Technical Note

Environmental DNA (eDNA) Water Testing

What is eDNA?

Environmental DNA (eDNA) can be defined as 'genetic material obtained directly from environmental samples without any obvious signs of the biological material'. All organisms (bacteria, fish, invertebrates, etc.) shed DNA into the environment. eDNA testing can provide information about the species present without disrupting the environment.

Principles of water eDNA testing

The eDNA analysis process typically involves the following steps:

1. **Sample Collection, Filtering and Preserving**
   Water samples are collected from a single or multiple points of a water body. This process can target specific areas of a water body or be distributed across a site. Filtering water samples enables the genetic material from organisms living in or passing through the water to be captured and analysed. Following filtering, a preservation solution is added to the sample.

2. **DNA Extraction**
   On arrival at the laboratory, the DNA preservation solution is removed from the syringe filter unit sample and DNA extracted.

3. **PCR Amplification and Sequencing**
   A series of Polymerase Chain Reaction (PCR) assays are used to amplify specific genetic markers from the extracted DNA. These markers are usually short conserved genomic regions that provide taxonomic information for each group of species included in the test (e.g. bacteria, plants, macroinvertebrates, etc). At Hill Labs, sequencing is completed using an Illumina MiSeq instrument.

4. **Data Analysis**
   The resulting sequencing data is processed through a series of bioinformatics tools. This involves several quality control checks, before matching final sequence results against reference databases, to assign species identities for each sequence result.

What's included in an eDNA test?

The following taxonomic groups are included in the Hill Labs comprehensive fresh water eDNA test:

Target Group	Molecular Target
Vertebrates (including fish, mammals, reptiles & birds)	12S
	16S
Plants	rbcL
	trnL
Microeukaryotes	18S
Bacteria	16S
Invertebrates	COI
Venerid clams (including *Corbicula*)	16S

For more information, freephone 0508 HILL LAB 44 555 22 mail@hill-labs.co.nz hill-labs.co.nz

How are eDNA Results Reported?

Hill Labs eDNA results are reported in three separate csv files containing 1) detailed results, 2) summary results, and 3) sample metadata (GPS coordinates, volume of water filtered, sample collection site, index scores etc.), along with a summary pdf report.

If any 'flagged' organisms are identified in a sample, a fourth csv entitled 'eDNA-Flagged' will also be reported.

For full details relating to the reporting of results, please refer to our Technical Note: Interpretation of Result Files for eDNA Water Testing, available on our website.

Some information relating to index scores and 'flagged' organisms is below:

1. Taxon-Independent Community Index (TICI) Score

A taxon-independent community index (TICI) score is calculated for river/stream samples. The TICI model uses a total of 3000 DNA sequences, each assigned a specific indicator/tolerance value using a Chessman rank iterative process. TICI covers a much broader range of taxa, compared to New Zealand's macroinvertebrate community index (MCI). Hill Labs recognises the importance of standardising the reporting of eDNA results. We have specifically developed our test to calculate TICI scores, following the criteria published by Wilkinson *et al*, 2024.

2. Identification of 'Flagged' Organisms

New Zealand has a wealth of unique flora and fauna. The early detection of harmful pests and diseases plays a vital role in the Biosecurity system of New Zealand. Under the Biosecurity Act 1993 (section 44 and 46), Hill Labs is legally required to inform the New Zealand Ministry for Primary Industries (MPI) of any suspect detections of notifiable organisms that are identified in eDNA samples. These results are reported in an additional csv file entitled 'eDNA - Flagged', in order to highlight these results. Unwanted organisms, along with notifiable organisms, are included in the 'eDNA-Flagged' report.

What do my eDNA Results Mean?

eDNA testing is a powerful tool for assessing the biodiversity and health of freshwater environments. eDNA testing provides many advantages over traditional monitoring schemes, however it is not without its limitations. Several factors must be taken into consideration when interpreting eDNA results. Some key benefits and limitations of eDNA testing are outlined below:

