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Popular Article

DNA Barcoding - a useful tool for taxonomists

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DNA barcoding approach started way before in 2003 by Paul Hebert. DNA barcode is one or more short gene sequences (generally 200–900 base pairs) taken from a standardized portion of the genome to aid species identification at taxonomic level. Thus, the fundamental function of this genetic tool seeks to compare barcode sequences to reference databases to efficiently and effectively assign any biological sample to its species regardless of the visual classification of the sample. International initiatives have been launched across hundreds of countries to evaluate the world's bio-diversities using this new taxonomic tool, and more than 3,21,000 species, covering animals, plants, fungi and others, have been barcoded so far.

The Barcode of Life Data System (BOLD) is a bioinformatics platform serving for the acquisition, storage, analysis and publication of DNA barcode records. Consortium for the Barcode of Life (CBOL) is the only consortium made on Bio-Barcoding and was launched in May 2004.

Molecular Markers for DNA Barcoding:

For DNA barcoding, it is important to have a suitable marker DNA sequence that satisfies a number of conditions. It should be easily and reliably amplified. It must be sufficiently variable in order to distinguish closely related species. One more important factor with respect to DNA barcoding is the absence of variability in length, which can lead to problems with alignment and identification. Markers are usually selected from a minor part of the cell genome, namely, the genomes of haploid organelles (mitochondria and plastids).

Molecular markers used for animal's DNA barcoding are:

Region	Marker	Gene description
Mitochondrion	12S	12S ribosomal RNA
	16S	16S ribosomal RNA
	atp6	ATP synthase F0 subunit 6

	COI	Cytochrome c oxidase subunit I
	cytb	Cytochrome b
	D-loop	Mitochondrial displacement loop region
	ND1	NADH dehydrogenase subunit 1
	ND2	NADH dehydrogenase subunit 2
Nucleus	28S	28S ribosomal RNA
	ITS	Internal transcribed spacer
	Rag1	Recombination activating 1
	Rag2	Recombination activating 2
	WG	Wingless

DNA Barcode of Plants: it is more challenging task compared to animals. Since, plants mitochondrial genes are unsatisfactory for DNA barcoding. Also, the low rate of nucleotide substitution in plant mitochondrial genomes precludes the use of COI as a universal plant barcode. The most satisfactory results have come from the gene maturase K (matK). The matK barcode has discriminated 90 percent of plant species.

DNA Barcode of Bacteria: The 16S rRNA gene is used as DNA barcode marker, as it is highly conserved for each and every species of bacteria.

DNA Barcode of Fungi: The mitochondrial gene encoding Cytochrome c oxidase is used as bio-barcode marker. But, in some classes like Oomycota, the Internal Transcribed Spacer (ITS) region is suitable for identification. In exceptional cases, sequence of large subunit of the nuclear ribosomal RNA (LSU) is used.

DNA Barcode of Virus: Molecular diversity of viral genome is complex. The identification and explanation of molecular entities of virus should be the major objective of DNA barcoding. Very few works have been incited to identify the pathogenically important viruses. And till today, no marker DNA or RNA has been developed for viruses.

DNA Barcode of Protists: The D1–D2 or D2–D3 regions at the 5′ end of 28S rDNA is used as bio-barcode marker. This was successfully used in ciliates, haptophytes, acantharians and diatoms. Also, the large subunit of ribulose-1,5-biphosphate carboxylase–oxygenase gene (rbcL) and the chloroplastic 23S rRNA gene for photosynthetic protists has been used.

DNA barcoding for spotting food adulteration:

The globalization of the food trade demands reliable information about the origin, manufacturing methods, authenticity, and traceability of foodstuffs. Major challenge today is

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deliberate fraudulent replacement, mislabelling of food and its contents or food packaging. Recently, a number of technologies have made it possible to identify food fraud, including DNA detection (such as DNA barcoding), protein quantification (such as ELISA), near-infrared spectroscopy, nanosensors, etc. One among them is DNA barcoding, that analyses and classifies food items using 400–800 bp long standardized unique DNA sequences of mitochondrial (e.g., COI), plastidial (e.g., rbcL), or nuclear (e.g., ITS) origin that are typically evaluated or quantified in order to validate the authenticity and traceability of food. Numerous protein-based techniques, such as immunological, electrophoretic, and chromatographic assays, have been used to assess food quality for evaluating fresh goods, however they are inefficient for assessing heavily processed meals. In such situations, a range of foods can be successfully tested using DNA-based approaches.

