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Role of DNA Fingerprinting in Crime Detection: Techniques, Applications, and Future Directions

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ABSTRACT:

DNA fingerprinting, also referred as genetic fingerprinting or DNA Analysis, has brought about a significant transformation in the domain of forensic science and has brought about a revolutionary change in the detection of criminal activities. The molecular signature of each person is distinguishable due to the vast polymorphic variations present in the DNA sequence inherited from biological progenitors, which remains conserved in all somatic/gamete cells. However the majority of human DNA sequences exhibit a 99.9% similarity across all individuals, there are sufficient variations within the DNA to enable differentiation between individuals, unless they are twins with monozygotic DNA. The process of DNA profiling involves the effective use of repetitive sequences, which exhibit a high degree of variability; commonly referred to as variable number tandem repeats (VNTRs) specifically, short tandem repeats (STRs). The VNTR loci exhibit a high degree of homogeneity among closely related human specimens, whereas the heterogeneity is so pronounced that the probability of unrelated individuals sharing the same VNTRs is exceedingly low. The uses of DNA profiling within the criminal justice system is a crucial matter for forensic scientists presently. This research paper presents an in-depth exploration of the principles, techniques, and applications of DNA fingerprinting in crime detection. It examines the historical development of DNA fingerprinting and its significance in forensic science.

Keywords: DNA fingerprinting, Variable number tandem repeats (VNTRs), Short tandem repeats (STRs), Crime Detection, monozygotic DNA, DNA Analysis

INTRODUCTION

The technique of DNA profiling was initially documented in 1986[1] by Sir Alec Jeffreys, a researcher at the University of Leicester in the United Kingdom.[2] Currently, it serves as the foundation for numerous national DNA databases. The commercialization of Dr. Jeffrey's genetic fingerprinting technique occurred in 1987, facilitated by Imperial Chemical Industries (ICI) which established a blood testing facility in the United Kingdom.[3] The human body is composed of approximately 6 x 10^13 cellular units. A diploid human cell is comprised of 23 pairs of chromosomes, totaling 46, with each parent contributing half of the genetic material. The haploid cellular content of DNA in humans is quantified at 3 x 109 base pairs. Approximately 99.9% of the nucleotide sequence within the human genome exhibits homology across individuals. Merely 0.1% of the genome remains, serving as the foundation for DNA profiling, and this segment is situated within the non-coding region. The non-coding DNA, also known as "junk DNA," comprises 97% of the nuclear DNA, with the remaining 3% being coding DNA.[4]

A significant percentage of non-coding DNA exhibits a peculiarity. The DNA is composed of extensive series (alleles) of tandemly repeated nitrogenous bases. This repetitive DNA makes up around 50% of the human genome. The DNA sequences adjacent to the repeated segments are referred to as flanking DNA. The variation in the number of tandem repeats among individuals is referred to as tandem repeat polymorphism. This characteristic is utilized in the process of DNA profiling.

DNA fingerprinting is a significant technical advancement in crime investigation, comparable to the discovery of fingerprinting a century ago. When DNA fingerprinting is performed on biological specimens, it is certain that an innocent suspect will be eliminated out and the guilty person will be linked. When DNA testing is done carefully, the results are highly repeatable and clear enough to show whether a suspect is guilty or innocent and to link the guilty to a crime. When DNA analysis is done carefully, the results are very repeatable and clear enough to show a suspect's guilt or innocence beyond a reasonable query. DNA fingerprints or profiles can be used in a wide range of criminal investigations, such as paternity tests, murder, rape, identifying people, etc. The conventional application of immunological and polymorphic enzyme system is optimal for cases of exclusion, with an inclusion probability not surpassing 99.7%. DNA profiling aims to aid law enforcement in solving crimes and facilitate the pursuit of justice within the judicial system. By considering the world population, estimated to be around 5x10^16, the probability of finding an identical DNA pattern between two randomly selected individuals is approximately 1 in 10^14 to 10^10, rendering DNA profiles highly unique, excluding cases of identical twins (monozygotic twins)

Deoxyribonucleic acid (DNA)

