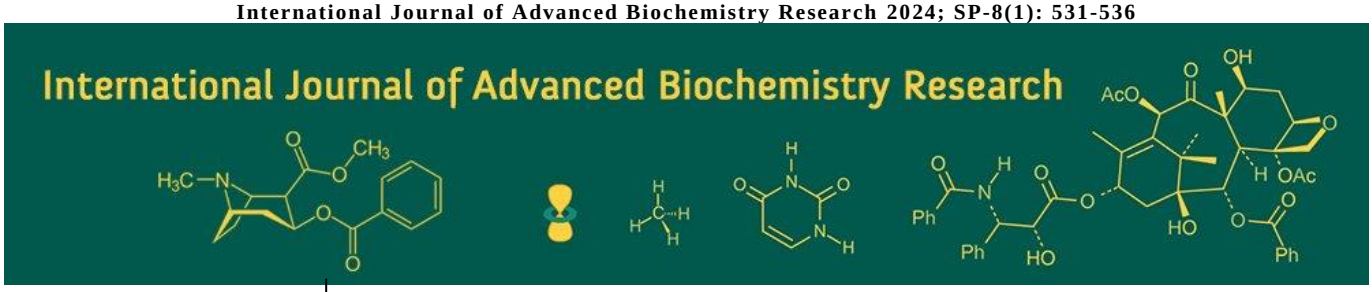

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DNA fingerprinting: Similarities or dissimilarities in your DNA?

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Abstract
Every individual, with the exception of identical twins, possesses a distinctive genetic signature that can be visualized through recombinant DNA techniques. In the DNA of every individual, there exists approximately 0.1% variation, while 99.9% similarity is shared among all individuals. Despite this, the DNA sequence resembles a fingerprint, uniquely identifying each person. It is a revolutionary technique, unveils individual genetic profiles using specific DNA markers. DNA fingerprinting is a technique used to identify and analyse unique patterns within an individual's DNA or to identify the individual by characteristics of their DNA which was introduced by Alec Jeffrey in 1985. The current standard methods in DNA profiling involve both PCR and non-PCR based methods. DNA fingerprinting or profiling find extensive applications in forensic analysis and paternity testing. Beyond law enforcement, DNA fingerprinting permeates medical diagnostics, facilitating disease identification, and tracking hereditary traits. In agriculture, it shapes breeding programs by Analyzing plant and animal lineages. This versatile tool's precision and broad utility underscore its pivotal role in understanding genetic diversity, individual identity and number of biological applications. Therefore, DNA fingerprinting has got considerable attention as a promising method to rapidly evaluate the degree of genetic diversity in both human and animal genetics.

Keywords: DNA, similarities, non-PCR, technological

Introduction
The evolution of DNA fingerprinting involves several key milestones and technological advancements that have refined the techniques used to analyse DNA samples. Alec Jeffrey's seminal work in identifying hyper variable repeat motifs in the human beta-globin gene was a paradigm shift in the field of biology giving birth to forensic biology and the numerous applications developed thereafter (Jeffreys *et al.*, 1985a) [15].

DNA fingerprinting, also known as DNA profiling or genetic fingerprinting, is a technique used to identify and analyse unique patterns within an individual's DNA. DNA fingerprinting techniques have evolved over time, offering various methods to analyse and characterize DNA. It involves examining specific regions of an individual's genetic code to create a genetic profile that is highly distinctive to that individual. Unique DNA patterns emerge when an individual's tandem repeat loci undergo digestion by restriction enzymes. Within individuals from sexually outbreeding populations, the resulting multilocus DNA profiles usually exhibit variability and distinctiveness. These multilocus 'minisatellite' DNA repeats, also termed Variable Number Tandem Repeats (VNTRs), generally comprise repetitive units spanning 10 to 60 base pairs (Nybom *et al.*, 2014) [24]. These segments vary significantly in length, mirroring the variation in restriction enzyme fragments. Detection often involves hybridizing radiolabelled VNTR probes to genomic DNA that has been digested by restriction enzymes and subsequently separated by size. Jeffrey's technique was primary developed as a tool for its application in forensic science but later found its way into various fields like parentage testing, biomedical research, anthropology and population genetics, phylogenetic studies, wildlife and ecology, veterinary science and medical diagnostics (Gill and Werrett, 1987) [9]. This paper aims to trace back the origins, evolution and expected future advancements in DNA fingerprinting technology.

