RESEARCH PAPER

A Review on Emerging Trends in Forensic DNA Profiling and Analysis

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ABSTRACT
Forensic DNA profiling has undergone remarkable advancements over the past few decades, evolving from conventional short tandem repeat (STR) analysis to highly sophisticated genomic and bioinformatic approaches. Emerging technologies such as next-generation sequencing (NGS), rapid DNA analysis, forensic investigative genetic genealogy (FIGG), probabilistic genotyping, forensic DNA phenotyping, epigenetic analysis, and artificial intelligence are transforming the field of forensic science. These innovations enable improved identification from degraded, mixed, and low-template samples while increasing analytical sensitivity, accuracy, and speed. Additionally, RNA profiling and mitochondrial DNA analysis have expanded the scope of biological evidence interpretation in criminal investigations and disaster victim identification. Despite these advancements, several ethical, legal, and privacy concerns remain, particularly regarding genetic databases, data protection, algorithm transparency, and admissibility in court. This review highlights the recent developments, applications, advantages, and challenges associated with emerging trends in forensic DNA profiling and analysis. The study emphasizes the importance of scientific validation, regulatory oversight, and ethical considerations for the responsible implementation of advanced forensic genomic technologies in modern criminal justice systems.

Keywords: *Forensic DNA profiling; Next-generation sequencing; Rapid DNA analysis; Forensic genetic genealogy; Probabilistic genotyping; DNA phenotyping; Epigenetics; RNA profiling; Artificial intelligence; Forensic genomics*
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1. INTRODUCTION

Forensic DNA profiling has become one of the most important scientific tools in modern criminal investigations, offering highly accurate methods for human identification. Since the discovery of DNA fingerprinting by Sir Alec Jeffreys in 1985, forensic genetics has undergone significant advancements, transforming the criminal justice system worldwide **[1]**. DNA analysis is now widely used in criminal investigations, paternity testing, disaster victim identification, missing person investigations, and the exoneration of wrongly convicted individuals. The progress in molecular biology, genomics, and computational technologies has greatly improved the sensitivity, specificity, and efficiency of forensic DNA analysis **[2]**.

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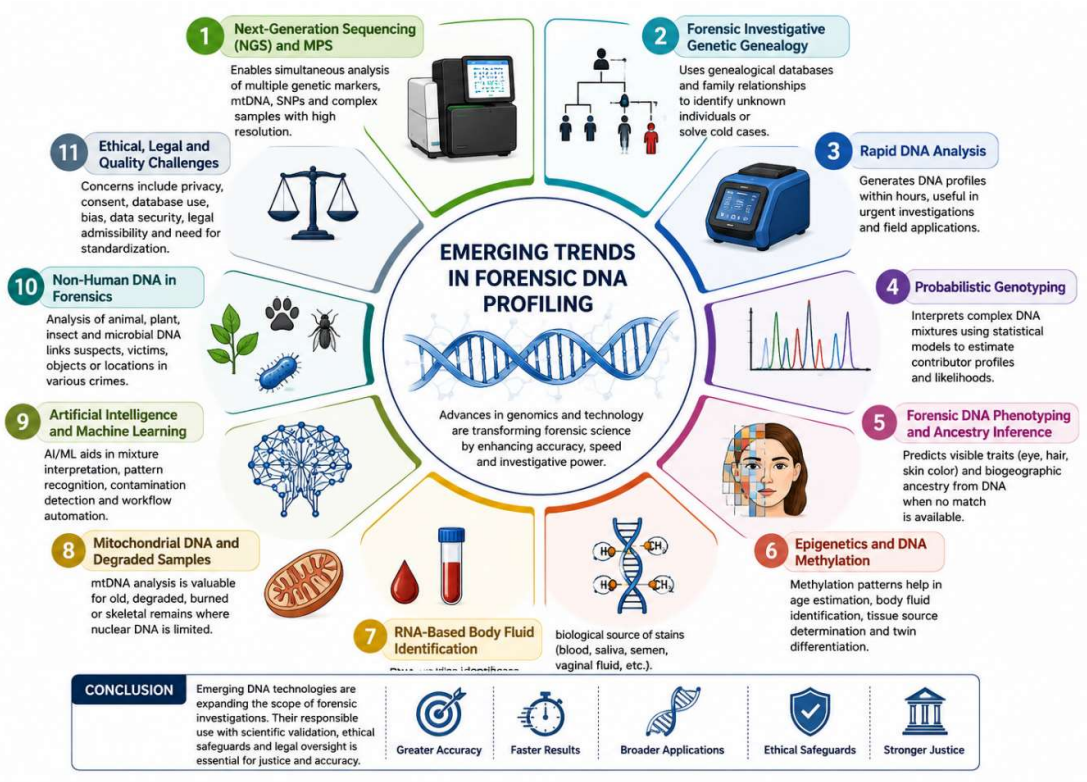

Figure 1: Emerging Trends in Forensic DNA

DNA, the hereditary material present in almost all living organisms, is unique to every individual except identical twins, making it highly valuable for forensic purposes. Biological materials such as blood, saliva, semen, hair roots, bones, and skin cells serve as important sources of DNA evidence. Early forensic DNA analysis mainly relied on restriction fragment length polymorphism (RFLP), which required large amounts of high-quality DNA **[3]**. However, the introduction of polymerase chain reaction (PCR) technology revolutionized forensic science by enabling the amplification of small and degraded DNA samples. Subsequently, short tandem repeat (STR) profiling became the gold standard for forensic identification because of its high discriminatory power and compatibility with national DNA databases. Despite its effectiveness, conventional STR analysis faces limitations when analyzing degraded samples, mixed DNA profiles, or low-template DNA evidence **[4]**.

Recent technological developments have introduced advanced approaches in forensic DNA profiling. Next-generation sequencing (NGS), also known as massively parallel sequencing, allows simultaneous analysis of multiple genetic markers, including STRs, single nucleotide polymorphisms (SNPs), mitochondrial DNA, and ancestry markers. Rapid DNA technology has further improved forensic investigations by enabling automated DNA profiling within a few hours **[5]**. In addition, forensic investigative genetic genealogy (FIGG) and forensic DNA phenotyping have expanded investigative capabilities by identifying familial relationships and predicting physical characteristics from DNA evidence. Probabilistic genotyping software, artificial intelligence, epigenetics, and RNA profiling are also improving the interpretation of complex forensic samples **[6]**.

