

FORENSIC DNA ANALYSIS: ADVANCEMENTS AND OBSTACLES IN ITS APPLICATION AT CRIME SCENES

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ABSTRACT

Forensic DNA testing has transformed contemporary investigative work, becoming an essential crime scene examination, suspect identification, and court tool. Technologically, since its introduction in the 1980s, the discipline has grown exponentially—through Restriction Fragment Length Polymorphism (RFLP) to Polymerase Chain Reaction (PCR), Short Tandem Repeats (STR), and now Next-Generation Sequencing (NGS). These have improved sensitivity, speed, and capacity to examine degraded or low-yield samples. Modern technology like Rapid DNA testing, touch DNA typing, mitochondrial DNA profiling, and single nucleotide polymorphism (SNP) panels has significantly improved forensic capability, making DNA testing more accessible and affordable even in field or resource-poor situations. Though successful, the science also faces deep-seated challenges. Technical challenges like contamination, allele drop-out, and interpretive problems with mixed or low-template DNA samples remain persistent challenges. Touch DNA and Rapid DNA technologies, though positive, are concerns from an aspect of reliability, particularly in juridical environments where proof has to be highly admissible. Increased application of mass forensic DNA databases has also triggered the very serious ethical concerns around individual privacy, informed consent, data protection, and threat of misuse or discrimination. Legal frameworks have to deal with these through good policy and transparent governance models. Moreover, there are operational impediments—like resource limitations, laboratory congestion, and differential procedural norms—that impede the functionality of forensic work globally. Overcoming these issues requires investment in infrastructure, expert training, and cross-disciplinary collaboration between scientific, legal, and policy-making communities. This report points to the historical advancements and continuing pitfalls of forensic DNA testing. It invokes sustained technological advancement, ethical awareness, and institutional support in order to achieve the highest potential of DNA evidence and protect the rights and confidence of the public. By such efforts, forensic DNA testing can continue to be the pillar of sound and fair criminal investigations.

Keywords: Forensic DNA typing, STR profiling, Next-generation sequencing, Touch DNA, Rapid DNA, Forensic databases, Criminal investigation.

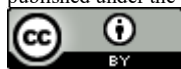
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INTRODUCTION

Forensic DNA analysis is a cornerstone of modern forensic science, widely recognized for its precision in identifying individuals involved in criminal cases. The technique originated in 1985 when British geneticist Sir Alec Jeffreys discovered that certain

regions of human DNA—later termed minisatellites—exhibited significant variability among individuals. This breakthrough led to the development of DNA fingerprinting, first applied in a criminal investigation in 1986 during a double

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homicide case in Leicestershire, UK. In that case, DNA evidence was instrumental in exonerating an innocent suspect and convicting the true perpetrator, Colin Pitchfork. This marked the beginning of forensic DNA as a legal tool with transformative potential for the justice system [1].

Since its inception, forensic DNA analysis has undergone significant technological evolution. The introduction of Polymerase Chain Reaction (PCR) in the late 1980s revolutionized the field by allowing DNA amplification from minute or degraded samples, expanding the scope of biological evidence that could be examined. By the 1990s, Short Tandem Repeat (STR) profiling became the gold standard due to its high discriminatory power, reproducibility, and ability to analyze multiple loci simultaneously. Further advancements have enabled the use of mitochondrial DNA for cases involving degraded samples and Y-chromosome STRs for male-specific identification. More recently, Next-Generation Sequencing (NGS) has allowed for deeper genetic analysis, opening doors to phenotypic prediction and ancestry inference in forensic contexts. These innovations have not only improved the accuracy of criminal investigations but also facilitated the exoneration of wrongfully convicted individuals, reinforcing the role of DNA as both a scientific and ethical instrument in legal proceedings [2].

Scope and Significance

Forensic DNA typing is a continually developing discipline that must be brought up to date regularly to include recent advances, highlight pitfalls, and direct future development. The review here seeks to summarize the evolutionary past, current methods, and future directions in forensic DNA profiling. It covers the analytical techniques employed—from initial Restriction Fragment Length Polymorphism (RFLP) to modern Short Tandem Repeat (STR) analysis and the latest Next-Generation Sequencing (NGS) technology. The review also alludes to specialized uses like mitochondrial DNA typing, Y-STR typing, and forensic phenotyping. By a consideration of the scientific developments and their legal and ethical implications, this book offers an overview for researchers, practitioners, and policy-makers.

The importance of the review stems from the key position DNA evidence occupies in contemporary criminal justice systems. DNA profiling not only transformed criminal investigations to become more accurate and reliable but also has largely been accountable for post-conviction exonerations and cold case solves. The Innocence Project states that over 375 individuals in the United States have been exonerated through DNA evidence, and most of them had spent several years on life sentence or under death sentence [3]. In addition, with the increasing global availability of forensic databases, adding enormous amounts of DNA data raises

urgent concerns about privacy, consent, and potential abuses. These concerns highlight the importance of critically examining forensic DNA practice not just from a technical but also a social and legal point of view [4]. By describing fundamental principles and new advances, this review contributes to knowledge about how forensic DNA typing continues to inform the practice of forensic science and influence legal systems across the globe.

