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Popular Article

DNA BARCODING - A Diagnostic Tool for Parasitic Diseases

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DNA Barcode is short gene sequence taken from standardized portions, of the genome used to identify species on the basis of their nuclear 18S and 28S rRNA genes, ITS1 and 2 regions, mitochondrial 12S , 16S ,30 rRNA, COI, COII, cytB, and ND1 gene etc. (Chan1 *et al*., 2022). These tpe of species identifications were also done by using molecular markers (e.g., allozymes, rDNA, and mtDNA) from long back (Avise, 2004).

Origin of DNA barcode

- Ribosomal RNA (small subunit 16sRNA of prokaryotes and 18s RNA of eukaryotes)
- ITS-90.5% species discrimination
- Cytochrome-c oxidase 1 (CO1 gene)-Standard DNA Barcode for animals
- Distinguishes 95% species

Why CO1 for DNA Barcoding?

- ✓ Greater difference among species in mitochondrial cytochrome oxidase,
- ✓ Protein distinguishes closely related- species,
- ✓ High Copy number 100-10,000.

Why DNA Barcoding?

- ✓ Identification of species using standardized DNA fragment is fast and reliable.

- ✓ The ideal DNA Barcoding procedure starts with well-curated voucher specimens deposited in natural history collections, ends with a unique sequence deposited in a public reference library of species identifiers that assign unknown sequences to known species.
- ✓ Can works with fragments.
- ✓ It will work for all stages of life.
- ✓ Unmasks looks-alike.
- ✓ Reduces the ambiguity.
- ✓ Electronic handheld field guide for future references.
- ✓ Value of collection etc.

Tools for DNA Barcoding

- DNA barcoding utilizes one or more standardized short DNA regions for taxon identification by new sequencing techniques, such as Next-generation sequencing (NGS), ONT MinION nanopore sequencing, Pac Bio sequencing, metagenomics etc.
- Consortium for Barcode of Life (CBOL) identify the universal barcode gene, such as CO1 in metazoans; rbcL, matK, and ITS in plants; ITS in fungi; 16S rRNA gene in bacteria and archaea creating a reference DNA barcode library (Antil *et al.,*2023) etc.

Related Terminology

- **Metabarcoding:** It is a rapid method of high-throughput, DNA-based identification of multiple species from a complex and possibly degraded sample of eDNA or from mass collection of specimens. The metabarcoding approach is often applied to microbial communities, but can be also applied to meiofauna or even megafauna.
- **Operational Taxonomic Unit (OTU):** The taxonomic level selected to be used in a study, such as individuals or bacterial strains, populations, species, or genera.

Demerits of metabarcoding

DNA barcoding involves sequencing one well-curated individual at a time while, metabarcoding entails massive parallel sequencing of complex bulk samples for which morphological identification and curation is not practical (Y. J i*et al.,* 2014)

Criteria of DNA barcode

- **Discrimination**-DNA locus should be differed in species
- **Universality**-Primers should be amplified consistently
- **Robustness**-Locus should amplify reliably and sequences well etc.

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Barcode of Life-data system

The BOLD data system is central to the DNA barcoding approach.

The specificities of BOLD are-

- To assemble standard information on voucher specimens using common description fields (DNA tag, specimen taxonomy, specimen details, collection information, voucher pictures)
- Dynamic status that allows taxonomic revisions and reassignment of the deposited sequences

DNA Barcoding Organizations

Variations among different species are big challenge to handle the about 19 million species DNA Barcode data. Registry in the biorepositories is very much necessary. The ICI alliance of researchers and organizations is working with the leading 21 countries for the handling of these types of data to store them. As examples-

- CBOL-Consortium for the Barcode of Life- Assigning the unidentified species to known Taxonomy collection for any similarity.
- iBOL-International barcode of life.
- CCDB-Canadian Centre for DNA Barcoding
- ABBI- **All Birds Barcoding Initiative**
- FISH-BOL-Fishes Barcode of Life
- MBI-Mosquito Barcoding Initiative

