



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L315

eDNA Laboratory Processing

Standard Operating Procedure

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Table of contents

1 Purpose	1
2 Scope	2
3 Responsibilities	2
4 Definitions	2
5 Safety and Hazards	3
5.1 Required PPE	3
5.2 Emergency Procedures	3
6 Equipment and Materials	3
6.1 Equipment	3
6.2 Reagents and Consumables	3
7 Procedure	4
7.1 Contamination Control and Spatial Separation	4
7.2 Phase A: Membrane Vacuum Filtration	4
7.3 Phase B: Genomic eDNA Extraction	5
7.4 Phase C: Internal Control Inhibition Testing	5
7.5 Cleanup and Storage	6
8 Quality Control and Acceptance Criteria	6
9 Waste Disposal	6
10 Maintenance	6
11 References	6
12 Training Record	8

1. Purpose

This SOP describes contamination-controlled processing protocols for environmental DNA (eDNA) from aquatic collection matrices. It outlines vacuum membrane filtration, total genomic extraction, and subsequent PCR inhibition testing steps (Goldberg et al., 2016).

2. Scope

- **Applies to:** Laboratory processing of aquatic eDNA samples, including filtration, DNA extraction, and inhibition screening prior to PCR amplification.
- **Does not apply to:** Field collection of eDNA water samples, PCR amplification and sequencing workflows, or bioinformatic analysis of sequence data.
- **Location:** Dedicated Pre-PCR clean room, physically separated from post-amplification facilities.

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, report deviations
New Students	Complete training before independent use

4. Definitions

- **Amplification inhibition:** Suppression of PCR or ddPCR amplification efficiency by co-extracted substances such as humic acids, tannins, heavy metals, or residual ethanol; detected by comparing amplification of a known-concentration internal positive control (Jane et al., 2015; McKee et al., 2015).
- **Contamination control:** Practices that prevent introduction of exogenous DNA into eDNA samples, including use of UV-decontaminated surfaces, negative-pressure rooms, dedicated pre-PCR equipment, and frequent glove changes (Goldberg et al., 2016).
- **eDNA (environmental DNA):** Genetic material shed into the environment by organisms through mucus, feces, skin cells, gametes, and other biological material; collected from water, sediment, or air samples without direct organism capture (Ficetola et al., 2008).
- **Environmental DNA extraction:** The process of lysing filter-captured cells or particles and purifying nucleic acids from co-extracted inhibitors; produces a DNA extract suitable for PCR-based detection (Qiagen, 2024).
- **Extraction blank (negative control):** A reagent-only sample carried through the full extraction procedure without biological material; detects reagent or cross-contamination; must yield no amplification of target sequences (Goldberg et al., 2016).
- **Field blank:** A filter processed through the collection and preservation steps at the site but with no water sample passed through it; detects airborne or handling contamination in the field (Goldberg et al., 2016).
- **Inhibition testing:** A diagnostic assay in which a known-positive control is co-amplified with the sample to detect PCR inhibition; common approaches include an internal amplification control (IAC) or serial dilution (McKee et al., 2015).
- **Sterivex filter:** A polysulfone capsule filter (0.22 or 0.45 μm) used to concentrate eDNA from large water volumes by inline pumping; sealed and stored at -20 °C or -80 °C for later extraction.

- **Vacuum filtration pressure:** Vacuum applied to the filter manifold during eDNA filtration; should be kept below 150 mmHg to minimize cell lysis and DNA shearing on the filter membrane.

5. Safety and Hazards

Hazards

- **Chemical:** 10% bleach solution (0.5% sodium hypochlorite) is corrosive to skin and eyes. Lysis buffers contain chaotropic salts (e.g., guanidinium thiocyanate) that are harmful if ingested or inhaled. Ethanol-based wash buffers are flammable.
- **Biological:** Environmental water samples may harbor unknown pathogens. Treat all samples as potentially infectious.
- **Physical:** Vacuum filtration apparatus operates under reduced pressure; ensure flasks are borosilicate and crack-free.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Dedicated Pre-PCR laboratory coat (do not wear in post-amplification areas)
- ☐ Clean nitrile gloves (change frequently between sample groups)

5.2. Emergency Procedures

In case of chemical spill or exposure, refer to [SOP-L002 Laboratory Safety](#) or call campus safety. For bleach or lysis buffer contact with eyes, flush immediately with water for at least 15 minutes.

