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Review Article

DNA profiling in forensic investigation – A review

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ABSTRACT

DNA Profiling is a revolutionary method for individual and relationship analysis, crime investigation, hereditary disorders, etc. It is a universal method used to establish accurate results during the process of forensic investigation. DNA profiling techniques, which are based on repetitive sequences within DNA, have proven to be of paramount importance, albeit the complete utilization of knowledge still remaining unexplored. Even a hair strand, blood drop or even skin flakes can be used to identify DNA sequences. It has a wide range of applications both in forensics and law. Because of the advancement in the field of forensics in the past four decades, DNA evidence now stands as one of the most reliable forms of proof in a court of law. In the following article, the authors explore the main concepts of DNA Profiling, and the techniques which are widely used in forensic laboratories such as RFLP, VNTR, STR, AFLP, mtDNA analysis, Y-chromosome analysis and gender typing.

1. Introduction

It has always been the main objective of forensic scientists and criminal investigators of being able to identify the origin of biological evidence eat the crime scene with certainty. DNA profiling allows the investigation of human biological material at its elemental level – the DNA molecule, that is located in every living cell within the biological system, which contains the genetic material that distinguishes an individual from others. DNA from a biological sample, such as semen, blood, or tissue, is extracted and analysed as part of the DNA profiling procedure. DNA Profiling stands out due to its exceptionally low probability of false positives. DNA Profiling evidence should be as credible as any other type of scientific evidence presented in court, provided that stringent laboratory standards are meticulously followed. Furthermore, it is obvious that DNA Profiling marks a significant advancement in science's ability to determine if two body samples collected at separate times are from the same person.[1]

Forensic identification becomes particularly challenging in cases of defragmented, decomposing, or burned bodies. Since nearly 0.1% of human DNA sequences are unique to each person and can be used for research, DNA analysis is essential for overcoming such circumstances. Human Genome Project (HGP), an extraordinarily massive collaborative scientific project in the world to sequence the human genome, states that just 1.5% of the human genome's DNA actually codes for proteins, with the majority being referred to as "junk DNA." Variable number tandem repeats (VNTRs) or minisatellites are specific repeating sequences that makes up a significant amount of junk DNA. An inheritable pattern known as "DNA fingerprints" was created in 1984 by geneticist Alec Jeffreys using these distinctive tandem repeats, and the technique is now widely known as DNA fingerprinting. One of the most

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ground-breaking discoveries of the 20th century which was the discovery of DNA fingerprinting, has been used predominantly in the forensic science for the identification of criminal suspects by using biological samples and also for medical uses, for example, detecting diseases that are inherited, finding suitable tissue donor for organ transplantation, linking kinship and blood relatives and so on. In this article, we highlight and discuss the different types of DNA fingerprinting/profiling techniques applied in forensic science.[2]

2. Relevance for the Law

DNA profiling has proven to be a noteworthy application to the criminal law because of the possibility that it offers of determining whether human biological samples such as blood, saliva, semen etc. discovered at the scene of a crime come from a person suspected of having committed the crime. This is especially vital when looking into sexual offences. A prominent feature of DNA profiling over more traditional tests, like serological tests for blood, is that the DNA profile obtained from the crime scene can be matched with the sample of a suspect which is tested with certainty. Whenever an assailant is injured or leaves any evidence at the scene of a crime (such as hair, saliva, etc.), the DNA profile has the capability to identify the person from his/her DNA extracted. It can also acquit the suspect. DNA profiling provides the possibility of identifying victims of 'unidentified crimes' such as hit-run cases. Moreover, it can give a clear indication whether the same person is responsible for a series of crimes, such as serial killings. DNA profiling has also been used extensively for paternity testing. The application of such information is wide, varying from family-related disputes, to resolving the identity of the father of the a child in case of rape victims, to rights to social security benefits, to inheritance issues. It is also of major significance to governments seeking to confine immigration to family reunion categories.[1]

