



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-F700

eDNA Field Sampling and Preservation

Standard Operating Procedure

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1. Purpose

This SOP describes field procedures for collecting, filtering, and preserving environmental DNA (eDNA) samples from surface waters. eDNA consists of DNA shed into the environment by organisms through skin cells, mucus, feces, gametes, and decomposing tissue. Detecting eDNA in

water samples allows non-invasive detection of aquatic species (fish, amphibians, invertebrates, pathogens) without direct observation or capture (Goldberg et al., 2016). Because eDNA is present at extremely low concentrations, preventing contamination during field collection is critical. This SOP covers the field side of the workflow; laboratory processing (filtration, extraction, inhibition testing) is covered in [SOP-L700 DNA Extraction](#).

2. Scope

- **Applies to:** Collection and preservation of water samples for eDNA analysis at all EA Program surface water sites (streams, lakes, ponds, wetlands, stormwater basins)
- **Does not apply to:** Soil eDNA or soil microbial sampling (see [SOP-F701](#)); tissue biopsy or organism capture for DNA; airborne eDNA sampling; lab-side filtration, extraction, and molecular analysis
- **Location:** All EA Program surface water field sites

3. Responsibilities

Role	Responsibility
PI / Field Lead	Approve sampling design; ensure permits are in place
Trained Personnel	Collect and preserve eDNA samples per this SOP
New Students before participating in eDNA sampling	Complete training (including contamination awareness)

4. Definitions

- **Contamination (eDNA):** The introduction of exogenous DNA from sources other than the target organism into a sample or filter; the single largest source of false positives in eDNA surveys; prevented by sterile technique, glove changes between samples, and decontamination of reusable equipment (Goldberg et al., 2016).
- **eDNA (environmental DNA):** Genetic material shed into the environment by organisms through mucus, feces, gametes, skin cells, and other biological material; collected from water without direct capture of the target organism; detectable at extremely low concentrations using PCR-based methods (Goldberg et al., 2016).
- **Equipment blank:** A control sample consisting of deionized water rinsed through decontaminated reusable equipment (e.g., a pump) and collected as a sample; detects carry-over contamination between sites when dedicated equipment is not used.
- **Field blank:** A sterile bottle of molecular-grade deionized water opened and handled at the field site under identical conditions to real samples; filtered and preserved like a field sample; detects airborne or handling contamination during field work; must yield no target amplification (Goldberg et al., 2016).

- **Filter pore size:** The nominal diameter of pores in the filter membrane; 0.45 µm is the standard for eDNA capture from surface water, retaining bacteria, cells, and particulate-bound DNA.
- **Longmire's buffer:** A non-freezing preservation solution (containing EDTA, Tris, NaCl, SDS, and sucrose) used to preserve eDNA on filters in the field when immediate freezing is not possible; inactivates nucleases and inhibits microbial degradation (Renshaw et al., 2015).
- **Open-channel filtration:** A filtration method using a funnel and vacuum pump or syringe; the filter membrane is exposed to the environment during collection, increasing contamination risk compared to closed-capsule methods.
- **PCR inhibitor:** A substance co-extracted with eDNA that suppresses PCR amplification efficiency; common in natural water samples (humic acids, tannins, metal ions); detected by running a dilution series of the extract and checking for concentration-dependent amplification; managed by diluting the extract or using commercial inhibitor removal kits.
- **Sterivex filter:** A commercially available enclosed 0.22 µm polysulfone cartridge filter; preferred for eDNA because the sealed design minimizes contamination, allows direct addition of preservation buffer, and is compatible with standard extraction kits (Renshaw et al., 2015).
- **Target species detection:** The determination of presence or absence of a specific taxon in a water sample based on amplification of taxon-specific eDNA; sensitivity depends on eDNA shedding rate, water volume filtered, PCR method, and inhibitor load in the extract (Goldberg et al., 2016).
- **Volume filtered:** The total volume of water passed through a filter during sample collection; a required reporting variable for calculating eDNA concentration; record for every filter regardless of whether filtration was complete or the filter clogged (Goldberg et al., 2016).

5. Safety and Hazards

Hazards

- **Physical:** Wading and sampling near water carry slip, fall, and drowning hazards. Work in pairs.
- **Chemical:** DNA preservatives (ethanol, Longmire's buffer) are flammable (ethanol) or contain EDTA and SDS (Longmire's). Handle with gloves.
- **Biological:** Water may contain pathogens. Wear gloves and avoid ingestion.

5.1. Required PPE

- ☐ Nitrile gloves (change between every sample)
- ☐ Safety glasses
- ☐ Wading boots or waders (if entering the water)
- ☐ Sun protection (hat, sunscreen)

5.2. Emergency Procedures

Refer to [SOP-F001 Field Safety](#).