Although these advancements offer significant benefits, ethical and legal concerns such as genetic privacy, database security, informed consent, and admissibility of evidence remain major challenges. Therefore, continued scientific validation, ethical regulation, and policy development are essential to ensure the responsible application of forensic genomic technologies in criminal justice systems **[7]**. This review article aims to provide a comprehensive overview of the emerging trends in forensic DNA profiling and analysis. The review discusses recent technological advancements, their forensic applications, advantages, limitations, and future prospects. In addition, the article highlights the scientific, ethical, and regulatory challenges associated with advanced forensic genomic technologies and emphasizes the need for continued research, validation, and policy development to ensure their effective and responsible implementation in forensic science.

2. NEXT-GENERATION SEQUENCING AND MASSIVELY PARALLEL SEQUENCING

Next-generation sequencing (NGS), also referred to as massively parallel sequencing (MPS), has emerged as a revolutionary advancement in forensic DNA profiling and analysis. Conventional forensic DNA analysis primarily relies on capillary electrophoresis (CE)-based short

tandem repeat (STR) profiling, which identifies alleles based on fragment length variation **[8]**. Although this method has demonstrated excellent reliability and discriminatory power, it possesses certain limitations, particularly in the analysis of degraded samples, mixed DNA profiles, and low-template DNA. NGS technology overcomes many of these limitations by enabling simultaneous sequencing of millions of DNA fragments and generating sequence-level information from multiple genetic markers in a single analysis **[9]**.

Unlike traditional STR typing, which only measures fragment size, NGS determines the exact nucleotide sequence of DNA fragments. This allows identification of sequence variations within STR alleles that may appear identical in size during capillary electrophoresis. Consequently, NGS enhances discrimination power, improves mixture interpretation, and increases the accuracy of forensic identification. Furthermore, the technology permits simultaneous analysis of STRs, single nucleotide polymorphisms (SNPs), mitochondrial DNA (mtDNA), insertion-deletion polymorphisms (InDels), ancestry markers, and phenotype-associated markers within a single workflow **[10]**.

2.1 Principles and Workflow of NGS Technology

The NGS process involves several important steps, including DNA extraction, library preparation, amplification, sequencing, and bioinformatic analysis. Initially, DNA samples are fragmented into smaller pieces, and specialized adapter sequences are attached to the DNA fragments during library preparation. These adapters facilitate amplification and sequencing reactions. The prepared libraries are then amplified and sequenced simultaneously in massively parallel reactions, producing millions of sequences reads within a short time **[11]**.

Advanced bioinformatics tools are subsequently used to process and analyze the generated data. Sequence alignment, quality filtering, allele calling, and statistical interpretation are performed using computational software. Compared with conventional CE-based analysis, NGS produces a significantly larger amount of genetic information, requiring specialized software and computational infrastructure for accurate interpretation **[12]**. Several NGS platforms are currently used in forensic science, including Illumina sequencing systems, Ion Torrent technology, and Oxford Nanopore sequencing. Illumina platforms are among the most widely adopted in forensic laboratories because of their high sequencing accuracy and reproducibility. Nanopore sequencing, on the other hand, offers portability and real-time sequencing capabilities, making it potentially useful for field-based forensic applications.

2.2 Applications of NGS in Forensic DNA Analysis

NGS technology has a wide range of forensic applications due to its ability to analyze multiple genetic markers simultaneously. One of the most important applications is enhanced STR analysis. Sequence-based STR typing provides additional genetic variation within alleles of identical length, thereby increasing discrimination power and improving individual identification **[13]**.

NGS is also highly effective in analyzing degraded and low-template DNA samples. Crime scene evidence is frequently exposed to environmental conditions such as heat, humidity, ultraviolet radiation, and microbial contamination, leading to DNA degradation. Since NGS can analyze shorter DNA fragments, it is more suitable for highly degraded forensic samples such as old skeletal remains, burned tissues, hair shafts, and ancient biological materials **[14]**.

Another important application is mitochondrial DNA sequencing. Mitochondrial DNA is present in higher copy numbers than nuclear DNA and is therefore valuable in cases involving degraded or limited biological material. NGS enables complete mitochondrial genome sequencing, improving discrimination and maternal lineage analysis compared with conventional mitochondrial DNA testing methods. In addition, NGS supports forensic investigative genetic genealogy, ancestry inference, and forensic DNA phenotyping through SNP analysis. These approaches help investigators identify unknown individuals, predict biogeographical ancestry, and estimate externally visible characteristics such as hair color, eye color, and skin pigmentation. Such information can provide valuable investigative leads when no suspect or database match is available.

3. FORENSIC INVESTIGATIVE GENETIC GENEALOGY

Forensic Investigative Genetic Genealogy (FIGG), also known as forensic genetic genealogy (FGG), is an emerging and rapidly evolving field in forensic science that combines advanced DNA analysis with traditional genealogical research to identify unknown individuals. This approach has gained significant attention in recent years due to its success in solving cold criminal cases, identifying unidentified human remains, and locating missing persons. Unlike conventional forensic DNA profiling, which relies mainly on direct matches in criminal DNA databases, FIGG uses single nucleotide polymorphism (SNP)-based genetic analysis and publicly accessible genealogical databases to identify distant biological relatives of an unknown individual **[15]**.

The growing popularity of direct-to-consumer genetic testing services has contributed significantly to the development of forensic genetic genealogy. Millions of individuals worldwide have voluntarily uploaded their genetic information to genealogy databases to trace ancestry and identify relatives. Forensic investigators can compare unknown DNA profiles with these databases to identify potential familial relationships and construct family trees that may eventually lead to the identification of suspects or unidentified individuals **[16-17]**.

3.1 Principles and Workflow of Forensic Genetic Genealogy

The fundamental principle of FIGG is based on the inheritance of shared DNA segments among biologically

related individuals. Unlike traditional forensic STR profiling, which focuses on a limited number of STR markers, FIGG relies on the analysis of hundreds of thousands of SNPs distributed across the genome. SNPs provide detailed genetic information that allows detection of distant familial relationships, including third, fourth, or even fifth cousins **[18]**.