BOLI (Barcoding of Life Initiative)

Barcoding of Life Initiative (BOLI)/ International Barcoding of Life Initiative is a horizontal approach to genomics, examining short, standardized genome segments across the sweep of eukaryotic life, all 10 million species which also deals the evolution and speciation along with disclosing the cryptic species and a rapid survey of taxon diversity in groups. Life history stages to known species and estimate of species ages, Key features of the mitochondrial genome, acting to flag taxonomic groups or species with unusual nucleotide composition or evolutionary rates, sequence variability within species is generally much lower than divergence among species (barcoding gap) etc. are some other assignments of BOLI and it was established at the rise of the genomics era.

New throughput technologies [next-generation sequencing (NGS)] and new adapted technologies such as matrix-assisted laser desorption–ionization time-of-flight (MALDI–TOF) mass spectrometry open new avenues (Ilina *et al*., 2009; Michelet *et al*., 2014). Among the

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available new technologies, MALDI–TOF) mass spectrometry starts to be widely used as a new tool for barcoding (Diarra *et al*., 2017). Although this technique should be based on respect to biodiversity targets (i.e. Aichi targets), national biodiversity strategies and action plans, monitoring, indicators and assessments, and invasive alien species (Vernooy *et al*., 2010).

Global open access to barcode data

- GenBank
- EMBL-European Molecular Biology Laboratory
- DDBJ-DNA Databank of Japan
- NCBI-National Center for Biotechnology Information
- GBIF- Global Biodiversity Information Facility
- Barcode records in INSDC-The International Nucleotide Sequence Database Collaboration (INSDC) is a long-standing foundational initiative that operates between DDBJ, EMBL-EBI and NCBI.

Cullicoides

DNA barcoding based on the mitochondrial gene (cox1) is used as a rapid and authentic tool for species identification (Sarkar et al., 2008). DNA barcoding for species-level identification employs a small portion (≈ 658 bp) of the cox1 gene to assign a specimen sequence to a voucher species library (Hebert et al., 2003).

Eimeria

Partial (780 bp) mitochondrial (COI) and near complete nuclear 18SrDNA (1,780 bp) sequences compared to assess as markers for species identification and phylogenetic analysis of coccidian parasites (phylum Apicomplexa). Unlike in the 18S rDNA-based phylogenetic reconstructions, *Eimeria necatrix* and *Eimeria tenella* formed monophyletic clades based on partial COI sequences (Ogedengbe *et al*., 2011).

Plasmodium

Martinsen *et al*. (2008) used mitochondrial COI sequences in addition to both plastid and nuclear genes to study the evolutionary relationships and events leading to host switching and diversification in *Plasmodium* spp. of birds, mammals and squamate reptiles.

Filarial nematode: Dataset B (that encompasses all the *coxI* sequences of filarioid nematodes available in GenBank) has been used to perform DNA barcoding and DNA taxonomy with a tree basedmethod.

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Toxoplasma and Trypanosoma

A versatile CRISPR-based method to barcode protozoa, which we successfully apply to *Toxoplasma gondii* and *Trypanosoma brucei.* Using libraries of barcoded *T. gondii* evaluated shifts in the population structure from acute to chronic infection of mice. Contrary to expectation, most barcodes were present in the brain one-month post intraperitoneal infection in both inbred CBA/J and outbred Swiss mice. These data establish the first, robust molecular barcoding approach for protozoa and evidence that the blood-brain barrier does not represent a major bottleneck to colonization by *T. gondii (*Wincott *et al*., 2022).

Challenges in DNA Barcoding

- DNA Barcoding based on mitochondrial genes (COX1) may overestimate the number of species due to the presence of pseudogenes (Song *et al*., 2008).
- Hybridization is the second issue in barcoding. Introgession of mitochondrial DNA due to hybridization and/or incomplete lineage sorting of mitochondrial DNA haplotypes may lead to misidentification (Nesi *et al*., 2011; Pages *et al*., 2013).

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