



Conference Abstract

# A Novel Metagenomic Workflow for Biomonitoring across the Tree of Life using PCR-free Ultra-deep Sequencing of Extracellular eDNA

Shivakumara Manu ‡

‡ CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India

Corresponding author: Shivakumara Manu ([smanu@ccmb.res.in](mailto:smanu@ccmb.res.in))

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## Abstract

Biodiversity is declining on a planetary scale at an alarming rate due to anthropogenic factors. Classical biodiversity monitoring approaches are time-consuming, resource-intensive, and not scalable to address the current biodiversity crisis. The environmental DNA-based next-generation biomonitoring framework provides an efficient, scalable, and holistic solution for evaluating changes in various ecological entities. However, its scope is currently limited to monitoring targeted groups of organisms using metabarcoding, which suffers from various PCR-induced biases. To utilise the full potential of next-generation biomonitoring, we intended to develop PCR-free genomic technologies that can deliver unbiased biodiversity data across the tree of life in a single assay. Here, we present a novel metagenomic workflow comprising of a lysis-free extracellular DNA enrichment protocol from large-volume filtered water samples, a completely PCR-free library preparation step, an ultra-deep next-generation sequencing, and a pseudo-taxonomic assignment strategy using the dual lowest common ancestor algorithm. We demonstrate the utility of our approach in a pilot-scale spatially-replicated experimental setup in Chilika, a large hyper-diverse brackish lagoon ecosystem in India. Using incidence-based statistics, we show that biodiversity across the tree of life, from microorganisms to the relatively low-abundant macroorganisms such as Arthropods and Fishes, can be effectively detected with about one billion paired-end reads using our reproducible workflow. With decreasing costs

of sequencing and the increasing availability of genomic resources from the earth biogenome project, our approach can be tested in different ecosystems and adapted for large-scale rapid assessment of biodiversity across the tree of life. \*1

## Keywords

Environmental DNA, Extracellular DNA, Next-Generation Biomonitoring, PCR-free, Shotgun Sequencing, Metagenomics, Tree of Life

## Presenting author

Shivakumara Manu

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## Conflicts of interest

None

## Endnotes

\*1 A preprint manuscript of this work is available on [Authorea](#).