

Application of Molecular Techniques in Forensic Identification: From DNA Profiling to Next-Generation Sequencing

¹Nidhi Rajesh Yadav*, ²Bhanupratap Vishwakarma

^{1,2}ZSCT's Thakur Shyamnarayan Degree College (Department of Microbiology), Kandivali East, Mumbai - 400101.

Email - nry2031@gmail.com, <https://orcid.org/0009-0008-2041-4434>
va057670@gmail.com, <https://orcid.org/0009-0004-6788-8072>

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Abstract

Forensic identification has experienced a profound transformation with the advent of molecular biology techniques, shifting from traditional morphological and serological methods to highly sensitive and specific DNA-based approaches. This review provides a comprehensive overview of the evolution of molecular techniques in forensic science, beginning with conventional DNA profiling methods such as Restriction Fragment Length Polymorphism (RFLP) and Short Tandem Repeat (STR) analysis, and extending to advanced methodologies including mitochondrial DNA (mtDNA) analysis, Y-chromosome markers, and Next-Generation Sequencing (NGS).

The review critically examines the principles, applications, advantages, and limitations of these techniques in diverse forensic contexts, including crime scene investigation, human identification, kinship analysis, and mass disaster victim identification. Furthermore, it highlights recent advancements in forensic genomics, epigenetics, and microbiome-based approaches, along with the growing role of bioinformatics and artificial intelligence in data interpretation. Despite significant progress, challenges such as data complexity, ethical considerations, and the need for global standardization remain key concerns. Overall, this review underscores the pivotal role of molecular techniques in modern forensic science and emphasizes their continued evolution toward more precise, rapid, and comprehensive identification systems.

1. INTRODUCTION

Forensic science serves as a vital bridge between science and the legal system, enabling the objective analysis of evidence to support criminal investigations and judicial decisions. Over the years, the discipline has evolved from relying primarily on physical and observational methods such as fingerprint analysis and blood typing to incorporating highly sophisticated molecular approaches (1). Among these, molecular biology has become one of the most influential and dependable tools in forensic investigations due to its ability to provide precise, reproducible, and individual-specific results (2). A major turning point in forensic science occurred in 1985, when Alec Jeffreys introduced the concept of DNA fingerprinting. This groundbreaking discovery demonstrated that variations within the human genome could be

used to distinguish individuals with remarkable accuracy (3). Since then, DNA-based technologies have transformed forensic identification, making it possible to analyze even minute or degraded biological samples that were previously considered unsuitable for examination (4).

Biological evidence, such as blood, saliva, semen, hair, bones, and teeth can now be subjected to molecular analysis to extract genetic information. These advancements have significantly improved the sensitivity and specificity of forensic investigations, allowing for reliable identification in complex scenarios, including mass disasters, missing person cases, and cold cases (5). Moreover, the integration of molecular techniques has reduced dependence on eyewitness testimony, thereby strengthening the scientific foundation of legal proceedings. In recent decades, the field has witnessed rapid technological progress, moving from early methods like restriction fragment analysis to more advanced techniques such as polymerase chain reaction (PCR)-based amplification and short tandem repeat (STR) profiling (6). More recently, the emergence of next-generation sequencing (NGS) has further expanded the capabilities of forensic genetics by enabling large-scale, high-throughput DNA analysis with greater resolution and accuracy (7).

Despite these advancements, challenges such as DNA degradation, contamination, mixed samples, and ethical concerns surrounding genetic data remain important considerations. Addressing these issues requires continuous refinement of analytical methods, strict quality control measures, and the development of standardized protocols (8). This review aims to present a comprehensive and critical overview of molecular techniques used in forensic identification. It traces the evolution of these methods from conventional DNA profiling approaches to cutting-edge sequencing technologies, while also discussing their applications, limitations, and future potential in modern forensic science.

