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REVIEW

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Molecular biotechnology techniques in DNA fingerprinting: A stepwise overview

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ABSTRACT

DNA fingerprinting is a molecular-based identification method that relies on the detection of polymorphic regions within the human genome, particularly short tandem repeats (STRs) and variable number tandem repeats (VNTRs). Originally developed using restriction fragment length polymorphism (RFLP), the methodology has evolved into a highly sensitive and specific system driven by PCR amplification and capillary electrophoresis. The standard workflow includes biological sample collection, DNA extraction, quantification, amplification of target loci, and fragment analysis, providing high-resolution genetic profiles suitable for individual discrimination.

Technological advancements have significantly extended the capabilities of DNA profiling. Real-time PCR offers quantification of nuclear and mitochondrial DNA and detection of PCR inhibitors, while digital PCR enables precise analysis of low-template and mixed samples. Next-generation sequencing (NGS) introduces sequence-level resolution of STR alleles, mitochondrial genome analysis, and single nucleotide polymorphism (SNP) profiling for biogeographic ancestry inference and identity testing in degraded specimens. Portable sequencing platforms and automated rapid DNA systems support deployment in field investigations and booking stations, although implementation requires rigorous validation.

Despite these developments, the method faces limitations. Sample contamination remains a critical concern, especially in low-copy-number contexts, requiring strict contamination control protocols. Ethical concerns involving informed consent, secondary data use, and genetic privacy continue to raise regulatory challenges. Inter-laboratory inconsistencies necessitate validated workflows, proficiency testing, and adherence to guidelines for statistical interpretation.

This review consolidates the molecular basis, procedural advances, applications, and regulatory considerations of DNA fingerprinting, reaffirming its relevance in forensic science, kinship analysis, conservation genetics, and medical diagnostics.

KEYWORDS

DNA fingerprinting; DNA extraction; Forensic science; Sample contamination; DNA profiling

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Introduction

Deoxyribonucleic acid (DNA) functions as the molecular blueprint of life, encoding the hereditary information necessary for cellular function, development, and reproduction. While the majority of the human genome is conserved among individuals, certain regions particularly tandem repeat sequences exhibit high inter-individual variability. These polymorphic loci, including Variable Number Tandem Repeats (VNTRs) and Short Tandem Repeats (STRs), form the genetic basis of DNA fingerprinting. DNA fingerprinting, also referred to as DNA profiling or genetic typing, is a molecular biotechnology-based method for individual identification through the analysis of polymorphic genomic loci [1]. The standard workflow involves the extraction of genomic DNA from a biological specimen, selective amplification of target loci using Polymerase Chain Reaction (PCR), and fragment separation using gel electrophoresis or high-resolution capillary electrophoresis with fluorescent-labeled primers. The resulting electropherogram or banding pattern represents an individual's genetic profile, which can then be compared against reference samples or genetic databases [2].

Since the initial demonstration by Sir Alec Jeffreys in 1984, which involved detecting hypervariable minisatellite regions using restriction fragment length polymorphism (RFLP), DNA fingerprinting has undergone significant development. Early labor-intensive and DNA-demanding protocols have been largely replaced by STR-based multiplex PCR systems compatible with degraded or low-template samples [3]. The adoption of standardized marker panels, such as the Combined DNA Index System (CODIS), has further improved inter-laboratory consistency and judicial acceptance. Modern systems also incorporate automated genotyping and probabilistic statistical models, improving interpretation accuracy even in complex DNA mixtures [4].

Despite its precision, DNA fingerprinting is susceptible to limitations including sample degradation, low-template DNA-induced stochastic variation, allelic drop-out, and potential contamination. Additionally, interpretation of mixed DNA profiles remains technically challenging. Ethical concerns have also emerged regarding the collection, storage, and use of genetic data, with implications for privacy, consent, and data security [5].

This review aims to systematically describe the principles and methodology of DNA fingerprinting, with an emphasis on the molecular biotechnology techniques that underpin each

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step. It further examines current applications, recent technological advancements, and limitations, providing a critical assessment of the technique's utility in research and applied contexts.

Basic Molecular Steps in DNA Fingerprinting

Sample collection and preservation

The quality and reliability of DNA fingerprinting largely depend on the condition and handling of biological samples. Commonly used sources include blood, buccal swabs, saliva, semen, hair follicles, and epithelial cells. The selection of sample type is influenced by the context of investigation [6].

