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Environmental DNA Monitoring Information Sheet

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Introduction

The environment is a critical part of the nation, and monitoring the biodiversity for conservation and biosecurity purposes informs its care. Monitoring provides data that can be compared year-on-year to identify areas of potential improvement, stability, or decline. These comparisons allow for action to be taken in the areas, where necessary, in a proactive manner to advance conservation efforts and mitigate biosecurity threats.

The recent advancement of molecular technology enables monitoring via the use of environmental DNA (eDNA) - which is DNA that has been shed by living organisms into the environment.

For example, instead of catching fish in a river to know what species are there, it is possible to take a sample of the river's water and extract DNA from it, thereby identifying the fish that swim in it. This method has become known as eDNA sampling; defined as capturing complex mixtures of DNA (i.e., DNA from multiple species) from the environment which includes water, soil, air and even faecal matter.

Monitoring using eDNA sampling is increasingly used and important for detecting certain species, quantifying biodiversity, and assessing ecosystem health. A typical eDNA workflow includes 1) extracting the DNA from the environmental

Advantages	Limitations
• Scalable, fast and cheap whole-ecosystem monitoring • High sensitivity for rare species • Wide taxonomic scope • Improved taxonomic resolution • Sampling consistency • Future-proof samples and data • Anyone can undertake sampling	• Presence/absence tool – can't measure exact population size • Incomplete DNA reference libraries can lead to some species not being detected, or species being confused for another species • False negatives – eDNA monitoring can still miss some species • False positives – eDNA samples are easily contaminated leading to the detection of species that aren't present

Table 1: Potential advantages of using eDNA monitoring rather than other methods of monitoring, and limitations of eDNA monitoring

sample, 2) targeting the barcode gene regions for sequencing, 3) matching the resulting sequences against a DNA reference library, and 4) identifying species from the environmental samples (Figure 1). eDNA monitoring has many benefits compared to other methods of biodiversity monitoring, which include reduced time spent at one location and a decrease in costs to identify species (Table 1). However, to accurately identify species in an eDNA sample, a good DNA reference library for the biodiversity in the environment of interest is required (Figure 2).