Evolution of DNA Profiling Technologies

Restriction Fragment Length Polymorphism (RFLP) was the first widely adopted method for forensic DNA profiling, developed in the mid-1980s by Sir Alec Jeffreys. RFLP relies on the variability in the length of DNA fragments produced by the action of restriction enzymes that cut DNA at specific recognition sites. These variable-length fragments—caused by differences in the number of repeated DNA sequences (minisatellites)—are separated by gel electrophoresis and then transferred to a membrane for hybridization with DNA probes. The resulting banding pattern is unique for each individual and can be used to establish identity with a high degree of accuracy [5].

The major strength of RFLP lies in its high discriminatory power, especially when using multiple loci. This made it ideal for early forensic applications, including paternity testing and criminal investigations. However, RFLP requires a relatively large amount of high-quality, undegraded DNA—typically 50–100 ng. As such, its use was limited in cases where biological samples were old, degraded, or limited in quantity. The entire process is also time-consuming, often taking several days to complete, and requires strict laboratory conditions to ensure accuracy [2]. Despite these limitations, RFLP played a crucial role in validating DNA profiling as a forensic tool. Its application in high-profile cases during the 1980s and early 1990s demonstrated the reliability of DNA evidence in criminal justice systems, paving the way for more advanced, faster, and sensitive techniques. While RFLP has largely been replaced by PCR-based methods like STR analysis, it remains a landmark in the history of forensic science, marking the beginning of the DNA era in forensics.

Second Generation: PCR-based Techniques

Polymerase Chain Reaction (PCR)-based methods form the second generation of DNA forensic analysis and were much of a better improvement over previous methods such as RFLP. PCR was first designed in the late 1980s and enables amplification of selected DNA segments from even very degraded or minute biological samples. PCR-based methods are therefore very useful in forensic investigations where sample quality and quantity tend to be compromised. PCR reaction can exponentially amplify target DNA segments so that DNA profiles

can be produced from trace material like a single hair root, skin cell, or deteriorated tissue [6].

Of the PCR-based methods, Short Tandem Repeat (STR) is presently the standard for forensic DNA typing. STRs are extremely polymorphic segments of tandem-repeated 2–6 base pair nucleotide units. The variation in the number of repeats among individuals offers high discrimination, including among family members. Multiplex PCR enables the simultaneous amplification of several STR loci and significantly enhances the efficiency and power of forensic DNA typing. It also accommodates automated capillary electrophoresis systems and supports high-throughput and precise analysis [2]. PCR-based approaches also opened the door to specialized forensic uses, including Y-STR typing of male-specific DNA and typing for mitochondrial DNA using highly degraded samples, e.g., bones or hair shafts. Additionally, the production of stable commercial kits standardized procedures in laboratories, rendering them more reproducible and court-admissible. PCR-based DNA profiling is quicker, more sensitive, and more adaptable than its predecessors and has become a routine technique in contemporary forensic genetics.

The Advent of Short Tandem Repeats

The discovery of Short Tandem Repeats (STRs) in the 1990s revolutionized forensic DNA typing. STRs are short segments of repeating 2–6 base pairs at typical loci within the human genome. Their extensive polymorphism—through variations in repeat unit number—makes them suitable genetic markers for identification of individuals. STR analysis avoided the majority of pitfalls of the older methods such as RFLP to allow high-resolution profiling from small, usually degraded DNA samples, which are common in forensic cases [7]. STRs became the basis of forensic genotyping due to being stable, sensitive, and responsive to amplification using the Polymerase Chain Reaction (PCR). Unlike RFLP, which needed pure DNA in large amounts, STR analysis was able to provide profiles from trace evidence such as skin cells, saliva, and old biological materials. Multiplex PCR methods even enhanced efficiency by enabling the amplification of more than one locus of STR within a single reaction. The resulting DNA profiles are then processed using capillary electrophoresis, resulting in allele patterns that are extremely discriminative and statistically robust [2]. Standardization of the STR markers also made possible the creation of national and international DNA databases like CODIS (Combined DNA Index System) in the US. These databases have transformed forensic investigations with the ability of making quick comparisons between retained profiles and crime scene evidence. STRs have also been used to solve missing persons, disaster victim identification, and paternity cases. The evolution of STR analysis not only enhanced the validity and

accuracy of forensic science but also helped free wrongly accused persons, thereby heightening its legal as well as moral reputation.

RECENT ADVANCES IN DNA ANALYSIS

Next-Generation Sequencing (NGS)

Next-Generation Sequencing (NGS) is an evolutionary leap in the fields of molecular and genomic biology. NGS enables the parallel sequencing of millions of DNA fragments at much higher speed, volume, and cost compared with Sanger sequencing, which has been the traditional method up to this point. NGS has transformed many clinical diagnostic, cancer genomics, infectious disease monitoring, and forensic testing applications.

One of the major strengths of NGS is its flexibility. It can be used for whole genome sequencing (WGS), whole exome sequencing (WES), gene panels, and RNA sequencing (RNA-Seq) and is thus amenable to numerous applications. In clinical genetics, for example, NGS permits the identification of disease-causing mutations in rare and complex disorders. In cancer, NGS permits the detection of somatic mutations, gene fusions, and copy number variation, which can be utilized for targeted therapy and prognosis [8].

The NGS process involves a number of critical steps, such as DNA/RNA extraction, library construction, sequencing, and bioinformatics analysis. Each of these steps has to be highly optimized to get good-quality data and reproducibility. Technological developments very rapidly, for instance, benchtop sequencers and robots for library preparation, have made NGS available in medium-scale research laboratories.

