eDNA Preparation and Sampling: Designing a Rigorous Study

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Contents

Introduction 1

Designing an eDNA Study 2
- What's the research question? 2
- Choosing the right marker 2
- Consider DNA persistence 2

Sampling Environments 2
- Aquatic Sampling 2
- Terrestrial and Air Sampling 2
- Museum Settings 2

Filtration and Preservation 3
- Filter Types 3
- Volume Considerations 3
- Preservation Options 3

Contamination Control 3
- Field Protocols 3
- Controls 3

Metadata Collection 3
- Essential Fields 3
- Museum Context 4

Field Kits and Logistics 4

Preservation Challenges in Museum Samples 4
- Ethanol Preservation 4
- Formalin Fixation 4
- Considerations for Museum Researchers 4

Foundational Reading 4

Summary 5

Introduction

Before collecting a single sample, it's important to think through the full design of your eDNA project. This section introduces the considerations and protocols for preparing and collecting high-quality eDNA samples in both field and museum environments.

Designing an eDNA Study

What's the research question?

- Are you detecting a specific species or surveying communities?
- Temporal and spatial scales matter.

Choosing the right marker

- Based on your taxa of interest (e.g., COI for animals, 18S for eukaryotes).
- Consider ecosystem and sample type (water vs. sediment vs. swabs).

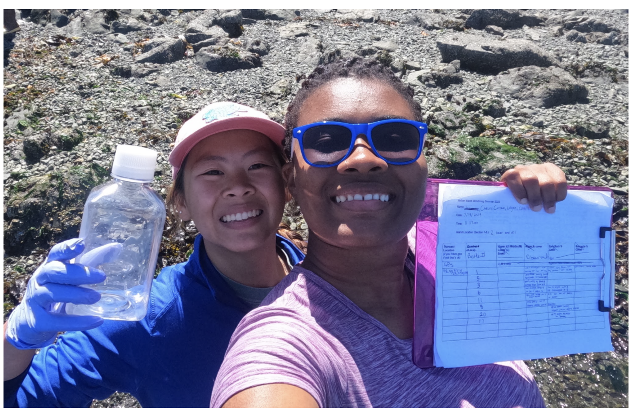
Consider DNA persistence

- Environmental DNA degrades over time.
- Degradation influenced by UV light, temperature, pH, microbial activity.

Sampling Environments

Aquatic Sampling

- Surface water, mid-column, sediment cores.
- Marine and freshwater protocols differ in filtration volume and salinity handling.



Terrestrial and Air Sampling

- Soil or snow sampling using sterile scoops or syringes.
- Air filters or cyclone samplers for airborne DNA.

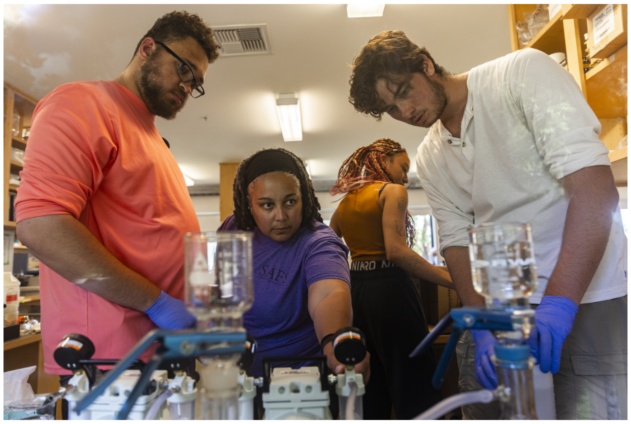
Museum Settings

- Swabbing collection surfaces, containers, or tools.
- Extracting DNA from ethanol or residual fluids.

Filtration and Preservation

Filter Types

- Sterivex (enclosed, on-site filtration)
- Glass fiber or cellulose nitrate filters (lab filtration)



Volume Considerations

- 250 mL to 2 L common for water samples
- Use peristaltic pumps or syringes with luer-lock filter holders

Preservation Options

- Ethanol or Longmire's buffer
- Dry preservation (silica desiccant)
- Freeze at -20°C or lower ASAP

Contamination Control

Field Protocols

- Wear gloves and change them between samples
- Sterilize gear with 10% bleach + rinse
- Use single-use consumables when possible

Controls

- Field blanks (filtered water from the field site)
- Filtration blanks (no sample passed through filter)
- Lab blanks (control throughout extraction process)

Metadata Collection

Essential Fields

- GPS coordinates

- Date and time
- Water temperature, salinity, turbidity, pH
- Weather and tides (for marine)

Museum Context

- Specimen ID and accession numbers
- Container type, date of preservation
- Preservation fluid composition

Field Kits and Logistics

- Pre-label tubes and filters
- Bring extra gloves, sample tubes, waste containers
- Clean workspace for filtering (e.g., in the car, pop-up lab tent)

Preservation Challenges in Museum Samples

Ethanol Preservation

- Ethanol can preserve DNA fairly well, especially at high concentrations (95–100%).
- However, over time, ethanol can **cause hydrolysis**, leading to DNA fragmentation.
- Repeated ethanol changes or evaporative concentration can alter preservation effectiveness.
- DNA yield is typically lower in aged ethanol than fresh environmental samples.

Formalin Fixation

- Formalin **crosslinks proteins and nucleic acids**, severely degrading or blocking PCR amplification.
- DNA in formalin-fixed samples is often:
  - Highly fragmented
  - Chemically modified (e.g., methylol adducts)
  - Difficult to extract without specialized protocols (e.g., reversal treatments or harsh lysis)

Considerations for Museum Researchers

- Prioritize ethanol-preserved materials when possible
- Use extraction protocols designed for **low-yield or fragmented DNA**
- Document preservative type and history in metadata

Foundational Reading

- **Hofreiter et al. (2001)** – Ancient DNA from museum specimens
  Nature Reviews Genetics, 2(5), 353–359.
  https://doi.org/10.1038/35072071

- **Zimmermann et al. (2008)** – DNA damage in preserved tissue
  Biotechniques, 44(5), 619–626.
  https://doi.org/10.2144/000112812

- **Raxworthy & Smith (2021)** – Museum genomics: New uses for old collections
  Trends in Ecology & Evolution, 36(8), 707–718.
  https://doi.org/10.1016/j.tree.2021.04.007
- **Díez-del-Molino et al. (2018)** – High-throughput sequencing of museum specimens
  Trends in Ecology & Evolution, 33(7), 633–648.
  https://doi.org/10.1016/j.tree.2018.05.005

Summary

Good eDNA starts with good planning. Clear protocols, contamination controls, and rich metadata ensure high-quality results and reproducibility.