2. CONVENTIONAL DNA PROFILING TECHNIQUES

2.1 Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP) represents one of the earliest molecular techniques applied in forensic DNA profiling and played a foundational role in the development of modern forensic genetics. This method is based on the principle that variations in DNA sequences among individuals can lead to differences in the lengths of DNA fragments produced after digestion with specific restriction enzymes (9). In practice, genomic DNA is first extracted from biological samples and then treated with restriction endonucleases, which cut the DNA at particular recognition sites. The resulting fragments are subsequently separated using gel electrophoresis and visualized, producing a pattern unique to an individual. RFLP analysis is known for its high discriminatory power and reliability when high-quality, non-degraded DNA samples are available, making it historically valuable in forensic casework (10). However, the technique has several notable limitations that have reduced its use in contemporary practice. It requires relatively large quantities of intact DNA, making it unsuitable for degraded or limited samples commonly encountered at crime scenes (11). Additionally, the procedure is time-consuming, labor-intensive, and involves complex laboratory workflows. As a result, RFLP has largely been replaced by more rapid and sensitive PCR-based methods, although its contribution to the evolution of forensic DNA analysis remains significant (12).

2.2 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) marked a significant advancement in forensic science by enabling the amplification of extremely small quantities of DNA, thereby overcoming one of the major limitations of earlier techniques like RFLP. This method allows specific regions of DNA to be copied exponentially through a series of temperature-controlled cycles, making it possible to generate millions of copies from minimal or partially degraded genetic material. As a result, PCR has become an indispensable tool in forensic investigations, particularly in cases where only trace amounts of biological evidence are available (13). It is widely applied in the analysis of degraded samples, such as aged or environmentally exposed materials, as well as in low-copy number DNA analysis, where the starting genetic material is extremely limited. The technique is highly sensitive and capable of producing results rapidly, which significantly enhances the efficiency of forensic workflows. Despite its advantages, PCR also requires careful handling to prevent contamination, as its high sensitivity can amplify even minute amounts of extraneous DNA. Overall, PCR has revolutionized forensic DNA analysis by improving both the accessibility and reliability of genetic evidence (14).

2.3 Short Tandem Repeat (STR) Analysis

Short Tandem Repeat (STR) analysis is widely regarded as the gold standard in modern forensic DNA profiling due to its high accuracy, sensitivity, and robustness. This technique focuses on specific regions of the genome where short DNA sequences are repeated in tandem, with the number of repeats varying significantly between individuals (15). By analyzing multiple STR loci simultaneously, a highly distinctive genetic profile can be generated, making it an extremely powerful tool for human identification. STR analysis is extensively used in criminal investigations to match suspects with biological evidence, in paternity and kinship testing to establish familial relationships, and in national DNA databases such as CODIS for large-scale comparison and identification (16). One of the key strengths of STR profiling is its high reproducibility across laboratories, ensuring consistent and reliable results. Additionally, because STR regions are relatively short, they can be successfully analyzed even in partially degraded DNA samples, which are commonly encountered in forensic casework. These advantages have made STR analysis the backbone of forensic genetics, although ongoing advancements such as next-generation sequencing are beginning to complement and enhance its capabilities (17).

3. ADVANCED MOLECULAR MARKERS

3.1 Mitochondrial DNA (mtDNA) Analysis

Mitochondrial DNA (mtDNA) analysis has become an essential tool in forensic investigations, particularly in situations where nuclear DNA is either severely degraded or completely unavailable. Unlike nuclear DNA, which is inherited from both parents, mtDNA is transmitted exclusively through the maternal lineage, meaning it remains relatively unchanged across generations (18). Additionally, each cell contains hundreds to thousands of copies of mtDNA, compared to only two copies of nuclear DNA, significantly increasing the chances of successful DNA recovery from compromised or limited biological samples. This characteristic makes mtDNA especially valuable in the analysis of hair shafts that lack roots, as well as in aged skeletal remains, burned tissues, and other highly degraded materials often encountered in forensic and archaeological contexts (19).