Even with these advances, there are challenges. Data interpretation involves a high degree of bioinformatics sophistication, and the management of large datasets requires a robust computational platform. Regulator and ethical concerns, particularly of data privacy and incidental findings, must be addressed within the clinical setting.

NGS is constantly advancing with the development of real-time sequencing technologies and long-read sequencing machines. It is hoped that the technologies have the potential to improve accuracy further and broaden applications in research and clinical diagnostics [9].

Rapid DNA Technology

Rapid DNA technology is the laboratory-automated process of creating a DNA profile from a biological sample within two hours. In contrast to existing processes that need laboratory space and time-consuming processing, Rapid DNA machines are portable and designed for police station, border crossing site, and even crime scene application. This technology is revolutionizing the way law enforcement approaches suspect identification and criminal investigations.

The core advantage of Rapid DNA lies in its speed and simplicity. By enabling near real-time identification, it allows law enforcement agencies to match arrestees' DNA profiles against national databases such as the FBI's Combined DNA Index System (CODIS) during booking. This immediate feedback can prevent the release of suspects linked to unsolved crimes and reduce wrongful arrests [10]. Aside from expediting suspect processing, Rapid DNA is also useful in disaster victim identification and human trafficking. Its capability to identify kin relationships at speed can be applied to reunite missing individuals or identify corpses in mass casualty cases. Yet, the technology is far from perfect. Rapid DNA systems perform best with high quality single-source samples such as buccal swabs. Mixture or degraded crime scene evidence still needs to be analyzed in accredited forensic labs in order to enable proper interpretation. In addition, there are still legal and ethical concerns regarding storage and utilization of DNA data, particularly outside criminal and forensic settings [11]. In spite of such limitations, Rapid DNA is a valuable tool for contemporary forensic science. With continued improvement and judicious regulation, its application on a large scale throughout justice systems globally could render criminal investigations more efficient, more accurate, and more global.

Mini STRs and SNPs

DNA typing in forensic genetics for identification of human beings under conditions of degradation of the biological sample exists. Mini STRs and single nucleotide polymorphisms (SNPs) are two good alternatives to the ubiquitous STRs. Mini STRs are abbreviated forms of typical STR loci. Placing the primers further towards the repeat regions reduces the overall amplicon size, improving amplification success with degraded or low-quality DNA. Mini STRs are as polymorphic and discriminating as typical STRs but are more tolerant of degraded samples like those found in mass disasters, old human remains, or trace body evidence [12]. Commercial kits now contain special Mini STR loci designed for problematic forensic samples. SNPs are bi-allelic markers with smaller amplicon lengths (50–100 bp), and they can be used to analyze even more degraded DNA. Even though individual SNPs are not as discriminating as STRs, panels of 50–100 SNPs in combination can be as good as or even better than STR profiling. SNPs are also used in ancestry inference, phenotype prediction, and mixture deconvolution because they are dense and evenly scattered throughout the genome [13].

Both SNPs and mini STRs widen the forensic repertoire, especially for applications in degraded samples. Mini STRs provide compatibility with current STR databases, while SNPs provide future genomic utility prospects, especially as coupled with next-generation sequencing.

TOUCH DNA AND LOW-TEMPLATE ANALYSIS

Touch DNA refers to DNA that is transferred when one touches something or a surface. The biological sample is normally restricted to a few cells, where there is challenge and potential for use in forensic investigations. Low template DNA analysis, or low copy number (LCN) DNA profiling, is applied when amounts of DNA are less than 100–200 picograms. This technique enables the DNA profiling of low or degraded samples like fingerprints, firearms, or clothing [14].

Touch DNA is of greater value in situation working where there is no traditional biological evidence. The much lower amounts of DNA, however, are fraught with risks of contamination, stochastic events, and allele drop-out. To mitigate such problems, higher sensitivity methods, e.g., additional PCR cycles and post-amplification clean-up procedures, have been adopted. Nevertheless, the methods increase the risk of spurious detection of DNA from secondary transfer, and thus strict laboratory protocols and guidelines for interpretation are required [15].

In spite of such constraints, LTDNA analysis has revolutionized cold cases, trace evidence analysis, and property crime cases. Using probabilistic genotyping software is also helpful for forensic analysts in the assessment of degraded or complex profiles from LTDNA. With advancement in forensic science, ethical and legal concerns of interpreting low-level DNA profiles also must be managed successfully to avoid miscarriages of justice.

Capabilities and Limitations

Capabilities

Touch DNA offers the capability to extract genetic material from less than a negligible contact with objects or surfaces. It can extract live DNA profiles from a matter of dozens of skin cells and is therefore best suited in invisible biological evidence-poor cases. The method is useful for solving cold cases, pinpointing suspects from the objects touched by them momentarily, and building physical connection to crime scenes [14]. Touch DNA is also applied in non-invasive sampling with evidence integrity preserved and the ability to do multiple tests. Greater sensitivity of new amplification technologies increases the success rate even for low-template samples, making touch DNA a useful tool in current forensic science.

