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The role of DNA in forensic science: A comprehensive review

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Abstract

Forensic genetics, leveraging molecular tools and scientific applications, has witnessed significant advancements in DNA analysis over the last three decades. These progressions have enhanced the discrimination power, speed, and sensitivity of DNA profiling methods, enabling the analysis of challenging samples. This article explores the significance of forensic genetics in criminal investigations, traces the historical evolution of DNA analysis techniques, and presents recent developments in the field. The article aims to provide a comprehensive understanding of the crucial role of forensic genetics in criminal investigations and sheds light on the latest trends and breakthroughs in this area. The evolution of DNA typing from ABO blood typing to the current standard of short tandem repeat (STR) analysis is discussed, along with alternative DNA analysis methods, such as Y-chromosome analysis and single nucleotide polymorphism (SNP) typing. Massively parallel sequencing (MPS) represents a groundbreaking advancement, enabling whole genome sequencing and addressing complex cases. The article also covers recent innovations, including DNA methylation analysis, body fluid identification, forensic DNA phenotyping, and genetic genealogy, highlighting their potential benefits in forensic investigations. Despite these advancements, standard STR profiling remains the gold standard due to its established protocols and databases. Ethical considerations regarding data privacy and cost implications are crucial as these technologies continue to progress in their pursuit of justice.

Keywords: Forensic science; Forensic genetics; DNA; DNA Typing; DNA analysis; DNA profiling; Restriction Fragment Length Polymorphism (RFLP); Short Tandem Repeat (STR); STR Typing; Massively Parallel Sequencing (MPS); Next-Generation Sequencing (NGS)

1. Introduction

Forensic genetics is an area that leverages molecular tools and scientific applications to resolve criminal and civil cases [1]. Over the last three decades, substantial advancements have been achieved in forensic DNA analysis. These advancements include enhancements in the discrimination power, speed, and sensitivity of DNA profiling methods, as well as the ability to process difficult samples.

This article aims to underscore the significance of forensic genetics as a tool in forensic investigations, chart the historical evolution of DNA analysis techniques, and present recent developments in the field. Through an exploration of these themes, the article seeks to offer a comprehensive understanding of how forensic genetics plays a crucial role in criminal investigations and to shed light on the latest trends and breakthroughs in this area.

1.1. Key points

- Forensic genetics has undergone significant advancements in DNA analysis techniques, including the adoption of Short Tandem Repeat (STR) analysis as the gold standard for DNA profiling.

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- Massively Parallel Sequencing (MPS) or Next-Generation Sequencing (NGS) allows simultaneous sequencing of thousands of genomic regions, enhancing genetic information and resolution in complex cases.
- Alternative DNA analysis methods, such as Y-chromosome analysis and Single Nucleotide Polymorphism (SNP) typing, address challenges with male-specific identification and degraded DNA analysis.
- Advancements in forensic genetics include DNA methylation analysis, body fluid identification, forensic DNA phenotyping, and genetic genealogy, offering potential benefits in age estimation, tissue identification, and suspect linkage.
- Ethical considerations regarding data privacy, security, and genetic discrimination are crucial as forensic genetics continues to progress toward a more just and secure society.

2. Forensic Genetics

Forensic genetics derives its etymology from the Latin word "forensis," signifying "of or before the forum," a term rooted in the ancient Roman marketplaces where public affairs, criminal cases, and court proceedings took place. In the modern context, forensic genetics primarily encompasses the use of DNA analysis in criminal investigations [2].

The evolution of forensic genetics has been driven by historical advancements and scientific breakthroughs. It originated with the identification of ABO blood groups by Landsteiner in 1900 [3]. This early discovery led to the adoption of blood typing for identification purposes, marking the inception of forensic genetics in the scientific era. In 1910, French criminologist Edmond Locard introduced the Locard's exchange principle, postulating that "every contact leaves a trace," which laid the foundation for modern forensic science [4]. Building on this groundwork, Thomas Hunt Morgan's gene theory in 1926 provided the basis for the development of forensic genetics [5].

A groundbreaking milestone in forensic genetics occurred in 1953 with the revelation of the double-helical structure of DNA, propelling research in this field to the molecular level [5]. Subsequently, in 1984, Alec Jeffreys made the seminal discovery of "DNA fingerprinting" or DNA typing. This technique involved identifying fragments representing unique combinations of DNA repetitive elements, enabling the identification of individuals and kinship lineages [6-7]. Its initial applications spanned across paternity, immigration, and forensic genetics cases [7-10], heralding a new era in forensic DNA typing.

In the contemporary landscape, forensic genetics plays a pivotal role at the convergence of law and science. These two institutionalized pursuits are considered the most crucial contemporary sources and guardians of social order [11]. As forensic genetics continues to advance, it brings forth opportunities and challenges. Ethical considerations surrounding privacy, data security, and genetic discrimination warrant careful consideration, ensuring responsible and equitable application as the field progresses in its pursuit of truth and justice. [11].

3. An Overview of DNA

The remarkable double helix structure of DNA enables it to carry biological information across generations (Figure 1) [12]. Within eukaryotic organisms, DNA resides in the cell nucleus, organized into tightly packed chromosomes. During reproduction, each parent contributes 23 pairs of chromosomes to their offspring [12]. Additionally, cells contain mitochondrial DNA (mtDNA), primarily found in structures responsible for energy production from food [13].