The FIGG process typically begins with collection and extraction of DNA from crime scene evidence or unidentified human remains. The extracted DNA is then subjected to SNP genotyping or next-generation sequencing to generate a dense genetic profile. This SNP profile is subsequently uploaded to approved genealogy databases where it is compared with profiles of database participants to identify potential relatives **[19]**.

After identifying possible genetic relatives, forensic genealogists perform extensive genealogical research using public records such as birth certificates, census records, marriage records, obituaries, and family history databases. Family trees are constructed to determine potential connections between the unknown individual and the identified relatives. Investigators then narrow down possible candidates based on demographic information, geographic location, age, and other case-related evidence **[20]**. Confirmation of identity is ultimately achieved using conventional forensic DNA analysis, such as STR profiling, by comparing reference samples from suspects or relatives with the original forensic evidence **[21]**.

3.2 Ethical, Legal, and Privacy Concerns

Despite its remarkable success, forensic genetic genealogy has generated substantial ethical, legal, and social concerns. One of the primary concerns involves genetic privacy. Individuals who upload their DNA profiles to genealogy databases may unknowingly expose genetic information about their relatives who have not provided consent for forensic use **[22]**.

Another major issue is informed consent and database access. Many genealogy databases were originally created for recreational ancestry analysis rather than forensic investigations. The use of these databases by law enforcement agencies raises questions regarding user consent, data ownership, and permissible use of genetic information **[23]**.

Potential misuse of genetic data and risk of genetic surveillance are also significant concerns. Improper handling or unauthorized access to sensitive genetic information may lead to discrimination, stigmatization, or privacy violations. Additionally, population bias within genealogy databases may affect the accuracy and fairness of investigations, particularly among underrepresented ethnic groups **[24]**. Legal admissibility and regulatory oversight are equally important challenges. Different countries have varying regulations regarding forensic genealogy, and the absence of universal legal frameworks may create inconsistencies in forensic practice. Therefore, strict ethical guidelines, transparency, quality assurance, and judicial oversight are essential for responsible implementation of FIGG.

4. RAPID DNA ANALYSIS

Rapid DNA analysis is an emerging advancement in forensic science that enables automated generation of DNA profiles within a few hours rather than the several days or weeks required in conventional laboratory-based forensic DNA analysis. Rapid DNA systems integrate DNA extraction, amplification, separation, detection, and profile interpretation into a single automated instrument with minimal human intervention. The development of this technology has significantly transformed forensic investigations by providing faster identification of individuals in time-sensitive situations such as criminal investigations, border security operations, disaster victim identification, and missing-person cases **[25]**.

Traditional forensic DNA profiling involves multiple laboratory procedures, including sample preparation, DNA extraction, quantification, amplification using polymerase chain reaction (PCR), capillary electrophoresis, and data interpretation. These processes require specialized laboratory facilities and trained forensic personnel. In contrast, rapid DNA technology automates these steps within compact instruments capable of producing STR profiles directly from biological samples such as buccal swabs. This automation reduces turnaround time and enhances the efficiency of forensic investigations **[26]**.

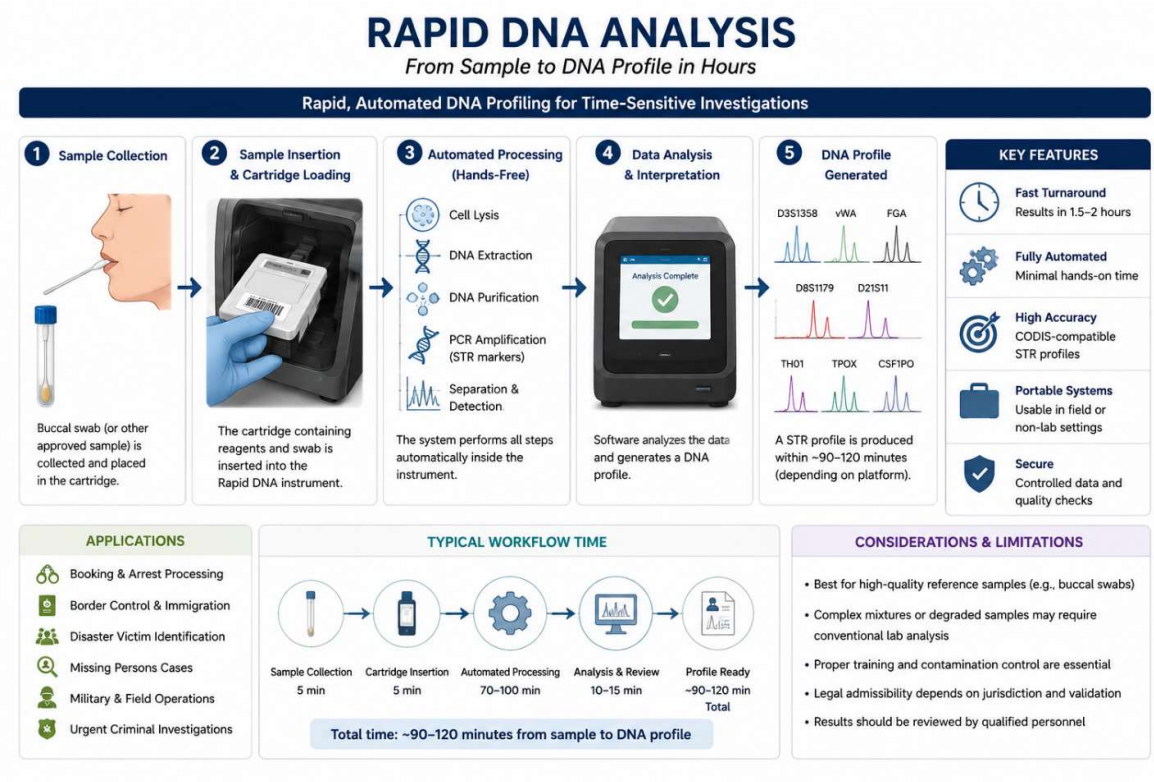

Figure 2: Rapid DNA Analysis

4.1 Principles and Workflow of Rapid DNA Technology

Rapid DNA systems are designed to perform fully automated STR analysis using integrated microfluidic and PCR-based technologies. The workflow generally begins with insertion of a biological sample, usually a buccal swab, into a disposable cartridge containing all necessary reagents for DNA extraction and amplification. The instrument subsequently performs cell lysis, DNA purification, PCR amplification of STR markers, electrophoretic separation, detection, and profile generation without requiring manual laboratory processing **[27]**.

