reagent that arrests nucleic acid degradation at ambient temperature for up to 7 days; an alternative to cryogenic preservation when dry ice is unavailable; add to the sample immediately after collection, invert gently, and store at room temperature or on ice.
- **Microbial community composition:** The relative abundances of different microbial taxa (bacteria, archaea, fungi) in a soil sample; highly sensitive to sample handling time, temperature, and oxygen exposure; shifts within minutes of soil disturbance (Frosteg aard et al., 1999; Knight et al., 2018).
- **Nuclease:** An enzyme that degrades DNA or RNA; active in soil samples at ambient temperatures; inactivated by low temperature ($< 4^{\circ}\text{C}$), chemical preservatives (LifeGuard buffer, ethanol), or rapid freezing.
- **Rhizosphere:** The narrow zone of soil immediately surrounding plant roots; has a distinct microbial community influenced by root exudates; sampled by collecting soil adhering directly to roots after gentle removal from the surrounding bulk soil.
- **Sterile technique:** A set of aseptic practices that prevent contamination of molecular samples; in soil microbial sampling, these include autoclaving or 70% ethanol sterilization of all metal tools, use of single-use sterile tubes and gloves, and minimizing exposure time to air (Frosteg aard et al., 1999).
- **Time from extraction to preservation:** A critical metric for molecular soil samples; ideally less than 2 minutes from the moment soil is removed from the ground to placement in ice, dry ice, or preservative; longer times cause measurable shifts in microbial community composition (Knight et al., 2018).

5. Safety and Hazards

Hazards

- **Biological:** Soil may contain pathogens. Wash hands thoroughly after handling. Treat all samples as potentially infectious.
- **Physical:** Repetitive strain from augering; standard field hazards (heat, ticks, uneven terrain).
- **Chemical:** Liquid nitrogen causes severe cryogenic burns. Dry ice causes frostbite on direct skin contact. Handle with insulated gloves.

5.1. Required PPE

- ☐ Nitrile gloves (change between samples)
- ☐ Closed-toed shoes or field boots
- ☐ Insulated cryogenic gloves (if using liquid nitrogen or dry ice)
- ☐ Safety glasses (if using liquid nitrogen)
- ☐ Sun protection (hat, sunscreen)

5.2. Emergency Procedures

Refer to [SOP-F001 Field Safety](#). For liquid nitrogen or dry ice burns, warm the affected area gently with lukewarm water. Do not rub.

6. Equipment and Materials

6.1. Sampling Equipment

Item	Notes
Soil auger (bucket-type, stainless steel)	Autoclaved or ethanol-sterilized before each event
Stainless steel trowel or spatula	Autoclaved or flame-sterilized
Ethanol (70%) in spray bottle	For field sterilization between samples
Lighter or butane torch	For flame-sterilizing metal tools
DI water squeeze bottle	For rinsing tools after ethanol sterilization

6.2. Sample Containers and Preservation

Item	Notes
Sterile 50 mL Falcon tubes or Whirl-Pak bags	Pre-labeled; one per sample
Cooler with ice packs (4 °C)	Minimum preservation for DNA samples
Dry ice (solid CO ₂)	For flash-freezing at -78 °C; preferred for RNA work
Insulated container for dry ice	Styrofoam cooler or dewar
LifeGuard Soil Preservation Solution	Optional; stabilizes DNA and RNA at ambient temperature for up to 7 days
Ziplock bags (gallon size)	For double-bagging frozen samples

7. Procedure

7.1. Pre-Field Preparation

1. Autoclave or ethanol-sterilize all metal sampling tools. Wrap in aluminum foil after sterilization.
2. Pre-label all sample tubes or bags: project ID, site, sample number, date, depth.
3. If using LifeGuard, pre-aliquot approximately 5 mL per tube.
4. Pack the cooler. If using dry ice, load the insulated container.
5. Include at least one field blank tube per sampling event.