Proper storage conditions are critical to preserving DNA integrity. Liquid blood samples are typically collected in tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and stored at 4°C for short-term preservation. For longer storage durations, freezing at –20°C is adequate, though –80°C is preferable for archival purposes. Dried biological specimens, such as swabs or blood stains, should be stored in breathable containers to prevent moisture accumulation and microbial growth. In humid environments, desiccants and temperature control are essential to prevent DNA degradation [7].

In forensic settings, adherence to a documented chain of custody is essential. Each stage of sample handling must be logged with time-stamped identifiers to ensure traceability and maintain the admissibility of evidence in legal proceedings. Any lapse in chain-of-custody protocols can compromise the evidentiary value of DNA profiles [8].

DNA extraction and purification

Efficient extraction and purification of high-quality genomic DNA are fundamental to downstream fingerprinting accuracy. Several protocols are commonly employed based on sample type, throughput, and required purity [9].

The phenol-chloroform extraction method involves organic phase separation to remove proteins and lipids. While effective, it requires hazardous reagents and is time-intensive. More widely adopted are silica-based spin column methods, which utilize chaotropic salts to facilitate DNA binding to a silica membrane, followed by wash and elution steps [10]. These kits are favored for their speed, reproducibility, and automation compatibility. Alternatively, magnetic bead-based methods employ DNA-binding beads and magnetic separation, making them ideal for high-throughput and automated workflows. However, both commercial methods may be cost-restrictive in low-resource settings [11].

Quantification and quality assessment

Post-extraction, DNA quantification and quality assessment are essential to ensure optimal input for amplification. Spectrophotometric methods estimate nucleic acid concentration via absorbance at 260 nm, with A260/A280 ratios near 1.8 indicative of pure DNA. However, these readings can be affected by RNA or protein contamination [12].

Fluorometric quantification offers greater specificity by using DNA-binding fluorescent dyes, thereby providing accurate measurements even at low concentrations. Agarose gel electrophoresis is routinely used to assess DNA integrity, where intact genomic DNA appears as a high-molecular-weight band, while smearing indicates fragmentation or degradation [13].

Biotechnological Techniques

Restriction enzymes and restriction fragment length polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP) was among the earliest methods used in DNA fingerprinting, particularly before PCR-based methods gained prominence. The technique relies on the use of restriction endonucleases, enzymes that recognize and cleave DNA at specific palindromic sequences. These sequence-specific cuts generate DNA fragments of variable lengths due to polymorphisms in the target regions among different individuals [14].

Following enzymatic digestion, the resulting DNA fragments are separated by agarose gel electrophoresis and transferred to a nylon or nitrocellulose membrane by southern blotting. The membrane is then hybridized with a radiolabeled or chemiluminescent probe that binds to a specific DNA sequence of interest. Autoradiography or chemiluminescent imaging reveals the hybridized bands, forming a unique fragment pattern for each individual [15] (Figure 1).

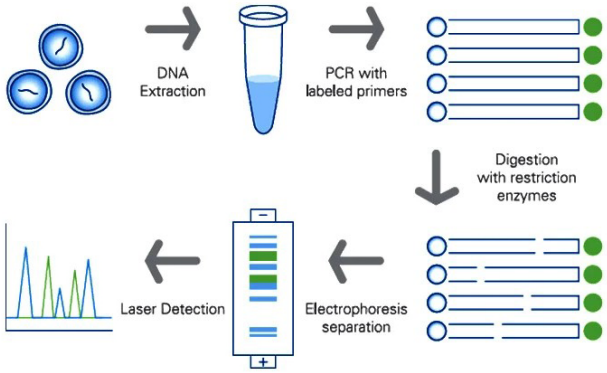

Figure 1. Detailed process of Restriction Fragment Length Polymorphism. [**Source**. https://microbenotes.com/restriction-fragment-length-polymorphism-rflp/]

Despite its historical significance, RFLP has largely been replaced due to its limitations. It requires relatively large amounts of undegraded DNA (typically >1 µg), involves lengthy hybridization and washing steps, and lacks sensitivity in mixed or degraded samples. However, it laid the groundwork for understanding DNA-based polymorphisms and remains a valuable reference in molecular diagnostics and population genetics [16].