Limitations

While it is beneficial, touch DNA has its flaws. One of the biggest drawbacks is secondary transfer—DNA may be transferred indirectly from an individual to an item through another individual or surface, into the question of origin and potentiality of the DNA that is collected. This can be misleading to investigations and incorrectly implicate innocent people [16]. The yield of DNA recovered is

extremely variable and depends upon a variety of factors, including the shedding rate, surface type, environmental conditions, and time since contact of the individual. Low levels of DNA compromise partial profiles and allelic drop-out, therefore compromising evidentiary value. Contamination during sampling or processing is also a major risk considering that samples are trace in nature. Mixed profiles from multiple donors complicate analysis even more, and probabilistic genotyping is often necessary. These issues require stringent control and interpretation in context to avoid reliance on potentially fallacious genetic evidence.

Application in High-Sensitivity Cases

Touch DNA is now a routine weapon in the examination of high-sensitivity forensic cases with little or no biological evidence. Its major use is in serious crime like rape, murder, and kidnap, where the offenders are likely to touch surfaces, clothes, or victims only briefly. Because it can produce profiles from trace cell deposits, it's possible for investigators to find out who the suspects are even without body fluids or visible traces [17].

In sexual assault, touch DNA is able to extract genetic material from the surface of clothing or skin that can prove vital when semen or blood samples are unavailable. Touch DNA extracted from weapons, ligatures, or victim fingernails in homicides can be used to confirm direct or indirect contact between victim and suspect. It is also used in child abuse cases where there is no physical injury or biological evidence, but where toy, clothing, or furniture shared by victim and abuser can be examined.

Additionally, touch DNA is priceless when it comes to re-investigating cold cases. Unresolved crimes are being re-opened using new DNA technologies that can re-analyze previously unuseable traces of evidence decades after the crime was even committed. Touch DNA can allow stored DNA evidence to be re-analyzed, perhaps to identify previously unknown offenders [18]. Despite the difficulties of secondary transfer and contamination, proper utilization of touch DNA within rigorous forensic controls can still significantly contribute weight to the probative value of the evidence at court. It provides critical investigative leads and corroborates other forms of evidence in complex and sensitive legal cases.

BIOINFORMATICS AND DNA DATABASES

Bioinformatics serves a key function in the analysis, management, and interpretation of DNA data, particularly forensic science. Advanced computer algorithms and tools enabled by bioinformatics allow for the rapid processing of vast amounts of gene information. Its most critical usage is in the establishment and maintenance of DNA databases, such as DNA profiles from suspects and suspects' crime scenes, and unidentified bodies.

Bioinformatics-dependent forensic DNA databases like the Combined DNA Index System (CODIS) depend upon bioinformatics to quickly and correctly match DNA profiles. Bioinformatics-based systems assist in linking suspects to crimes, finding repeat offenders, and linking multiple crime scenes. Sequence alignment, mutation discovery, and statistical analysis are supported by bioinformatics tools for correct matching and false positives reduction [19].

In addition to criminal investigation, DNA databases enabled by bioinformatics are essential in the recovery of victims of disasters, missing individuals, and human victims of mass disasters. High-level data mining and pattern recognition functions enable rapid comparison of thousands of profiles even from incomplete and degraded samples. New bioinformatics methods also enable the addition of phenotypic and ancestry-informative markers to the equation, enhancing the prospects of suspect identification on genetic characteristics [20]. But applying bioinformatics to forensic DNA databases is ethically and legally problematic. Privacy, consent, and data protection are most critical, particularly as databases grow larger to encompass more people and non-criminal profiles. Responsible leadership and good bioinformatics models must provide data integrity and trust in government.

Data Analysis Tools and Software Platforms

Software platforms and data analysis tools are at the core of deciphering complicated DNA data in forensic genomics. These platforms simplify the process of transforming raw genetic data into usable profiles, with precision and pace, in forensic investigation.

STR mix is a broad range probabilistic genotyping system using Bayesian modeling to read complex mixtures of DNA. It issues likelihood ratios to determine quality of evidence, enabling conclusions to be found acceptable in court [21]. Gene Mapper ID-X is another popular choice in forensic labs that interprets STR data automatically by reading capillary electrophoresis data and making high-quality allele calls with minimal user interaction.

Bio Numerics provides a general analytical framework that can incorporate different forms of molecular data, such as STRs, SNPs, and sequencing data. It has phylogenetic analysis and database management capabilities and can be applied to both forensic casework and population studies. Likewise, open-source solutions like Galaxy enable users to build their own pipelines for DNA sequence analysis, enabling advanced bioinformatics without a high level of programming skills.

Cloud-based tools like Illumina's Base Space and Thermo Fisher Connect are increasingly utilized for scalability, automation, and collaboration capabilities. Such tools can manage big genomic data through secure, auditable environments that are crucial for forensic compliance. However, the

success of these tools also depends on valid validation, user training, and adherence to forensic standards. As DNA evidence becomes more complex, the employment of machine learning and AI in these platforms will most likely improve interpretation accuracy and reduce human error [22].

CRIME SCENE PROTOCOLS FOR DNA EVIDENCE

The integrity of DNA evidence begins with proper crime scene protocols. Ensuring the accurate collection, preservation, and documentation of biological evidence is essential for its admissibility in court and for minimizing contamination risks.

The first step is securing the crime scene to prevent unauthorized access. This reduces the chance of introducing foreign DNA and preserves the scene's original condition. Investigators then perform a systematic search to identify potential sources of DNA, such as blood, saliva, hair, skin cells, or other biological materials. Documentation, including photography and detailed notes, must accompany each item of evidence to maintain a clear chain of custody. Proper use of personal protective equipment (PPE) is critical. Crime scene personnel must wear gloves, masks, and disposable suits to avoid shedding their own DNA. Gloves should be changed frequently, especially between handling different items, to reduce cross-contamination [23].