Origins

The discovery of DNA double helix by Watson, Crick and Franklin in 1953 can be considered as the first step in the field of molecular biology. In the early stages of 1985, Jeffreys and colleagues (Jeffreys *et al.*, 1985b) [16] presented the initial development of multilocus DNA fingerprints and suggested that these distinctive DNA patterns unique to individuals might offer a robust method for individual identification and paternity testing. Initially, there were concerns about the prolonged implementation of these applications and the legal challenges that might arise as DNA evidence transitioned from the research laboratory to the courtroom. However, subsequent events proved this prediction overly pessimistic.

As early as April 1985, the first case, involving a UK immigration dispute, was effectively resolved using DNA fingerprinting (Jeffreys *et al.*, 1985a) [15]. Shortly thereafter, DNA evidence was admitted in a UK civil court for a paternity dispute. The introduction of DNA typing in criminal investigations occurred in October 1986 during the Enderby murder case, where the investigation led to the release of a prime suspect proven innocent by DNA evidence (Gill and Werrett, 1987) [9]. By 1987, DNA typing results were admissible as evidence in criminal courts in both the UK and the USA. Subsequently, in 1988, the UK Home Office and Foreign and Commonwealth Office, officially approved the use of DNA fingerprinting to resolve immigration disputes related to contested family relationships. In 1989, the USA witnessed the first significant challenge to the procedural and scientific validity of DNA typing in forensics (Lander, 1989 and 1991). This led to a substantial independent review by the US Congress Office of Technology Assessment in 1990. The assessment concluded that DNA-based identification was scientifically sound provided appropriate technology, quality control, and quality assurance procedures were in place.

A) Non-PCR Based Method

1. Restricted fragment length polymorphism

Restricted fragment length polymorphism (RFLP) was the first method used for DNA Fingerprinting by Alec Jeffrey. It involved the detection of variations in DNA sequences among individuals by using restriction enzymes. These enzymes recognize specific DNA sequences and cut the DNA at those sites, resulting in fragments of varying lengths. The cut DNA fragments are separated by size using gel electrophoresis, creating a pattern of bands. Radioactive or fluorescent probes, complementary to specific DNA sequences, are used to identify and bind to the fragments of interest. The location and intensity of the bands on the gel are visualized using X-ray films or fluorescent imaging, revealing the unique pattern of DNA fragments. The resulting pattern of DNA fragments, observed as bands on the gel, constitutes the individual's DNA fingerprint. Variations in the number and size of fragments among individuals create distinct patterns that can be compared for identification or genetic analysis purposes. RFLP analysis was initially used in various fields, including forensic science, paternity testing, genetic research, and population studies. However, due to limitations such as the need for a large DNA sample, susceptibility to degradation, and being labour-intensive (Brettschneider, 1998) [2]. RFLP has largely been replaced by more advanced and sensitive DNA fingerprinting techniques, such as PCR-based methods (Jeffreys *et al.*, 1985a) [15].

B) PCR Based Methods

Polymerase Chain Reaction (PCR) is a fundamental molecular biology technique used to amplify a specific segment of DNA, creating millions of copies of that particular DNA sequence. This method, developed by Kary Mullis in the 1980s, revolutionized genetic research, diagnostics, and greatly optimised DNA fingerprinting.

2. Variable number tandem repeat

Variable Number Tandem Repeat (VNTR) analysis is a DNA profiling technique that examines variations in the number of repeated DNA sequences, known as variable number tandem repeats or minisatellites. These repeats consist of sequences of DNA typically ranging from 10 to 60 base pairs, repeated in tandem arrays within the genome. Although the high degree of length polymorphism among minisatellites indicates that they are fast evolving sequences, most of them are in fact quite stable, and neomutated alleles have been observed only at a few loci (Vergnaud and Denoeud, 2000) [29]. VNTR analysis involves; DNA extraction via saliva, blood, hairs etc., then specific regions of the DNA containing VNTR sequences are amplified using Polymerase Chain Reaction (PCR). Primers targeting the flanking regions of the VNTR segments enable selective amplification. The amplified DNA fragments are separated by size using gel electrophoresis or other fragment analysis techniques. The number of repeats at each VNTR locus is determined based on the size differences of the resulting fragments. The pattern of different-sized fragments obtained from VNTR analysis provides a unique genetic profile specific to each individual (Vergnaud and Porcel, 2006) [30]. VNTR analysis was historically used in forensic investigations, similar to Short Tandem Repeat (STR) analysis, to create DNA profiles for identifying individuals and comparing DNA evidence from crime scenes. However, due to technical limitations and the development of more advanced methods like STR analysis, VNTR analysis is less commonly used in forensic practice today.