6. Equipment and Materials

6.1. Equipment

Item	Notes
Vacuum filtration manifold	Dedicated to Pre-PCR room
Vacuum pump or line	Regulated to < 150 mmHg
Digital dry bath or shaker	Capable of 56 °C incubation
Microcentrifuge	10,000 × g
qPCR thermocycler	For inhibition testing (Phase C)
Freezer (−20 °C)	For deferred extraction storage

6.2. Reagents and Consumables

Item	Notes
Sterile membrane filters	0.45 µm cellulose nitrate or glass fiber
Sterile single-use plastic forceps	
2.0 mL microcentrifuge tubes	Sterile, DNA-free
Genomic extraction kit	e.g., DNeasy Blood & Tissue or PowerWater (Qiagen, 2024)
Proteinase K	Supplied with extraction kit
Lysis buffer	Supplied with extraction kit
Ethanol-based wash buffers	Supplied with extraction kit
Elution buffer	10 mM Tris-HCl or molecular-grade water
10% bleach solution	0.5% sodium hypochlorite final
70% ethanol	For surface decontamination
Molecular-grade water	
IPC primer/probe set	Internal Positive Control for inhibition testing (McKee et al., 2015)
Inhibitor removal columns	e.g., Zymo OneStep PCR Inhibitor Removal
Silica gel desiccant	If filters are stored dry before extraction

7. Procedure

7.1. Contamination Control and Spatial Separation

! Contamination Prevention

Environmental DNA exists at extremely low fragment densities and is exceptionally vulnerable to cross-contamination. Strict spatial separation and decontamination protocols are mandatory (Goldberg et al., 2016; Turner et al., 2015).

1. All eDNA filtration and extraction work must occur in the dedicated Pre-PCR room, structurally separated from post-amplification thermocycling facilities.
2. **Decontamination regimen:** Before and after each protocol sequence, wipe all benchtops, pipettes, and manifold bodies with 10% bleach solution. Allow a 10-minute contact window, then rinse with 70% ethanol and molecular-grade water.
3. Wear a lab coat dedicated to the Pre-PCR room. Put on clean nitrile gloves and replace them frequently (e.g., between processing separate filtration manifolds or extraction groups).

7.2. Phase A: Membrane Vacuum Filtration

1. Sanitize the filter manifold station completely using 10% bleach solution and rinse as described in §6.1.
2. Using sterile, single-use plastic forceps, center a sterile 0.45 µm cellulose nitrate or glass fiber filter disc onto the clean manifold support pad. Mount the upper funnel collar tightly.

3. Agitate the water sample bottle and pour a precise volume (typically 500 mL to 2 L) into the funnel. Apply low vacuum pressure (< 150 mmHg) to avoid cell lysis on the filter.

Vacuum Pressure

Keeping vacuum pressure below 150 mmHg minimizes mechanical disruption of captured cells and reduces DNA loss through the filter membrane. If the filter clogs before the target volume is processed, record the actual volume filtered and proceed to step 4.

4. Once filtration completes, use sterile forceps to roll or fold the filter disc inward (preventing target loss on exterior surfaces) and insert it into a sterile 2.0 mL microcentrifuge tube containing lysis buffer or silica gel desiccant. Freeze at –20 °C if extraction is deferred.

7.3. Phase B: Genomic eDNA Extraction

This phase uses standard commercial spin-column kits (e.g., DNeasy Blood & Tissue or PowerWater kits (Qiagen, 2024)).

1. Add 50 µL Proteinase K and the target volume of lysis buffer directly to the microcentrifuge tube containing the folded filter disc. Incubate on a digital dry bath shaker at 56 °C for at least 2–3 hours (or overnight) to thoroughly digest captured cellular structures.
2. Centrifuge to pellet filter debris. Pass the clear supernatant lysate through a silica spin column. Wash sequentially with ethanol-based buffers to remove protein impurities. Elute clean genomic fragments using a low-ionic-strength buffer (10 mM Tris-HCl or molecular-grade water).

Elution Volume

A smaller elution volume (e.g., 50–100 µL) increases DNA concentration but may reduce total yield. Choose based on downstream assay sensitivity requirements.

7.4. Phase C: Internal Control Inhibition Testing

Environmental matrices contain co-extracted PCR inhibitors (humic acids, fulvic acids, tannic compounds) that can produce false negative results (Jane et al., 2015; McKee et al., 2015).

