



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L706

eDNA Metabarcoding Lab Workflow

Standard Operating Procedure

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1. Purpose

This SOP describes the end-to-end laboratory workflow for environmental DNA (eDNA) metabarcoding, from receipt of field-collected water filter samples through DNA extraction, PCR amplification, library preparation, sequencing, and bioinformatics analysis. eDNA metabarcoding combines

universal or group-specific primers with high-throughput sequencing to detect and characterize aquatic biodiversity from water samples without capturing organisms (Deiner et al., 2017; Taberlet et al., 2012).

This SOP serves as the integrating document that ties together the field collection procedures in [SOP-F700](#) with the molecular and computational SOPs in the L700 series. It does not repeat detailed procedures described in other SOPs but provides the eDNA-specific modifications, QC requirements, and decision points that distinguish eDNA metabarcoding from standard amplicon sequencing of soil or tissue samples.

2. Scope

- **Applies to:** Laboratory processing of eDNA water samples for multi-species detection via amplicon sequencing on Illumina platforms; target taxa include fish (12S), amphibians (16S/12S), macroinvertebrates (COI), and general biodiversity surveys (18S, COI)
- **Does not apply to:** Species-specific eDNA detection by qPCR or ddPCR (single-species assays); soil microbial community analysis (see [SOP-L704](#) and [SOP-L705](#)); field collection procedures (see [SOP-F700](#))
- **Location:** Molecular biology bench, SGM 133

3. Responsibilities

Role	Responsibility
Lab PI	Approve target markers, primers, and sequencing plan
Trained Personnel	Process samples per this SOP; maintain contamination controls
New Students	Complete eDNA-specific contamination training before participating

4. Definitions

- **Amplification blank (no-template control, NTC):** A PCR reaction containing all reagents except sample DNA; run with every PCR plate; any amplification in the NTC indicates contamination and invalidates the entire plate.
- **Blocking primer:** A modified oligonucleotide added to a PCR reaction to selectively suppress amplification of abundant non-target DNA (e.g., host DNA in gut or tissue eDNA samples); allows rare target sequences to be amplified competitively.
- **eDNA metabarcoding:** A method that uses universal or group-specific primers to amplify and sequence a short, taxonomically informative DNA region (barcode) from environmental DNA, yielding a community-level inventory of taxa present in a sample.
- **Environmental DNA (eDNA):** Genetic material shed into the environment by organisms through mucus, feces, skin cells, eggs, and other biological material; collected non-invasively from water, sediment, or air.

- **Extraction blank:** A tube containing only extraction reagents and no biological material, processed through the full extraction procedure; detects reagent or handling contamination; must yield no target amplification.
- **Field blank:** A filter or collection vessel processed through all field collection steps but with no environmental sample; detects airborne or handling contamination during field work.
- **Metabarcoding locus:** The specific genomic region amplified for community identification; common loci include 12S rRNA (fish), 16S rRNA (bacteria, invertebrates), 18S rRNA (eukaryotes), COI (invertebrates, fish), and ITS2 (fungi, plants).
- **Occupancy model:** A statistical model that accounts for imperfect detection in eDNA surveys by estimating the probability that a species is truly present given detection/non-detection across replicate samples or PCR replicates.
- **PCR replicate:** One of multiple independent PCR reactions run from the same DNA extract; increases detection sensitivity for rare targets and allows estimation of false-negative rates; standard eDNA practice is 3–8 replicates per sample.
- **Sequence read depth (coverage):** The total number of sequencing reads assigned to a sample; directly affects the number of rare taxa detected; EA Program target is a minimum of 10,000 reads per sample after quality filtering.

5. Safety and Hazards

Hazards

- **Chemical:** Lysis buffers (guanidine salts), ethanol, SPRI beads, and DNA stains. See individual SOP safety sections for details.
- **Biological:** eDNA samples may originate from water bodies containing pathogens. Handle with standard precautions.
- **UV radiation:** If using UV transilluminator for gel imaging.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves (changed frequently; mandatory at all steps)

5.2. Emergency Procedures

Refer to [SOP-L003 Hazardous Materials Handling](#).