DNA possesses a unique genetic code for each individual, akin to fingerprints, remaining constant throughout their lifetime, with the exception of homozygous twins. Exploiting this uniqueness, DNA profiling, also referred to as DNA testing or DNA typing, employs biological samples to identify individuals [14].

The Human Genome Project, an international endeavor, aimed to sequence the entire human genome, containing approximately 3 billion base pairs and 20,000 to 25,000 protein-coding genes distributed across 23 pairs of chromosomes [15]. Scientists have observed repetitive sequences in the non-coding regions of the genome, including single-locus satellites and multilocus satellite elements, also known as short tandem repeats (STRs) [16]. Single-locus satellites are found at specific sites on human chromosomes, while multilocus satellite elements or STRs are dispersed throughout the genome [17].

4. Repetitive DNA

The eukaryotic genome contains repetitive DNA sequences, which can be either moderately or highly repetitive and are categorized as either tandem or dispersed. Repetitive DNA is further classified into two classes: satellite DNA, consisting

of short tandem repeats arranged in tandem and making up about one-third of DNA repeats [19]. Examples of satellite DNA include minisatellites and microsatellites, which form highly repetitive tandem sequences or variable numbers of tandem repeats (VNTRs) [20].

Polymorphisms in the loci arise from variations in the number of repeat units, and the mutation rate in the VNTR region is significantly higher (10 to 100,000 times) than the average mutation rate at other genomic sites [21]. Minisatellites differ from microsatellites in their structure and function, with minisatellites having a heterogeneous arrangement of 10-100 base pair tandem repeats extending to 1-15 kilobases [22]. On the other hand, microsatellites or STRs (short tandem repeats) are a homogeneous set of short tandem repeats, typically 2-7 base pairs in length, with a total repeat size of less than or around 1 kilobase [2,23].

Although minisatellite polymorphisms have been studied, STR markers are the preferred method for forensic investigations due to their abundance and compatibility with the polymerase chain reaction (PCR) [20].