Most rapid DNA instruments are based on capillary electrophoresis or microfluidic electrophoresis systems and are capable of generating CODIS-compatible STR profiles. The entire process can typically be completed within 90 minutes to two hours, depending on the platform used. Advanced software integrated into these systems automatically interprets genetic data and generates DNA profiles for comparison with forensic databases **[28]**. Several commercial rapid DNA platforms have been developed, including portable and field-deployable systems suitable for law enforcement and military applications. These instruments are designed to operate outside traditional forensic laboratories while maintaining high analytical accuracy and reliability.

4.2 Applications of Rapid DNA Analysis

Rapid DNA analysis has a broad range of applications in forensic and security-related investigations. One of the primary applications is use in police booking stations for rapid identification of suspects. Generating DNA profiles shortly after arrest can accelerate criminal investigations and help identify repeat offenders or link suspects to unsolved crimes **[28]**.

Another important application is border security and immigration control. Rapid DNA systems can assist in verification of biological relationships during immigration investigations and identification of trafficking victims or separated family members. The technology also has potential applications in military operations and national security. Rapid DNA analysis is particularly valuable in disaster victim identification (DVI) and mass casualty incidents. Natural disasters, terrorist attacks, aircraft accidents, and armed conflicts often involve large numbers of unidentified victims requiring rapid identification. Portable rapid DNA systems can provide timely identification of victims, thereby assisting humanitarian and legal processes **[29]**.

The technology is also useful in missing-person investigations and humanitarian forensic cases. Rapid identification of unidentified remains can facilitate reunification of families and improve efficiency of forensic investigations in remote or resource-limited settings.

4.3 Advantages and Limitations of Rapid DNA Technology

Rapid DNA technology offers several significant advantages over conventional forensic DNA analysis. The

most important advantage is reduced turnaround time. Traditional forensic analysis may require days or weeks due to laboratory backlog and multiple analytical procedures, whereas rapid DNA systems can generate profiles within hours **[30]**.

Automation is another major advantage. Minimal manual handling reduces the risk of human error and contamination while simplifying forensic workflows. The portability of some rapid DNA instruments further enables on-site forensic analysis in field conditions, police stations, military environments, and disaster zones. Rapid DNA analysis also improves operational efficiency by reducing dependency on centralized forensic laboratories. Faster generation of DNA profiles can support immediate investigative decisions and enhance public safety responses **[31]**.

Despite these advantages, rapid DNA technology also has several limitations and challenges. One major concern is sample quality. Rapid DNA systems are most effective with high-quality reference samples such as buccal swabs, whereas degraded, mixed, or environmentally compromised forensic samples may still require conventional laboratory analysis **[32]**.

Contamination control and quality assurance are additional concerns, particularly when instruments are used outside controlled laboratory environments. Inadequate handling or poor operational practices may compromise profile accuracy and reliability. Another important limitation is the inability of some rapid DNA systems to accurately interpret complex DNA mixtures or low-template samples. Such cases often require expert forensic review and advanced laboratory analysis. Furthermore, legal admissibility and regulatory approval remain significant considerations, as different countries have varying standards governing the use of rapid DNA technologies in criminal justice systems **[33]**.

4.4 Future Perspectives of Rapid DNA Analysis

Future developments in rapid DNA technology are expected to focus on improving analytical sensitivity, portability, automation, and integration with forensic databases. Advances in microfluidics, nanotechnology, and artificial intelligence may further enhance the capability of rapid DNA systems to analyze complex forensic samples with greater accuracy **[34]**.

Integration of rapid DNA instruments with mobile forensic laboratories and real-time database connectivity may strengthen field-based forensic investigations. Improvements in contamination prevention systems and automated quality control mechanisms are also expected to enhance reliability and legal acceptance. As validation studies and international guidelines continue to evolve, rapid DNA analysis is likely to become an increasingly important component of modern forensic science, particularly in time-sensitive and high-throughput forensic applications **[35]**.

5. PROBABILISTIC GENOTYPING

Probabilistic genotyping is a modern computational approach used in forensic DNA analysis to interpret complex DNA mixtures using statistical and mathematical models. In forensic casework, biological evidence frequently contains DNA from multiple contributors, making interpretation challenging using conventional manual analysis methods. Traditional DNA interpretation often relies on threshold-based visual examination of electropherograms, which may produce subjective or inconclusive results, particularly in low-template or degraded samples. Probabilistic genotyping software addresses these challenges by applying statistical algorithms to estimate the likelihood of different contributor combinations and improve the accuracy and objectivity of forensic DNA interpretation **[36]**.

The increasing complexity of forensic DNA evidence, including mixed samples from multiple individuals, low-level DNA, stochastic effects, allele dropout, and degraded biological material, has accelerated the adoption of probabilistic genotyping systems in forensic laboratories worldwide. These computational methods have significantly enhanced the reliability and sensitivity of forensic DNA analysis and are increasingly accepted in criminal investigations and court proceedings **[37]**.

5.1 Principles and Methodology of Probabilistic Genotyping

Probabilistic genotyping systems use mathematical and statistical models to evaluate DNA evidence and estimate the probability of different genetic scenarios. Instead of relying solely on manual interpretation, the software analyzes peak heights, allele frequencies, stutter patterns, degradation effects, and amplification variability to calculate likelihood ratios for competing hypotheses **[38]**.

The process generally involves comparing two hypotheses: one representing the prosecution scenario, in which a specific individual contributed to the DNA mixture, and another representing the defense scenario, in which the DNA originated from unknown individuals unrelated to the suspect. The software calculates a likelihood ratio (LR), which quantifies how strongly the DNA evidence supports one hypothesis over the other. Probabilistic genotyping approaches are commonly classified into semi-continuous and fully continuous models. Semi-continuous models primarily consider the presence or absence of alleles and account for allele dropout, whereas fully continuous models additionally incorporate quantitative information such as peak height data and signal intensity. Fully continuous systems generally provide greater sensitivity and accuracy in interpreting complex DNA mixtures **[39]**.