Benefits of Water eDNA Testing

- **Non-Invasive:** Water eDNA testing does not require the capture of or direct interaction with species, minimising any disruption of their natural environment.
- **Simple Sample Collection:** Sample collection for aquatic monitoring is straightforward, requiring minimal technical expertise. Following collection, a preservation solution is added and the sample can be stored at room temperature prior to being sent to the lab for analysis.
- **Cost-Effective and Efficient:** Traditional methods of species monitoring, such as visual surveys, trapping, or netting, can be labour intensive and expensive. eDNA testing is more cost-effective, enabling large-scale monitoring with fewer resources.
- **Detection of Rare and/or Unwanted Organisms:** Water eDNA testing can detect organisms that are present in low numbers, including elusive or rare organisms that may be difficult to find using traditional methods. Invasive and/or pest organisms can also be identified, potentially helping to mitigate their ecological impact.
- **Biodiversity Assessment:** eDNA provides an extra layer of confidence in biodiversity studies and environmental monitoring, as it delivers extensive biodiversity data easily.

Limitations of Water eDNA Testing

- **Variable DNA Shedding:** Organisms shed DNA at different rates. For example, in water, fish shed a lot of DNA, whereas terrestrial organisms such as birds, lizards, etc. leave less DNA as they typically only occasionally interact with water.
- **Presence Only:** The presence of eDNA confirms that a species has been present, but does not provide any detail relating to the exact time a species was present in a water body, or for how long. In addition, the detection of eDNA does not necessarily confirm the presence of live individuals.
- **DNA Degradation:** The rate at which DNA degrades in the environment is highly variable. Factors such as temperature, pH, UV, etc. can all influence the rate at which eDNA degrades in a water body.

- **Not Quantitative:** Results provide an indication of relative abundance only and are not quantitative.
- **Sequence Database Coverage:** Accurate species identification relies on robust reference databases. Gaps in databases may limit the ability to identify species, particularly those that are poorly represented and/or recently discovered.

Frequently Asked Questions

Do Hill Labs offer eDNA sampling kits?

Yes, we offer kits for single and replicate analysis. Kits can be purchased containing either 1.2 or 5µm syringe filters. Please contact our environmental client services team to order kits, email: env.csm@hill-labs.co.nz

Please note our kits are for active syringe filtering only. We do not currently provide passive filter sample collection kits.

How do I collect eDNA samples?

Please follow our guide, *Sampling Instructions: Water eDNA test kits*, available on our website.

For replicate sampling, how many replicates should I take?

Previous studies completed in New Zealand, have shown that six sample replicates at a single site were optimal to maximise the detection rates for fish, macroinvertebrates and other taxa (Smith *et al*, 2024). Increasing the number of replicate samples analysed also reduces the risk of false negative results.

How long will it take to get my results?

The turnaround time for results can vary, but we aim to report eDNA results within 10 working days following day of receipt at the laboratory.

How will my results be reported?

eDNA results are reported in csv file format. Up to four csv files are reported, containing detailed sample results, summarised sample results, metadata (including TICI scores) and flagged results for notifiable/unwanted organisms (where appropriate).

Why are some of my sequencing result reported as unknown?

Accurate species identification relies on robust databases. Gaps in databases may limit the ability to identify species, particularly those that are poorly represented and/or recently discovered. We are continuously updating and improving our reference databases, but if you notice a species missing in your results that you expect to be present please contact us.

What are the storage options for my eDNA samples?

Samples will be stored for a maximum of three months, after which time un-analysed samples will be disposed.

Following analysis, DNA extracts are stored for at least 12 months from the date of receipt, in case additional testing is requested.

A copy of all eDNA data is retained for at least 7 years, or for a longer period if required for any legal, regulatory, compliance or accreditation purposes.

Additional storage services of samples and DNA extracts can be arranged on request. Please contact our team to discuss.

Need something else? Please contact our client service team, email: env.csm@hill-labs.co.nz, who will be happy to discuss your needs.

References

Smith J, David B, Hicks A, Wilkinson S, Ling N, Fake D, Suren A, Gault A. Optimizing eDNA Replication for Standard Application in Lotic Systems in Aotearoa, New Zealand. 2024. Environmental DNA, 2024; 6:e70017, http://doi.org/10.1002/edn3.70017

Wilkinson SP, Gault AA, Welsh SA, Smith JP, David BO, Hicks AS, Fake DR, Suren AM, Shaffer MR, Jarman SN, Bunce M. 20024. TICI: a taxon-independent community index for eDNA-based ecological health assessment. *PeerJ* **12**:e16963 http://doi.org/10.7717/peerj.16963