

# Low-cost, portable sequencing enables experimental environmental DNA monitoring.

## Monitoring plant biodiversity in aquatic eDNA with low-cost Nanopore Flongle sequencing

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### Premise of the study

Cellular and cell-free DNA obtained from environmental samples of soil, water, and air can detect local biodiversity. Environmental DNA (eDNA) sequencing typically relies on standard molecular techniques and resources. Here we present a fully portable workflow designed to work with limited infrastructure for the collection, isolation, extraction, sequencing, and analysis of aquatic eDNA for the monitoring of plant biodiversity.

### Methods

We extracted aquatic eDNA from an open pond and a seasonal wetland. Samples were amplified targeting 350bp to 890bp segments from *psbA3*, *rbcL*, *ITS2*, *18S*, *trnL* genomic regions. Amplified DNA was barcoded, pooled up to twelve reactions per run, and sequenced on the Oxford Nanopore MinION sequencer using the low-throughput Flongle flowcells. Amplicon sequences were classified using

Kraken2 and BLAST followed by a lowest common ancestor algorithm using the NCBI taxonomy. Figure 1

The full protocol is available  
[dx.doi.org/10.17504/protocols.io.bc4wiyxe](https://doi.org/10.17504/protocols.io.bc4wiyxe)

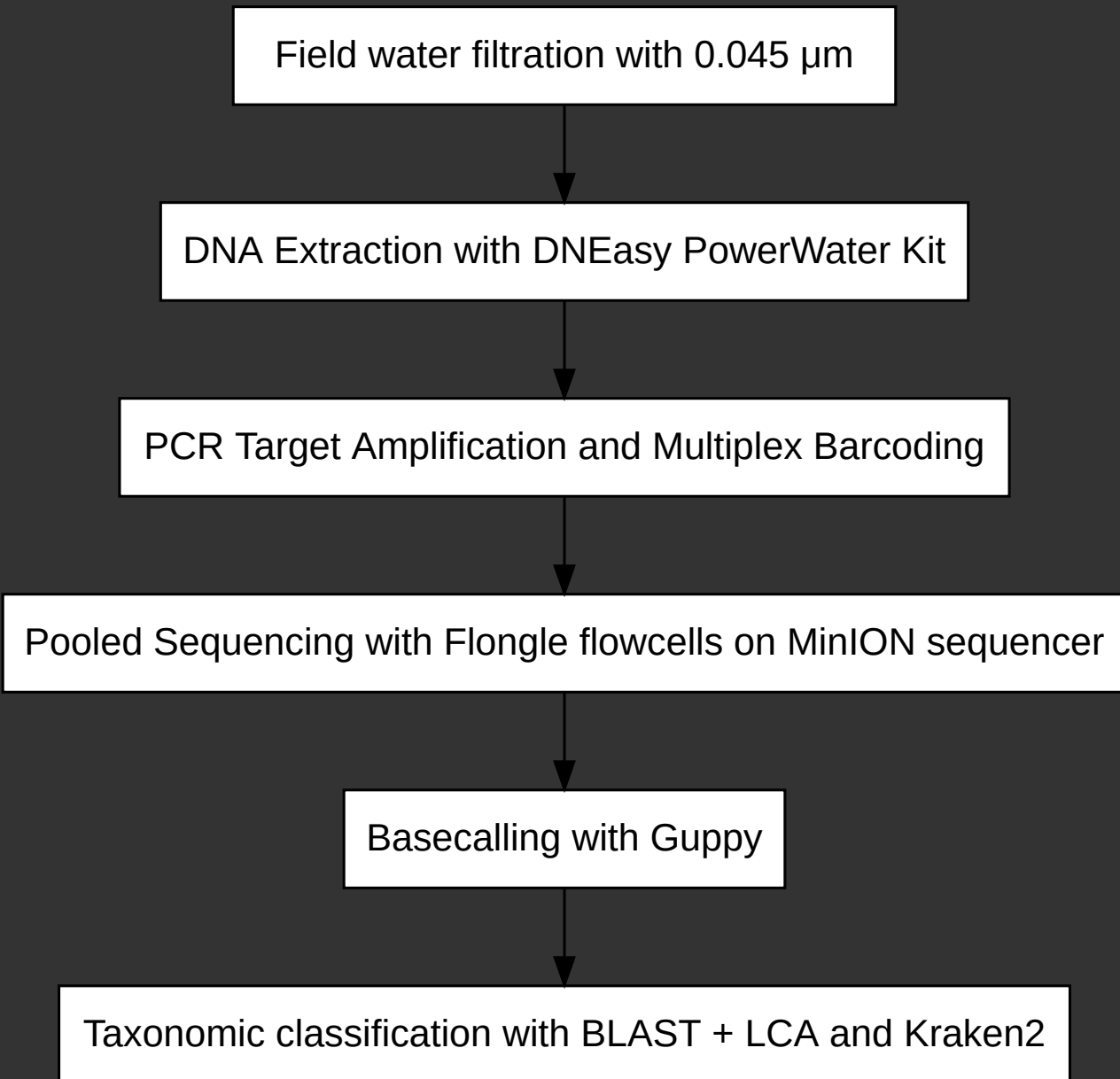


Figure 1: General Methodological Workflow

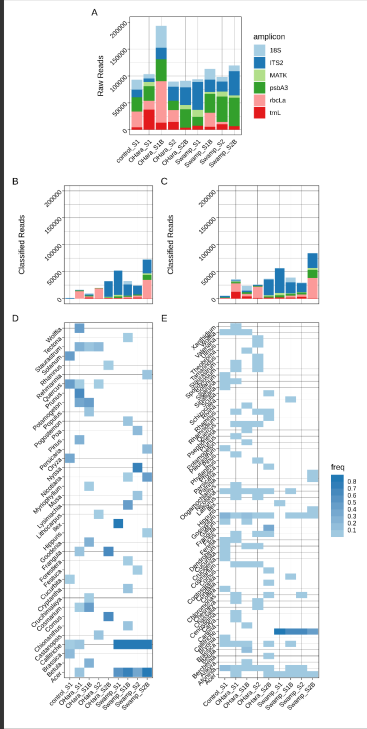


Figure 2: eDNA Classification Results

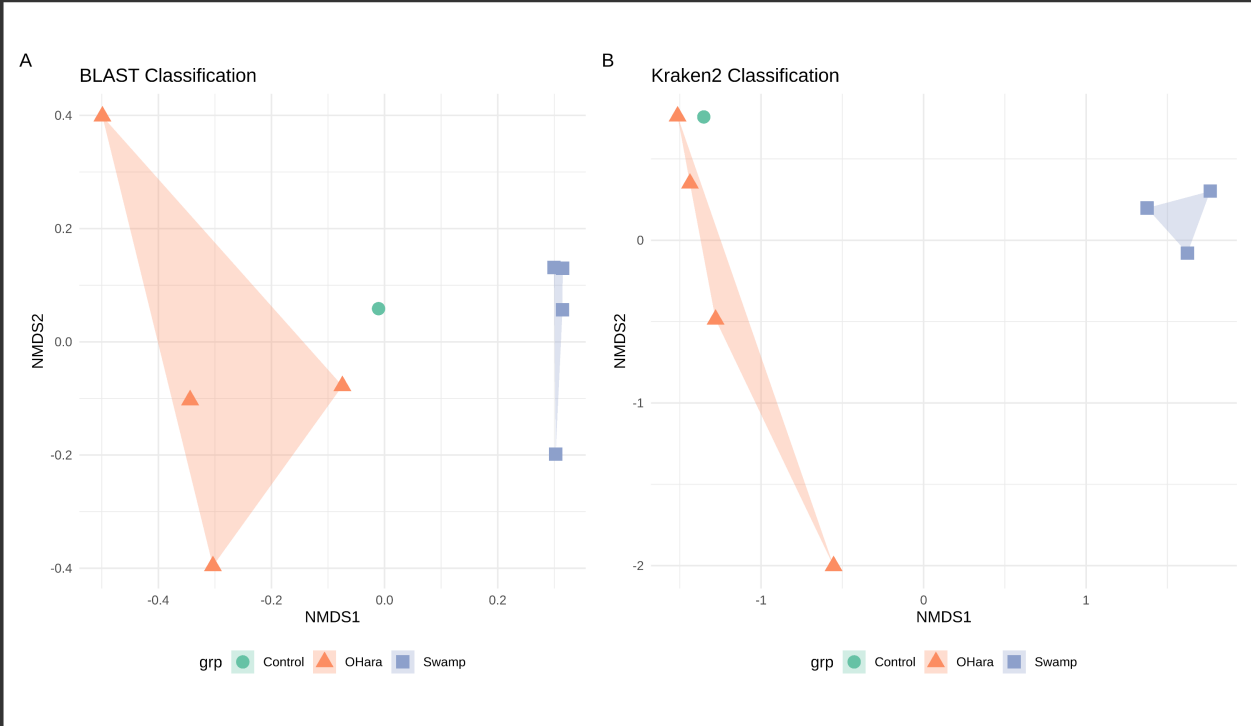


Figure 3: Ordination plots

### Results

Sequence analysis reveals highly local differences in plant diversity including both aquatic and terrestrial plant taxa Figure 2 and Figure 3.

The workflow built around this platform provides end-to-end amplicon-based sequencing that can be used to detect nearby plant diversity. The Oxford Nanopore MinION sequencer and the low throughput Flongle flowcell provides a sequencing platform that combines portability, low cost, and ease of use that are essential features for field applications of amplicon metabarcoding.

### Resources

- Code Repository
- NCBI BioProject: PRJNA488629