When it comes to collecting and handling evidence at the crime scenes, it is essential to bear in mind that proper care is taken for the samples collected, in manner of maintaining integrity of the crime scene, wearing face masks during investigation of the scene, as improper handling of the evidence can lead to serious consequences. In worse cases, cross-contamination leads to an extreme level of sample degradation and this can confuse or avert the final result of evidence for submission at court of law.[3] Four decades after the development of DNA fingerprinting, DNA analysis has remained a bridge to link suspects to evidence found at crime scene and to identify individuals during crimes and disasters. DNA profiling has also been used to diagnose hereditary disorders and diseases.[4]

3. An Overview of DNA

For many years, scientists debated over which molecule carried the instructions for life. The majority of scientists thought DNA was far too basic a chemical to play such a crucial part. Instead, scientists hypothesised that proteins' higher complexity and wider range of forms made them more likely to perform this crucial task.[5] After the infamous series of ground-breaking experiments conducted by Hershey and Chase in 1952, it was evident how important DNA was as the genetic material. In 1953, thanks to the work of James Watson, Francis Crick, Maurice Wilkins and Rosalind Franklin, on X-ray diffraction patterns, a significant contribution was made towards unravelling the double helical structure of DNA; a structure that enables it to carry biological information from generation to generation.[5]

As it is widely known, DNA is situated in the cell nucleus (nuclear DNA), but a fragment of DNA can also be found in the mitochondria (mitochondrial DNA or mtDNA).[6] Similar to fingerprints, everyone has a DNA signature that is unique and remains unchanged throughout their lives. DNA testing, broadly called DNA profiling, exploits the fact that, with the except the case of homozygous twins, the genetic material of a person is unique and is an omnipresent residue that trails us wherever we go.[7] The human genome project validated what forensic researchers already knew, that the noncoding sections of the genome contain, among other things, stretches of repetitive sequences . The single-locus satellites are located at a specific site of a particular human chromosome, while multi-locus satellite repeats or generally known as STRs, are spread throughout the entire genome.[8]

4. Forensic DNA Analysis

Forensic genetics has progressed owing to the analysis of human genetic variations. Alec Jeffreys's revelation of hypervariable polymorphisms in the human genome, also known as variable nucleotide tandem repeats (VNTRs) or minisatellites, provided the impetus to the use of multilocus probes for DNA fingerprinting. This proved for match probabilities to be so low that only mono-zygotic twins could in a sense, share DNA fingerprints. VNTRs are also the foundation of the restriction fragment length polymorphism (RFLP) method. However, this simple pattern match which is more resilient, technically unmanageable, requiring minute quantities of DNA led to the utilization of single locus probes (SLP) that analyse unique minisatellite species under harsher conditions. Polymerase chain Reaction (PCR) based methods on the other hand, were used to convey qualitative and quantitative issues in forensic samples as well as the length of time needed for SLP Analysis. Short tandem repeats (STRs) or a microsatellite consisting of short repeating motifs contained

within a minute fragments are, however, proven more suited for PCR.[8]

It has been almost 40 years since Dr. Alec Jeffreys, an English geneticist, first introduced the concept of "DNA fingerprinting", or DNA Typing (Profiling), as it is now known. Evett and Buckleton, however, pushed for a modification to DNA profiling that has gained widespread support.[9] DNA Profiling has improved the system's sensitivity, reproducibility in terms of results, as well as computer database compatibility. It has also rapidly established itself as the global standard forensic DNA Technology for paternity testing and criminal casework.[10]

4.1. DNA isolation methods

Forensic DNA analysis is a sensitive technique and samples from the scene of crime or a mass disaster may contain only minute amounts of DNA, which makes it increasingly problematic and sensitive to handle since it may include polymerase chain reaction (PCR)-inhibitors as well. For analysis of such samples and in order to achieve optimum results, efficient DNA extraction procedures as well as accurate DNA quantification methods are critical steps involved in the process of successful DNA analysis of such samples.[10]

4.1.1. Organic extraction (phenol e chloroform method

This method was introduced by Barker et al in 1998.[3] It is a conventional and widely used method,[11] albeit being labour-intensive and time consuming.[12] The phenol-chloroform method is the oldest and most delicate approach for isolating DNA from a wide range of forensic evidence.[10] Organic extraction yields high quality, double stranded DNA, in many biological samples such as tissue, teeth, bones, blood etc., and therefore can be utilized in situations where PCR typing is performed.[13]