6. Workflow Overview

The eDNA metabarcoding workflow consists of six stages. Each stage references a detailed SOP.

Stage	Description	SOP Reference
1	Field collection and preservation	F700
2	DNA extraction from filters	L700
3	PCR amplification and verification	L703
4	Library preparation and sequencing	L704
5	Bioinformatics (QIIME 2 / DADA2)	L705
6	Taxonomic interpretation and reporting	This SOP, Section 6.6

7. Procedure: eDNA-Specific Modifications

7.1. Sample Receipt and Logging

1. Upon receipt from the field, verify that all samples match the chain of custody form per [SOP-F008](#).
2. Inspect Sterivex capsules or filter bags for integrity (no leaks, caps intact, preservative present).
3. Log each sample in the lab notebook and [Freezer Inventory Google Sheet](#).
4. Store preserved filters at -20 C (short-term) or -80 C (long-term) until extraction.

7.2. DNA Extraction from Filters

Follow [SOP-L700](#) with the following eDNA-specific modifications:

- **Sterivex capsules:** Open the capsule aseptically using a sterile razor blade or the Sterivex extraction adapter. Transfer the membrane into the PowerBead Pro tube using sterile forceps. If Longmire's buffer was used as preservative, add the buffer to the bead tube as well.
- **Open membrane filters:** Transfer the folded filter directly into the bead tube using sterile forceps.
- **Extraction blanks:** Process one extraction blank per batch. For eDNA, this means running an empty bead tube through the full protocol.
- **Field blanks:** The field blank filter (from [SOP-F700](#)) is extracted alongside samples and serves as the primary contamination control for the entire field-to-lab chain.

7.3. Inhibition Testing

eDNA extracts from natural waters frequently contain PCR inhibitors (humic acids, tannins, metal ions) (McKee et al., 2015). Test for inhibition before committing to full library preparation:

1. Run an internal positive control (IPC) qPCR assay on a subset of extracts (or all extracts, if the batch is small). The IPC uses a synthetic DNA target spiked into the PCR reaction at a known concentration.
2. If the IPC C_t value shifts by more than 2 cycles compared to the uninhibited control, the extract is inhibited.
3. For inhibited samples, dilute the extract 1:5 or 1:10 with nuclease-free water and retest. Alternatively, clean the extract using a OneStep PCR Inhibitor Removal Kit (Zymo Research).

7.4. Primer Selection for eDNA Metabarcoding

Target Taxa	Marker	Primers	Amplicon	Reference
Fish	12S rRNA	MiFish-U-F / MiFish-U-R	~170 bp	Miya et al. (2015)
Amphibians	16S rRNA	16Smam / 16Smam-R (modified)	~250 bp	Vences et al. (2005)
Macroinvertebrates	COI	mlCOIintF / jgHCO2198	~313 bp	Leray et al. (2013)
General eukaryotes	18S rRNA	Euk1391f / EukBr	~130 bp	Amaral-Zettler et al. (2009)
General eukaryotes	COI	fwhF2 / fwhR2n	~205 bp	Vamos et al. (2022)

Note

Primer choice determines which taxa can be detected. Discuss target taxa and primer selection with the PI before beginning library preparation. Multi-marker approaches (e.g., 12S + COI) provide broader taxonomic coverage but require separate library preparations and sequencing runs (Deiner et al., 2017).