Despite these advantages, mtDNA analysis has certain limitations that must be considered. Its primary drawback is its lower discriminatory power compared to STR profiling, since individuals from the same maternal lineage share identical or very similar mtDNA sequences (20). Consequently, mtDNA cannot uniquely identify an individual in the same way as nuclear DNA markers, but is highly effective for excluding individuals or establishing maternal relationships. Therefore, it is often used as a complementary technique alongside other molecular methods in forensic casework (21).

3.2 Y-Chromosome STR (Y-STR) Analysis

Y-chromosome STR (Y-STR) analysis is a targeted molecular approach designed for male-specific identification, making it particularly useful in forensic scenarios involving mixed DNA samples. The Y chromosome is passed directly from father to son with little genetic recombination, preserving paternal lineage markers across generations. This unique inheritance pattern allows forensic scientists to trace male ancestry and identify male contributors in biological evidence (22).

One of the most important applications of Y-STR analysis is in sexual assault cases, where male DNA is often present in very small quantities compared to a large excess of female DNA. In such cases, conventional autosomal STR analysis may fail to distinguish the male component effectively. Y-STR profiling overcomes this limitation by selectively amplifying only Y-chromosome-specific regions, enabling clear identification of the male genetic profile. Additionally, this method is useful in paternal lineage studies, missing persons investigations, and cases involving related male individuals (23). Similar to mtDNA analysis, Y-STR profiling has reduced discriminatory power because all males within the same paternal lineage tend to share identical or highly similar Y-STR profiles. As a result, while it is highly effective for narrowing down suspects or confirming lineage, it may not always provide definitive individual identification (24).

3.3 Single Nucleotide Polymorphisms (SNPs)

Single Nucleotide Polymorphisms (SNPs) are the most common form of genetic variation in the human genome, involving a change in a single nucleotide base at a specific position in the DNA sequence. Due to their abundance and widespread distribution across the genome, SNPs have emerged as powerful markers in forensic genetics, particularly in cases where DNA is highly fragmented or degraded (25). Unlike STRs, which require longer DNA fragments, SNP analysis can be performed on very short DNA sequences, making it highly suitable for challenging forensic samples such as old bones, teeth, or environmentally exposed biological materials (26).

Beyond their utility in identification, SNPs offer additional capabilities that extend the scope of forensic investigations. They can be used to infer biogeographical ancestry, helping to provide information about an individual's population background (27). Moreover, SNP-based analysis plays a key role in forensic phenotyping, where genetic data is used to predict externally visible characteristics such as eye color, hair color, and skin pigmentation. This information can be particularly valuable in cases where no direct DNA match is found in forensic databases, assisting investigators in generating leads (28). However, SNP analysis also presents certain challenges, including the need for advanced sequencing technologies, complex data interpretation, and the requirement for robust statistical models. Despite these limitations, SNPs are increasingly being integrated with next-generation sequencing

platforms, positioning them as a crucial component of the future of forensic molecular biology (29).

4. NEXT-GENERATION SEQUENCING (NGS) IN FORENSIC SCIENCE

Next-Generation Sequencing (NGS), also referred to as massively parallel sequencing, represents a major advancement in forensic molecular biology by enabling the simultaneous sequencing of millions of DNA fragments in a single run. Unlike traditional methods that analyze one genetic region at a time, NGS allows comprehensive examination of multiple genetic markers, including STRs, SNPs, and mitochondrial DNA, within a single workflow. This high-throughput capability has significantly expanded the scope and depth of forensic DNA analysis, providing more detailed and informative genetic profiles (30).

One of the key applications of NGS in forensic science is the analysis of complex DNA mixtures, where biological samples may contain genetic material from multiple individuals. Conventional techniques often struggle to accurately resolve such mixtures, but NGS offers improved sensitivity and resolution, allowing for better separation and interpretation of individual contributors (31). Additionally, NGS plays a crucial role in forensic phenotyping, where genetic data is used to predict externally visible characteristics such as eye color, hair color, and, to some extent, facial features. This can provide valuable investigative leads in cases where no direct match is available in DNA databases. Another important application is ancestry inference, which helps determine the biogeographical origin of an individual, thereby assisting in narrowing down potential suspects or identifying unknown remains (32).