Evidence should be collected using sterile tools, such as swabs or forceps, and each item must be packaged separately in paper envelopes or bags—not plastic—to allow airflow and prevent DNA degradation due to moisture. Labels must include time, date, collector's name, and item description. Items should be stored in cool, dry conditions until processed in the laboratory. Transport of DNA evidence must be timely and secure. Documentation of each transfer step is vital to demonstrate that evidence was not tampered with. Any deviations from standard protocols can be grounds for evidence exclusion in legal proceedings. Ultimately, adherence to meticulous protocols is vital for maximizing the evidentiary value of DNA. Continuous training and strict compliance with forensic guidelines help ensure the reliability of results and uphold judicial integrity [24].

Collection, Storage, and Preservation

Greatest significance in current forensic analyses, and collection, storage, and preservation of DNA evidence in a proper way are the foundation for evidentiary integrity. DNA degradation caused by exposure to the environment, contamination, or mishandling has an impact on the admissibility and credibility of forensic findings.

Collection Practices

The DNA evidence assembly must be carried out in a manner that will avoid contamination. The researchers must wear gloves, masks, and coverall protective garments, and use sterilized, disposable

tools for each sample. Sterile distilled water on cotton swabs is widely utilized to collect biological materials such as blood, saliva, or epithelial cells from surfaces. Each item must be individually packaged with aerated paper containers, not plastic, so there will be no build-up of moisture to initiate premature DNA decay [25].

Storage Conditions

Temperature and humidity control are essential in the storage of DNA evidence. Biological samples should be dried well prior to storage to avoid microbial contamination. Cold, dry storage is necessary for long-term storage; storage under frozen conditions at -20°C or below is ideal for liquid biological samples. Room temperature humidity-controlled storage is ideal for dried stains or swabs to achieve stability of DNA over time [26].

Preservation and Chain of Custody

Preservation encompasses physical and procedural protection. Regular checking for deterioration, e.g., quantification or DNA integrity testing, allows suspect samples to be identified at an early point. In addition, strict chain of custody controls maintains the evidentiary value from crime scene to court. Labels are required to carry case identifiers, dates, and initials of handlers, and transfers must be recorded carefully [27].

Strategies to Prevent Contamination of the Scene

Prevention of contamination within the crime scene is essential to ensuring forensic evidence integrity. Contamination is capable of disqualifying investigations by introducing extraneous material that masks or misinforms the evidence, resulting in miscarriages of justice. Effective prevention of contamination involves controlled procedures, trained staff, and compliance with forensic standards.

Securing Scene and Access Control

The immediate closing of the crime scene is the first step in contamination control. Physical segregation, such as police tape and limited access, serves to restrict entrance to trained personnel only. A log should be kept in order to document everyone entering or leaving the scene so that it can be traced and accounted for [28].

Application of Personal Protective Equipment (PPE)

Persons entering the scene must wear PPE composed of gloves, masks, disposable coveralls, and shoe covers. These protective barriers minimize the possibility of losing DNA or leaving foreign substance on the scene. PPE must be changed between scene locations or when they touch separate evidence items [29].

Sterile Equipment and Single-Use Tools

All the apparatus employed in the collection of evidence should be sterilized and, if possible, single-use. Reusable apparatus should be decontaminated properly between utilization. Packaging material should be sterile, and evidence should be placed in

distinct packaging in order to prevent cross-contamination [14].

Evidence Collection Protocols

Evidence must be gathered in an order of logic—usually from the least stable or most temporary evidence towards the higher stability ones. Instruments used to gather one item should not be used to gather another. Sampled items should be labeled, sealed, and documented in order to maintain the chain of custody.

Training and Supervision

Continuing education in contamination prevention and constant audit of crime scene practices are necessary to provide best practice. Forensic units need to be thoroughly trained on measures to control contamination and guidelines for handling evidence.

Interpretation Challenges

Forensic DNA analysis is a pillar of contemporary criminal evidence, yet interpretation is plagued by problems that can impact reliability of biological evidence results. One of the main obstacles to interpretation is the mixed DNA profile—samples containing genetic contributions from two or more individuals. Analysts are compelled to utilize probabilistic models to estimate contributor ratios and identities but statistical outcomes depend on software algorithms and analyst skill, thus producing conflicting interpretations [30].

The second critical problem is that of low-template DNA (LTDNA), where there is scarcely any DNA in a minute quantity. LTDNA is very prone to random events such as allele drop-out or drop-in, and thus it is extremely challenging to reconstruct a full and true genetic profile. Mis- or over-interpretation in such a case is possible, especially when analysts try to make conclusions from incomplete data sets [31].

Secondary transfer of DNA also complicates the interpretation. Presence of an individual's DNA at the crime location does not inherently imply involvement in the criminal act, as DNA can indirectly be transferred by contact with a person or object. Without proper contextual and environmental examination, secondary transfer can be taken wrongly as primary evidence, leading to incorrect conclusions of culpability [32].