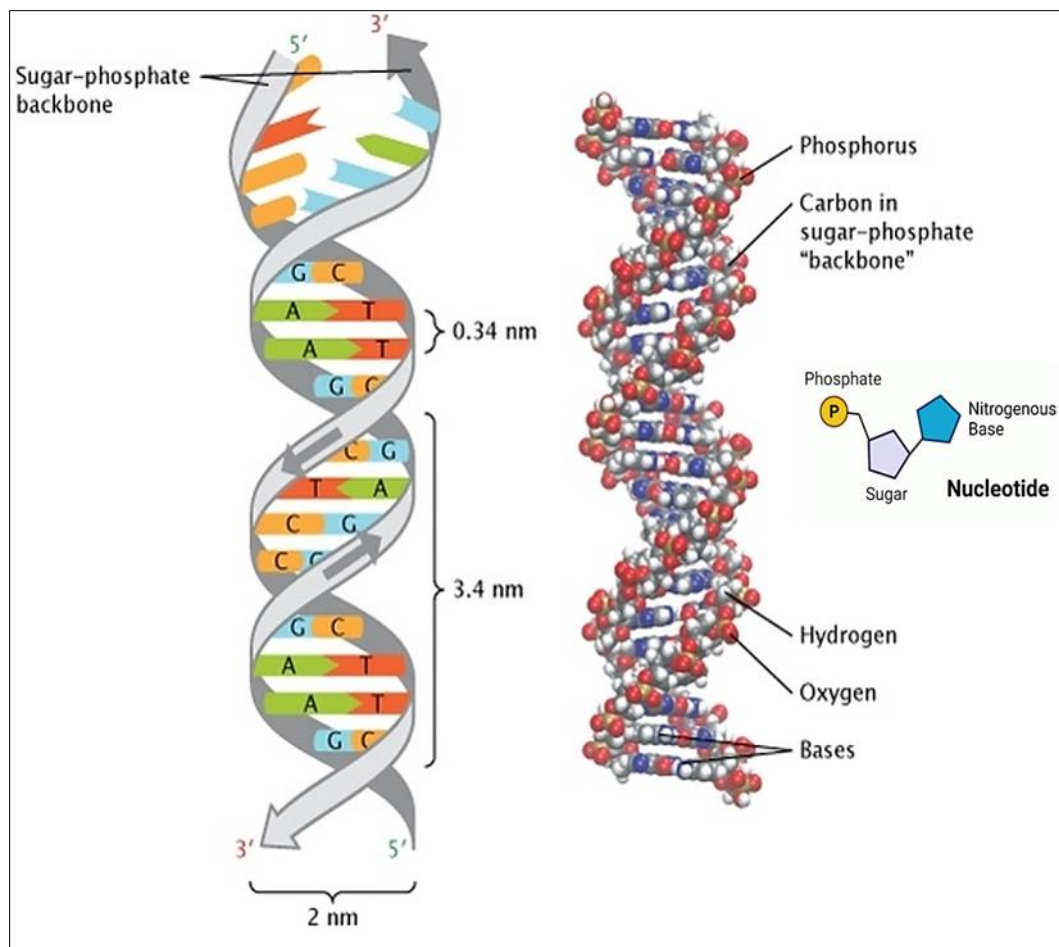


Figure 1 Double helix structure of DNA, which comprises complementary nitrogen bases held together in pairs by hydrogen bonds [18]. The fundamental building blocks of DNA are nucleotides, each consisting of a sugar group, a phosphate group, and one of four nitrogen bases. These nucleotides link together in a continuous chain to form a DNA strand. The four nitrogen bases present in DNA are adenine (A), thymine (T), guanine (G), and cytosine (C). The specific arrangement of these bases determines the biological instructions encoded within the DNA strand. For instance, a sequence like ATCGCT might encode for brown eyes, while a different sequence such as ATCGTT could code for blue eyes. The sequence of nitrogen bases along the DNA strand serves as the genetic blueprint for an individual's traits and characteristics

5. DNA as Forensic Evidence

The foundation of modern forensic crime investigation lies in Locard's exchange principle, which posits that every contact leaves a trace. When two objects come into contact, there is an exchange of material that leaves behind identifiable evidence. Traditional identification methods based on anthropological and physical characteristics of victims can prove ineffective or inconclusive, particularly when crime scenes involve intermixed or fragmented remains along with multiple pieces of evidence [24-25]. As a result, DNA profiling has emerged as the gold standard in resolving forensic cases, facilitating victim identification, and establishing links to suspects.

Reports reveal that nearly 99.9% of the DNA sequence is identical in all human beings, with only approximately 0.1% variation [26]. The probability of two unrelated individuals having the same DNA sequence is a staggering 1 in 594.1 trillion persons, rendering DNA testing a formidable tool for exonerating the innocent and convicting the guilty [26-27]. As a result, forensic science has widely adopted DNA molecular biology tools, making it the preeminent field leveraging DNA analysis [28].

In contemporary times, forensic DNA analysis has become a routine practice in investigating crime scenes, determining paternity, and identifying human remains [29]. Its unrivaled accuracy and discriminatory power have transformed forensic investigations, enabling law enforcement to solve complex cases with unprecedented precision and bringing justice to both victims and society as a whole.

DNA analysis has significantly elevated the efficacy of forensic evidence, aiding in the resolution of seemingly inscrutable cases and safeguarding against wrongful convictions. By harnessing the power of DNA, forensic science has redefined the landscape of criminal investigations, fostering a more just and secure society. As technology continues to advance, DNA analysis is expected to remain at the forefront of forensic investigations, continually refining its capabilities and enhancing its impact on the pursuit of truth and justice.

6. The Evolution of DNA Typing: Past, Present, and Beyond

The field of forensic science has witnessed remarkable progress over time, driven by numerous discoveries and technological advancements. Table 1 provides a comprehensive overview of the differences, advantages, and drawbacks of past and current forensic technologies, while Figure 2 presents a chronological timeline of developments in DNA typing technologies from 1900 to the present.

ABO blood typing, credited to Landsteiner's discovery in 1900, marks a significant milestone as the earliest form of human identification. Notably, in 1915, it was even employed in an Italian court to resolve a paternity case by analyzing different blood groups [30-32]. While ABO blood grouping was revolutionary in its time, it had limitations from a forensic standpoint, requiring substantial biological material for analysis. Nevertheless, it yielded a few phenotypes (1 in 10) [33], making it a noteworthy achievement in forensic biology.

Genetic markers play a crucial role in forensic genetics, serving as identifiable phenotypes of genotypes with features such as robust polymorphisms, codominant expression, and ease of observation and recording. The utilization of genetic markers has progressed steadily alongside advancements in genetics, undergoing four key phases: morphological markers, cytological markers, biochemical markers, and molecular markers.

The field of DNA typing, in particular, has witnessed exponential growth and transformation. From the basic ABO blood typing of the past to the highly sophisticated DNA profiling methods of today, forensic science has come a long way. The advent of DNA technology has revolutionized criminal investigations, allowing for unparalleled accuracy in victim identification and suspect linkage.

Looking ahead, the future of DNA typing holds even greater promise. With ongoing advancements in genomics, sequencing techniques, and analytical tools, forensic science is poised to achieve new levels of precision and sensitivity. These advancements will not only enhance the efficiency of forensic investigations but also contribute to the swift administration of justice and the protection of innocent individuals.

As we continue to unravel the mysteries of human genetics and push the boundaries of forensic science, the potential applications of DNA typing are boundless. The continuous pursuit of knowledge and innovation will undoubtedly pave the way for a future in which forensic DNA analysis becomes an even more indispensable tool in solving complex criminal cases and safeguarding society's well-being.