This method includes using risky organic solvents, requires quite some time, and any leftover phenol or chloroform could have an influence on PCR or other downstream applications.[11]

4.1.2. Silica based DNA extraction methods

One of the most demanding DNA extractions is from bones and teeth due to the robustness of the material and the relatively low DNA content. The greatest challenge is due to manifold nature of the material, which is defined by the various factors including age, storage, environmental conditions, and contamination with inhibitors.[14] Silica-based nucleic acid purification methods employ a simple bind-wash-elute process.[11] Manjunath et al. claimed that silica-based extraction methods showed better results in nuclear STR typing from degraded bone samples than a commonly used phenol/chloroform method.[10]

4.1.3. Chelex 100

Walsh et al. came up with the first protocol for DNA extraction using Chelex 100, which enables rapid extraction of DNA from trace amounts of biological samples. Procedures devised to extract DNA from human biological samples such as hard tissue (bones, teeth), and biological fluids (blood, semen, saliva etc.) for use with the PCR, include Chelex100 chelating resin. This procedure is relatively easy to work with as there is no need for several tube transfers and there is no use of organic solvents. Chelex 100 is said to be as effective as or more than Organic extraction method.[15]

4.1.4. Commercial DNA extraction kits

With the advancement of technology and demand for molecular tests increased, new automate methods of DNA extraction have developed to handle larger samples (16). DNA extraction kits allow a much higher throughput of samples along with being less labour intensive and produces PCR-ready DNA.[16] The PrepFiler Forensic DNA Extraction kit enables the researcher to be able to extract exceptional yield of DNA from most forensic sample types, i.e., bodily fluids, stains etc. by utilizing magnetic particles with an optimised multi-component surface chemistry. The kit is designed to help improve yield and concentration of the DNA thus recovered, all the while enabling removal of PCR inhibitors. This calls for much larger quantities of highly purified DNA for Genotyping results.[17]

4.2. DNA profiling

DNA profiling uses a variety of DNA typing systems including Restriction Fragment Length Polymorphism (RFLP), Variable Number Tandem Repeat (VNTR), Short Tandem Repeat (STR), Single Nucleotide Polymorphism (SNP) typing, Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Y-chromosome Analysis, Mitochondrial DNA (mtDNA) analysis, and Gender typing.

4.2.1. Restriction fragment length polymorphism (RFLP)

In Restriction Fragment Length Polymorphisms (RFLP) technique, DNA is subjected to one or more restriction enzymes, or molecular scissors, which cleaves the DNA at specific sites, particularly known as unique restriction site. This results in generation of numerous DNA fragments of various lengths.[18] An RFLP probe, is basically a labelled DNA sequence which hybridizes with the DNA sample, which upon separation by gel electrophoresis, reveals a unique blotting pattern. This pattern is specific to a specific genotype at a specific locus.[19] After the extracted DNA is has been digested by the enzyme, the fragments of DNA are separated according to their size using gel electrophoresis.[20] The DNA can then be visualised by a variety of methods, yielding a pattern of bands, often described as similar to

a supermarket bar code.[18] If a difference in patterns is found between the extracted DNA from sample at the crime scene to the DNA of the suspect, the suspect is exonerated. However, if the patterns match, then by law, court can use this fact as admissible evidence to link the suspect to the crime scene.[20]

4.2.2. Variable number of tandem repeat (VNTR

The human genome is comprised of long stretches of tandemly repeated short DNA sequences. In a given locus, the quantity of these short sequence repeats varies greatly between unrelated individuals.[18] It was Dr. Jeffrey who described this repetition of sequences and thus, these repeated sequences are known as variable number of tandem repeat sequences (VNTR).[21] VNTRs are classified into mini- and micro-satellites based on the size of the repeated blocks. The sequence repeat unit of a microsatellite is 2-9 bp long, whereas mini-satellites is between 9 to 100 base pairs long. The technique used for the analysis the VNTRs is now called restriction fragment length polymorphism (RFLP) due to ability of restriction enzyme cutting the DNA regions surrounding the VNTRs.[22] Moreover, many aspects of variability mechanisms, its interaction with evolutionary processes and its biological roles yet remain obscure.[23] VNTR is a genotyping method that offers data in a simple, obscure format based on the number of repeated sequences.[24]

