eDNA Topic:

eDNA Preservation: Proper Sample Preservation

Author: Jonah Ventures

Introduction

Effective preservation of environmental DNA (eDNA) is essential to maintain the integrity of the genetic material for analysis. Once collected, eDNA is vulnerable to several degradation processes that can compromise the quality of the samples.

eDNA Degradation

Degradation can occur through biological, chemical, and physical mechanisms:

- Biological Degradation: Enzymatic activity from nucleases naturally present in the environment is a primary biological factor that breaks down DNA.
- Chemical Degradation: Low pH and reactive chemicals can fragment DNA or alter its bases, leading to chemical degradation.
- Physical Degradation: UV radiation, extreme temperatures, and repeated freeze-thaw cycles can cause physical degradation of the DNA.

Understanding these degradation pathways is critical for developing effective preservation strategies that maintain the integrity of eDNA samples for accurate analysis.

Preservation Techniques

There are various techniques that experimenters can use to preserve eDNA samples, with the most common methods involving desiccation, chemical buffering, and freezing. Some environmental samples are more suited to specific preservation methods:

- Aquatic filters are often preserved by freezing, either dry or in a preservative solution.
- Air filters can be preserved through desiccation or by freezing to protect the eDNA collected on them.
- Swabs are typically frozen or stored in a buffer solution to maintain the integrity of the eDNA.

Selecting the appropriate preservation method is essential for ensuring that the eDNA remains intact for accurate downstream analysis.

eDNA Preservation through Desiccation

Desiccation, or drying, is an effective method for preserving eDNA as it inhibits microbial growth and reduces enzyme activity that degrades DNA. However, it is essential to ensure that during desiccation, temperatures remain below the DNA denaturation threshold (typically around 95°C), to maintain the integrity of the DNA helix. Proper desiccation requires that the sample dries completely, and that enough desiccating agent is used to absorb all moisture. Common desiccating agents include silica gel or other absorbent materials, while air-drying in a controlled environment is another option. Some products, like the water filters from Smith-Root, are self-preserving as they are encased in water-absorbing material that effectively dries the filters.

eDNA Preservation through Chemical Buffers

Chemical buffers are another effective method for preserving eDNA by stabilizing its environment. These buffers help maintain pH levels and protect DNA from degradation. Common preservation buffers include:

- **Ethanol:** High-concentration ethanol (95-100%) is widely used for eDNA preservation, as it dehydrates DNA and inhibits enzymatic activity that could degrade the material.
- **RNAlater:** A commercial solution designed for preserving genetic material, RNAlater stabilizes RNA and DNA at room temperature, making it ideal for field collection when refrigeration is unavailable.
- **Tris-EDTA (TE) Buffer:** TE buffer preserves DNA by maintaining a stable pH and chelating metal ions, protecting the DNA from degradation. It consists of:
    - **Tris (tris(hydroxymethyl)aminomethane):** A buffer that promotes nucleic acid stability and acts as a chelating agent to inhibit enzymatic activity.
    - **EDTA (Ethylenediaminetetraacetic acid):** Binds divalent metal ions, necessary cofactors for DNA-degrading enzymes, preventing enzymatic degradation.
- **Detergents (e.g., Sodium Dodecyl Sulfate/SDS):** Detergents disrupt cell membranes, releasing DNA into solution. They also denature proteins, including degradative enzymes that destroy DNA, and solubilize membrane proteins and lipids, aiding in DNA extraction.
- **CTAB (Cetyltrimethylammonium Bromide):** CTAB is particularly effective in preserving DNA from samples containing polysaccharides or other contaminants that interfere with extraction and purification.

Regardless of the buffer used, it is important to ensure that enough solution is added to fully saturate the eDNA-containing material. However, avoid adding excessive amounts, as it can dilute the DNA, reducing detection sensitivity. Always consult the DNA extraction protocol to ensure the chosen preservation method is compatible with the extraction technique.

eDNA Preservation through Freezing

Freezing eDNA samples at -20°C is a commonly used preservation method because it effectively slows down enzymatic reactions and microbial activity that could cause DNA degradation. At this temperature, the metabolic processes of DNA-degrading enzymes are essentially halted. Freezing at -80°C or using dry ice is usually unnecessary until after the DNA is extracted. However, care should be taken with automatic defrost freezers, as repeated freeze-thaw cycles can degrade the DNA. Additionally, eDNA collected from highly saline environments may benefit from the removal of salts before freezing, as salts can depress freezing points and interfere with DNA extraction. Samples preserved in buffer solutions can also be safely frozen for long-term storage.

Example Protocol: Preservation of Aquatic eDNA in Syringe Filters

1. ### Dry filter

   After completing water filtration, use a syringe to push air through the filter to remove any excess water. This step helps to dry the filter and ensures that as much eDNA as possible is retained on the filter surface for subsequent analysis.

2. ### Inject preservative

   Using a 1-mL syringe, slowly inject Longmire's preservation buffer (a Tris-EDTA preservation buffer) into the filter housing. Continue injecting until most of the air in the filter housing is displaced and the filter is fully saturated with the buffer. This step ensures proper preservation of the eDNA on the filter for downstream analysis.

3. ### Put on lock caps

   Apply luer lock caps to both the male and female ends of the syringe filter to securely seal it. This step helps to prevent contamination and preserve the eDNA sample until further processing.

4. ### Store

   Store preserved filters in a cool, dark place to maintain their integrity. If long-term storage is needed, the filters can be frozen indefinitely to ensure the eDNA remains preserved for future analysis.

Frequently Asked Questions

1. What is the best method to preserve aquatic eDNA on filters?

   The best method for preserving eDNA on filters typically involves either using a preservation solution or freezing the filters. Common preservation solutions include ethanol, Longmire's solution, and proprietary solutions such as RNA*later*. Alternatively, filters can be stored dry and frozen. The choice of preservation method depends on the specific needs of the analysis and the logistics of fieldwork, such as transport conditions and the availability of refrigeration.

2. Do eDNA filters need to be stored at -80°C?

   While storing eDNA filters at -80°C is ideal for long-term preservation, as it minimizes DNA degradation, it is not always necessary. For shorter-term storage, -20°C is typically sufficient. The key is to freeze the samples as soon as possible after collection to prevent degradation and maintain the integrity of the eDNA for future analysis.

3. Are some eDNA preservation fluids toxic?

   Yes, some eDNA preservation fluids can be toxic, but they are generally chosen to have low toxicity. Non-toxic preservative fluids, such as Longmire's solution and RNA*later*, are often preferred for fieldwork where safety and ease of transport are important considerations (though note that Longmire's solution is regulated in the EU under SDS requirements). As a general safety precaution, preservative fluids should be handled carefully to avoid contact with eyes or skin and to prevent them from entering the environment.

4. How quickly does DNA degrade if not preserved?

   The rate of unpreserved DNA degradation can vary significantly depending on environmental conditions. In warm, enzyme-rich environments, DNA can start to degrade within hours, while in cooler, less biologically active settings, it may last longer. However, even in favorable conditions, DNA typically begins to break down within days. Therefore, prompt preservation is essential for maintaining DNA integrity and ensuring reliable analysis.

5. Does DNA degrade in water after collection, but before preservation?

   Yes, DNA can degrade in water after collection if not preserved promptly. Enzymatic activity, microbial action, and physical factors like UV exposure all contribute to DNA

degradation. To prevent this, it is crucial to minimize the time between collection and preservation, ensuring the DNA remains intact for accurate analysis.

Further reading

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