Several probabilistic genotyping software systems have been developed for forensic applications, including STRmix, TrueAllele, EuroForMix, and Lab Retriever. These systems use advanced computational algorithms and Bayesian statistical frameworks to analyze complex forensic DNA evidence.

5.2 Applications in Forensic Casework

Probabilistic genotyping has become particularly important in the interpretation of mixed DNA samples, which are common in violent crimes such as sexual assault, homicide, physical assault, and robbery cases. Biological evidence recovered from crime scenes frequently contains DNA from multiple contributors, making conventional interpretation difficult. Probabilistic genotyping improves mixture deconvolution and assists in identifying contributors with greater confidence **[40]**.

Another important application is analysis of low-template and degraded DNA samples. Trace amounts of DNA or environmentally degraded samples often produce incomplete or weak genetic profiles. Probabilistic models account for stochastic effects such as allele dropout and peak imbalance, thereby improving interpretation accuracy. The technology is also valuable in cold case investigations where older or compromised evidence must be reanalyzed using modern analytical methods. In many cases, probabilistic genotyping has enabled successful reinterpretation of previously inconclusive forensic evidence **[41]**.

In addition, probabilistic genotyping is increasingly used in kinship analysis, disaster victim identification, and missing-person investigations involving partial or mixed DNA profiles.

5.3 Advantages and Challenges of Probabilistic Genotyping

One of the primary advantages of probabilistic genotyping is improved objectivity in forensic DNA interpretation. Traditional manual analysis may involve subjective decision-making, whereas statistical models provide standardized and reproducible interpretations. Another major advantage is enhanced sensitivity for complex mixtures and low-template samples. The technology allows forensic scientists to extract more information from challenging DNA evidence and improve the probability of identifying contributors **[42]**.

Probabilistic genotyping also strengthens the statistical evaluation of forensic evidence by generating quantitative likelihood ratios that can support courtroom testimony and legal decision-making. The approach enhances scientific rigor and reduces the likelihood of incorrect exclusions or inclusions. Despite these benefits, several challenges remain associated with probabilistic genotyping. One major concern is the complexity of software algorithms and lack of transparency in some proprietary systems. Defense attorneys and legal experts have raised concerns regarding limited access to source codes and difficulties in independently evaluating software reliability **[43]**.

Validation and standardization are also critical issues. Different software systems may produce varying likelihood ratios for the same evidence depending on model assumptions and analytical parameters. Therefore, rigorous validation, quality assurance, and standardized interpretation guidelines are essential **[44]**.

Another challenge involves communication of statistical results in court. Likelihood ratios and probabilistic interpretations may be difficult for judges, juries, and legal professionals to understand. Effective expert testimony and clear scientific explanation are therefore necessary to ensure proper interpretation of forensic evidence.

5.4 Future Perspectives of Probabilistic Genotyping

Future developments in probabilistic genotyping are expected to focus on improving computational accuracy, transparency, user accessibility, and integration with artificial intelligence and machine learning technologies. Enhanced algorithms may further improve interpretation of highly complex DNA mixtures involving multiple contributors and degraded samples. Open-source and transparent software systems may help address concerns regarding reproducibility and legal scrutiny. Advances in high-performance computing and bioinformatics are also expected to reduce analysis time and improve efficiency **[45]**.

As forensic laboratories continue to adopt advanced statistical approaches, probabilistic genotyping is likely to become a standard component of routine forensic DNA analysis. Continued research, validation, training, and legal education will be essential to ensure accurate interpretation and responsible use of probabilistic genotyping technologies in forensic science and criminal justice systems **[46]**.

6. FORENSIC DNA PHENOTYPING AND ANCESTRY INFERENCE

Forensic DNA phenotyping (FDP) and ancestry inference are emerging branches of forensic genomics that aim to predict externally visible characteristics and biogeographical ancestry from biological evidence obtained at crime scenes. Unlike conventional forensic DNA profiling, which primarily focuses on identifying individuals through direct DNA matches, forensic DNA phenotyping provides investigative intelligence when no suspect or database match is available. This technology assists law enforcement agencies by generating predictive information about the physical appearance and ancestral background of an unknown individual based on genetic analysis **[47]**.

Recent advances in genomics and next-generation sequencing have significantly improved the ability to analyze genetic markers associated with human appearance and ancestry. Single nucleotide polymorphisms (SNPs) play a major role in forensic phenotyping because certain SNPs are strongly associated with traits such as eye color, hair color, skin pigmentation, facial morphology, and geographical ancestry. The analysis of these markers enables forensic scientists to estimate probable physical characteristics and ancestral origins of individuals from DNA evidence **[48]**.

6.1 Principles of Forensic DNA Phenotyping

Forensic DNA phenotyping is based on the relationship between genetic variations and phenotypic traits. Specific genes influence externally visible characteristics. For

example, variations in the HERC2 and OCA2 genes are strongly associated with blue or brown eye color, while genes such as MC1R, TYR, and SLC24A5 contribute to hair color and skin pigmentation. The process generally begins with extraction of DNA from biological evidence followed by amplification and sequencing of selected SNP markers. Bioinformatic algorithms and statistical models are then used to predict phenotypic characteristics from the obtained genetic data. Predictions are usually presented as probabilities rather than exact determinations because phenotypic traits are influenced by multiple genes and environmental factors **[49]**.

Ancestry inference similarly uses ancestry-informative markers (AIMs), which are SNPs that exhibit population-specific frequency differences across geographical regions. By comparing unknown DNA profiles with reference population databases, forensic scientists can estimate the likely ancestral origin of an individual **[50]**.

6.2 Applications in Forensic Investigations

One of the most important applications of forensic DNA phenotyping is generation of investigative leads in criminal cases where no suspect has been identified. Predicted physical traits such as eye color, hair color, and skin pigmentation may help narrow the pool of potential suspects and support investigative efforts. Ancestry inference is also useful in missing-person investigations and identification of unidentified human remains. Determining probable ancestral background can provide valuable demographic information and assist in narrowing identification possibilities **[51]**.

In addition, forensic DNA phenotyping has applications in disaster victim identification, historical investigations, and humanitarian forensic projects. The technology may also complement traditional forensic methods such as fingerprint analysis, eyewitness testimony, and conventional DNA profiling **[52]**.