Table 1 Comprehensive comparison of DNA profiling technologies, highlighting their variations, benefits, and drawbacks spanning the historical to the contemporary era

Analysis technique	Basis of differentiation	Advantages	Disadvantages
Restriction Fragment Length Polymorphism (RFLP)	Restriction site sequence and fragment length	– High power of discrimination – Reproducible – No prior sequence information required – Can differentiate between homozygotes and heterozygotes	– Time-consuming – Partial digests – Need at least 10–25 ng of DNA – Genetic mutations only identified at restriction cut sites – Not ideal for whole genome variation identification – requires radioisotopes
Short Tandem Repeat (STR)	STR fragment length	– Fast – Highly reproducible – High level of discrimination, codominant alleles – Standardized across forensic laboratories – Uses low DNA amounts for amplification – Database of genetic profiles and allelic frequencies for statistical comparisons	– Mixture deconvolution not easy – PCR artifacts can complicate results – Challenges with highly degraded or low template DNA
Sanger Sequencing	Sequences every base	– Gold standard for sequence analyses – Uses capillary electrophoresis techniques	– Low throughput – Only 500–700 bases sequenced at a time – Cannot sequence mixtures without cloning
SNaPshot™	Single base changes	– Detects bi-allelic and multi-allelic SNP markers – Able to distinguish between heterozygotes and homozygotes – Human SNP database for statistical comparisons	– Time-consuming – Need to know SNP sequence in advance to design primers – Multiple markers required for high level of discrimination
Next-Generation Sequencing (NGS)	Massive parallel sequencing using various technologies	– High throughput – Deconvolve mixtures – Sequence entire genomes/metagenomes – Simultaneous detection of STR amplicon lengths and SNPs within the amplicon – Used for any DNA (human, non-human, viral, microbes)	– Massive data output that may be challenging to analyse – Analysis algorithms not standardised – Difficult with some technologies to analyse metagenomes to species level

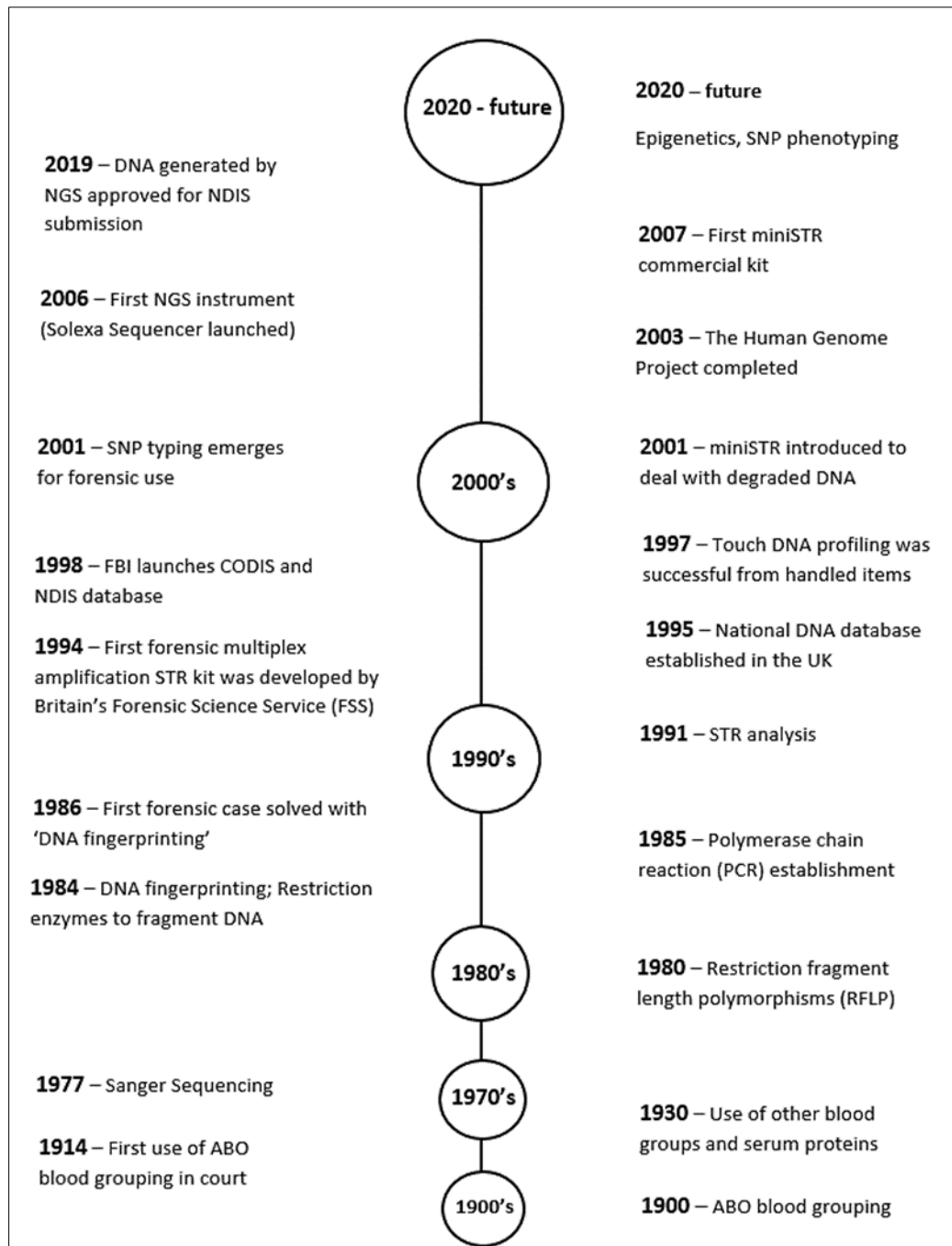


Figure 2 Chronological representation of the evolutionary journey of DNA profiling technologies, spanning from the 1900s to the modern era

6.1. The Birth of Forensic Genetics: Restriction Fragment Length Polymorphism (RFLP)

The field of forensic genetics traces its origins to the groundbreaking discovery of DNA fingerprinting by Jeffreys, who observed distinct patterns in specific regions of an individual's DNA that could be used for differentiation [7]. Jeffreys employed a unique hybridization technique, involving restriction enzymes to fragment DNA, leading to the emergence of Restriction Fragment Length Polymorphism (RFLP) patterns [6]. Building on this concept, various DNA analysis methods were subsequently developed, incorporating electrophoretic fragment separation [34].