6. Equipment and Materials

6.1. Sampling Equipment

Item	Notes
Sterile 1 L HDPE sample bottles	One per sample; single-use
60 mL sterile syringes	For Sterivex filtration
Sterivex 0.22 µm filter capsules	Preferred; one per sample
0.45 µm PES open filter funnels	Alternative to Sterivex
Longmire's buffer (1–2 mL per filter)	For filter preservation without freezing (Renshaw et al., 2015)
Molecular-grade ethanol (95%)	Alternative preservative for Sterivex
Molecular-grade deionized water	For field blanks (1 bottle per event)
Cooler with ice packs (4 °C)	For sample transport if not filtering on-site

7. Procedure

7.1. Pre-Field Preparation

1. Prepare all equipment in the lab using UV-decontaminated surfaces and dedicated pre-PCR equipment.
2. Pre-label all bottles and filters with project ID, site, replicate number, date.
3. Pack a cooler with ice packs or dry ice.
4. Include at least one sterile bottle of molecular-grade DI water per sampling event for field blanks.

7.2. Water Collection

1. Put on fresh nitrile gloves.
2. Open the sterile bottle only when ready to collect.
3. Submerge the bottle to 10–20 cm below the water surface, facing upstream, away from your body (Goldberg et al., 2016).
4. Fill to 1 L and cap immediately.
5. For composite samples: collect 250 mL from each of 4 microhabitats and combine in one bottle.
6. Record the GPS coordinates, collection time, and site conditions.

7.3. Field Filtration (If Filtering On-Site)

Sterivex capsule method:

1. Attach a sterile 60 mL syringe to the Sterivex filter inlet.
2. Draw water from the sample bottle and push through the filter. Repeat until the filter clogs or 1 L has been filtered. Record the total volume filtered.

3. Expel remaining water from the capsule.
4. Add 1–2 mL of Longmire's buffer or fill with molecular-grade ethanol using a syringe (Renshaw et al., 2015). Cap both ends of the Sterivex.
5. Label the capsule and place in a ziplock bag on ice.

Open filter funnel method:

1. Pour the sample through a sterile 0.45 μm PES filter unit.
2. Use sterile forceps to fold the filter membrane in half (sample side inward) and place in a labeled cryovial.
3. Add preservative (ethanol or Longmire's buffer) to cover the filter. Seal.
4. Place on ice immediately.

7.4. If Not Filtering On-Site

Keep whole-water samples on ice (4 °C) and filter in the lab within 6 hours of collection. Exceeding 6 hours significantly increases DNA degradation (Goldberg et al., 2016).

7.5. Field Blanks and Controls

- **Field blank:** Open the sterile DI water bottle at one site per event, filter it using the same method as field samples, and preserve. This blank detects contamination during field handling.
- **Equipment blank:** If any reusable equipment contacts the sample, collect a deionized water rinse blank after decontamination.
- Process blanks through the entire lab workflow alongside samples.

7.6. Decontamination Between Sites

1. Spray or soak all reusable equipment with 10% bleach solution for at least 1 minute.
2. Rinse with clean water.
3. Change gloves.
4. Single-use items (bottles, filters, syringes) are discarded after each sample.

See [SOP-F007](#) for general decontamination procedures.

8. Sample Transport and Storage

1. Keep all samples and preserved filters on ice (4 °C) during transport.
2. Deliver to the lab the same day. If overnight storage is unavoidable, store at -20 °C.
3. For long-term storage (> 1 week before extraction), store preserved filters at -20 °C or -80 °C.
4. Complete the chain of custody form per [SOP-F008](#).

9. Quality Control

- Minimum 1 field blank per sampling event; 1 equipment blank if reusable equipment is used.
- Collect 3 replicate samples per site unless the study design specifies otherwise.
- Record the total volume filtered for each sample.
- Any field blank positive for target DNA invalidates samples from that event unless below a pre-established threshold. Consult the PI.
- Log all glove changes, bottle openings, and deviations in the field notebook.

10. References

- Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., Spear, S. F., McKee, A., Oyler-McCance, S. J., Cornman, R. S., Laramie, M. B., Mahon, A. R., Lance, R. F., Pilliod, D. S., Strickler, K. M., Waits, L. P., Fremier, A. K., Takahara, T., Herder, J. E., & Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, 7(11), 1299–1307. <https://doi.org/10.1111/2041-210X.12595>
- Renshaw, M. A., Olds, B. P., Jerde, C. L., McVeigh, M. M., & Lodge, D. M. (2015). The room temperature preservation of filtered environmental DNA samples and assimilation into a phenol–chloroform–isoamyl alcohol DNA extraction. *Molecular Ecology Resources*, 15(1), 168–176. <https://doi.org/10.1111/1755-0998.12281>

11. Related SOPs

SOP	Title	Relationship
F001	Field Safety	Required prerequisite
F007	Field Decontamination	Between-site decon
F008	Sample Labeling and COC	Sample ID and chain of custody
F308	eDNA Water Collection	Habitat-specific collection strategies
F701	Soil Microbial Sampling	Companion soil DNA protocol
L700	DNA Extraction	Lab-side extraction

12. Training Record

Before collecting eDNA samples independently, personnel must:

1. Read this SOP, [SOP-F007](#), and [SOP-L700](#) in full
2. Demonstrate proper sterile technique (glove changes, bottle handling, filter preservation) during a supervised practice session
3. Successfully process a field blank with a negative result
4. Be signed off by the PI or field lead