6.3 Limitations and Ethical Concerns

Despite its growing importance, forensic DNA phenotyping faces several scientific and ethical limitations. Prediction accuracy varies depending on the trait being analyzed. Eye color prediction is generally more accurate than prediction of facial morphology or skin pigmentation because many physical traits involve complex interactions among multiple genes and environmental influences. Another important limitation is the possibility of overinterpretation. Phenotypic predictions are probabilistic estimates and should not be considered definitive evidence of identity. Incorrect interpretation may lead to misidentification or investigative bias **[53]**.

Ethical concerns associated with forensic phenotyping are particularly significant. The use of ancestry inference may contribute to racial profiling, discrimination, or stigmatization of certain populations. Privacy concerns also arise because genetic analysis may reveal sensitive personal or familial information beyond physical characteristics **[54]**.

Therefore, careful regulation, scientific validation, ethical oversight, and legal guidelines are essential to ensure responsible use of forensic DNA phenotyping and ancestry inference in criminal investigations.

7. EPIGENETICS AND DNA METHYLATION

Epigenetics refers to heritable modifications in gene expression that occur without altering the underlying DNA sequence. Among various epigenetic mechanisms, DNA methylation is one of the most widely studied in forensic science. DNA methylation involves the addition of methyl groups to cytosine nucleotides, particularly at CpG sites, resulting in regulation of gene activity and cellular function **[55]**.

Recent research has demonstrated that DNA methylation patterns vary according to tissue type, age, environmental exposure, lifestyle, and physiological conditions. These variations have created new opportunities in forensic science beyond traditional identity matching. DNA methylation analysis is increasingly being explored for applications such as body fluid identification, tissue source determination, forensic age estimation, estimation of lifestyle factors, and differentiation of monozygotic twins **[56]**.

7.1 Principles of DNA Methylation Analysis

DNA methylation analysis generally involves treatment of DNA with sodium bisulfite, which converts unmethylated cytosines into uracil while leaving methylated cytosines unchanged. Subsequent PCR amplification and sequencing allow forensic scientists to identify methylated and unmethylated regions of DNA. Different tissues exhibit distinct methylation patterns because gene expression varies among cell types. Similarly, certain methylation markers show predictable age-related changes, allowing estimation of an individual's approximate chronological age **[57]**.

The analysis may be performed using PCR-based methods, pyrosequencing, microarrays, or next-generation sequencing technologies. Bioinformatic tools are subsequently used to analyze methylation patterns and generate forensic interpretations **[58]**.

7.2 Applications in Forensic Science

One of the major applications of forensic epigenetics is body fluid identification. Different biological materials such as blood, saliva, semen, vaginal fluid, and menstrual blood possess characteristic methylation signatures that can help determine the origin of forensic stains. Forensic age estimation is another important application. Specific DNA methylation markers demonstrate strong correlations with chronological age, allowing investigators to estimate the approximate age of individuals from biological evidence left at crime scenes **[59]**.

DNA methylation analysis also shows promise in distinguishing monozygotic twins, who possess nearly identical DNA sequences but may develop different epigenetic profiles due to environmental and lifestyle influences. This represents a significant advancement

because traditional DNA profiling cannot reliably differentiate identical twins. Additional applications include tissue source determination, lifestyle inference, estimation of postmortem interval, and disease-associated forensic investigations **[60]**.

7.3 Challenges and Future Perspectives

Despite its promising applications, forensic epigenetics faces several challenges. Environmental conditions, disease states, smoking, diet, stress, and other lifestyle factors may influence methylation patterns and affect predictive accuracy. Standardization of methylation markers and analytical methods remains limited, and large-scale validation studies are still required before routine forensic implementation. Furthermore, epigenetic analysis requires highly sensitive instrumentation and advanced computational interpretation **[61]**.

Future research is expected to improve the identification of robust methylation biomarkers and develop more accurate prediction models. Advances in sequencing technologies and bioinformatics are likely to expand the role of epigenetics in forensic investigations.

8. RNA-BASED BODY FLUID IDENTIFICATION

RNA-based body fluid identification is an emerging forensic technique used to determine the biological source of forensic evidence. In many criminal investigations, identifying whose DNA is present at a crime scene is not sufficient; investigators also need to determine how the biological material was deposited and which body fluid or tissue it originated from. RNA profiling provides this contextual information by distinguishing among blood, saliva, semen, vaginal fluid, menstrual blood, skin cells, and other biological materials **[62]**.

RNA molecules are involved in gene expression and exhibit tissue-specific expression patterns. Certain RNA markers are uniquely or predominantly expressed in particular body fluids, making them useful for forensic body fluid identification. Messenger RNA (mRNA), microRNA (miRNA), ribosomal RNA (rRNA), and circular RNA (circRNA) are among the RNA species investigated for forensic applications **[63]**.

8.1 Principles of RNA Profiling

RNA profiling involves extraction of RNA from biological samples followed by reverse transcription into complementary DNA (cDNA). Tissue-specific RNA markers are subsequently amplified and analyzed using PCR, quantitative PCR, or sequencing methods. Different body fluids express characteristic RNA transcripts. For example, semen contains semen-specific RNA markers, while blood and saliva express different tissue-associated transcripts. By detecting these markers, forensic scientists can identify the biological source of forensic stains **[64]**.

MicroRNAs are particularly valuable because of their small size and relatively high stability under degraded forensic conditions **[65]**.

8.2 Applications in Forensic Investigations

RNA-based body fluid identification is especially important in sexual assault and violent crime investigations. Determining whether a stain contains blood, semen, saliva, or vaginal fluid can provide critical contextual information regarding the sequence of events and type of contact involved **[66]**.

RNA profiling is also useful for analyzing mixed body fluid samples, which are common in forensic casework. The technology may help identify multiple biological components within a single stain. Additional applications include tissue identification, wound-age estimation, postmortem investigations, and forensic pathology studies **[67]**.

9. MITOCHONDRIAL DNA AND DEGRADED SAMPLES

Mitochondrial DNA (mtDNA) analysis is an important component of forensic genetics, particularly in cases involving highly degraded, old, or limited biological samples. Unlike nuclear DNA, which is present in only two copies per cell, mitochondrial DNA exists in hundreds to thousands of copies within each cell because mitochondria are abundant cellular organelles responsible for energy production. The high copy number of mtDNA makes it more resistant to degradation and significantly increases the probability of successful DNA recovery from compromised forensic evidence **[68]**.