In 1986, the revolutionary potential of DNA fingerprinting in forensic science was realized when Dr. Alec J. Jeffreys was approached by the police to create a DNA profile of a suspect in the tragic rape and murder case of 15-year-old Dawn Ashworth in Leicestershire, UK [35]. Jeffreys' DNA fingerprinting method boasted an incredibly high degree of discrimination, reaching 1×10^{11} [7]. However, it came with certain limitations, such as being time-consuming and

requiring a substantial amount of DNA (at least 10-25 ng) to yield reliable results [36]. Consequently, the RFLP technique was primarily applicable to testing fresh samples available in ample quantities, such as blood or semen [37]. Despite its groundbreaking potential, the RFLP technique faced practical challenges in forensic cases, especially with difficult samples, such as degraded or minute ones [38]. As a result, the application of RFLP was not always feasible in every scenario, prompting the need for further advancements in DNA profiling technologies.

As we delve into the realm of forensic genetics, the evolution of DNA profiling techniques has paved the way for more rapid, sensitive, and versatile methodologies, enabling forensic scientists to overcome previous limitations and handle a broader array of forensic samples with greater efficiency.

6.2. Short Tandem Repeat (STR) Typing

Following the groundbreaking discovery of DNA fingerprinting, Kary Mullis revolutionized DNA analysis in 1985 with the invention of Polymerase Chain Reaction (PCR), a technique enabling DNA amplification [39]. Subsequent improvements in PCR-based technology led to the adoption of Short Tandem Repeat (STR) analysis within the forensic community [40-41]. This powerful technique utilizes PCR to amplify highly polymorphic, repetitive DNA regions, which are then separated based on amplicon length using capillary electrophoresis. STRs are short repeated sequences (2-7 bp) found in specific loci, often in non-coding genetic regions, frequently exhibiting tetranucleotide repeats [2]. Comprising approximately 3% of the human genome [42], the number of repeat units in STRs varies significantly between individuals, providing a remarkably high degree of discrimination for identification purposes [43]. Consequently, STRs have become the prevailing standard for human DNA typing [41].

In 1994, the Forensic Science Service (FSS) in Britain developed the first forensic multiplex amplification STR kit, incorporating four genetic loci - TH01, vWA, FES/FPS, and F13A1 [44]. Despite its advancements, analyzing highly degraded or low-template DNA samples presented challenges. To address these limitations, mini-STR kits were introduced, utilizing shorter versions of STR cores and adhering to strict regulations for forensic cases [45-46]. Presently, commercial STR kits can typically detect 15-20 STR loci simultaneously, with six-color fluorescent marker STR kits allowing up to 25-30 STR loci to be detected. The International Society for Forensic Genetics (ISFG) has published a guide for the forensic validation of STR kits, outlining high standards and guidance for their forensic application.

Maintaining a clear and unbroken chain of custody is of paramount importance in DNA analysis within the context of law and courts. The chain of custody ensures the integrity and reliability of DNA evidence, guaranteeing that the samples collected from the crime scene or individuals remain untampered and accurately documented throughout the entire process [47]. Adhering to stringent chain of custody protocols establishes the trustworthiness and admissibility of DNA evidence in court proceedings, reinforcing the validity of the results presented. This meticulous approach not only safeguards the rights of the accused but also enhances the overall credibility of forensic science in the pursuit of justice. Without a secure chain of custody, there is a risk of evidence contamination or mishandling, which could lead to potential misinterpretations and jeopardize the outcome of legal proceedings [48]. As such, upholding the chain of custody is a cornerstone in ensuring the fairness and accuracy of DNA analysis within the judicial system.

The process of STR typing analysis generally follows a specific methodology recommended by the manufacturer of the commercial kit used in a forensic laboratory. Key steps include DNA extraction, quantification of the extracted DNA, amplification of STR loci using PCR technology, separation of PCR amplicons by DNA electrophoresis using a genetic analyzer, data analysis using bioinformatics, and comparison of the sample data to reference DNA profiles or databases of previously generated STR sets [49-50] (Figure 3). Statistical analysis is then performed to determine the likelihood of a match for the court, with today's standard technology typically yielding a likelihood of one in billions for a random match.

The advent of STR multiplex kits also facilitated the establishment of forensic DNA databases, which store DNA profiles of crime scene samples or suspects. These databases have become indispensable investigative tools in modern criminal justice systems [51-52]. The first DNA database, the National DNA Database (NDNAD), was introduced in the UK in 1995, containing DNA profiles from crime scene samples as well as personal DNA profiles [53-54]. In 1998, the FBI established the Combined DNA Index System (CODIS), a national DNA database in the United States. Criminal DNA databases facilitate matches between stored DNA profiles of suspects, convicted criminals, victims, and DNA evidence found at crime scenes, as permitted by the laws of each country. Currently, about 69 countries have national forensic DNA databases, with an additional 34 countries either expanding or developing their databases [55].