Polymerase chain reaction

The polymerase chain reaction (PCR), introduced by Kary Mullis in 1983, revolutionized molecular biology by enabling the exponential amplification of specific DNA sequences from minute amounts of template DNA. PCR is based on a three-step thermal cycling process: denaturation (usually at 94-95°C), annealing of sequence-specific primers (50-65°C), and extension by a thermostable DNA polymerase, typically Taq polymerase, at 72°C. The cycle is repeated 25-35 times to generate millions of copies of the target sequence [17] (Figure 2).

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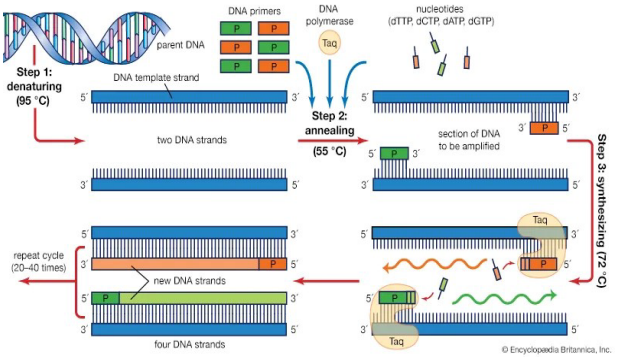

Figure 2. Detailed process of Polymerase Chain Reaction.
[**Source**. https://www.britannica.com/science/molecular-biology#/media/1/388110/18071]

PCR's sensitivity and rapid amplification capability make it indispensable in DNA fingerprinting, particularly for forensic casework where samples may be degraded, contaminated, or available in trace amounts. Its utility extends to the amplification of STR loci and mitochondrial DNA, as well as in microbial forensics and ancestral lineage tracing [18].

Inhibitors such as heme, humic acid, and certain detergents can interfere with PCR efficiency. Therefore, DNA extraction protocols are optimized to remove these inhibitors, especially in forensic workflows.

Short tandem repeat analysis

STRs are tandemly repeated DNA motifs, typically 2–6 base pairs in length, found abundantly throughout the human genome. These loci exhibit high allelic diversity due to variability in the number of repeat units, making them highly informative for individual identification. STR loci are inherited in a Mendelian fashion, making them suitable for both identity testing and kinship analysis [19].

In forensic and population genetics, STR loci are amplified via multiplex PCR using fluorescently labeled primers. The amplified fragments are then size-separated via capillary electrophoresis. Each STR allele is represented by a specific fragment length, which is interpreted through software to produce an electropherogram, where each peak corresponds to one allele [20].

The U.S. Federal Bureau of Investigation's CODIS (Combined DNA Index System) currently utilizes a core set of 20 STR markers, which collectively yield a random match probability of less than 1 in 10^18 in unrelated individuals. The high discrimination power, stability, and reproducibility of STRs have made them the global standard for DNA profiling [21].

Agarose gel electrophoresis

Agarose gel electrophoresis is a routine method for separating DNA fragments based on size. DNA, being negatively charged, migrates toward the positive electrode through a porous agarose matrix when an electric field is applied. Smaller DNA fragments move more rapidly than larger ones [22].

Post-electrophoresis, DNA bands are visualized using intercalating dyes such as ethidium bromide, SYBR Safe or GelRed, which fluoresce under UV or blue light. While agarose gel electrophoresis is widely used for qualitative analysis such as assessing PCR products or genomic DNA integrity, it lacks the resolution and sensitivity required for accurate STR profiling [23] (Figure 3).

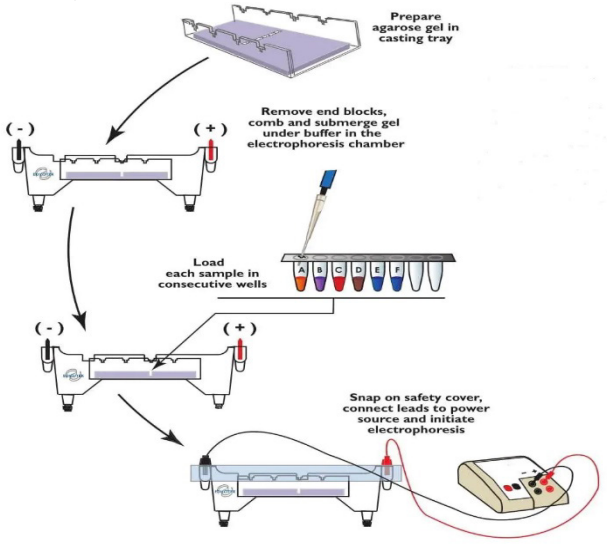

Figure 3. Detailed process of Agarose Gel Electrophoresis.
[**Source**. https://people.wou.edu/~courtna/ch462/Gel%20Electrophoresis.pdf]

In DNA fingerprinting, it primarily serves as a preliminary tool to verify the presence and approximate size of amplified DNA, prior to high-resolution analysis by capillary electrophoresis.