7.5. PCR and Library Preparation

Follow [SOP-L703](#) for initial PCR screening and [SOP-L704](#) for library preparation, with these eDNA-specific considerations:

- **Replicates:** Run 3 independent PCR replicates per sample to improve detection probability for rare species (Ficetola et al., 2015). Pool the replicates after PCR1 before cleanup and indexing.
- **Cycle number:** Use 35 cycles for PCR1 (higher than standard 16S amplicon work) because eDNA concentrations are typically very low. Be aware that additional cycles increase the risk of chimera formation and tag-jumping (Schnell et al., 2015).
- **Blocking primers (optional):** If non-target taxa (e.g., human DNA) dominate the PCR product, consider using blocking primers or PNA (peptide nucleic acid) clamps to suppress amplification of non-target sequences.
- **Controls:** In addition to the standard NTC and extraction blank, include the field blank and (if available) a positive control consisting of a mock community of known species at known concentrations.

7.6. Bioinformatics and Taxonomic Assignment

Follow [SOP-L705](#) for denoising and taxonomic classification, with these modifications:

- **Reference database:** Use a curated database matched to the marker and target taxa:
 - 12S fish: MitoFish or MIDORI2

- COI: BOLD / MIDORI2
- 16S/18S: SILVA or GenBank nt
- **Species-level assignment:** For eDNA, species-level identification is the primary goal (unlike microbiome studies where genus-level is often sufficient). Use BLAST-based classification (`classify-consensus-blast` in QIIME 2) in addition to or instead of the Naive Bayes classifier for improved species-level resolution (Goldberg et al., 2016).
- **Decontamination:** Use the `decontam` R package to identify and remove contaminant ASVs using the field blank and extraction blank data (Davis et al., 2018).
- **Detection criteria:** An ASV is considered a positive detection for a species only if:
 1. It matches a reference sequence at greater than or equal to 97–99% identity (marker-dependent)
 2. It is absent from all negative controls (field blank, extraction blank, NTC), or its abundance in the sample exceeds 10x the abundance in the highest-read control
 3. It is detected in at least 2 of 3 PCR replicates (if replicates were sequenced independently) (Ficetola et al., 2015)

7.7. Reporting

eDNA metabarcoding results are reported as a species detection list per site with the following metadata:

- ASV sequence and percent identity to reference
- Number of reads per ASV per sample
- Detection across replicates (e.g., detected in 2/3 replicates)
- Negative control results
- Any taxa flagged as potential contaminants

! Important

eDNA metabarcoding provides presence/detection data. Quantitative interpretation of read counts as species abundance is not recommended without careful validation, because PCR amplification bias, primer affinity, and DNA shedding rates vary among taxa (Kelly et al., 2019).

8. Quality Control

- **Field blanks:** Must be negative for all target taxa. Any target taxon detected in a field blank compromises the detection claim for that taxon across all samples in the same sampling event.
- **Extraction blanks:** Must be negative. Positive extraction blanks indicate lab contamination; the entire extraction batch is suspect.
- **NTC:** Must be negative. Positive NTCs indicate PCR contamination.
- **Positive control (mock community):** Must recover all expected species. Failure to detect a known species in the mock community indicates primer bias or PCR failure.

- **Replication:** A minimum of 3 PCR replicates per sample is recommended for occupancy-based detection analyses.
- Document all control results in the project data file and report alongside species detections.

9. Waste Disposal

Refer to waste disposal tables in [SOP-L700](#), [SOP-L703](#), and [SOP-L704](#).

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11. Related SOPs

SOP	Title	Relationship
F700	eDNA Field Sampling	Upstream: field collection
F008	Sample Labeling and COC	Chain of custody
L700	DNA Extraction	Stage 2: extraction
L703	PCR and Gel Electrophoresis	Stage 3: amplification
L704	Amplicon Library Prep	Stage 4: library prep
L705	Bioinformatics Pipeline	Stage 5: sequence analysis
L801	RStudio and GitHub	Data management

12. Training Record

Before processing eDNA samples independently, personnel must:

1. Read this SOP and all related SOPs (F700, L700, L703, L704, L705)
2. Demonstrate aseptic filter handling and extraction under supervision
3. Complete an eDNA-specific contamination awareness session covering pre-PCR/post-PCR separation, filter handling, and control interpretation
4. Process a supervised batch including field blank, extraction blank, and positive control with clean results
5. Be signed off by the PI or lab manager