Analyzing trace or touch DNA can be particularly challenging due to several factors that can affect the amount of DNA obtained [56-57]. These factors include the type of surface, collection and extraction methods [58], as well as time and environmental conditions [59]. Interpretation of DNA profiles becomes complex, especially when dealing with partial profiles or mixtures from multiple sources [60-68]. However, advancements in software technologies such as STRmix™ have eased the interpretation of DNA data, enhancing its usefulness in DNA databases [69].

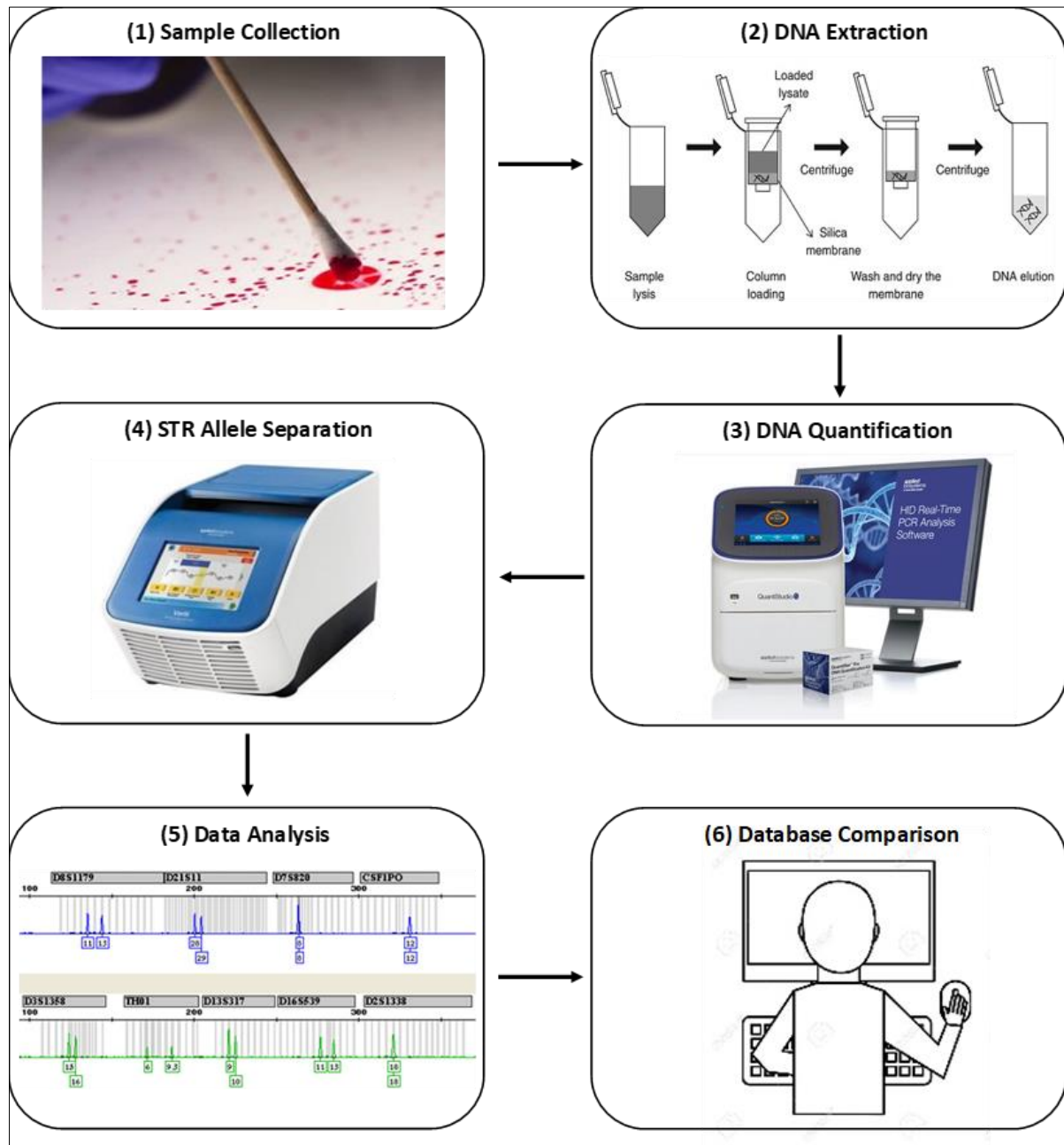


Figure 3 Step-by-step workflow of DNA profiling, encompassing key stages involved in extracting and analyzing genetic information from biological samples. This systematic process transforms raw genetic material into informative DNA profiles, providing crucial support for forensic investigations and the administration of justice. The stages include Sample Collection, DNA Extraction, DNA Quantification, Multiplex PCR Amplification, STR Allele Separation (Electrophoresis), Data Analysis, and Database Comparison

6.3. Alternative Forensic DNA Analysis Methods

Advanced DNA analysis methods have been developed as alternatives or supplements to standard STR profiling protocols, especially in cases where the DNA is severely degraded. One such method involves the utilization of Y-chromosome analysis, which is unique to males and consists of over 400 STRs and roughly 60 million base pairs [63,70].