Deoxyribonucleic acid, commonly referred to as DNA, serves as the genetic blueprint in humans and nearly all living entities. It is a macromolecule that exhibits a double helical configuration, which is assembled from four distinct nucleotides. Each nucleotide contains a sugar moiety, a phosphate group (which collectively constitute the DNA backbone), and a nucleobase. The discovery of deoxyribonucleic acid (DNA) was initially discovered by Dr. Friedrich Miescher, a Swiss physician, in 1869. The double helical conformation of the DNA discovered in 1953, By James Watson and Francis Crick. Since the elucidation of the DNA structure, significant advancements have been made in mastering the human genome and the vital function of DNA in sustaining life and promoting well-being. The DNA structure comprises solely of four distinct nucleobases, namely Adenine (A), Guanine (G), Cytosine (C), and Thymine (T).[5] All somatic cells, excluding erythrocytes and gametes, harbour the complete genetic blueprint of an organism within their deoxyribonucleic acid (DNA). The program is inscribed by four nucleobases, or subunits, namely Guanine, Cytosine, Adenine, and Thymine (frequently abbreviated as G, C, A, and T), which are arranged into extremely elongated sequences. Triplet nucleobase sequences, referred to as codons, encode the 20 amino acids, the fundamental constituents of life. Subsequently, these amino acids are polymerized to form proteins.

The stop codons provide signals for the termination of the amino acid sequence. Despite the comprehensive understanding of the genetic code, molecular biologists have yet discovered every aspect of its expression. Although each cell within an individual contains identical genetic information, the human genome is composed of a vast nuclear genome with over 26,000 genes and a minute circular mitochondrial genome with only 37 genes. The nuclear genome is dispersed among 24 linear DNA molecules, one for each of the 24 distinct human chromosomes (Organisation of the Human Genome). The genome comprises of the complete set of DNA molecules within an organism, and in humans, this encompasses the DNA present in the 23 pairs of chromosomes in the nucleus in addition to the relatively small mitochondrial genome. Humans possess a diploid genome, inheriting one set of chromosomes from each parent. A complete and functional diploid genome is necessary for normal development and the maintenance of life.

The human genome, which consists of around 3 billion base pairs, contains genetically relevant information that is critical for the identification of each person. Genetically relevant information is believed to account for less than 10% of the human genome. This tiny portion of the gene-coding DNA has been exposed to evolutionary pressure and selection processes, allowing highly organised creatures to arise. The remaining 90% of the genome is junk DNA, a misnomer since its roles are yet unclear rather than useless. Repetitive sequences make up a portion of this non-coding DNA. Mini- or micro-satellites are highly polymorphic locations in these non-coding regions that are characterised by repetitive blocks of DNA. Single-locus satellites are found at a particular location on a human chromosome, while multi-locus satellite elements or short tandem repeats (STRs) are found across the genome.

The genome has a tremendous amount of diversity. During evolutionary process, the process of selection entails non-directed mutations that can persist if they result in a neutral or beneficial trait, while mutations are typically eliminated. The non-coding regions of the human genome do not follow the same rules of selection and maintenance, unless they have an impact on the individual's survival abilities. This is the underlying cause of the accumulation of mutations that ultimately result in the creation of genetic variation within the non-coding regions of the genome. Polymorphisms occurring in gene-coding regions are exceptions that demonstrate a remarkable genetic stability and a significantly low frequency of mutation.

Classification of tandem repeats[6]

1. STRs [Short tandem repeats or micro-satellites]: the length of the repeating unit ranges from 1 to 6 base pairs, the sequence is referred to as short tandem repeats (STRs).

2. VNTRs [Variable number tandem repeats or mini-satellites]: if the length of the repeating unit falls within the range of 7-100 base pairs, this specific sequence is referred to as variable number tandem repeats.

3. Satellite DNA: if the repeated segment ranges from 100 to multiple thousands of base pairs, it is known as satellite DNA.

DNA Fingerprinting Process:

Extraction of DNA from samples:

The process of DNA extraction involves the use of physical and/or chemical techniques to isolate DNA from a sample, thereby separating it from cell membranes, proteins, and other cellular constituents. The DNA analysis procedure is initiated by obtaining a reference sample of an individual's DNA. The best method for obtaining a reference sample is through the proper utilisation of a buccal swab, as it minimises the likelihood of contamination. In cases where access to a biological sample is restricted (e.g. due to legal requirements), alternative approaches must be employed to obtain a specimen of blood, saliva, semen, or other suitable bodily fluid or tissue retrieving from a personal item (e.g. toothbrush, razor) or from stored samples (e.g. banked sperm or biopsy tissue).