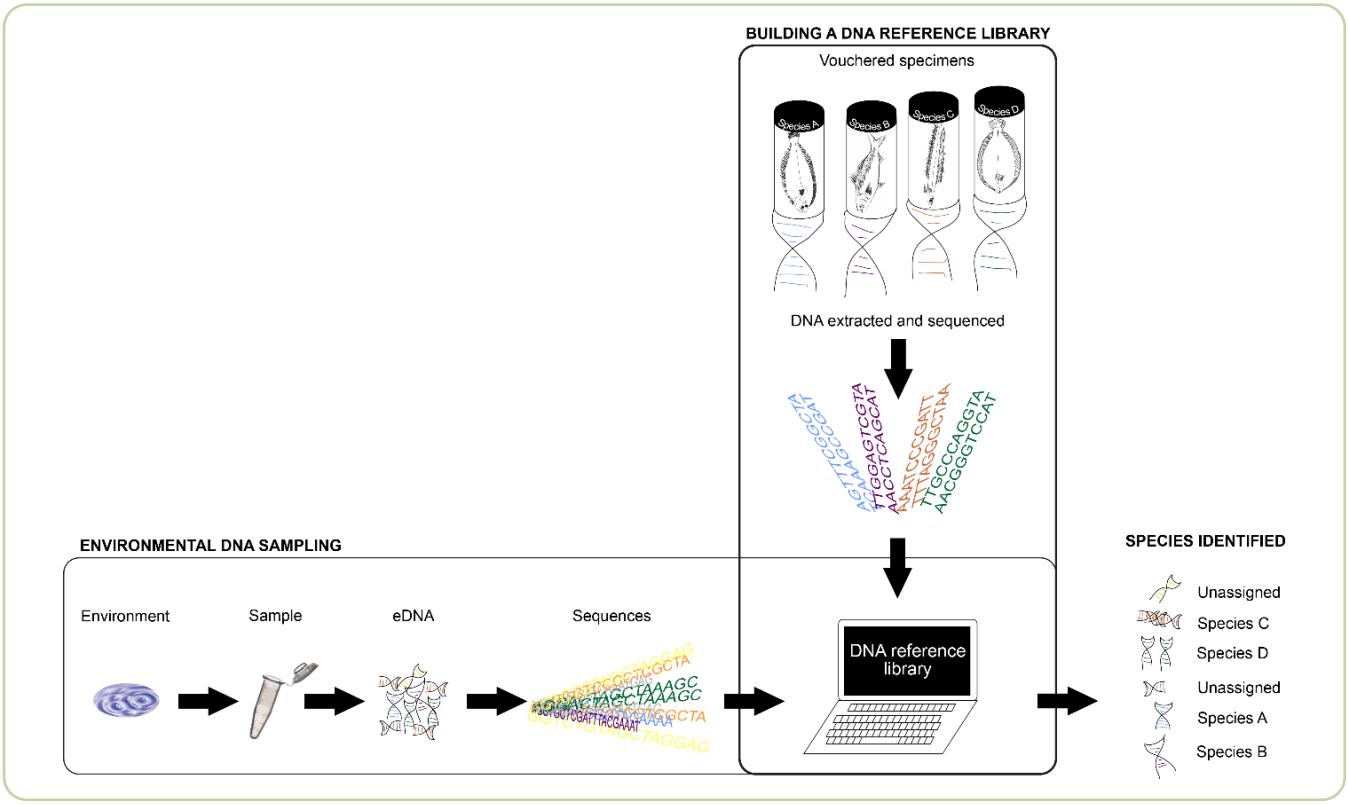

Figure 1. eDNA monitoring requires eDNA sampling and the use of a DNA reference library that has been created in order to identify species. Both these activities require sampling, for which there should be appropriate consultation and permissions granted by Māori communities and landowners. DNA reference libraries should have governance by Māori who can authorise appropriate use and benefit-sharing. See "*eDNA, DNA reference libraries, and Kaitiakitanga Info Sheet*" and "*Te Nohonga Kaitiaki Guidelines Info Sheet*" for more information. Figure created by Aimee van der Reis

DNA Reference Libraries

A DNA reference library holds digital DNA sequences for known species. These DNA sequences act as 'barcodes' that are unique to each species and are used to distinguish the species identities based on the DNA found in an eDNA sample. These DNA barcodes are typically short gene regions that provide only sufficient detail for species identification.

To build a DNA reference library, tissue samples are taken from vouchered[1] specimens held at a museum or other reputable collections (e.g., a university collection; Figure 1). DNA is then extracted from the tissue samples and barcode regions are sequenced to generate a digital text string that constitutes the DNA barcode. Each vouchered specimen is then linked with their respective DNA barcode sequences as a representative for their species. This DNA sequence matching between the DNA reference library and those found in the eDNA sample, is an essential foundation for eDNA monitoring, ensuring the identification and quantification of biodiversity in the sample.

1 The sample specimen has undergone morphological examination by an expert taxonomist who then assigns, or confirms, the species identification.

To be useful for eDNA monitoring, a good reference library must be both comprehensive and accurate. If species are missing from the DNA reference library, they are not able to be identified precisely. Identification of the species at a higher taxonomic level such as Genus, Family or Class can sometimes be attained, but it can also lead to an incorrect eDNA result. For example, if an Australian species is present in the DNA reference library but a closely related species from Aotearoa is absent from the library, the DNA in the eDNA sample may be identified as the Australian species by mistake and prompt an expensive incursion response.

If non-vouchered or incorrectly identified specimens are used to generate the DNA barcodes in the reference library, the eDNA results can include inaccurate detections as well. Garbage in, garbage out! Nonetheless, as DNA reference libraries become more comprehensive, the sequences previously generated from eDNA

Want to know more about international efforts in this space?
In the last two decades there has been considerable effort by the scientific community to add to DNA reference libraries for species identification. This effort was largely driven by the International Barcode of Life initiative (https://ibol.org/) that initially focused on the cox1 gene region. The Barcode of Life Datasystems database (BOLD) contains more than 9.7 million public records for this gene region (http://www.boldsystems.org/index.php/databases). Besides cox1, other gene regions commonly used as DNA barcodes include 12S, 16S, 18S, 23S, rbcL and tufA. The largest DNA barcode public repository is the GenBank sequence database which is maintained by the National Centre for Biotechnology Information as part of the International Nucleotide Sequence Database Collaboration (https://www.ncbi.nlm.nih.gov/genbank/).

Figure 2: A hypothetical DNA reference library, where expertly identified organisms have been sampled from the environment to represent their species. The DNA reference library retains the digital DNA sequence information (made up of A, T, C, and G), much like a barcode, alongside the species name that the organism represents. Figure sourced from wilderlab.co.nz.

samples can be re-matched to DNA sequences of known species identified in these updated DNA reference libraries – creating a consistent way to monitor environments year-on-year, increasing accuracy but also ensuring compatibility among datasets.

Best practice in eDNA monitoring

To ensure that eDNA monitoring delivers the best outcomes, there are several ethical, cultural and technical aspects during both eDNA sampling and the generation of a DNA reference library that should be considered. For example, consultation with Māori and any stakeholders prior to sampling of the environment, or of specimens, including explanation regarding the intent of the sampling and how the generated data will be stored and used is important.

This enables consent to be given by Māori. In the case of the DNA reference library, it is important that information regarding where the specimen was collected, and the relevant permissions is retained with the specimen and the generated DNA sequences, and that all this information is well cared for in a permanent collection. Historical or existing, older specimens that are held in museums may not have a recording of the consent provided. In these cases, the potential cultural sensitivity of the generated data should be respected and disclosed, and an invitation to tangata whenua made to reconnect with their taonga (see eDNA, DNA reference libraries, and Kaitiakitanga Info Sheet).

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