4.2.3. Short tandem repeat (STR)

Short Tandem Repeats (STRs),which make up around 3% of the human genome, are short repetitive DNA sequences.[25] When it comes to evaluation for identification purposes, the number of repeat units is highly variable between individuals, resulting in significant power of discrimination. It is largely believed that since STRs are non-coding in nature, they are not involved in regulating gene expression.[26] 13 autosomal STR loci are now recognised as the system used for forensics in the United States.[27] The probability of an unrelated person having a perfect match for all 13 STRs given a strong crime scene DNA sample as well as adequate data for all 13 STRs is around 1 in 1 billion. Hanson et al. claimed that a perfect match has a probability of roughly 1 in 50 thousand according to an experimental work using a very reliable set of 30 Y-STR loci.[28] STR analysis is widely used as means of identification, and the uniqueness of an individual's STRs serves as a marker to identify not only human remains but to establish or exclude paternity and even match a suspect to a crime scene sample.[27]

4.2.4. Single nucleotide polymorphisms (SNPs)

Single Nucleotide Polymorphisms or SNPs are variations in the DNA sequence that arises due to change in a single nucleotide (A, T, C, or G) in the genome sequence. SNPs are emerging as new markers of interest to forensic science due to their small amplicon size, which are utilised in analyses of degraded samples, relatively low mutation rate compared to STRs, are abundant in the human genome and has the ability to acquire specific information about ancestry, lineage, evolution, or phenotype, as well as determine sex.[29] There has also been the possibility of automating the analysis with high-throughput technologies.[30] When STR Analysis fails to produce a promising result or yields only a partial profile, SNP typing is likely to be an adjunct forensic technology. Several SNP typing strategies have been introduced like SNaP shot, SNPstream Ultra High Throughput System, electrospray ionization mass spectrometry etc.[31] Some studies have considered that SNPs will replace STRs in forensic investigation,[32] owing to its advantages over STRs such as more stable genetic markers, low mutation rates and low probability of changes over generations which plays an important role in paternity cases.[33]

4.2.5. Random amplified polymorphic DNA (RAPD)

Many exceptionally valuable DNA markers have been developed for the identification of genetic polymorphism as a result of advancements in molecular biology techniques. One of the most popular molecular methods for developing DNA markers over past decade has been the RAPD technique, which is based on PCR analysis. While the dependability of RAPD analysis is still a centre of debate, the technique has been proven useful due to its low cost, efficiency in development of large number of DNA markers in short amount of time and demand for less specialised instrumentation.[34] The RAPD analysis has captured the interest of many researchers due to its ease of use. The greatest factor, however, contributing to the success of RAPD analysis is the gain of large number of genetic markers that need only modest amount of DNA, without the need for cloning, sequencing, or any other type of molecular characterization of the genome of species of interest.[34] RAPD is an approach that aids in detection of polymorphism by utilising PCR and subjecting the resulting product to band pattern detection.[35] However, one disadvantage of RAPD is its total reliance on PCR-based enzymatic reactions.[36] Therefore, the reaction parameters, including the temperature at which the primer anneal, number of PCR cycles, the concentration of PCR components, and the template DNA, altogether influence the result of this technique.[37]

4.2.6. Amplified fragment length polymorphism (AFLP)

One of the newest and promising methods in forensics is the Amplified-fragment Length Polymorphism (AFLP) analysis, developed by Keygene BV, Wageningen, Netherlands. For AFLP, only a minute amount of purified genomic DNA is required.[38] AFLP is used to detect polymorphisms in DNA often when there is no information

about the genome available. After digestion of the DNA with restriction enzymes, a subset of DNA fragment is selected which is then subjected to PCR amplification and visualization. A unique fingerprint is produced for a particular genome. Originally, AFLP was first developed for plant studies, however, now it has been widely used in field of forensic science.[39] Amplified fragment length polymorphisms (AFLPs) were initially the starting point for the application of PCR in forensic science. The PCR of AFLP system used the specific locus and the method was useful because small and degraded samples could be analysed.[40] Some of the crucial characteristics of AFLP analysis includes its robustness, reliability, and quantitative nature.[41]