1. Run a dual-plex qPCR assay using an artificial Internal Positive Control (IPC) DNA target sequence and matched primer/probe set alongside your environmental samples.
2. **Analysis threshold:** If the IPC amplification curve shifts to the right ($\Delta C_t > 3.0$ cycles) relative to the control baseline water standard, inhibition is present (McKee et al., 2015).
3. **Remediation:** For inhibited extracts, either pass the genomic sample through a secondary commercial inhibitor-removal column (e.g., Zymo OneStep PCR Inhibitor Removal) or perform a 1:10 dilution with molecular-grade water before final target gene amplification. Re-test IPC performance after remediation (McKee et al., 2015).

7.5. Cleanup and Storage

1. Dispose of used lysis buffer, wash buffer, and flow-through in the appropriate chemical waste stream (§8).
2. Wipe down all work surfaces with 10% bleach, followed by 70% ethanol and molecular-grade water.
3. Store extracted DNA at -20°C (short-term) or -80°C (long-term archival).

8. Quality Control and Acceptance Criteria

QC Element	Requirement
Field blank	Filtered molecular-grade water collected alongside field samples; must show no target amplification (Goldberg et al., 2016)
Extraction blank	Lysis buffer processed through full extraction without a filter; must show no target amplification
IPC ΔC_t	Must be ≤ 3.0 cycles relative to baseline; samples exceeding this threshold require inhibitor remediation (McKee et al., 2015)
Positive extraction control	Known positive tissue or synthetic DNA standard carried through extraction; must amplify at expected C_t
No-template control (NTC)	Molecular-grade water in place of template during qPCR; must show no amplification

9. Waste Disposal

Waste Type	Container	Disposal Method
Lysis buffer and flow-through	Chemical waste carboy	EH&S pickup
Used filters and tubes	Biohazard bag	Autoclave and solid waste
Bleach rinse waste	Drain (if neutralized)	Per local discharge rules
Ethanol wash waste	Flammable waste container	EH&S pickup

10. Maintenance

- **Each use:** Decontaminate manifold, benchtops, and pipettes per §6.1.
- **Weekly:** Check vacuum pump oil level and integrity of tubing connections.
- **Monthly:** Verify dry bath temperature accuracy with a calibrated thermometer.
- **As needed:** Replace UV bulbs in the Pre-PCR room (if UV decontamination is used).

11. References

- Goldberg et al. (2016)

- Turner et al. (2015)
- McKee et al. (2015)
- Jane et al. (2015)
- Ficetola et al. (2008)
- Qiagen (2024)
- [SOP-L002 Laboratory Safety](#)
- [SOP-F700 eDNA Field Sampling](#)

- Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology Letters*, 4(4), 423–425. <https://doi.org/10.1098/rsbl.2008.0118>
- 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>
- Hinlo, R., Gleeson, D., Lintermans, M., & Furlan, E. (2017). Methods to maximise recovery of environmental DNA from water samples. *PLoS ONE*, 12(6), e0179251. <https://doi.org/10.1371/journal.pone.0179251>
- Jane, S. F., Wilcox, T. M., McKelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., Letcher, B. H., & Whiteley, A. R. (2015). Distance, flow, and PCR inhibition: eDNA dynamics in two headwater streams. *Molecular Ecology Resources*, 15(1), 216–227. <https://doi.org/10.1111/1755-0998.12285>
- McKee, A. M., Spear, S. F., & Pierson, T. W. (2015). The effect of dilution and the use of a post-extraction nucleic acid purification column on the accuracy, precision, and inhibition of environmental DNA samples. *Biological Conservation*, 183, 70–76. <https://doi.org/10.1016/j.biocon.2014.11.031>
- Qiagen. (2024). *DNeasy blood and tissue kit handbook*. <https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/dneasy-blood-and-tissue-kit>
- Turner, C. R., Uy, K. L., & Everhart, R. C. (2015). Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biological Conservation*, 183, 93–97. <https://doi.org/10.1016/j.biocon.2014.11.017>

 Missing bibliography entry: hinlo2017

The previous version of this SOP cited @hinlo2017 for the < 150 mmHg vacuum pressure recommendation. This key does not exist in **references.bib**. The full citation is:

Hinlo, R., Gleeson, D., Lintermans, M., & Furlan, E. (2017). Methods to maximise recovery of environmental DNA from water samples. *PLOS ONE*, 12(6), e0179251. <https://doi.org/10.1371/journal.pone.0179251>

Please add this entry to **references.bib** using the BibTeX block in the supplemental bib additions file, then replace this callout with the inline citation [@hinlo2017] in the Phase A filtration step.

12. Training Record

Training is documented in the lab training log. Before working independently, personnel must:

1. Read this SOP in full
2. Observe a trained operator perform the procedure
3. Perform the procedure under supervision
4. Be signed off by the PI or designated trainer