3. Short Tandem Repeats

Short tandem repeats (STRs), also called microsatellites, comprise patterns of 1–6 base pairs (bp), constituting approximately 3% within the human genome. Their repetitive structure leads to DNA replication errors, causing frequent mutations in the number of repeats. Consequently, STRs display mutation rates substantially higher than other genetic variations, contributing significantly to human genetic diversity. The method of Short Tandem Repeat (STR) analysis is employed in DNA profiling to identify individuals based on unique variations within specific repetitive DNA sequences known as short tandem repeats or microsatellites. These sequences, found in non-coding regions of the human genome, are inherited from both parental sources. In forensic applications, scrutiny of Short Tandem Repeat (STR) loci is routine. Amplification of small DNA regions facilitates successful outcomes, especially from heavily degraded material where the DNA fragment length may be < 500 base pairs. This method supersedes conventional analysis utilizing single locus probes (SLPs). However, dimeric STR loci often manifest

stutter artifacts; thus, in casework, preference is given to STRs limited to tri or tetrameric loci (Gymrek, 2017) [13]. The application of STR analysis extends across forensic science, paternity verification, and human identification due to its exceptional discriminatory ability, precision and reliability. Its basis lies in the significant variation in the number of repeats at each STR locus among individuals, making it highly improbable for two unrelated persons to share an identical STR profile. Apart from its established roles in forensics, paternity testing, anthropology and medical research, STR analysis has recently gained traction in commercial genetic testing services. These services facilitate ancestry testing and genealogical exploration by comparing STR profiles, enabling individuals to trace their ancestral lineage and familial connections.

4. Amplified fragment length polymorphism

The AFLP method stands as a potent DNA fingerprinting tool applicable across various organisms, requiring no prior sequence information. Initially designed to craft dense linkage maps for gene positional cloning and molecular breeding, this technique follows a protocol encompassing distinct stages. Firstly, the DNA undergoes restriction and adapter ligation, subsequently advancing to selective amplification of restricted fragments via PCR. In this step, primer annealing targets the adapter and restriction site sequences, facilitating PCR amplification of specific fragments. The selective amplification relies on primers that extend into the restriction fragments, allowing only the amplification of fragments aligning with the primer extensions and flanking nucleotides. This approach enables visualization of fragment sets via PCR, bypassing the need for nucleotide sequence knowledge. While the method facilitates specific co-amplification of numerous restriction fragments, the quantity analysed simultaneously depends on the detection system's resolution. Typically, denaturing polyacrylamide gels accommodate the amplification and detection of 50-100 restriction fragments (Vuylsteke *et al.*, 2007) [33]. AFLP emerges as an innovative and robust DNA fingerprinting technique adaptable to DNAs of diverse origins or complexities. Its reliability, robustness and quantitative nature make it significant. The quantitative aspect has been leveraged for co-dominant scoring of AFLP markers, especially in sample collections like F2 or back-cross populations, utilizing tailored AFLP scoring software.

5. Mitochondrial DNA (mtDNA) Analysis

Mitochondrial DNA differs from nuclear DNA by being solely inherited from the mother. The endosymbiont hypothesis stands as the primary explanation for the origin of the mitochondrial genome and the intricate structure and functions of mitochondria themselves (Gray and Doolittle 1982) [11]. This theory suggests that mitochondria evolved as bacterial endosymbionts that eventually merged with a host cell, contributing to the nuclear genome. While mitochondria maintain some autonomy by retaining a distinct genome that undergoes replication and expression, they are unable to exist independently. In forensic investigations, mitochondrial DNA (mtDNA) analysis becomes valuable when nuclear DNA is inaccessible, deteriorated or insufficient for identification purposes (Gray, 1983) [12]. It aids in identifying missing individuals, establishing familial relationships and scrutinizing evidence collected from crime scenes. In Population Genetics, the analysis of Mitochondrial DNA offers insights into human evolutionary paths, migration patterns and the genetic diversity among diverse populations. It facilitates the tracing of maternal lineages and provides comprehension of ancient population migrations (Max Taylor, 1987) [1]. Due to its distinct inheritance pattern and resilience when dealing with degraded samples, mitochondrial DNA analysis remains an invaluable tool across various scientific domains, mtDNA analysis serves as a means to investigate inherited mitochondrial disorders, explore diseases associated with mitochondrial dysfunction, and delve into potential therapeutic interventions in medical research.