Autosomal multiplex kits have traditionally included AMEL as an indicator of sex, but to avoid cases of AMELY null in casework, additional markers, usually Y-STRs, such as Yindel, DYS391, DYS570, and DYS576, are being incorporated. Leading Y-STR multiplex kits, offered by two manufacturers, can examine up to 23 and 27 loci, respectively [71]. Y-STR profiling is particularly valuable in sexual assault cases involving a mixture of male and female components as it enables the separation of male-specific information from the mixture [72-74]. This technique is also beneficial when the male individual does not produce spermatozoa since a DNA profile can be extracted from the male epithelial cells present, which are typically dominated by female epithelial cells in traditional STR analysis [70]. Y-STRs are also utilized in human remains identification and paternity investigations [75].

In addition to standard STR profiling protocols, alternative DNA analysis methods have been established, particularly for degraded DNA samples. One such method involves the analysis of single nucleotide polymorphisms (SNPs), which are sequence variations resulting from single base pair mutations [63,76]. Unlike STR loci, SNP sites have a lower mutation rate of approximately 10^{-8} , and the amplification products of individual SNP sites can be very short, making them suitable for analyzing highly degraded forensic samples [2,77-78]. Commercially available SNP kits, such as SNaPshot™ Multiplex, can identify known SNPs using single base extension (SBE) technology [79-80]. SNPs are utilized in four forensically relevant classes: identity-testing, phenotype informative, ancestry informative, and lineage informative. SNPs are particularly valuable for identifying individuals after mass disasters [63]. While SNP analysis offers advantages over STR analysis, transitioning from STRs to SNPs may require considerable time.

Another potential forensic application of SNP technology involves the analysis of mitochondrial coding regions for haplotyping [81]. Unlike nuclear DNA located in the cell nucleus, mitochondrial DNA (mtDNA) is found in the mitochondria organelles within the cell cytoplasm. MtDNA has several differences compared to nuclear DNA, including the presence of multiple copies in each cell. Each cell contains a few hundred to a thousand mitochondria, with each mitochondrion having 2 to 10 copies of mtDNA, and each copy being identical for that individual, except for any mutations [82-83]. Human mtDNA is a 16,569 bp double-stranded, closed-circular DNA molecule, encoding 13 polypeptides, including two rRNAs and one set of 22 tRNAs required for protein synthesis in mitochondria [84]. Within the mtDNA D-loop, two polymorphic regions known as Hypervariable regions I and II (HVI and HVII) are useful for forensic exploitation, with a mutation rate five to ten times higher than nuclear genes [85]. The primary advantage of mtDNA in forensic science is its prevalence in extremely old or degraded samples [86-87]. However, a major disadvantage of mtDNA analysis is that the mtDNA sequence is not unique to an individual because mtDNA is maternally inherited, and all maternally related individuals will have the same mtDNA sequence, except for mutations [76].

6.4. Massively Parallel Sequencing (MPS)

Massively parallel sequencing (MPS), also known as next-generation sequencing (NGS), has garnered significant attention in the field of forensic genetics due to its capacity to simultaneously sequence thousands of genomic regions. This technology enables whole genome sequencing, metagenomic sequencing, or targeted amplicon sequencing, offering extensive genetic information [88]. In contrast to STR typing, which relies on capillary electrophoresis to detect length differences, NGS can identify variations in the internal sequence and flanking structure of STRs. This advancement enhances the amount of obtainable genetic information and presents novel approaches to address challenging cases involving complex kinship identification and the resolution of DNA mixtures, which can be one of the main challenges in DNA profile interpretation. Furthermore, NGS technology enhances the discriminatory power of STR alleles by utilizing the intrinsic genetic microhaplotypes of SNPs, which involve combinations of 2–4 closely related SNPs within an allele [89-90]. However, it should be noted that the approval of analysis programs to deconvolve mixtures has not been regulated to the same level as it has for STRs.

At present, diverse NGS technologies are available, each utilizing slightly different methodologies to sequence DNA. For the use of human forensic genomics, Verogen has developed kits using Illumina's MiSeq FGx system [91-92]. Notably, in 2019, DNA profiles generated by Verogen's forensic technology received approval for upload into the National DNA Index System (NDIS), marking the first NGS technology to be approved for a forensic database [93]. The introduction of MPS represents a significant advancement in forensic genetic analysis, offering improved resolution and expanded possibilities for addressing complex cases.

6.5. Advancements in Forensic Genetics and Related Innovations

Advancements in molecular biology technologies have led to rapid progress in forensic DNA analysis. These cutting-edge techniques are unlocking the potential of unconventional evidence, providing answers to previously unanswerable questions through traditional DNA analysis and shedding light on the donor of a biological sample [41,94]. Some of the notable advancements in forensic genetics include DNA methylation analysis and epigenetics, body fluid identification, forensic DNA phenotyping, and genetic genealogy.

Epigenetics and DNA methylation markers offer promising tools for estimating a person's age, identifying tissue types, and distinguishing between monozygotic twins [95]. However, it is important to consider environmental influences, as epigenetic patterns can be altered by various factors, especially when designing prediction models like age estimation [96].