4.2.7. Y- Chromosome analysis

In forensic DNA analysis, the male-specific region of the human Y-chromosome is widely utilized, especially when standard autosomal DNA profiling is not informative. For determining the biological sex of a crime scene trace donor, a fragment of Y-chromosome is used. In a scenario where a male and female has contributed to the same trace, such as in case of sexual assault, haplotypes composed of Y-chromosomal STR polymorphisms (Y-STRs) are used to characterise paternal lineages of unknown male trace donors.[42] When it comes to crime scene investigation, Y-STR haplotyping can not only rule out the involvement of male suspects in the crime, reveal the paternal lineage of male perpetrators, highlight multiple male contributors to a trace, but also provide investigative leads for finding unknown male perpetrators. Male child paternity disputes, other sorts of paternal kinship testing, including historical cases, as well as unique cases of missing person and disaster victim identification specifically involving men, all include the use of Y-STR haplotype analysis.[42]

4.2.8. Mitochondrial DNA (mtDNA) analysis

MtDNA has proven to be a useful tool for forensic identification due to its high copy number, maternal inheritance, and high degree of sequence variability. In case of missing person investigations, it can be crucial to compare the mtDNA profile of unidentified remains to the profile of a likely maternal relative.[29] The mtDNA is maternally inherited, contrarily to the nuclear DNA, which explains that apart from mutations, mtDNA sequence of siblings and all maternal relatives is identical. This can be very helpful in forensic cases, such as analysis of the remains of a missing person, where the known maternal relative can provide a sample for reference for a direct comparison.[43] Furthermore, Silva and Passos (2007) noted that only ancient tissues such as bones, teeth, and other biological sample like hair, in which nuclear DNA cannot be analysed, are appropriate for mitochondrial DNA investigation for forensic purposes. Direct sequencing of its nitrogenous bases is used for this evaluation, albeit being a very expensive method since it makes use of a highly specialised technology. Additionally, mtDNA is exclusively matrilineal, making it less informative. Thus this analysis is not employed often in all forensic laboratories especially directed at solving crimes and identification of people.[44,45]

4.2.9. Gender typing

In the event of accidents, explosions of chemical or nuclear bombs, natural disasters, criminal investigations, and ethnic studies, determination of a person's sex becomes the first priority in the process of identification which is done by a forensic investigator. A great problem arises for the forensic investigator to determine the sex or gender using skeletal remains, when only fragments of body are retrieved. Features of hard tissue like teeth such as its morphology, crown size, and length of roots are distinguishable for both males and females. The patterns on the skull vary as well. A forensic odontologist, can use these in order to determine the sex of the person. Development of Polymerase Chain Reaction (PCR) amplification has helped in providing accurate determination of sex from the remains.[46]

In an experiment conducted by Tsuchimochi et al., Chelex method was utilized to extract DNA from dental pulp and was amplified with PCR along with typing at Y chromosomal loci in order to study how temperature affects the sex determination of the teeth.[47] Hanaoka and Minaguchi also conducted a series of experiment in order to determine sex from blood and teeth using amplification of X-specific (131bp) and Y-specific (172 bp) sequences in males and Y-specific sequences in females. It was proven to be a useful method in order to determine sex of an individual.[48] In another study conducted by Das et al., it was observed that up to four weeks after death, determining sex can be achieved accurately from studying X and Y chromosome, keeping in mind the variation of temperature and humidity.[49] An important matrix protein present in the human enamel is called amelogenin, or AMEL. The AMEL gene that codes for female amelogenin is situated on the X-chromosome and AMEL gene that encodes for male amelogenin is situated on Y chromosome respectively. Unlike the male, who has two different AMEL genes, the female has two identical AMEL genes, or alleles. This can be used as an indispensable tool to determine the sex of the remains, even with minute traces of DNA.[50]

