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MOLECULAR TOOLS IN CONSERVATION GENETICS :- DNA Barcoding, MICROSATELLITE, eDNA

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Abstract

Conservation geneticsrelies on molecular tools to study genetic variation within and among populations of species. These tools help inform conservation efforts by providing insights into population dynamics, genetic diversity, and species interactions. Environmental DNA (eDNA) refers to the genetic material present in environmental samples such as sediment, water, and air. It includes whole cells, extracellular DNA, and potentially even whole organisms. Organisms shed DNA into their environments naturally, and eDNA analysis allows us to detect and study this genetic material without disrupting the ecosystem. Analysis has become a valuable tool for detecting the presence of species in their natural habitats. Microsatellites are short, repetitive DNA sequences found throughout the genome. They are highly polymorphic and exhibit variation in the number of repeats. Microsatellites are commonly used to assess genetic diversity, population structure, and relatedness among individuals within a population. DNA barcoding is a technique used in conservation genetics to identify and classify species based on short, standardized DNA sequences. This method relies on analyzing specific regions of an organism's genome that exhibit high variation between species but remain relatively conserved within species.Unlike physical surveys, eDNA analysis doesn't disrupt the ecosystem. Provides insights into both bacterial and eukaryotic species in various sample types (aquatic, soil, etc.). DNA barcoding provides a rapid and accurate means of identifying species, particularly in cases where traditional morphological identification is challenging due to cryptic species,incomplete specimens, or taxonomic uncertainties. By comparing the DNA barcode of an unknownsample to a reference database of known species, researchers can quickly determine its species identity.

Key Words – eDNA, DNA Barcoding, Conservation, Microsatellite.

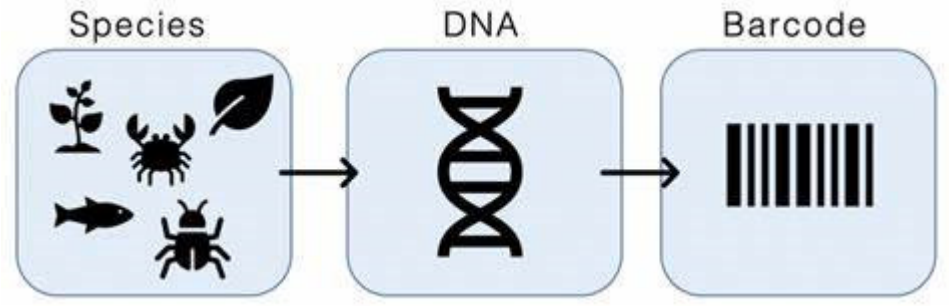
INTRODUCTION

Environmental DNA (eDNA) analysis has become a powerful tool for understanding ecological and evolutionary processes. It allows for the identification of common species, endangered, invasive, and elusive species, generates quantitative indices for species, community diversity, and dynamics, and offers novel opportunities through time-serial samples and unprecedented sensitivity for detecting rare or difficult-to-sample taxa. Despite technical challenges, eDNA is progressing at a rapid pace, with key areas requiring improvement and potential future developments.Conservation efforts to preserve biodiversity rely on biological monitoring to obtain precise data on species distributions and population sizes. Traditional methods, such as visual surveys and counting individuals using distinct morphological characters, often fail due to phenotypic plasticity and closely related species. This results in species databases with errors. Traditional monitoring techniques can also be invasive, such as marine surveys that use destructive techniques. Morphological identification relies heavily on taxonomic expertise, which is

often lacking or in decline. These limitations have led to the demand for alternative approaches to biodiversity monitoring, as traditional methods often fail to accurately represent species distributions and population sizes.

DNA BARCODING

DNA barcoding is a method of species identification using a short section of DNA from a specific gene or genes. It allows for unique identification of an organism to species by comparing it with a reference library of DNA sections. This method is used to identify unknown species, catalog taxa, or compare with traditional taxonomy to determine species boundaries. Similar to how supermarket scanners use UPC barcodes, DNA barcoding helps in identifying and cataloging diverse species.



Barcoding can be performed from tissue from a target specimen, a mixture of organisms (bulk sample), or DNA from environmental samples like water or soil, with varying methods for sampling, preservation, or analysis.To barcode a tissue sample, use a small piece of skin, scale, leg, or antenna. Sterilize used tools between samples to prevent contamination. Collect two samples from one specimen for archive and barcoding, and ensure sample preservation to prevent DNA degradation. This method is recommended for specimens of varying sizes.