DNA barcoding in identifying food adulteration:

1) **Authentic:** If the product has a DNA barcode for a tested product's primary constituent.
2) **Contamination:** If, in addition to the genuine product barcode, a DNA barcode for a species different than the principal constituent is discovered.
3) **Substitution:** If the tested product's primary constituent may be replaced with a DNA barcode for a different species.
4) **Filler:** If a product, such as rice, wheat, or soybean, has a DNA barcode for the known product. Fillers can be substituted for or used to contaminate substances.

DNA barcoding of meat:

The cytochrome c oxidase subunit I (COI) gene of processed beef and poultry items was sequenced using conventional full-length DNA barcoding (650 bp) and minibarcoding (220 bp) in order to verify food products produced from animals, such as meat, poultry, and fish and for classifying tested processed goods. Full barcoding has been proven to be more reliable.

Advancements of DNA Barcoding:

As an extension of DNA barcoding, mini-barcodes are introduced with higher efficiency than regular barcodes owing to their reduced size (typically ≤ 200–300 bp). The advent of high-throughput sequencing (HTS) technologies facilitates the emergence of DNA metabarcoding and revolutionizes our ability to barcode life. By taking advantage of the multiplex nature of next-generation sequencing (NGS) and the third-generation sequencing platform, metabarcoding not only enables assignment of multiple species using DNA barcodes in a mixed sample and makes the data output magnitudes more reliable, but also allows simultaneous processing of DNA barcodes for thousands of diverse specimens in a single sequencing run. A recent work with the real-time MinION sequencer, a portable third generation sequencer, has just achieved great barcode sequencing throughput at a cost of less than 10 cents, showing a promising future in this direction.

Utilities of DNA Barcoding:

DNA barcoding is a chief component of the modern diagnostic toolbox with increasing applications in taxonomy, systems biology and ecological studies. Besides traditional way by

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sampling separate individuals, barcode technology, especially metabarcoding, can be adopted for assessment to dietary items using gut contents and scats of animals, or utilized for analysing environmental samples, such as soil, water and even air that possibly contain DNA materials from life, for biomonitoring and disease screening. Another potentially valuable utility of combining metabarcoding with mini-barcodes is to analyse invertebrate derived DNA (iDNA). It is not surprising that barcoding is highly desirable for customs and national authorities in the conservation area of rare wildlife.

Advantages of DNA barcoding:

1) Economical
2) Time efficient
3) To recognize species at any stage of life even from sparse or imperfect samples, such as stomach contents or faecal remnants.
4) Integration of powerful phylogenetic calculations with web-based barcoding databases (like CBOL).
5) Beneficial for extensive sampling efforts.
6) Used in a variety of scientific fields, including agronomy, ecology, biomedicine, epidemiology, evolutionary biology, biogeography, conservation biology, and the bioindustry.

Limitations of DNA barcoding:

1. Hybridization in mitochondrial DNA can lead to confusion about species boundaries.
2. Rarity of mitochondrial DNA can produce sequence variations.
3. Heteroplasmy - presence of nucleotide differences within a single specimen.
4. Unintentional amplification of nuclear pseudogenes of mitochondrial origin (NumtS) may lead to inaccuracy.

Conclusion:

DNA barcoding no doubt holds great promise for potentially widespread scientific and practical benefits. Although DNA sequencing methods have noticeably advanced since their introduction, the traditional direct approach is as well limited by low speed of morphological analysis. At the same time, next-generation sequencing methods make it possible to accelerate this process fundamentally with the reverse approach. Thus, it is possible to reach the stated global goals of DNA barcoding without exact reference to taxonomy. With the exploration of mini-barcodes and metabarcoding in DNA-based species delineation, it is believed that barcode techniques will be further integrated into a wider context of scientific, political, economic and social areas. To sum up, what we know today is that no single classification approach can be applied universally for all species. DNA barcodes in conjunction with traditional taxonomic tools for sure are more rapid and more reliable than any method alone for disclosing cryptic and overlooked biodiversity.

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