Besides, there is no standardization of DNA interpretation in forensic laboratories, particularly the application of thresholds of inclusion, exclusion, and statistical reporting. This absence of uniformity will lower the reproducibility and comparability of DNA evidence across jurisdictions. Lastly, the explanation of complicated probabilistic results to courts is another challenge, as lay people may misinterpret the statistical significance of DNA evidence.

Ethical and Legal Considerations

Forensic DNA analysis has dramatically changed the criminal justice process by allowing for proper identification and exoneration. Use, however, is

extremely problematic ethically and legally with respect to privacy, consent, data protection, and abuse. All of these issues require strict policies that balance the interests of justice and individual rights. A primary ethical concern is the taking and storage of DNA samples. DNA carries very intimate information such as genetic vulnerabilities and family relationships. When DNA samples are taken from suspects, victims, or even innocent bystanders (e.g., during mass screening), the legal line between investigation and violation of privacy is crossed. Indefinite storage of DNA profiles in national data bases, particularly those of non-convicted persons, constitutes an invasion of the right to privacy and bodily integrity [33].

Consent also creates legal concerns. In most jurisdictions, individuals may not be informed to what extent their genetic data will be processed and stored. Moreover, familial searching—where investigators look for partial matches to identify relatives of a suspect—raises ethical concerns regarding the involvement of uninformed and unrelated individuals in criminal investigations [34]. And yet another serious concern is the risk of discrimination and bias. When forensic databases disproportionately represent minority groups, there are dangers of institutionalizing current systemic imbalances in the justice system. Jurisdictional protections need to provide equal treatment and protection against misuse of genetic data, especially in employment, insurance, and public surveillance applications [35].

To counteract these concerns, ethical principles and legal statutes like Europe's General Data Protection Regulation (GDPR) and national forensic acts of nations have been enforced to regulate the gathering, storage, and application of DNA information. These principles focus on responsibility, transparency, and minimizing harm. Finally, as valuable as forensic DNA typing is to contemporary investigations, it must be regulated by robust ethics and laws so as to safeguard civil liberties while at the same time ensuring justice.

Case Applications and Impact

DNA analysis in forensic science has evolved as an awesome tool in contemporary criminal justice, offering effective tools for suspect identification, acquitting the innocent, and closing cold cases. What is most notable about DNA analysis is its use in celebrity crime cases, where old evidence might be unavailable or inconclusive.

A seminal case demonstrating the utility of DNA evidence is the 1987 exoneration of Gary Dotson, an innocent man wrongly convicted of rape. It was the first case in the United States where DNA testing was applied to reverse a conviction, establishing a precedent for court admissibility and showing its value in post-conviction analysis [36]. In the same vein, the Innocence Project has indicated that DNA evidence has been used to exonerate more than 375

wrongful convictions within the United States alone, of which most are eyewitness misidentification or faulty forensic science cases [37].

In active criminal investigations, DNA analysis has provided valuable leads. In the case of the "Golden State Killer," a public genealogy database was utilized by detectives to identify a suspect decades later. This method, genetic genealogy, combines DNA analysis and genealogical research to uncover family connections and track down suspects, the expanding scope of forensic science in the digital world [38].

The effect of forensic DNA analysis is not only in the individual case. It has initiated reforms in the handling of evidence, enhanced criminal databases accuracy, and introduced national DNA databases in most countries. The ethical issues of privacy and genetic information abuse are ongoing issues for the justice system.

In a nutshell, forensic DNA analysis has transformed criminal investigation to be more accurate, provide justice, and increase public faith in justice systems. With the development of technology, its use will be utilized to accomplish even more towards solving intricate forensic cases.

Emerging Forensic DNA Trends

Forensic DNA examination has undergone tremendous changes in the past decade, driven by genomics revolutions, miniaturization technology, and computational biology. One of the most important new trends might be the incorporation of Next-Generation Sequencing (NGS). In contrast to traditional STR (short tandem repeat) profiling, NGS yields dense SNP data enabling ancestry inference, phenotype prediction, and more robust mixture deconvolution. This revolution enlarges the evidentiary value of biological evidence, especially in degraded or complicated cases [39].

Touch DNA and trace DNA collection methods are also becoming more recent. Devices such as microfluidic platforms and advanced swabbing media enhance recovery of low-template DNA, which is present in the majority of minor contact traces. However, this has also given rise to issues with contamination and interpretation of complex mixtures, requiring stringent quality assurance controls [40].

Another is the application of Rapid DNA technology. It is an in-the-field system used to streamline DNA profiling in under 90 minutes and that is being tested in police stations and border

enforcement security. Quicker and more convenient to use in the field, its application remains questionable due to problems over reliability and admissibility to the courts [41].

Artificial intelligence and machine learning are also moving towards forensic DNA interpretation. They support mixture analysis, pattern recognition, and probabilistic genotyping and provide more reproducible and accurate results. With growing forensic databases and a heightened sense of privacy, legal and ethical challenges must be addressed simultaneously by these technological improvements. Generally, future forensic DNA technology is moving towards more inclusive, quicker, and smarter systems of interpretation of biological evidence. Therefore, the forensic community will be presented with new problems and prospects for preservation of the integrity and admissibility of DNA evidence.