Mitochondrial DNA is maternally inherited and does not undergo significant recombination, allowing maternal lineage tracing across generations. Although mtDNA possesses lower discriminatory power than nuclear STR profiling, it remains highly valuable in forensic investigations involving old skeletal remains, burned tissues, hair shafts without roots, ancient biological materials, and disaster victim identification. Recent advancements in sequencing technologies, particularly next-generation sequencing (NGS), have greatly improved mitochondrial genome analysis by enabling complete sequencing of the mitochondrial genome rather than focusing only on limited hypervariable regions **[69-70]**.

9.1 Structure and Characteristics of Mitochondrial DNA

Mitochondrial DNA is a small circular genome approximately 16,569 base pairs in length and is located within the mitochondria of cells. It contains genes involved in cellular respiration and energy metabolism. Unlike nuclear DNA, mtDNA is inherited exclusively from the mother, meaning maternally related individuals generally share similar mitochondrial sequences **[71]**.

The mitochondrial genome contains both coding and non-coding regions. Traditional forensic mtDNA analysis primarily focused on sequencing the hypervariable regions (HV1 and HV2) within the control region because these areas exhibit relatively high mutation rates and sequence variability among individuals. However, modern sequencing technologies now allow complete

mitochondrial genome sequencing, thereby improving discrimination power and forensic accuracy **[72]**.

Another important feature of mtDNA is its enhanced resistance to environmental degradation. Since multiple copies are present within each cell, mtDNA may still be recoverable even when nuclear DNA is severely damaged or absent **[73]**.

9.2 Applications in Forensic Investigations

One of the most important applications of mitochondrial DNA analysis is the identification of highly degraded human remains. Skeletal remains recovered from archaeological sites, mass disasters, fires, explosions, and war zones often contain degraded nuclear DNA that is insufficient for conventional STR profiling. In such situations, mtDNA analysis may provide valuable genetic information for identification **[74]**.

Hair shaft analysis is another significant forensic application of mtDNA. Hair samples lacking roots usually contain little or no nuclear DNA, making STR analysis difficult or impossible. However, mitochondrial DNA can often be successfully extracted from hair shafts due to its high copy number **[75]**.

Mitochondrial DNA is also widely used in disaster victim identification (DVI) and missing-person investigations. Maternal relatives can provide reference samples for comparison because mtDNA is maternally inherited. This approach has been extensively applied in identifying victims of natural disasters, terrorist attacks, aircraft accidents, and historical conflicts **[76]**.

In addition, mtDNA analysis has important applications in anthropological and historical forensic studies. Ancient human remains, archaeological specimens, and historical figures can often be genetically analyzed using mitochondrial sequencing techniques. Modern next-generation sequencing technologies have further expanded the forensic utility of mtDNA by enabling complete mitochondrial genome analysis. Whole mitochondrial genome sequencing improves discrimination between individuals who share similar control-region sequences and enhances interpretation accuracy in forensic investigations **[77]**.

9.3 Limitations and Future Perspectives

Despite its importance, mitochondrial DNA analysis possesses several limitations. One major limitation is lower discriminatory power compared with nuclear DNA profiling because maternally related individuals generally share identical or highly similar mtDNA sequences. Consequently, mtDNA analysis cannot uniquely identify individuals in the same manner as STR profiling **[78]**.

Another challenge involves heteroplasmy, a condition in which multiple mitochondrial DNA variants exist within the same individual. Heteroplasmy may complicate sequence interpretation and comparison in forensic investigations. Contamination is also a significant concern because mtDNA analysis often involves highly sensitive sequencing of low-template or degraded samples. Strict laboratory quality control measures are therefore essential to avoid contamination and ensure accurate results **[79]**.

Future advancements in next-generation sequencing, nanopore sequencing, and bioinformatics are expected to improve sensitivity, discrimination power, and analytical efficiency of mitochondrial DNA analysis. Complete mitochondrial genome sequencing and integration with artificial intelligence-based analytical tools may further enhance forensic identification capabilities in degraded sample analysis. As forensic genomics continues to evolve, mitochondrial DNA analysis will remain a valuable complementary tool in cases where conventional nuclear DNA profiling is unsuccessful **[80]**.

10. ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING

Artificial intelligence (AI) and machine learning (ML) are rapidly emerging technologies that are transforming multiple scientific disciplines, including forensic DNA analysis. AI refers to computational systems capable of simulating human intelligence, while machine learning involves algorithms that learn patterns from data and improve performance through experience without explicit programming. In forensic science, AI and ML are increasingly being explored for automated data analysis, pattern recognition, probabilistic interpretation, and workflow optimization **[81]**.

The growing complexity of forensic genomic data generated by next-generation sequencing, probabilistic genotyping, forensic genealogy, and large DNA databases has created a demand for advanced computational tools capable of handling large-scale data interpretation efficiently and accurately. AI-driven forensic workflows have therefore emerged as an important area of research and development in modern forensic science **[82]**.

10.1 Applications of Artificial Intelligence in Forensic DNA Analysis

One of the most significant applications of AI in forensic genomics is DNA mixture interpretation. Crime scene evidence frequently contains DNA from multiple contributors, making interpretation challenging using conventional methods. Machine learning algorithms can assist in identifying contributor patterns, distinguishing allelic peaks from background noise, and improving probabilistic genotyping accuracy **[83]**.

AI is also useful for peak pattern recognition in electropherograms generated during STR analysis. Automated systems can identify true allelic peaks, stutter artifacts, pull-up peaks, and signal noise with greater consistency and reduced subjectivity compared with manual interpretation. Contamination detection represents another important application. AI-based algorithms can monitor laboratory workflows, identify unusual sequence patterns, and detect possible contamination events in forensic samples, thereby improving quality assurance and analytical reliability **[84]**.

In kinship analysis and forensic genealogy, machine learning models can analyze large genomic datasets to predict familial relationships and assist in genealogical reconstruction. AI may also support ancestry inference and forensic DNA phenotyping through analysis of complex genomic markers. Additionally, AI technologies are increasingly being integrated into laboratory automation systems for sample tracking, workflow management, report generation, and quality control. Automated forensic workflows can improve efficiency, reduce human error, and enhance consistency across forensic laboratories **[85]**.