5. DNA Database - CODIS

The establishment of DNA databases is another significant advancement in DNA profiling. The introduction of a national database in the United States allowed forensic scientists to enter unmatched DNA evidence found at the crime scene into a computerised system in order to make DNA matches.[40]

The CODIS is an acronym for Combined DNA Index System. It was started and supported by the Federal Bureau of Investigation (FBI) in 1990, and has since served as the foundation of the US DNA database. It was designed to enable open access forensic DNA testing facilities to build searchable databases of authorised DNA profiles. The CODIS programme enables laboratories in the United States to share and compare data. Apart from that, it also includes a central database with all DNA profiles from all the user laboratories. The 13 CODIS locations are TH01, TPOX, CSF1PO, vWA, FGA, D3S1358, D5S818, D7S820, D13S317, D16S539, D8S1179, D18S51, and D21S11.[29] The United States maintains the largest DNA database in the world: The Combined DNA Index System, with over 60 million records as of 2007.[29] CODIS was established to draw comparison between a target DNA record against the DNA records contained in the database. The laboratories involved in the match exchange information to confirm the match and create coordination between two agencies after the CODIS software identifies a match in order to establish a probable cause to obtain an evidentiary DNA sample from the suspect, the forensic DNA record must match the DNA record in the database. This documentation can then be used by the law enforcement agency to obtain a court order authorizing the collection of a known biological reference sample from the offender. The known biological sample can undergo a DNA analysis in casework laboratory so that the results can be presented as evidence in court.[51] According to Machado & Silva (2019), it was concluded that around 69 countries at present employ national forensic DNA databases; others are being expanded or established in at least 34 additional countries.[52]

5.1. Recent advancement

The science of DNA profiling has undergone many changes during these intervening years and will continue to do so in future. Even though several typing methods have been established and were used to deduce the identity or genetic linkage of individuals, other genetic markers are being used to determine certain phenotypic traits to a good degree of accuracy. Genetic testing such as next-generation DNA sequencing, adapted from medical and pharmaceutical sciences, will soon be applied to mainstream forensic science, opening new avenues in criminal investigations.[53] The forensic community, faces the question of direction towards which the DNA fingerprint technology will be developed. With the help of commercial instruments being developed and introduced in the market, time, another essential factor in police investigations will be considerably reduced in future applications of DNA profiling.[54] Like the Olympic motto "faster, higher, stronger", forensic DNA techniques can be predicted to become more rapid and sensitive and provide even stronger investigative potential. New Short Tandem Repeat (STR) loci have expanded the core set of genetic markers which plays an essential role in human identification in USA and Europe. Even familial DNA searching has expanded capabilities of DNA databases all around the world. Presently employed DNA profiling with STR markers involves use of fluorescent dyes in order to label PCR products and capillary electrophoresis (CE) in order to rapidly separate and analyse the dye-labelled PCR products respectively. Future advances in forensic DNA analysis will probably mirror genomic technology.[55] As forensic DNA typing techniques advance, forensic scientists will analyse more form of evidence in order to find answers for questions otherwise deemed unresolvable with traditional DNA analyses (56). For instance, Vidaki and Kayer (2018) studied how epigenetics as well as markers for DNA methylation were proposed to estimate age, determine tissue type, and even differentiate between monozygotic twins.[56]

6. Discussion

DNA profiling is one of the biggest achievements of the late 20^{th} Century. The analysis of DNA from biological evidence, which was first used in a criminal case in 1987, has completely revolutionised how forensic investigations are carried out. In the three decades thereafter, DNA profiling techniques have made significant furtherance in respect of their sensitivity, speed, and power.[54] Various number of unique approaches have been developed in the rapidly expanding field of forensic genetics in order to enable the analysis of exigent forensic samples and to provide intelligence about the donor of the biological sample.[57] This article puts forth the development of DNA profiling to a systemized investigative technique for use at court of law which has, since 1980s led to conviction of thousands of criminals and to exonerate many wrongfully suspected or convicted individuals.[58] DNA Profiling on its own could not bring down the crime rate in any of the many countries, however it helps to add unwavering scientific value to the evidence and increases the credibility of the legal system.