The advantages of NGS include its high throughput, the ability to generate large volumes of data rapidly, and its superior resolution compared to traditional STR-based methods. It can detect sequence-level variations within STR regions and analyze a broader range of genetic markers simultaneously, enhancing both accuracy and discriminatory power (33). However, despite these benefits, several challenges limit its widespread adoption in routine forensic practice. The technology involves high initial and operational costs, requires specialized equipment and technical expertise, and generates complex datasets that demand advanced bioinformatics tools for analysis and interpretation (34). Furthermore, there is an ongoing need for standardized protocols, validated methods, and universally accepted guidelines to ensure consistency and reliability across forensic laboratories. Overall, NGS is poised to play a transformative role in the future of forensic science, complementing existing techniques while offering new possibilities for more precise and comprehensive genetic analysis (35).

5. EMERGING TRENDS IN FORENSIC MOLECULAR BIOLOGY

5.1 Forensic Epigenetics

Forensic epigenetics is an emerging area that focuses on heritable changes in gene expression that do not involve alterations in the underlying DNA sequence. One of the most widely studied epigenetic modifications is DNA methylation, which involves the addition of methyl groups to specific regions of the genome (36). In forensic science, analysis of DNA methylation patterns has shown great promise in providing information beyond simple identification. For instance, specific methylation markers can be used to estimate the biological age of an individual with reasonable accuracy, which can be particularly useful in narrowing down suspect profiles (37). Additionally, epigenetic signatures can help determine

the tissue origin of biological samples, allowing investigators to distinguish whether the DNA originated from blood, saliva, semen, or other body fluids. Although still under development, forensic epigenetics has the potential to significantly enhance the interpretative value of forensic evidence (38).

5.2 Forensic Microbiomics

Forensic microbiomics is a rapidly developing field that examines microbial communities associated with the human body and the surrounding environment for forensic applications. After death, the human body undergoes predictable microbial changes, collectively referred to as the thanatomicrobiome. By analyzing these microbial succession patterns, scientists can estimate the postmortem interval (PMI), which is a critical factor in death investigations (39). Microbial signatures can also provide insights into the location and environmental conditions of decomposition, potentially linking remains to specific sites. This approach is particularly useful when traditional methods of PMI estimation are unreliable. However, factors such as temperature, humidity, soil composition, and environmental variability can influence microbial communities, making standardization and interpretation challenging. Despite these limitations, forensic microbiomics holds considerable promise as a complementary tool in forensic investigations (40).

5.3 Forensic Proteomics

Forensic proteomics involves the large-scale study of proteins for identification and investigative purposes, particularly in cases where DNA is too degraded or insufficient for analysis. Proteins are generally more stable than DNA and can persist in samples such as hair shafts, bones, and teeth for extended periods (41). By analyzing specific protein markers and their variations, forensic scientists can obtain valuable information regarding species identification, tissue type, and even individual characteristics in some cases. Proteomic approaches are increasingly being explored in disaster victim identification and archaeological investigations, where conventional DNA-based methods may fail. Although still an emerging technique, forensic proteomics provides an important alternative avenue for identification in challenging scenarios (42).

5.4 Bioinformatics and AI Integration

The rapid advancement of molecular techniques has led to the generation of large and complex datasets, particularly with technologies like next-generation sequencing. As a result, bioinformatics and artificial intelligence (AI) have become indispensable components of modern forensic science (43). Bioinformatics tools are used for sequence alignment, data processing, and interpretation of genetic information, ensuring accurate and efficient analysis. Meanwhile, AI and machine learning algorithms are being developed to enhance predictive modeling, automate pattern recognition, and improve the interpretation of complex forensic data, such as DNA mixtures and phenotypic predictions (44). These technologies not only increase analytical speed and accuracy but also reduce human error and subjectivity. As computational methods continue to evolve, their integration into forensic workflows is expected to further revolutionize the field, enabling more precise and data-driven decision-making.