6. Y-chromosome analysis

This technique focuses on specific regions of the Y chromosome, which are passed down from father to son. It's utilized in paternity testing, male lineage identification and forensic analysis involving male-specific DNA markers (Kayser, 2017) [18]. The sex-determining function of the Y chromosome means that it is paternally inherited and haploid. Because of this haploidy, most of the chromosome does not recombine with any other at meiosis. These properties have important consequences for its population genetics, since Y chromosomes are passed down from father to son unchanged except by the gradual accumulation of mutations. In principle it is possible to reconstruct the histories of paternal lineages by comparing modern Y-chromosomes using DNA polymorphisms (Jobling *et al.*, 1997) [17]. The aim is to build phylogenetic trees, and to find out about population histories. Such studies are now yielding useful information, and complement data on human evolution which come from mitochondrial DNA (mtDNA) and from autosomes.

7. Single nucleotide polymorphism analysis

SNP is defined as a nucleotide site, for which a high substitution rate has been shown among individual samples in a population. SNPs are single base pair positions in genomic DNA, at which different sequence alternatives (alleles) exist in normal individuals in some populations, herein the least frequent allele has an abundance of 1% or greater (Brookes, 1999) [4]. The restriction posed on frequency distinguishes SNPs from rare point mutations and implies the use of the former as genetic markers. Unlike the methods discussed before, SNP analysis is a microarray-based method, this implies that SNPs can be analysed simultaneously by application of DNA microarrays. Although in principle, any of the four nucleotide bases could be found at each position within a sequence, single nucleotide polymorphisms (SNPs) tend to exhibit a biallelic nature in practice. This is partly attributed to the infrequent occurrence of single nucleotide substitutions, estimated to be between 1×10^{-9} and 5×10^{-9} per nucleotide and per year at neutral positions in mammals. Consequently, the likelihood of two independent base changes happening at a single position is exceedingly low. Another contributing factor is a bias in mutation, resulting in the predominance of two distinct SNP types. Two primary methods were employed to generate high numbers of SNPs. The first involved shotgun sequencing of reduced genome representations. The second method compared sequences from overlapping regions of large insert BAC (bacterial artificial chromosome) clones sequenced during the Human Genome Project (HGP). By March 2001, a public database

contained 2.84 million deposited SNPs, with 1.65 million being non-redundant. SNP mapping utilized sequence comparison with the assembled human genome, resulting in a map of 1.42 million SNPs by February 2001, establishing an average density of one SNP per 1.91 kilobases. Several key conclusions emerge from this research. For instance, the normalized measure of heterozygosity (π) indicates the probability of a nucleotide position being heterozygous when comparing two randomly chosen chromosomes from the population. In the human genome, $\pi = 7.51\times10-4$, implying an expectation of one SNP occurring approximately every 1,331 base pairs when comparing two chromosomes. These dense SNP distributions enable comprehensive genome-wide studies, offering fresh perspectives on population and genome dynamics (Vignal *et al.,* 2002) [31]. SNPs exhibit distinctive characteristics, including their high frequency in genomes, averaging around one SNP per 1000 bases. They are found in both coding and non-coding regions, and a substantial number ranging from thousands to several hundred thousand can be genotyped in a single reaction. In comparison to microsatellites, the mutational mechanism of SNPs is better comprehended. Since 2015, SNPs have become the predominant molecular markers in the assessment of genetic diversity in local farm animals. Commercial high-throughput arrays are now available for the majority of livestock species, with two major options: (i) Illumina's Infiniumi Select Microarray or Bead Chip, utilizing single nucleotide extension or allele-specific primer extension, and (ii) Affymetrix's Gene Chip or Axiom Array, employing molecular inversion probe hybridization (Cortes *et al.,* 2022) [6].

In contrast to multiallelic markers, analysis of biallelic SNP markers can be practically fully automated. Thus, using modern technologies, the effectiveness of SNP analysis can be many times higher than that of other methods of DNA analysis. Moreover, none of other types of DNA polymorphisms has such diverse and numerous methods of analysis, as SNP. Various SNP microarray chips are being used in animals, in conjunction with progeny testing programmes for identifying regions responsible for better productivity for early selection and to reduce generation interval on organised farms (Saravanan *et al.,* 2022) [27].