Before any new procedure can be adopted in any accredited forensic laboratory, robust validation processes are required. In addition to this, selection of right DNA typing platforms that meet demands of sample size, coverage, cost and is accurate is also a fundamental requirement of forensic DNA applications. In the meantime, research in forensic science and improvement in forensic investigation continue to advance.[54] Even advance techniques like Next-Generation Sequencing has been employed to identify novel, and rare cancer mutations, detect familial cancer mutation carriers and allows greater insight obtained from biological evidence. For instance, challenges such as extremely degraded or DNA in low quantity can be overcome to increase recovery of informative profiles.[59] NGS provides deep coverage to

extract genetic information from minute quantity of mtDNA samples found in less than idea condition.[60]

With modern technology, the amount of DNA needed for analysis can now be extracted from even a minute biological sample, which enables relevant authorities to match suspects to evidence found at the crime scene. However, even an absolute "match" is not sufficient for concrete testament of guilt since forensic science is largely rooted in probabilities. Additionally, concerns with delayed sample entries, and individual genetic rights pose challenges to DNA databases which are designed to simply the process of connecting past offenders to recent crimes. This hinders usefulness of the databases. Even though forensic science is inarguably vital to the modern justice system, its ethical implications and personal implications are still debated in the legal, law enforcement, and scientific communities.[27]

7. Conclusion

DNA is the genetic code that is located in each and every cell of within an individual's body. Although only 99.9 percent of DNA sequences is similar in every person, it is the 0.1 percent of the DNA difference that is unique to the individual and the forensic scientists are interested in. Typically, forensic DNA analysis is carried out in following steps: Sample preparation, DNA extraction, DNA amplification, DNA quantitation, and DNA profile matching where the profile obtained from crime-scene evidence is either entered into DNA database for comparison or compared directly with that from the suspect to determine whether suspect contributed DNA to the crime scene or not. Forensic DNA analysis has played an increasingly crucial role in criminal justice system. But as the role expands, many social, legal and ethical concerns are also raised. Of these, prevention of unauthorized use of an individual's DNA profile, is of paramount importance.[61] Apart from this, reliability and robustness of DNA testing itself is an important concern, since errors can occur during evidence collection, storage of the samples, DNA extraction, etc., which could lead to accusation of the wrong person. DNA extraction methods have become more and more effective with regard to obtaining purified DNA from crime-scene evidence. Time continues to work against forensic sample analysis. This is because DNA tends to degrade under certain environmental conditions, and it becomes difficult for it to retain its integrity over an indefinite time of storage.

The most common challenges often faced by forensic DNA laboratories include the demand for a) faster DNA results from b) a vast number of samples which are collected from c) an ever increasing spectrum of substrates by the law enforcement agencies. Some laboratories have chosen to go for fully-automated methods in extraction process in order to reduce backlog of cases. Compared with the time-consuming gold standard organic extraction method, standardized rapid DNA and automated methods often lack a certain quality of sensitivity and robustness to handle wide range of samples, especially if it concerns low quantity of DNA. However, laboratories are making informed choices in expansion of arsenal of methods in their forensic DNA toolbox.[62] It is worth noting that even though numerous scientific improvements are sure to come, current methods employed are reliable and valid.

As more is understood about the functioning of the human genome, aspects of its new knowledge will be adapted for use in forensic science.[53] The DNA analysis has been proven to be a crucial tool in solving cases in forensics, such as establishing custody of a child through paternity or maternity tests, identification of victims from crimes or disasters, or exoneration innocent people convicted to prison.[8] The authors have demonstrated some of the established and cutting-edge methods in this concise review for the reader. This article presents a literature review referring to the main studies on DNA Profiling and makes an overview of the evolution of this technology in the last years, highlighting the importance of molecular biology in forensic science.

This review also highlights some of the important DNA Profiling techniques and their developments in the rapidly expanding field of forensic science. It will be crucially important that researchers consider how to harness the innovations thus produced by the dynamic field in order to ensure their implementation in forensic investigations. Many more exciting scientific and technological advances are still to be explored, and there is no doubt that the future landscape of forensic DNA analysis will look quite different from the one we see today.

8. Conflict of Interest

The authors declare that there is no conflict of interest.

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None.

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