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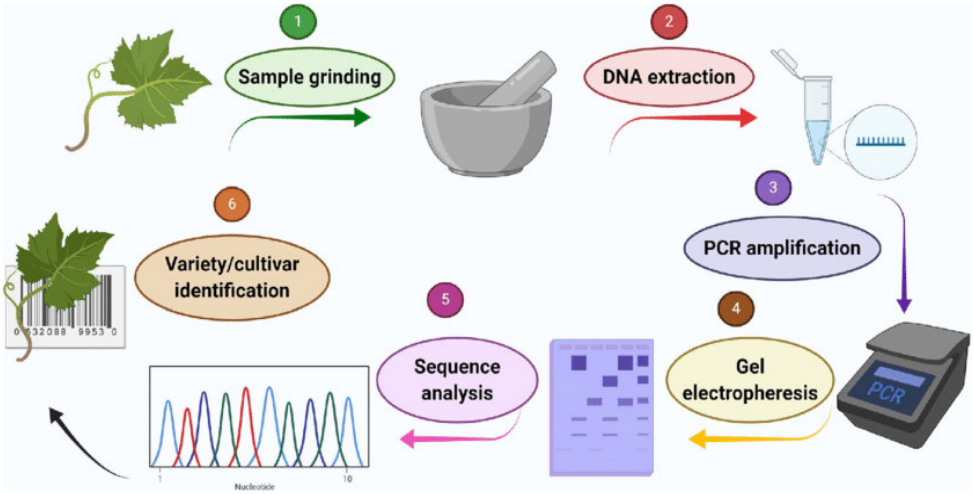


Fig. sequence of DNA extraction

DNA barcoding involves the initial step of collecting a sample from the organism of interest, which can be a tissue sample like leaf or fin tissue or a whole specimen like an insect or fish.

DNA Extraction

The next step involves extracting DNA from the sample using techniques like phenol-chloroform extraction or automated DNA extractors.

PCR Amplification

The extracted DNA is amplified using the polymerase chain reaction (PCR) to obtain enough for analysis, using specific primers designed to amplify the DNA region of interest.

DNA Sequencing

The amplified DNA undergoes sequencing to determine the exact order of its base pairs, employing techniques like Sanger sequencing and next-generation sequencing.

Data Analysis

The DNA sequence is compared to a database of known DNA sequences to determine the species' identity, using computer algorithms that match the DNA sequence to the closest matching species.

Fig. Showing DNA barcode

Potential Applications

DNA Barcoding is a method used to identify organisms from a sample containing DNA from multiple organisms, such as plant leaves, pollen collected from pollinating animals, insect larvae, or animal diets. DNA metabarcoding is used when barcoding identifies organisms from a sample containing DNA from multiple organisms, such as assessing water quality in diatom communities in rivers and streams.

Species identification

DNA barcoding is a method that can identify species in mixed samples, such as insects collected from a specific location, and confirm species identity in cases where traditional methods are difficult or impossible, such as when dealing with small or damaged specimens.

Biodiversity monitoring

DNA barcoding is being used to monitor changes in biodiversity over time, such as the presence or absence of particular species in an ecosystem.

To identify and classify various species, including insects (like moths, sand flies, and bugs), fish, and plants, to understand the biodiversity of different regions in Maharashtra, DNA barcoding is used.

Environmental impact assessment: DNA barcoding can be used to assess the impact of human activities on biodiversity, such as the effects of pollution or habitat destruction and also human-wildlife conflicts.

Conservation Efforts:

DNA barcoding supports conservation efforts by providing information about threatened or endangered species,as seen in studies on fish and plants.