Future Prospects of DNA Fingerprinting

The future of DNA fingerprinting holds several promising developments and advancements that are likely to reshape its applications and capabilities. Here are some anticipated trends and areas of development (Dash *et al.,* 2018) [7]:

1. **Enhanced Sensitivity and Accuracy:** Advancements in DNA sequencing technologies and analytical methods are expected to improve the sensitivity and accuracy of DNA fingerprinting. This could enable the analysis of smaller or more degraded samples, leading to more precise identifications in forensic investigations and other fields (Gill, 2001) [10].
2. **Next-Generation Sequencing (NGS):** NGS technologies are evolving rapidly, offering high-throughput sequencing capabilities at reduced costs. These advancements could revolutionize DNA fingerprinting by enabling comprehensive analysis of entire genomes, including non-coding regions, which might provide deeper insights into genetic diversity and inherited traits (Muzzey *et al.,* 2015) [23].
3. **Rapid DNA Analysis:** Continued development in miniaturization and automation could lead to portable, on-site DNA analysis devices. This could allow for real-time DNA profiling and identification in various settings, such as crime scenes, disaster response or clinical emergencies (Tomlinson *et al.,* 2005) [28].
4. **Single-Cell DNA Profiling:** Techniques for analysing DNA at the single-cell level are progressing. This could unlock new possibilities in understanding genetic mosaicism, tumour heterogeneity and complex cellular interactions, impacting medical diagnostics and research (Ge *et al.,* 2021) [8].
5. **Artificial Intelligence (AI) Integration:** Considering the current developmental trend in AI and machine learning algorithms and with the development of deep AI models like Google's Gemini with tremendous computational capabilities, these models are likely to play a more significant role in DNA fingerprinting data analysis. These technologies could improve pattern recognition, interpretation of complex data and prediction of genetic traits or disease predispositions from DNA profiles (Brezillon *et al.,* 1993 & Rigano, 2019) [3, 26].
6. **Ethical and Legal Considerations:** With the increasing use of DNA data, addressing ethical concerns related to privacy, consent and misuse of genetic information will be crucial. Regulations and guidelines will likely evolve to safeguard individual's genetic privacy and ensure responsible use of DNA data (Hicks *et al.,* 2010) [14].
7. **Personalized Medicine and Therapeutics:** DNA fingerprinting may contribute extensively to personalized medicine by identifying individual's genetic variations influencing drug responses, disease susceptibility and treatment outcomes. This could lead to tailored therapies and interventions (Collins, 2010) [5].
8. **Environmental DNA (eDNA) Analysis:** Utilizing DNA fingerprinting for environmental monitoring by analysing DNA shed by organisms into their environments is a burgeoning area. It allows for non-invasive species identification, biodiversity assessment and ecosystem monitoring (Rees *et al.,* 2014) [25].

Conclusion

The evolution of DNA fingerprinting, pioneered by Alec Jeffreys and colleagues, marked a pivotal moment in molecular biology, spawning numerous applications in forensic biology and beyond. From its roots in identifying hypervariable repeat motifs within the human beta-globin gene, DNA fingerprinting techniques have rapidly evolved. Early concerns about the transition of DNA evidence from the laboratory to the courtroom were dispelled by landmark cases demonstrating its effectiveness. The development of DNA fingerprinting methodologies such as RFLP, VNTR, STR analysis and AFLP, expanded its applications across diverse fields including forensic science, genetics, anthropology and medical diagnostics. However, the field has transitioned, favouring advanced techniques due to limitations of early methods, such as the need for large DNA samples or labour-intensive protocols. Advancements in Next-Generation Sequencing (NGS), single-cell DNA profiling and AI integration are poised to revolutionize DNA fingerprinting. NGS offers comprehensive genome

analysis, promising deeper insights into genetic diversity. The emergence of rapid, on-site DNA analysis devices and AI-driven data analysis heralds real-time profiling, enhanced pattern recognition and predictive genetic analysis, revolutionizing forensic investigations and medical diagnostics. Ethical considerations regarding genetic privacy, misuse of genetic information and regulatory frameworks will play a pivotal role in shaping the future landscape of DNA fingerprinting. The field's potential in personalized medicine, environmental DNA (eDNA) analysis and its contributions to biodiversity assessment and ecosystem monitoring emphasize its expanding scope and importance. In conclusion, the future of DNA fingerprinting holds immense promise. Its evolution, from its origins to the present, reflects a journey marked by technological advancements, expanding applications and ethical considerations. Anticipated advancements in DNA sequencing, AI integration, and personalized medicine are expected to reshape its capabilities, enabling more accurate identifications and innovative applications across various scientific disciplines. As the field continues to evolve, its impact on genetics, forensics, healthcare and environmental sciences will be profound.

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