7.2. Sampling Procedure

1. Navigate to the sampling location; record GPS coordinates.
2. Put on fresh nitrile gloves.
3. Sterilize the auger or trowel: spray with 70% ethanol, allow 30 seconds air-dry, optionally flame. Rinse with DI water.
4. Clear surface litter without disturbing mineral soil.
5. Collect soil from the target depth (typically 0–15 cm). Avoid large roots and rocks.
6. Using the sterilized trowel, transfer approximately 2–5 g of soil directly into the sterile tube. Minimize handling time; do not homogenize in a bucket.

! Speed Matters

Minimize the time between coring and preservation. The soil should be in the tube and on ice within 2 minutes of extraction. Prolonged exposure to air and ambient temperature causes shifts in microbial community composition and DNA degradation (Frosteg aard et al., 1999; Knight et al., 2018).

7. Immediately preserve:
 - **DNA only:** Place capped tube on ice (4 °C). Transport to lab within 6 hours or freeze at -20 °C.
 - **DNA and RNA:** Flash-freeze on dry ice or liquid nitrogen. Transfer to -80 °C freezer on return.
 - **LifeGuard:** Add 5 mL LifeGuard to the soil, cap, invert 3 times, store at ambient temperature up to 7 days.
8. Record in the field notebook: sample ID, GPS, depth, soil temperature, preservation method, time of collection, and any deviations.

7.3. Decontamination Between Samples

1. Spray tools with 70% ethanol; allow 30 seconds air-dry; optionally flame. Rinse with DI water.
2. Change nitrile gloves.
3. Between sites, decontaminate boots and waders per [SOP-F007](#).

8. Sample Transport and Storage

Preservation Method	Field Storage	Lab Storage
Ice (4 °C)	Cooler, ice packs	Process within 24 h or freeze at -20 °C
Dry ice (-78 °C)	Insulated cooler	Transfer to -80 °C freezer immediately

Preservation Method	Field Storage	Lab Storage
Liquid nitrogen (-196 °C)	Dewar	Transfer to -80 °C freezer immediately
LifeGuard buffer	Ambient, up to 7 days	Extract within 7 days; or freeze at -20 °C

Record storage location in the Freezer Inventory and in the lab notebook per [SOP-L106](#).

9. Quality Control

- Include at least one field blank per sampling event.
- Change gloves and sterilize tools between every sample.
- Record the time elapsed between soil extraction and preservation. Flag any sample with elapsed time > 5 minutes.
- Field blanks positive for target DNA invalidate the batch unless concentrations fall below the established threshold.
- Avoid sampling immediately after rainfall when soils are saturated.

10. References

- Frosteg aard, Aa., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F. ccois, Jeannin, P., Nesme, X., & Simonet, P. (1999). Quantification of bias related to the extraction of DNA directly from soils. *Applied and Environmental Microbiology*, 65(12), 5409–5420. <https://doi.org/10.1128/AEM.65.12.5409-5420.1999>
- Knight, R., Vrbanc, A., Taylor, B. C., Aksenov, A., Callewaert, C., Debelius, J., Gonzalez, A., Kosciolk, T., McCall, L.-I., McDonald, D., Melnik, A. V., Morton, J. T., Navas, J., Quinn, R. A., Sanders, J. G., Swafford, A. D., Thompson, L. R., Tripathi, A., Xu, Z. Z., ... Dorrestein, P. C. (2018). Best practices for analysing microbiomes. *Nature Reviews Microbiology*, 16(7), 410–422. <https://doi.org/10.1038/s41579-018-0029-9>

11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F007	Field Decontamination	Between-site decon
F008	Sample Labeling and COC	Sample ID scheme
F400	Soil Sampling	General soil collection
F700	eDNA Field Sampling	Water eDNA companion protocol
L700	DNA Extraction	Lab-side extraction
L701	ddPCR Primers	Downstream analysis

12. Training Record

Before collecting soil samples for molecular analysis independently, personnel must:

1. Read this SOP, [SOP-F400](#), and [SOP-L700](#) in full
2. Demonstrate sterile sampling technique (tool sterilization, glove changes, rapid transfer to preservative) during a supervised practice session
3. Successfully process a field blank with a negative extraction and PCR result
4. Be signed off by the PI or field lead