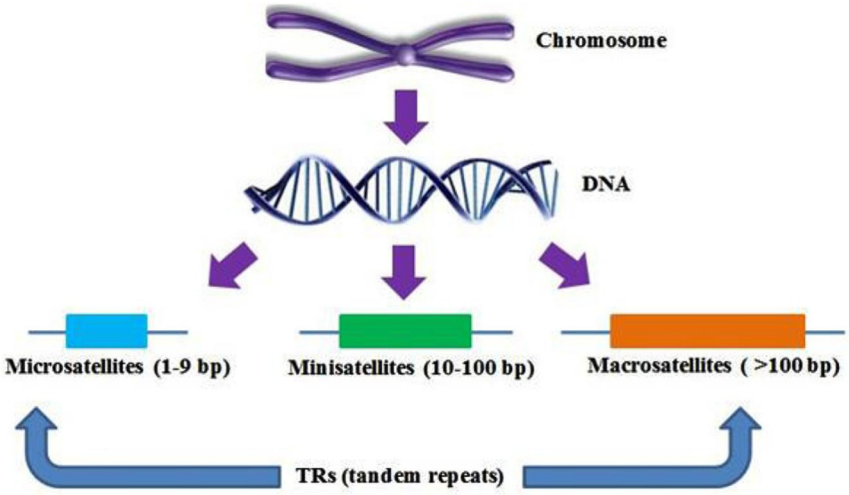
CASE STUDY

The FISH-BOL campaign was initiated in 2005, and currently has barcoded for the cytochrome c oxidase subunit I (COI) gene about 8,000 of the 31,000 fish species currently recognized. This includes the great majority of the world's most important commercial species. Results thus far show that about 98% and 93% of marine and freshwater species, respectively, are barcode distinguishable. One important issue that needs to be more fully addressed in FISH-BOL concerns the initial misidentification of a small number of barcode reference specimens. This is unsurprising considering the large number of fish species, some of which are morphologically very similar and others as yet unrecognised, but constant vigilance and ongoing attention by the FISH-BOL community is required to eliminate such errors. Once the reference library has been established, barcoding enables the identification of unknown fishes at any life history stage or from their fragmentary remains. The many uses of the FISH-BOL barcode library include detecting consumer fraud, aiding fisheries management, improving ecological analyses including food web syntheses, and assisting with taxonomic revisions.

Microsatellite

A **microsatellite** is a tract of repetitive DNA in which certain DNA motifs (ranging in length from one to six or more base pairs) are repeated, typically 5–50 times. Microsatellites occur at thousands of locations within an organism's genome. They have a higher mutation rate than other areas of DNAleading to high genetic diversity. Microsatellites are often referred to as **short tandem repeats** (**STRs**) by forensic geneticists and in genetic genealogy, or as **simple sequence repeats** (**SSRs**) by plant geneticists.

Microsatellites and their longer cousins, the minisatellites, together are classified as VNTR (variable number of tandem repeats) DNA. The name "satellite" DNA refers to the



early observation that centrifugation of genomic DNA in a test tube separates a prominent layer of bulk DNA from accompanying "satellite" layers of repetitive DNA.

They are widely used for DNA profiling in cancer diagnosis, in kinship analysis (especially paternity testing) and in forensic identification. They are also used in genetic linkage analysis to locate a gene or a mutation responsible for a given trait or disease. Microsatellites are also used in population genetics to measure levels of relatedness between subspecies, groups and individuals.

Microsatellites or simple sequence repeats (SSRs) are 2-6-bp DNA sequences that are tandemly repeated at each STR locus. The human genome contains 128,000 STRs, with 230 six-nucleotide repeat sites. STRs are polymorphic in populations, making them valuable in genetic mapping and forensics. PCR is the preferred method for analyzing STR polymorphic loci due to their relatively short overall length. Two alleles of an STR locus, one with 6 copies of the GATA repeat and the other with 10 copies, are shown. In a population, there will be many different length alleles at an STR locus. The analysis uses primers that flank the locus and produces DNA fragments of different lengths, consisting of the STR span plus the DNA from the STR to the left and right primers. For these two alleles, the DNA fragments differ by 16 bp due to the four-repeat difference in the repeat length. This PCR approach can distinguish homozygotes and heterozygotes, as well as define the actual copy number of each repeat, both from the lengths of the amplified DNAs.

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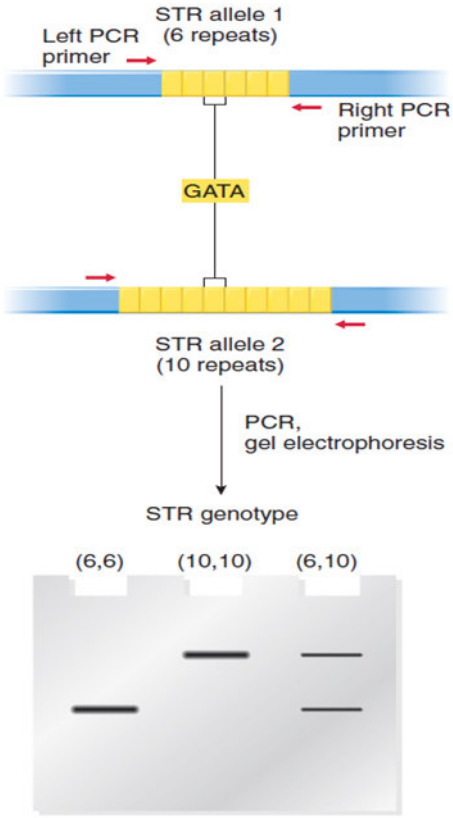


Potential Applications

Use to Monitor reintroduced species:

Microsatellites can be used to track the success of reintroduction programs by monitoring the genetic diversity and population structure of reintroduced populations.