Detecting specific human body fluids can be critical in investigations, as it provides valuable information about the events surrounding an incident and allows for the linkage of a DNA profile to a particular biological source. Current presumptive and confirmatory tests for body fluid identification have limitations, such as low sensitivity, specificity, and the potential destruction of limited samples when multiple tests are required [97]. To address these challenges, RNA analysis has been explored for body fluid identification. RNA can be co-extracted with DNA, enabling both body fluid testing and DNA profiling [98]. Initially, body fluid-specific messenger RNA (mRNA) markers were used in test assays, but advancements in multiplex technology now allow for the identification of single or multiple body fluid types, particularly useful in analyzing mixed samples [99].

When standard STR profiling fails to yield a match to known offenders or DNA databases, additional information about the donor of a DNA sample becomes invaluable. Forensic DNA phenotyping (FDP) has emerged to predict externally visible characteristics (EVCs), biogeographic ancestry, and age from DNA samples using epigenetic markers [100]. This technique utilizes small sets of SNPs associated with specific characteristics through genome-wide association research [101]. With statistical models, FDP can accurately predict EVCs of interest, aiding in narrowing the pool of suspects and providing assistance in missing person cases and body identification in mass disasters [101-102]. Among these approaches, the prediction of human pigmentation traits is considered the most advanced and effective [101].

Familial searching and genetic genealogy are innovative techniques that leverage genetic data to identify potential relatives of suspects. Familial searching involves searching forensic DNA databases to find close relatives, while genetic genealogy relies on large genetic datasets from Direct-to-consumer (DTC) genetic tests for genealogical research [103]. These tests examine autosomal SNP variants, with the results published on public platforms like GEDmatch, enabling testers to identify potential relatives [104-105]. Searching these platforms using profiles from biological samples recovered in criminal investigations can help identify relatives of potential offenders, leading to additional genealogical research and ultimately the identification of a suspect whose DNA can be compared to crime samples [106]. Nevertheless, this approach has raised concerns about data privacy and ethics [107].

Despite the potential benefits of these advancements in forensic genetics, multiplex STR profiling remains the gold standard in human forensic analysis due to its standardization of DNA markers, databases, and statistical analyses. These promising technologies have the potential to enhance intelligence gathering and human identification in forensic cases, but their establishment will require significant time and cost. In addition, the significance of touch or trace DNA cannot be underestimated in forensic investigations [108-116]. Even minute amounts of DNA left behind through contact with surfaces or objects can now play a crucial role in identifying suspects and linking them to crime scenes. Multiplex STR profiling has emerged as an incredibly valuable tool in harnessing the potential of touch DNA. This technique allows simultaneous analysis of multiple STR loci, enabling the detection of DNA profiles from limited or degraded samples. With the ability to generate more comprehensive DNA profiles from trace amounts of genetic material, multiplex STR profiling greatly enhances the success rate of identifying perpetrators and solving complex cases. Its sensitivity and efficiency have revolutionized forensic investigations, enabling law enforcement agencies to piece together crucial evidence that was previously undetectable. As technology advances, the continued utilization of touch DNA and multiplex STR profiling holds tremendous promise for the future of forensic science and its vital role in delivering justice.

7. Conclusion

Forensic genetics has undergone remarkable advancements over the years, transforming the landscape of criminal investigations and human identification. The foundational techniques of DNA profiling, represented by the double helix structure of DNA with its unique nucleotide sequence, have become the gold standard for resolving forensic cases. With the ability to carry biological information from one generation to the next, DNA serves as a powerful tool in victim identification and suspect linking.

The past, present, and future of DNA typing showcase the evolution of forensic science, from the early discovery of ABO blood typing to the revolutionary DNA fingerprinting and subsequent adoption of short tandem repeat (STR) analysis. As the technology progressed, new methods like massively parallel sequencing (MPS) emerged, enabling the sequencing of thousands of genomic regions simultaneously and providing invaluable support in complex cases.

Alternative forensic DNA analysis methods have been developed to address specific challenges, such as using Y-chromosomes for male-specific identification or single nucleotide polymorphisms (SNPs) for analyzing degraded samples. Epigenetics and DNA methylation markers offer potential in estimating age and identifying tissue types. Additionally, forensic DNA phenotyping and genetic genealogy offer valuable insights into externally visible characteristics and potential familial connections, respectively.

Throughout the DNA profiling workflow, from sample collection to database comparison, meticulous attention to detail and adherence to standardized protocols are essential to ensure accuracy and reliability in forensic analysis. Moreover, the use of commercial STR kits and software technologies like STRmix™ has enhanced the interpretability and efficiency of DNA data analysis.

While advancements in forensic genetics present exciting opportunities to improve intelligence gathering and human identification, traditional DNA profiling using STRs remains the cornerstone of forensic analysis. As technology continues to progress, it is crucial to maintain rigorous quality assurance and data privacy standards to uphold the integrity and ethical principles of forensic science.

In conclusion, the field of forensic genetics continues to evolve, offering new possibilities and expanding the capabilities of criminal investigations and human identification. The combination of established techniques and emerging methodologies is shaping the future of forensic science, ensuring a brighter and more effective path for justice and truth.

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