Capillary electrophoresis and DNA sequencing

Capillary electrophoresis (CE) represents a major advancement in high-resolution DNA separation. In this technique, DNA fragments labeled with fluorescent dyes are injected into narrow capillaries filled with a polymer matrix. Upon application of an electric field, DNA fragments migrate through the capillary at rates inversely proportional to their size. As fragments pass a laser detector, the emitted fluorescence is recorded, and data are translated into electropherograms [24].

CE offers several advantages over gel-based systems: greater resolution, faster run times, automation compatibility, and multiplexing capability using multiple dye colors. It is the current gold standard in forensic STR analysis and is used extensively in national DNA databases [25].

Sanger sequencing, often implemented using CE, is used for determining the nucleotide sequence of specific genomic regions. Though not used for STR profiling, it is critical for analyzing single nucleotide polymorphisms (SNPs) and rare mutations in biomedical and forensic investigations. For broader applications, high-throughput next-generation sequencing (NGS) is gaining attention, but CE remains dominant in STR-based profiling due to its regulatory acceptance and validated workflows [26].

Visualization and Analysis

Gel staining and interpretation

Gel electrophoresis, commonly using agarose as a matrix, is an essential preliminary step in molecular analysis for verifying

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DNA fragment size and PCR amplification success. Post-electrophoresis, DNA bands are visualized through the use of intercalating fluorescent dyes. Ethidium bromide (EtBr) has historically been the standard dye, intercalating between base pairs and fluorescing under UV light. However, due to its mutagenic nature, non-toxic alternatives such as SYBR Safe, GelRed, and Midori Green are increasingly employed in both research and clinical laboratories [27].

DNA molecular weight ladders containing fragments of known sizes are loaded alongside samples to serve as references for estimating the size of unknown fragments. Migration distance in the gel is inversely proportional to fragment size, allowing for approximate sizing by comparison to ladder bands. Though not used for quantitative analysis, band intensity can provide a semi-quantitative indication of DNA concentration when visualized under a UV transilluminator or blue-light system [28].

High-resolution gel imaging systems allow for digital documentation of electrophoresis results, essential for maintaining laboratory records, publications, or evidentiary purposes in forensic workflows. While useful for routine validation, this method does not possess the resolution or sensitivity required for individual STR genotyping, which necessitates capillary electrophoresis [29].

STR profile matching and probability calculations

In STR analysis, alleles are defined based on the number of repeat units at specific loci. These loci are amplified using fluorescently labeled primers in multiplex PCR, and the amplicons are separated via capillary electrophoresis. The output, displayed as an electropherogram, shows each allele as a peak whose position indicates fragment size and whose height correlates with the quantity of DNA [30].

Allele calling is performed using allelic ladders and internal size standards. An individual is homozygous if both alleles at a locus are the same; heterozygous if they differ. Analysts must differentiate true allelic peaks from stutter artifacts, pull-up peaks, or noise. Mixture interpretation often requires deconvolution and consideration of peak height ratios [31].

The strength of DNA evidence is quantified using the Random Match Probability (RMP), which estimates the probability that a randomly selected unrelated individual would have an identical STR profile. The product rule is applied across loci, assuming Hardy–Weinberg equilibrium and linkage equilibrium among loci. For the CODIS core loci, the RMP for a full match is typically less than 1 in 10^18 [32].

Forensic DNA profiles are routinely compared against databases such as CODIS. A profile match suggests identity or a common source, though it requires statistical confirmation. In complex cases involving DNA mixtures or partial profiles, likelihood ratios (LRs) and probabilistic genotyping software like STRmix and TrueAllele are employed to assess evidentiary weight more robustly, accounting for peak imbalances, allele dropout, and stochastic variation [33].