To Identify genetically distinct populations:

Microsatellites can help identify species populations that may be genetically distinct, even within the same species, which can be significant for conservation efforts.

Marker-assisted breeding:

Microsatellites are used to identify desirable traits in plants and animals, which can be used to improve breeding programs.

Use in Gene discovery:

It can be used to identify genes that are associated with specific traits or diseases.

CASE STUDY

The high variability, ease, and accuracy of assaying microsatellites make them the marker of choice for high-resolution population analysis . Microsatellites with only a few alleles are well suited for population genetic studies, while the more variable loci are ideal for genome mapping and pedigree analysis and the fixed or less polymorphic microsatellite loci are used to resolve taxonomic ambiguity in different taxa Highly polymorphic microsatellite markers have great potential utility as genetic tags for use in aquaculture and fisheries biology. They are powerful DNA markers for quantifying genetic variations within and between populations of species They may prove particularly valuable for stock discrimination and population genetics due to the high level of polymorphism compared with conventional allozyme markers. Microsatellite DNA markers are among the most likely to conform to the

assumption of neutrality and have proven to be powerful in differentiating geographically isolated populations and sibling species and subspecies. The qualities of microsatellites make them very useful as genetic markers for studies of population differentiation and stock identification , in kinship and parentage exclusion and in genome mapping. Microsatellites are also being used as genetic markers for identification of population structure, genome mapping, pedigree analysis, and to resolve taxonomic ambiguities in many other animals besides fishes.

eDNA

The discovery of diverse eDNA from macro-organisms has proven relevant in conservation settings, showing its relevance in various environments, including ancient and modern, terrestrial and aquatic. DNA from macro-organisms in environmental samples differs from targeting microbial organisms, as the former is present only as parts of the organism, while the latter can be detected by DNA from whole, living organisms present in the samples.

eDNA metabarcoding is an innovative method for assessing biodiversity, involving extracting DNA from environmental samples and amplifying it using polymerase chain reaction. Next-generation sequencing generates thousands to millions of reads, allowing species presence determination and overall biodiversity assessment. This interdisciplinary approach integrates traditional field-based ecology with molecular methods and advanced computational tools.

The process of eDNA analysis involves several steps:

Sample Collection: Collect an environmental sample of interest (e.g., water, soil).

DNA Extraction: Extract and purify the DNA from the sample.

Amplification: Using techniques like PCR , specific region of DNA will amplify. Amplified DNA is sequenced **next-generation sequencing (NGS)** associated with different species.

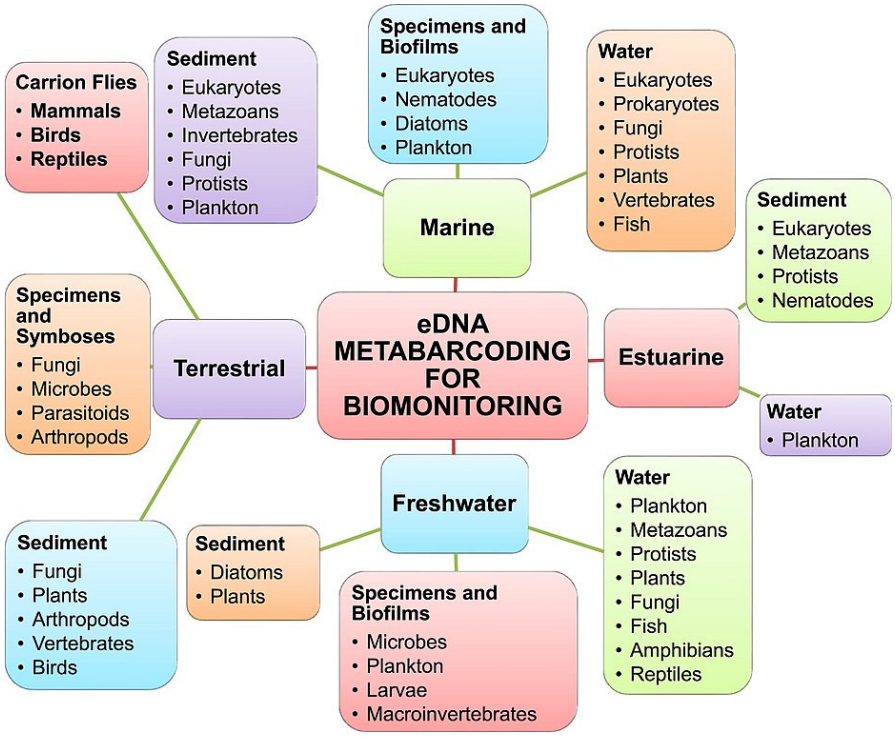
Species Identification: Analyze the sequenced DNA to identify the species present in the sample by comparing with available Database. eDNA can be used to monitor species throughout the year and is particularly useful in conservation monitoring.