CONCLUSION

Forensic DNA typing has grown to be a fundamental component of criminal investigations, evolving through the modes of STR profiling, PCR, and Next-Generation Sequencing. These have facilitated precise identification from poor or degraded samples and increased forensic capability with Rapid DNA and touch DNA analysis. Nevertheless, there are more significant challenges. Challenges like contamination, understanding complex mixtures, and secondary DNA transfer complicate results. Complicating forensic application further are ethical challenges related to privacy and control of the DNA databases. Furthermore, the limited infrastructure, absence of protocol standardization, and limited resources could further complicate forensic application in an effective and timely manner. In spite of these problems, continued innovation, strict verification, and cross-disciplinary cooperation can increase the scientific and legal integrity of DNA evidence. Forensic DNA testing will continue to be a cornerstone in the pursuit of justice and protection of citizens' rights if applied appropriately and continually refined.

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REFERENCES

1. Jeffreys AJ, Wilson V, Thein SL. Hypervariable 'minisatellite' regions in human DNA. *Nature*. 1985;314(6006):67–73. Available from: <https://doi.org/10.1038/314067a>
2. Butler JM. Forensic DNA typing: biology, technology, and genetics of STR markers. 2nd ed. Burlington (MA): Elsevier Academic Press; 2005. <https://www.elsevier.com/>
3. Innocence Project. DNA Exonerations in the United States [Internet]. New York: Innocence Project; 2024. Available from: <https://innocenceproject.org/dna-exonerations-in-the-united-states/>

4. Machado H, Silva S. Ethical challenges of forensic DNA databases: A European perspective. *Sci Eng Ethics*. 2019;25(4):1105–1122. Available from: <https://doi.org/10.1007/s11948-018-0032-y>
5. Jeffreys AJ, Wilson V, Thein SL. Individual-specific ‘fingerprints’ of human DNA. *Nature*. 1985;316(6023):76–79. Available from: <https://doi.org/10.1038/316076a0>
6. Mullis K, Faloona F. Specific synthesis of DNA in vitro via a polymerase chain reaction. *Methods Enzymol*. 1987;155:335–350. Available from: [https://doi.org/10.1016/0076-6879\(87\)55023-6](https://doi.org/10.1016/0076-6879(87)55023-6)
7. Edwards A, Civitello A, Hammond HA, Caskey CT. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *Am J Hum Genet*. 1991;49(4):746–756. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1683382/>
8. Goodwin S, McPherson JD, McCombie WR. Coming of age: ten years of next-generation sequencing technologies. *Nat Rev Genet*. 2016;17(6):333–51. <https://www.nature.com/articles/nrg.2016.49>
9. Van Dijk EL, Jaszczyszyn Y, Naquin D, Thermes C. The Third Revolution in Sequencing Technology. *Trends Genet*. 2018;34(9):666–81. DOI: [10.1016/j.tig.2018.05.008](https://doi.org/10.1016/j.tig.2018.05.008)
10. Gutiérrez-Hurtado IA, García-Acéves ME, Puga-Carrillo Y, Guardado-Estrada M, Becerra-Loaiza DS, Carrillo-Rodríguez VD, Plazola-Zamora R, Godínez-Rubí JM, Rangel-Villalobos H, Aguilar-Velázquez JA. Past, present and future perspectives of forensic genetics. *Biomolecules*. 2025 May 13;15(5):713. <https://doi.org/10.3390/biom15050713>
11. Zhu Q, Mao Z, Zhang J. DNA Mixture Deconvolution: A Four-Strategy Framework from Physical Separation to Database Searching. *Genes*. 2026 Apr 9;17(4):434. <https://doi.org/10.3390/genes17040434>
12. Coble MD, Butler JM. Characterization of new miniSTR loci to aid analysis of degraded DNA. *Journal of forensic sciences*. 2005 Jan 1;50(1):JFS2004216-11. <https://doi.org/10.1520/JFS2004216>
13. Kayser M, de Knijff P. Improving human forensics through advances in genetics, genomics and molecular biology. *Nat Rev Genet*. 2011;12(3):179–92. doi:10.1038/nrg2952.
14. Van Oorschot RAH, Ballantyne KN, Mitchell RJ. Forensic trace DNA: a review. *Investig Genet*. 2010;1(1):14. doi:10.1186/2041-2223-1-14. doi: [10.1186/2041-2223-1-14](https://doi.org/10.1186/2041-2223-1-14)
15. Gill P, Whitaker J, Flaxman C, Brown N, Buckleton J. An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Sci Int*. 2000;112(1):17–40. [https://doi.org/10.1016/S0379-0738\(00\)00158-4](https://doi.org/10.1016/S0379-0738(00)00158-4)
16. Meakin GE, Jamieson A. DNA transfer: Review and implications for casework. *Forensic Sci Int Genet*. 2013;7(4):434–443. <https://doi.org/10.1016/j.fsigen.2013.03.013>
17. Sweet D, Lorente JA, Lorente M, Valenzuela A, Villanueva E. An improved method to recover saliva from human skin: the double swab technique. *J Forensic Sci*. 1997;42(2):320–322. <https://doi.org/10.1520/JFS14120J>
18. Anslinger K, Bayer B, Eisenmenger W, Schön U. Application of DNA typing in two old cases of suspected homicide. *Int J Legal Med*. 2004;118(6):306–310. <https://pubs.acs.org/doi/10.1021/ac070871s>
19. Butler JM. Advanced topics in forensic DNA typing: methodology. Amsterdam: Elsevier Academic Press; 2012.
20. Pivetti M, Caggiano A, Cieri F, Di Battista S, Berti C. Support for the forensic DNA database and public safety concerns: an exploratory study. *The Open Psychology Journal*. 2017;10(1):104–17. DOI: [10.2174/1874350101710010104](https://doi.org/10.2174/1874350101710010104)
21. Bright JA, Taylor D, Curran JM, Buckleton JS. Developing allelic and stutter peak height models for a continuous method of DNA interpretation. *Forensic Sci Int Genet*. 2013;7(2):296–304. <https://doi.org/10.1016/j.fsigen.2012.11.013>
22. Alley SJ, Elkins KM. Artificial intelligence and machine learning in forensic DNA analysis. In *Investigative and Predictive DNA Testing 2026 Jan 1* (pp. 315–324). Academic Press. <https://doi.org/10.1016/B978-0-443-30128-5.00015-1>
23. Houck MM, Siegel JA. Fundamentals of forensic science. 3rd ed. Amsterdam: Elsevier; 2015.
24. Muqadas JN, Noor Ul Aien, Tasmia Fatima, Ansari MA. Crime scene to judgment: A review on identifying gaps in crime scene investigation and implementing solutions. *Forensic Insights and Health Sciences Bulletin*. 2025;3(1):1–6. <https://doi.org/10.56770/fi2025311>
25. Budowle B, Schutzer SE, Einseln A, et al. Building microbial forensics as a response to bioterrorism. *Science*. 2003;301(5641):1852–1853. <https://doi.org/10.1126/science.1090083>
26. Alaeddini R. Forensic implications of PCR inhibition—a review. *Forensic Science International: Genetics*. 2012 May 1;6(3):297–305. <https://doi.org/10.1016/j.fsigen.2011.08.006>