Applications of DNA Fingerprinting

Forensic science

DNA fingerprinting is a central method in forensic casework for the identification of individuals based on polymorphic loci, primarily STRs. Biological evidence collected from crime scenes such as blood, semen, or epithelial cells is subjected to STR analysis and compared to suspect profiles or entries in national forensic databases like the Combined DNA Index System (CODIS). The comparison is based on allelic matches across standardized STR loci, where a full match may provide a match probability of less than 1 in 10^{18}, depending on the number and diversity of loci examined [34].

The technique has been critical in resolving active investigations, revisiting cold cases, and exonerating wrongfully convicted individuals through post-conviction DNA testing. In partial profile or mixed DNA cases, analysts employ peak height ratios, stutter filters, and likelihood ratio modeling to assess match strength [35].

Paternity and maternity testing

Parentage testing employs STR genotyping based on Mendelian inheritance. A child's DNA must contain alleles present in either biological parent. Non-matching alleles across multiple loci lead to exclusion, while consistent inheritance patterns allow statistical inclusion. Legal standards often require a probability of paternity exceeding 99.99% for conclusive determination. Commercial and court-ordered tests use the same CODIS STR loci to ensure standardization and reproducibility [36].

Wildlife conservation

In conservation genetics, DNA fingerprinting is applied to assess genetic variation, delineate populations, identify individuals, and trace the origin of confiscated biological materials. STR markers have been used in species such as elephants to track ivory poaching routes. Individual identification supports wildlife tagging and monitoring, while population-level STR data informs breeding programs and reintroduction efforts. Non-invasive sampling allows for large-scale genetic surveys without direct animal handling. Molecular data also assists in enforcing CITES regulations through species and origin identification [37].

Medical genetics

While not typically used for mutation detection, DNA fingerprinting using STRs is applied in specific clinical contexts. Loss of heterozygosity (LOH) at tumor suppressor loci is a common event in cancer, detectable via STR analysis. Chimerism analysis post-allogeneic stem cell transplantation also utilizes STR profiling to monitor donor versus recipient cell populations. Additionally, HLA typing relies on genetic profiling for matching organ or bone marrow donors with recipients. STR analysis has also been employed in prenatal diagnostics, tissue origin confirmation, and twin zygosity determination when sequence-based methods are not required [38].

Recent Advances in DNA Fingerprinting

Real-time PCR and digital PCR

Real-time PCR (qPCR) and digital PCR (dPCR) have expanded the capabilities of DNA quantification and genotyping, especially in forensic and degraded sample contexts.

qPCR quantifies DNA by monitoring fluorescence emitted during the amplification process. It is primarily used to

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determine template quantity, assess degradation indices, and identify the presence of PCR inhibitors before STR typing. However, its accuracy is dependent on standard curve calibration, and its dynamic range is limited to approximately 10–10^6 copies of DNA [39] (Figure 4).

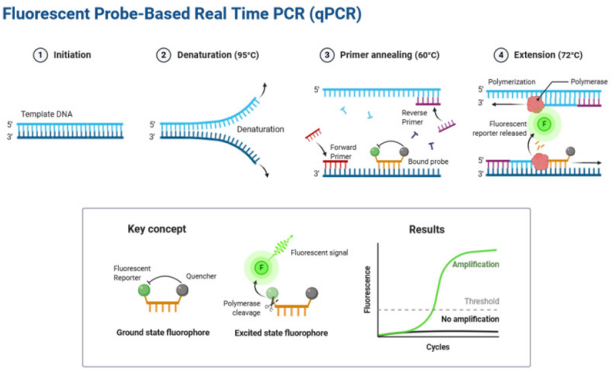

Figure 4. Detailed process of Agarose Gel Electrophoresis. [**Source**. https://people.wou.edu/~courtna/ch462/Gel%20Electrophoresis.pdf]

dPCR partitions the DNA sample into thousands of nanoliter reactions, allowing for absolute quantification without reliance on calibration curves. This is particularly valuable for low-template DNA, allele drop-in/drop-out detection, and minor contributor resolution in complex mixtures. dPCR has been increasingly applied in chimerism monitoring in stem cell transplantation and may have future utility in forensic mixture deconvolution [40].

Next-generation sequencing (NGS)-based profiling

Next-generation sequencing enables high-throughput, parallel sequencing of genomic markers, providing more detailed forensic information than traditional capillary electrophoresis (CE)-based methods.

STR Analysis by NGS offers both fragment length and sequence variation, enabling isoallele resolution, thereby increasing discriminatory power. Multiplexing dozens of STR loci, even in degraded samples, improves profile completeness and interpretability [41].