Challenges and Considerations:

Degradation: eDNA degrades over time, so timely sampling is crucial.

Background Noise: Environmental samples may contain DNA from other sources (e.g., decomposed organic matter).

Spatial Variation: eDNA distribution can vary within a habitat. eDNA analysis is a powerful tool for detecting species presence without disturbing their natural habitats. It contributes to biodiversity monitoring, conservation efforts, and our understanding of ecosystems molecular tools in Conservation genetics.



Potential Applications

In Maharashtra for **Fish Population Monitoring:**

To assess fish diversity and distribution in rivers, lakes, and coastal areas of Maharashtra, eDNA metabarcoding is used. This is particularly useful for identifying changes in fish populations due to pollution, habitat degradation, or other environmental factors.

Challenging the illegal wildlife trade

To determine the provenance of the ivory tusk or product, environmental DNA from ivory tusks can be compared to environmental DNA collected from wild elephant families. Because of the precision of these methods, the tusk's origin may be linked to the precise family of elephants as well as the precise area from whence the animal originated. For individuals looking to track out the international supply chains and trading routes of illicit goods, this kind of provenance data is essential.

Using environmental DNA to detect and track COVID-19 in wastewater

eDNA techniques were being used for disease detection before the pandemic, their use was significantly increased due to the rapid spread of COVID-19.Researchers discovered pieces of viral genetic material in wastewater, specifically RNA in the case of SARS-CoV-2. Several days before SARS-CoV-2 RNA is discovered by clinical surveillance, it can be found in environmental samples. This makes it a valuable method for tracking patterns over time and for giving early indications of emerging epidemics or clusters.

Using air capture eDNA methods to detect crop pests and pathogens

eDNA can be gathered, examined, and utilized to identify pests and diseases that could harm crops by using air filter devices.Air eDNA surveillance can identify fungal spores as they travel through the atmosphere before they generate symptoms of disease. Instead of spraying as a prophylactic measure, this might serve as an early warning signal, enabling farmers to only spray when early indications of infection are found.

Use to link suspects to crime scenes

Unique vegetation at a crime scene can be linked to plant eDNA found in the dirt on a suspect's shoe sole. Characterizing the microbiological fungi that grow on plant surfaces, such leaves, which are frequently specific to plants in a given area and ecosystem, is how this is accomplished. This can offer vital connections between crime scenes and suspects.

Using eDNA from honeybees to monitor ecosystem health

Since 2018, honey samples have been gathered from all over the United Kingdom with the assistance of beekeepers. Advanced eDNA barcoding techniques are used to analyze these samples and determine the type of plant pollen that is present. This provides us with information about the food sources for bees in various regions of the nation and throughout the year. This data aids in the identification of potential dangers to pollinating insects' floral supplies. Because they are especially vulnerable to environmental stressors including climate change, the appearance of new illnesses, pesticide usage, and changes in land use, bees and their honey provide ideal indicators for the health of an ecosystem as a whole.

REFERENCES

- DNA Barcoding- Definition,Types, Procedure, History, Applications
- MOLECULAR BIOLOGY / By sciencevivid / December 26, 2022
- IGenetics : a Mendelian approach : Russell, Peter J : Free Download, Borrow, and Streaming : Internet Archive
- Thomsen, Philip Francis; Willerslev, Eske (1 March 2015). "Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity
- Environmental DNA: An Emerging Tool for Understanding Aquatic Biodiversity
- Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity overlay panelPhilip Francis Thomsen, Eske Willerslev
- Microsatellites: Methods and Protocols
- Application of Microsatellite Markers in Conservation Genetics and Fisheries Management: Recent Advances in Population Structure Analysis and Conservation StrategiesP M Abdul-Muneer [1,2,*]
- FISH-BOL, a case study for DNA barcodesRobert D Ward
- chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://royalsociety.org/-/media/policy/projects/environmental-dna/environmental-dna-case-studies.pdf
- https://pmc.ncbi.nlm.nih.gov/articles/PMC10138012/
- ScienceDirect.com