27. Woollacott C, Goray M, van Oorschot RA, Taylor D. The transfer, prevalence, persistence, and recovery of DNA from body areas in forensic science: a review. *Forensic Sciences*. 2025 Feb 14;5(1):9. Doi: <https://www.mdpi.com/2673-6756/5/1/9#>
28. Roux C, Talbot-Wright B, Robertson J, Crispino F, Ribaux O. The end of the (forensic science) world as we know it? The example of trace evidence. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2015 Aug 5;370(1674). <https://doi.org/10.1098/rstb.2014.0260>
29. van Oorschot RA, Meakin GE, Kokshoorn B, Goray M, Szkuta B. DNA transfer in forensic science: recent progress towards meeting challenges. *Genes*. 2021 Nov 7;12(11):1766. <https://doi.org/10.3390/genes12111766>
30. Zhang R, Xue J, Tan M, Chen D, Xiao Y, Liu G, Zheng Y, Wu Q, Liao M, Lv M, Qu S. An MPS-based 50plex microhaplotype assay for forensic DNA analysis. *Genes*. 2023 Apr 4;14(4):865. <https://doi.org/10.3390/genes14040865>
31. Vajpayee K, Dash HR, Parekh PB, Shukla RK. PCR inhibitors and facilitators—Their role in forensic DNA analysis. *Forensic Science International*. 2023 Aug 1;349:111773. <https://doi.org/10.1016/j.forsciint.2023.111773>
32. Oosthuizen T, Howes L, White RO. Forensic DNA analysis and legislative provisions: Balancing rights in a time of scientific advancement. *Altern Law J*. 2023;48:178–184. <https://doi.org/10.1177/1037969X231190484>
33. Kaye DH. The ethics of DNA use in criminal justice. *Nat Rev Genet*. 2010;11(5):359-364.
34. Krimsky S, Simoncelli T. Genetic justice: DNA data banks, criminal investigations, and civil liberties. Columbia University Press; 2010 Dec 31. <https://doi.org/10.7312/krim14520>
35. Irfan M, Syed Aftab Haider, Muhammad Yasir, Muhammad Ammar Akram, Muhammad Adeel. Invisible witness: Innovations in the collection of touch DNA and the risk of forensic cross contamination. *Forensic Insights and Health Sciences Bulletin*. 2025;3(2):28-33. <https://doi.org/10.56770/fi2025321>
36. Connors E, Lundregan T, Miller N, McEwen T. *Convicted by Juries, Exonerated by Science: Case Studies in the Use of DNA Evidence to Establish Innocence After Trial*. U.S. Department of Justice; 1996.
37. Innocence Project. DNA Exonerations in the United States [Internet]. 2023 [cited 2025 May 18]. Available from: <https://innocenceproject.org/dna-exonerations-in-the-united-states/>
38. Greytak EM, Moore C, Armentrout SL. Genetic genealogy for cold case and active investigations. *Forensic science international*. 2019 Jun 1;299:103-13. <https://doi.org/10.1016/j.forsciint.2019.03.039>
39. Butler JM. *Advanced topics in forensic DNA typing: methodology*. Academic press; 2011 Jul 27.
40. Van Oorschot RA, Szkuta B, Meakin GE, Kokshoorn B, Goray M. DNA transfer in forensic science: a review. *Forensic Science International: Genetics*. 2019 Jan 1;38:140-66. <https://doi.org/10.1016/j.fsigen.2018.10.014>
41. Sijen T, Harbison S. On the identification of body fluids and tissues: a crucial link in the investigation and solution of crime. *Genes*. 2021 Oct 28;12(11):1728. <https://doi.org/10.3390/genes12111728>