

eDNA Topic:

Experimental Design: Deciding on qPCR vs. Metabarcoding

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Introduction

Environmental DNA (eDNA) refers to genetic material obtained from environmental samples. eDNA is generated when DNA is shed from organisms into the environment through skin cells, hair, bodily fluids, and other biological materials. As a result, eDNA can be collected from virtually any source, including water, air, soil, and both natural and artificial surfaces. Empirically, eDNA can also include DNA from entire organisms, such as bacteria, phytoplankton, and zooplankton, if these organisms are small enough to be collected using the same techniques. Once collected, eDNA can be analyzed to determine the presence and abundance of different species.



When considering an eDNA study, it is important to keep an open mind regarding environmental sampling. For example, aquatic eDNA can be collected from various water bodies, including puddles, rivers, and oceans. Airborne eDNA, though less commonly studied than aquatic eDNA, involves capturing DNA present in the atmosphere, potentially from pollen, spores, or skin fragments. Soil and sediment eDNA provide insights into both current and past biodiversity, capturing DNA fragments from decomposing organisms and microorganisms. However, sediment sampling can also capture eDNA that has been preserved for long periods of time. On general surfaces, eDNA can be gathered from places where organisms have made contact or where their biological residues have settled.

Designing an eDNA Study

Designing an eDNA study involves several key components:

1. Selecting an appropriate collection medium and technique:

An experimenter must account for the study's goals and weigh trade-offs such as the likelihood of successful capture, cost, effort, and representativity.

2. Preserving eDNA immediately after collection:

It is critical prevent degradation before analysis to ensure your collected sample is representative.

3. Performing DNA extraction:

Typically done in a laboratory, several DNA extraction protocols and kits are available. The experimental designer should choose the appropriate protocol or kit depending on the type of eDNA and the medium from which it is extracted.

4. Performing polymerase chain reaction (PCR):

PCR will generate billions of copies of a DNA segment. The experimenter will need to select primers that target and amplify a DNA region of interest. Quantitative PCR (qPCR) may also be relevant, as it can use amplification results to estimate the initial number of copies of target DNA in the extract.

5. Sequencing DNA:

PCR products must undergo DNA sequencing (e.g., Illumina or Oxford Nanopore sequencing).

6. Bioinformatics processing and analysis:

Computational methods are used to determine sequence quality and perform filtering steps, followed by additional software tools for analysis and data interpretation.

Additional Sampling Considerations

Setting up eDNA studies requires careful planning for how individual samples are collected, how multiple samples are distributed across space and time, and how negative control samples will be included for later analysis. For individual sample collection, consider the target ecosystem and the most effective method for capturing eDNA, whether through water filtration, soil sampling, or air filtration. Factors like sample volume, timing, and location should be optimized to maximize DNA yield and relevance.

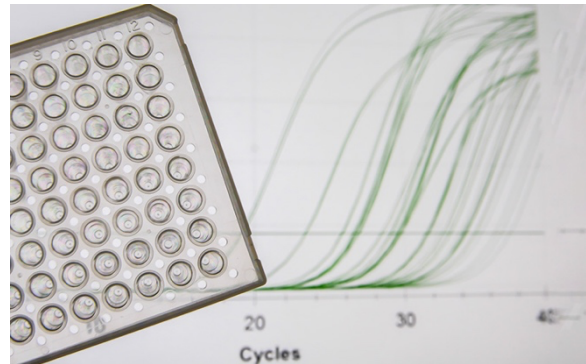
When arraying multiple samples, plan strategically to capture environmental contrasts, allowing for a comprehensive analysis of the area and species of interest. In some cases, collecting multiple samples from the same site can increase species representation or the likelihood of detecting a specific species. In other cases, distributing samples across gradients or environmental contrasts can provide a broader understanding of patterns at the cost of detailed knowledge of any one site. Incorporating negative controls is also important. These are samples processed without any environmental material to check for contamination and procedural errors.

Considerations for Downstream Analysis

When choosing between **qPCR** and **metabarcoding** for analyzing eDNA samples, several key factors should guide the decision, depending on the study's goals and the type of data needed.

Quantitative PCR (qPCR):

qPCR is a powerful technique for detecting a single species and/or quantifying the amount of its DNA in the environment. It amplifies DNA sequences using species-specific primers along with a fluorescent probe (e.g., Applied Biosystems TaqMan qPCR assays), enabling both detection and quantification of DNA from the target species. This method is highly sensitive, capable of detecting even small amounts of DNA. It is also cost-effective when focused on a single species. Detection sensitivity depends on lab replication, and while qPCR is not inherently more sensitive than metabarcoding, it can detect a single DNA copy in a sample. The success of qPCR depends on careful assay design and thorough testing to prevent false positives from closely related species.