6. APPLICATIONS IN FORENSIC IDENTIFICATION

Molecular techniques have become indispensable across a wide range of forensic applications, significantly improving the accuracy, sensitivity, and reliability of identification processes. In crime scene investigations, DNA analysis is routinely used to link suspects to biological evidence such as blood, saliva, semen, or hair. Even trace amounts of DNA can now be amplified and analyzed, allowing investigators to reconstruct events and establish connections that would otherwise be impossible using traditional methods alone (45).

In cases involving missing persons, molecular profiling plays a crucial role by comparing DNA obtained from unidentified remains with that of biological relatives. This approach is particularly valuable when visual identification is not possible due to decomposition or skeletal remains (46). Similarly, in mass disaster situations such as natural calamities, aviation accidents, or terrorist attacks, DNA-based identification provides a reliable means of identifying victims when bodies are fragmented or severely damaged. Techniques such as STR analysis, mitochondrial DNA profiling, and increasingly next-generation sequencing are commonly employed in these scenarios (47). Kinship and paternity testing represent another major application of forensic molecular biology. By analyzing inherited genetic markers, it is possible to establish biological relationships with a high degree of certainty. These analyses are widely used in legal disputes, immigration cases, and inheritance claims, where accurate determination of familial relationships is essential (48).

Beyond human identification, molecular techniques are also applied in wildlife forensics, an area that focuses on combating illegal poaching, trafficking, and trade of endangered species. DNA analysis can be used to identify species, determine geographic origin, and even link seized animal products to specific populations or habitats. This application has become increasingly important in supporting conservation efforts and enforcing environmental laws. Overall, the integration of molecular techniques into forensic science has expanded its scope far beyond traditional boundaries, enabling precise and reliable identification in diverse and often challenging scenarios (49).

7. CHALLENGES AND LIMITATIONS

Despite the remarkable advancements in molecular forensic techniques, several challenges and limitations continue to affect their routine application and interpretation. One of the most critical concerns is the risk of contamination, as modern methods such as PCR and next-generation sequencing are highly sensitive and can amplify even minute amounts of extraneous DNA. Contamination may occur during sample collection, handling, or laboratory processing, potentially leading to misleading or erroneous results if strict quality control measures are not maintained (50).

Another major challenge lies in the interpretation of mixed DNA samples, which contain genetic material from multiple individuals. Such mixtures are commonly encountered in forensic casework, particularly in violent crimes or shared environments. Although advanced analytical methods have improved the ability to deconvolute these mixtures, interpretation can still be complex and may require sophisticated statistical models and expert judgment, increasing the possibility of ambiguity (51).

Ethical and privacy concerns also represent significant limitations in the use of forensic genetic data. The expansion of DNA databases and the use of techniques such as forensic phenotyping and ancestry inference raise important questions regarding consent, data security, and potential misuse of genetic information. Balancing the benefits of these technologies with the protection of individual rights remains a key issue for policymakers and forensic practitioners (52).

Additionally, the lack of global standardization, especially for advanced technologies like next-generation sequencing (NGS), poses a challenge for consistent implementation across forensic laboratories. Differences in protocols, data interpretation methods, and validation standards can lead to variability in results and limit the comparability of findings between jurisdictions (53). Addressing these issues requires the development of universally accepted guidelines, rigorous validation studies, and continuous training of forensic professionals. While molecular techniques have greatly strengthened forensic science, overcoming these challenges is essential to ensure their reliability, ethical use, and global acceptance (54).

