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Metabarcoding: A Molecular Phylogenetic Tool for Large scale, Rapid Assessment of Species Diversity

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Abstract

Metabarcoding is a DNA barcoding process that allows for the simultaneous identification of several taxa within the same sample. This technique can be synonymously applied to environmental DNA (eDNA) barcoding. Primary difference of this technique and the barcoding is that metabarcoding does not focus on one specific organism whereas it aims to determine species composition within a sample. Unlike DNA barcoding, metabarcoding makes use of Next Generation Sequencing approaches for rapid assessment of species diversity.

Introduction

A DNA barcode consists of a short variable gene region that serves as a taxonomic assignment flanked by highly conserved gene regions which can be used for primer design. The idea of DNA barcode as that of one used in supermarkets as Universal Product Code (UPC) was putforth by Paul Hebert and his team from the University of Guelph, Ontario, Canada. Metabarcoding approaches are based on the work of Taberlet (Valentini *et al.*, 2009), taking advantage of Next Generation Sequencing (NGS) technologies for rapid assessment of species diversity in environmental samples.

Different genes have been studies as potential standard barcodes are used depending on studies with single species or metabarcoding several species. Major differences in metabarcoding lies in usage of DNA/RNA from several different organisms derived from one environmental or bulk sample unlike DNA barcoding that uses DNA from single species.

Environmental DNA or eDNA

Environmental DNA (eDNA) is collected from a wide variety of environmental samples such as soil, seawater, snow or even air rather than directly sampled from an individual organism. Since the organisms interact with the environment, the DNA is exuded out and accumulates in its surroundings from various sources which include but not limited to, feces, mucus, gametes, shed skin, carcasses and hair. These samples can be analyzed by high-throughput Next generation sequencing methods, for rapid monitoring and measurement of biodiversity.

Steps in Metabarcoding

1. DNA Extraction

Specific DNA extraction techniques are chosen to isolate DNA from substrates with partially degraded DNA like fossil samples, and those samples containing inhibitors, such as blood, faeces and soil. Normally extractions quality

and yield is expected to be low when done with ancient DNA facility, together with established protocols to avoid contamination with modern DNA.

2. PCR Amplification

DNA barcodes serve as primers for the polymerase chain reaction of the isolated DNA sampes. The most commonly used DNA barcode region for animals is mitochondrial gene cytochrome oxidase I (CO1) coding gene and other barcode regions used for species identification of animals are ribosomal DNA (rDNA) regions such as 16S, 18S and 12S and mitochondrial regions such as cytochrome B.

3. Sequencing

Normally done through the next generation technologies sequencing platforms like Illumina, PacBio Sequel, Ion torrent sequencing *etc*.

4. Data Analysis

A reference database containing barcodes generated from vouchered specimens deposited in a natural history museum or research institute is used Example include International Barcode of Life Project (iBOL) and Consortium for the Barcode of Life (CBOL). Other well-known barcode repositories are NCBI GenBank, DNA databank of Japan, European Molecular Biology laboratory, UK and the Barcode of Life Data System (BOLD) databases.

Workflow of DNA Metabarcoding

Samples are collected from a variety of different environments using appropriate sampling and collection techniques. DNA is then prepared from the samples in the laboratory by adopting standard extraction procedures depending on the source samples which shall be used to answer a variety of ecological questions. Metabarcoding answers questions about "who" is present in the environment whereas the function of communities or individuals in that particular microclimate is established using a metagenomics, single-cell genomics or metatranscriptomics studies only.

Metabarcoding workflow includes sampling from the environment, genetic analysis of the samples by PCR followed by sequencing, bioinformatics analysis of sequences against the databases and ecological analysis for understanding the diversity, community structure and population dynamics (Fig. 1)

Constraints Faced Working with eDNA Samples

• The samples of complex mixture normally contain degraded DNA.

• The barcode primers must be highly versatile to equally amplify the different target DNAs for species discrimination.

• Problem of the taxonomic resolution when using very short DNA barcodes.

• Problem of the reference database libraries when using non-standard barcodes for matching against the subject sequences.

• eDNA metabarcoding lack whole organisms, no *in situ* comparisons can be made whereas the sequences generated from community DNA metabarcoding can be taxonomically verified.

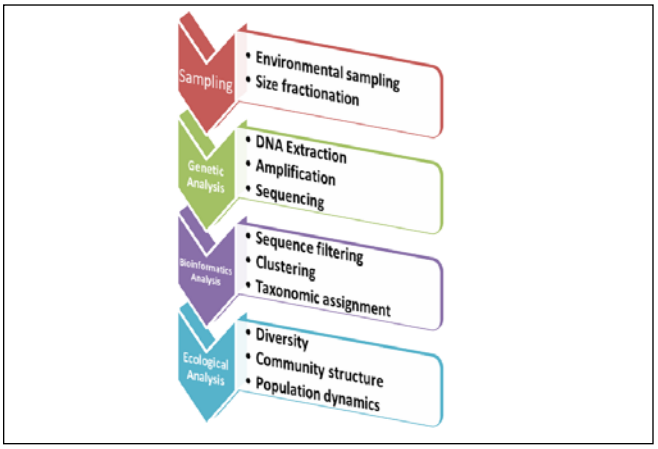

Figure 1: Metabarcoding Workflow (Adapted from Brant *et al*., 2020)

Applications of Environmental DNA Metabarcoding

eDNA barcoding has applications in aquatic and terrestrial ecosystems, which include-

1. Understanding ancient ecosystems.
2. Plant pollinator interactions.
3. Diet analysis.
4. Invasive species detection.
5. Pollution response.
6. Air quality monitoring.

Conclusion

In ecological studies potent challenge is always the data collection with high precision and accuracy to detect and manage dynamic global changes. However, a major hurdle with regard to this is time-consuming and challenging process of sorting and identification of organisms. This has been sorted out by DNA metabarcoding as a biodiversity observation tool which provides a potential solution. As Next Generation sequencing technologies become more rapid and cost-effective, in years to come a "big data" revolution is anticipated with greater sample processing capacity higher

and more accurate taxonomic resolution outputs, more efficient detection of species diversity in the environment.

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