Metabarcoding:

Metabarcoding provides a broader eDNA analysis by using universal primers to amplify a specific DNA region across multiple taxa. High-throughput sequencing is then used to identify a wide range of organisms, offering a comprehensive view of biodiversity. While metabarcoding can estimate relative abundance when DNA concentrations are high, absolute quantification can be achieved by adding a known quantity of unique DNA before PCR. In some cases, metabarcoding may even detect individual species more accurately than qPCR, since the amplified sequences are sequenced, reducing the risk of false positives from closely related species. Although more expensive than qPCR, metabarcoding can be cost-effective when detecting multiple species, as it avoids the need for running several separate qPCR assays.



Experimental Design Example – Sampling for Carp

In this hypothetical example, following are considerations you might follow to examine the presence of carp in a freshwater river. Sample collection is critical to the success of any eDNA study, as it underpins the reliability and confidence of the analysis. For example, when designing a protocol to collect river water for qPCR detection of carp, considerations include sample volume, timing, and location to ensure effective DNA capture and accurate results.

1. Selecting Sites

Choosing multiple locations along the river can ensure comprehensive coverage of the study area. Consider upstream, midstream, and downstream sites to capture any variations in carp distribution. Ensure that the selected sites are accessible and safe for water collection. The number of sites can be estimated if the probability of detection is known or adjusted based on budget or time constraints. Sites should be spaced to reflect the possible movement of carp and the dispersal of their DNA. Prioritize areas with known or suspected carp presence based on previous research or local insights. Additionally, include a few sites where carp are known to be absent to serve as negative controls.

2. Sampling Replicates

Once the number of sites is determined, maximize the number of within-site replicates while considering time and budget constraints. The more samples collected, the greater the statistical confidence in the results. Multiple replicates help reduce the effects of sampling errors and increase the reliability of detection. Within-site replicates can also account for any stratification or partitioning in DNA abundance, which may occur in areas where water is not well mixed. To capture this, collect samples from different flow conditions, such as slow-moving backwaters, main channel flow, and turbulent areas. Additionally, sample from varying depths and surface water to account for the vertical distribution of eDNA.

3. Water sampling

Prepare all equipment and consumables in advance. Wear sterile gloves throughout the process to prevent contamination. Ensure that each filter is uniquely labeled for proper identification. At each site, follow standard sampling protocols to collect and filter the water. Be sure to record important metadata for each sample, including the time and date of collection, geolocation coordinates, site name, and the volume of water filtered.

4. Filter Preservation

Once the water has been filtered, add a DNA preservative to the filters, desiccate the samples, or freeze them immediately to prevent DNA degradation. Preserved or

desiccated samples can usually be kept at ambient temperature until transported to the laboratory. Once there, samples should be stored at -20°C or colder for long-term preservation.

Frequently Asked Questions

1. How long does eDNA stay in water before it degrades?

eDNA typically persists in water for a few days to a few weeks, depending on environmental conditions such as temperature, pH, and microbial activity. Higher temperatures and microbial activity tend to degrade eDNA more quickly.

2. How much water should I sample?

The amount of water to sample can vary depending on the sensitivity required and the abundance of the target species. Commonly, volumes between 0.5 to 2 liters are sampled for eDNA analysis. Less water is necessary when sequencing common species. In general, filtering as much water as possible for a given filter is typically better.

3. Can I run both qPCR and metabarcoding on the same sample?

Yes, it's possible to use the same eDNA sample for both qPCR and metabarcoding analyses. A typical qPCR analysis will only use 10% of the extracted DNA, while a metabarcoding analysis could use 5-20% of the extracted DNA.

4. How many samples should I take at a site?

The number of samples needed at a site depends on the spatial variability, the habitat size, and the study objectives. Generally, multiple samples (e.g., 3–5) are recommended to account for potential spatial heterogeneity in eDNA distribution. Taking additional samples and archiving them in case more work is required is a good practice.

5. Is qPCR more sensitive than metabarcoding?

qPCR is generally more sensitive than metabarcoding for detecting low quantities of target DNA because it is designed to amplify a specific sequence. Metabarcoding can be as sensitive as qPCR in detecting specific DNA in low abundance if enough lab replicate samples are amplified.

6. Which technique is more likely to give false positive results?

Both techniques can give false positives, but qPCR may be more susceptible if there is contamination with non-target DNA that also amplifies under the chosen conditions.

Metabarcoding can misidentify species due to errors in DNA sequencing or misinterpretations of the genetic database. Cross-contamination can affect either equally.

7. What do I do if there is no qPCR assay for a species I want to detect?

If a qPCR assay is not available for a specific species, you might consider developing a new assay if you have the expertise and resources. Alternatively, metabarcoding can be used as it allows for the detection of a broader range of species without the need for species-specific assays.

Further reading

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