8. FUTURE PERSPECTIVES

The future of forensic identification is expected to be shaped by the continued integration of advanced genomic technologies, artificial intelligence, and portable analytical platforms. Rapid developments in sequencing technologies are making it possible to generate comprehensive genetic profiles with greater speed, accuracy, and efficiency than ever before (55). In particular, the transition from traditional DNA profiling methods to high-throughput genomic approaches is enabling deeper insights into genetic variation, ancestry, and phenotypic traits. One of the most promising directions is the incorporation of artificial intelligence and machine learning algorithms into forensic workflows. These tools have the potential to enhance the interpretation of complex DNA data, improve mixture deconvolution, and assist in predictive modeling for forensic phenotyping and ancestry analysis. As datasets grow larger and more complex, AI-driven systems will play a crucial role in ensuring accurate, rapid, and unbiased analysis (56).

Another important advancement is the development of portable and field-deployable sequencing technologies, such as handheld DNA sequencers. These devices could allow on-site analysis of biological evidence, reducing the time required for laboratory processing and enabling real-time decision-making during investigations (57). Rapid DNA analysis systems are also evolving, with the potential to provide near-instant genetic profiles in police stations, border control points, and disaster sites. In addition, future research is likely to focus on integrating multi-omics approaches combining genomics, epigenomics, proteomics, and microbiomics to provide a more comprehensive understanding of forensic evidence (58). This holistic approach could significantly enhance the ability to reconstruct events, estimate time since death, and generate investigative leads. However, alongside these technological advancements, there will be an increasing need for robust ethical frameworks, data protection policies, and international standardization (59). Ensuring the responsible use of genetic information while maintaining scientific accuracy and legal admissibility will be critical for the continued progress of forensic science. The future of forensic molecular biology is highly promising, with emerging technologies set to transform forensic identification into a faster, more precise, and more informative process (60).

9. CONCLUSION

Molecular techniques have fundamentally transformed the field of forensic identification, offering a level of accuracy, sensitivity, and reliability that was previously unattainable with conventional methods. The introduction of DNA-based analysis has not only strengthened the scientific basis of forensic investigations but has also significantly improved the ability to resolve complex cases involving degraded, limited, or mixed biological evidence. Techniques such as STR profiling continue to serve as the backbone of forensic genetics due to their robustness and widespread acceptance in legal systems. At the same time, emerging technologies, particularly next-generation sequencing (NGS) and advances in forensic genomics, are expanding the scope of what can be achieved through molecular analysis. These innovations enable more detailed genetic insights, including sequence-level variation, ancestry information, and phenotypic predictions, thereby enhancing both investigative and identification capabilities. As these technologies continue to evolve, they are expected to complement and, in some cases, redefine traditional forensic approaches.

However, the successful integration of these advanced methods into routine forensic practice will depend on addressing existing challenges such as standardization, cost, data interpretation, and ethical considerations. Establishing universally accepted protocols and ensuring proper training of forensic professionals will be essential to maintain the reliability and admissibility of molecular evidence in court. In conclusion, molecular forensic science is entering a new era characterized by rapid technological progress and interdisciplinary integration. With continued research, innovation, and global collaboration, these techniques will play an increasingly vital role in strengthening criminal justice systems and delivering accurate, science-based outcomes worldwide.

Ethical Statement

This review article is based solely on previously published literature and does not involve any new experiments on human participants or animals. Therefore, ethical approval and informed consent were not required. The authors confirm that all sources have been appropriately cited and that the manuscript is free from plagiarism. Artificial intelligence tools, including ChatGPT and Grammarly, were used only for language refinement, grammar correction, and improving clarity of expression. These tools did not contribute to the scientific content, data interpretation, or conclusions of the study. The authors take full responsibility for the accuracy, integrity, and originality of the work.

Author Contributions

Nidhi Rajesh Yadav conducted the literature review, collected and analyzed relevant published studies, and prepared the initial draft of the manuscript. Bhanupratap Vishwakarma was responsible for the conceptualization of the study, provided supervision throughout the work, and critically reviewed and edited the manuscript. Both authors contributed to refining the content and approved the final version of the manuscript.

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