Mitochondrial DNA (mtDNA) sequencing by NGS allows complete mitogenome analysis, which is useful in cases involving old, skeletal, or otherwise compromised remains. Despite limited discrimination due to maternal inheritance, mtDNA remains valuable in kinship analysis and missing person identification [42].

SNP-based profiling provides data for biogeographic ancestry inference and phenotype prediction through predictive models, such as HIrisPlex-S. These investigative leads are applied in no-suspect cases, though not for legal identification [43].

Portable and rapid DNA testing Kits

Field-deployable DNA technologies offer time-efficient alternatives for preliminary identification. MinION is a portable DNA sequencer using nanopore technology to perform long-read sequencing in real time. While its error rate of 5-15% limits definitive forensic use, it is applicable in species identification, environmental DNA (eDNA) studies, and wildlife forensics [44].

Rapid DNA systems like Thermo Fisher's RapidHIT and ANDE 6C automate DNA extraction, amplification, and STR analysis in under 2 hours. These systems are approved by the FBI for booking station deployment but are currently limited to reference samples due to chain-of-custody and validation requirements [45].

Challenges and Limitations

Sample contamination

Contamination poses a critical threat to the reliability of DNA evidence, particularly when working with low-template or degraded biological material. Contaminants may be introduced at the crime scene, during evidence collection, in transportation, or within the laboratory environment. Sources include operator DNA, aerosolized amplicons, and contaminated reagents.

Best practices to minimize contamination include:

- Use of dedicated pre- and post-PCR work areas, physical separation of clean and amplification zones, and unidirectional workflow.
- Frequent glove changes and use of barrier pipette tips.
- Surface decontamination using sodium hypochlorite or UV crosslinkers [46].

Ethical issues and data privacy

The use of DNA in forensic databases raises complex ethical issues related to consent, privacy, and potential misuse of genetic information. While informed consent is typically required for voluntary database inclusion, non-consensual collection from crime scenes or relatives creates ethical issues.

Data protection concerns include:

- Unauthorized secondary use of genetic material.
- Long-term retention of profiles from individuals not convicted of crimes.
- Re-identification of individuals via indirect genetic matching [47].

Standardization across laboratories

Variability in laboratory protocols, interpretation thresholds, and statistical reporting can affect the reproducibility and admissibility of DNA evidence.

To ensure reliability:

- Laboratories should follow guidance from SWGDAM and ENFSI for method validation, mixture interpretation, and stochastic threshold setting.
- STR kits must be validated per ISO/IEC 17025, and laboratory personnel must participate in regular internal and external proficiency testing.
- Probabilistic genotyping systems are increasingly used to standardize mixture analysis, reducing subjective bias in allele interpretation [48].

Conclusion

DNA fingerprinting remains an important technique in molecular biotechnology, offering precise identification based on the analysis of polymorphic loci such as short tandem

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repeats and variable number tandem repeats. Its evolution from early restriction-based methods to PCR-based and high-throughput sequencing approaches has significantly enhanced its sensitivity, specificity, and applicability across diverse sample types, including degraded or low-template DNA. Advancements such as real-time PCR, digital PCR, and next-generation sequencing have expanded the analytical capabilities of DNA profiling. These technologies enable detailed genotyping, mitochondrial DNA analysis, and sequence-level resolution of alleles, facilitating interpretation in complex forensic and kinship scenarios. Additionally, the development of portable and rapid DNA testing platforms has introduced new opportunities for real-time identification in field-based and time-sensitive contexts.

Despite its strengths, DNA fingerprinting has several challenges. Contamination remains a critical concern in forensic identification, while analyzing detailed quantities of DNA. Strict adherence to contamination control measures, standardized protocols, and quality assurance procedures is essential. Ethical considerations surrounding consent, data security, and the potential misuse of genetic information require comprehensive regulatory frameworks. Moreover, variability in laboratory practices and data interpretation underscores the need for method validation, proficiency testing, and harmonization of analytical standards.

DNA fingerprinting continues to be a reliable and evolving tool for identity analysis. Its future application depends on continued technological innovation, adherence to validated protocols, and the integration of ethical and legal safeguards to ensure its responsible and effective use in scientific, forensic, and medical domains.

Disclosure statement

No potential conflict of interest was reported by the authors.

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