10.2 Advantages and Challenges of AI-Based Forensic Systems

Artificial intelligence offers several important advantages in forensic DNA analysis. One major advantage is improved analytical speed and efficiency. AI systems can rapidly process large amounts of genomic data and assist forensic scientists in interpreting complex cases more efficiently **[86]**.

Another important benefit is increased consistency and reduction of human subjectivity. Manual interpretation of forensic evidence may vary among analysts, whereas AI-driven systems provide standardized computational analysis and reproducible results. AI also enhances scalability of forensic operations by enabling automated handling of large forensic databases and high-throughput sequencing data. Integration of AI with next-generation sequencing and probabilistic genotyping systems can further improve forensic sensitivity and interpretation accuracy **[87]**.

Despite these advantages, significant challenges remain associated with AI implementation in forensic science. One major concern is algorithm transparency and explainability. Many machine learning systems function as “black box” models, meaning their decision-making processes may not be fully understandable to forensic experts, legal professionals, or courts **[88]**.

Bias in training datasets is another important issue. If AI systems are trained on incomplete or unrepresentative genetic datasets, inaccurate or biased forensic interpretations may result. Such biases may disproportionately affect underrepresented populations. Validation and legal admissibility also represent critical concerns. AI-based forensic tools must undergo rigorous scientific validation, quality assurance testing, and peer review before routine courtroom implementation. Courts require forensic evidence to be scientifically reliable, transparent, and explainable **[89]**.

10.3 Future Perspectives of AI in Forensic Science

Future developments in artificial intelligence and machine learning are expected to further transform forensic DNA analysis. Integration of AI with next-generation sequencing, forensic genealogy, epigenetics, and probabilistic genotyping may improve interpretation of increasingly complex forensic evidence. Explainable AI systems, which provide transparent and interpretable reasoning for their outputs, are likely to become increasingly important for courtroom acceptance and public trust. Advances in deep learning, neural networks, and bioinformatics may also enhance predictive accuracy in forensic investigations **[90]**.

Portable AI-integrated forensic devices and cloud-based analytical platforms may facilitate real-time forensic analysis in field conditions and disaster response situations. Furthermore, AI may support predictive forensic intelligence through automated integration of genomic, demographic, and investigative data **[91]**. However, successful future implementation of AI in forensic science will require continuous validation, international standardization, ethical oversight, and legal regulation to ensure fairness, reliability, transparency, and responsible use of advanced computational technologies in criminal justice systems **[92]**.

11. NON-HUMAN DNA IN FORENSICS

Non-human DNA analysis is an expanding field within forensic science that involves the genetic examination of plants, animals, insects, fungi, and microorganisms for investigative purposes. Although traditional forensic genetics primarily focuses on human identification, non-human biological evidence has become increasingly important in modern forensic investigations. Plant, animal, insect, and microbial DNA can provide valuable links between suspects, victims, objects, and crime scenes, thereby contributing additional contextual and investigative information **[93]**.

Advancements in molecular biology, next-generation sequencing (NGS), environmental DNA (eDNA) analysis, and bioinformatics have significantly enhanced the ability to identify and analyze non-human biological materials. These technologies are increasingly applied in wildlife crime investigations, illegal trade detection, food fraud analysis, forensic botany, forensic microbiology, and forensic entomology **[94]**.

12. ETHICAL, LEGAL, AND QUALITY CHALLENGES

The rapid advancement of forensic DNA technologies has significantly enhanced the capabilities of criminal investigations and human identification. However, emerging forensic genomic technologies also raise major ethical, legal, and quality-related concerns. Technologies such as forensic genetic genealogy, next-generation sequencing, forensic DNA phenotyping, rapid DNA analysis, and artificial intelligence-based interpretation systems generate extensive genetic information that may affect privacy, human rights, and legal fairness **[95]**. Therefore, responsible implementation of advanced forensic DNA technologies requires careful consideration of ethical principles, legal regulations, scientific validation, and quality assurance standards **[96]**.

CONCLUSION

Forensic DNA profiling has evolved dramatically from conventional short tandem repeat (STR)-based identification into a broader and highly sophisticated discipline of forensic genomics. Emerging technologies

such as next-generation sequencing (NGS), rapid DNA analysis, forensic investigative genetic genealogy, probabilistic genotyping, forensic DNA phenotyping, epigenetics, RNA profiling, mitochondrial DNA sequencing, artificial intelligence, and non-human DNA analysis are transforming the landscape of modern forensic science.

These advancements have significantly improved the ability to analyze degraded, mixed, and low-template DNA samples while enhancing sensitivity, discrimination power, speed, and investigative efficiency. Modern forensic genomic approaches provide powerful tools for criminal investigations, disaster victim identification, missing-person cases, wildlife forensics, and humanitarian applications.

Among these innovations, next-generation sequencing has expanded forensic analysis beyond traditional STR profiling by enabling simultaneous examination of multiple genetic markers. Rapid DNA technology has accelerated identification processes in time-sensitive investigations, while forensic genetic genealogy has opened new possibilities for solving cold cases and identifying unknown individuals. Probabilistic genotyping and artificial intelligence have further improved interpretation of complex forensic evidence through advanced statistical and computational methods.

Emerging areas such as forensic DNA phenotyping, epigenetics, RNA-based body fluid identification, and non-human DNA analysis have broadened the scope of forensic investigations by providing additional contextual and biological information beyond simple identity matching.

Despite these remarkable advancements, significant ethical, legal, and scientific challenges remain. Issues related to genetic privacy, informed consent, database security, racial bias, algorithm transparency, and courtroom admissibility require careful consideration and regulatory oversight. Furthermore, rigorous validation, laboratory accreditation, standardization, and expert interpretation are essential to ensure reliability and maintain public trust in forensic genomic technologies.

Future developments in genomics, bioinformatics, artificial intelligence, and sequencing technologies are expected to further revolutionize forensic science by improving analytical precision, automation, and accessibility. However, successful integration of these innovations into forensic practice will depend on balancing scientific progress with ethical responsibility, legal accountability, and quality assurance. With continued research, international collaboration, and responsible regulation, forensic DNA analysis is likely to become increasingly comprehensive, rapid, and reliable in the future.

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