The method of DNA typing is presently the most validated technique for identifying the individuals bodily fluid stains obtained at crime scenes. In numerous genetic investigations, the commonly used technique involves isolating DNA from the nucleated cells found in peripheral blood. However, due to the invasive nature of this technique, obtaining samples from the study subjects may pose a challenge.[7,8]

The procedures followed for isolation are tedious, and the overall analysis duration has been quite lengthy. Additional options for DNA extraction include buccal cells, hair with follicles, and urine, which can be obtained non-invasively compared to the invasive method of blood collection.[8] The collection of buccal cells can be easily collected through the use of a cotton swab for buccal swabbing or by means of a mouthwash procedure.[9] The isolation of DNA through buccal swabs offers numerous advantages, including economical processing, reduced sample volume requirements, extended archival potential, and compatibility with self-collection. Buccal swabs are a preferred option for DNA collection as they offer greater patient comfort and yield sufficient DNA for PCR analysis, requiring only a small amount of nanograms of DNA.[9]Human hair is a frequently used biological material in forensic investigations and has been utilised for population studies based on statistics and DNA analysis in criminology.[10] The supreme method for DNA analysis is the examination of short tandem repeats in nuclear DNA.[11]

This can be obtained if the hair root segment and/or attached tissue is available. Telogen hairs, which are commonly found at crime scenes, may not have any nuclear material.[11] The mitochondrial DNA (mtDNA) and cellular mitochondria remain preserved, [11,12] in keratinized hair, while the nucleus undergoes degradation due to hardening of the hair shaft during keratinization. Therefore, mtDNA analysis can be performed on keratinized hair. However, due to the proteinaceous composition of hair samples, supplementary measures are necessary to disrupt the shaft and liberate the DNA molecules. These measures may include fragmenting the sample via (microscopic glass grinder, followed by an organic solvent extraction)[13-15] which may elevate the possibility of specimen contamination. The forensic analysis of human urine stains is a crucial aspect in determining the precise crime scene and manner of death.[16] Using a sample of human urine as a specimen for toxicological analysis is a viable option in doping and drug-screening examinations.[17]

The organic extraction method for DNA isolation is a procedure that requires significant effort and a considerable amount of time. The process of cell lysis can be achieved through the use of nonionic detergent such as sodium dodecyl sulphate, Tris-Cl, and Ethylene diamine tetraacetic acid (EDTA). Subsequently, followed by removal of cell debris through centrifugation. Protease treatment is employed to induce protein denaturation. Organic solvents, like chloroform, phenol, or a combination of phenol and chloroform (with a ratio of phenol/chloroform/isoamyl alcohol of 25:24:1), are commonly employed to denature and precipitate proteins from nucleic acid solutions. The denatured proteins are subsequently eliminated through centrifugation and washing procedures. RNAse treatment is a standard procedure used to eliminate unwanted RNA from a sample. The DNA can be concentrated by performing precipitation with ice-cold ethanol. A nucleic acid precipitate can be formed under moderate concentrations of monovalent cations (salt). The precipitate can be retrieved through centrifugation and subsequently re-dissolved in TE buffer or doubly-distilled water.

Alternative techniques involve the use of silica-based methodology, where DNA adheres to silica beads or particles under specific pH conditions in the presence of particular salts. Magnetic separation is another method, where DNA forms a reversible bond with magnetic beads coated with DNA-binding antibodies. Additionally, anion exchange technology, salting out, and cesium chloride density gradients are also employed.

The assessment of DNA quality and yield is usually carried out using either spectrophotometry or gel electrophoresis techniques. The process of spectrophotometry is applied to determine the concentration of DNA by quantifying the absorption of light at specific wavelengths in the sample. The nucleic acids exhibit an absorption peak at approximately 260 nanometers. The ratio of A260/A280 is approximately 1.8 for double-stranded DNA. A ratio of less than 1.7 is indicative of protein contamination. Subsequently, the reference specimen is subjected to examined to generate the individual's DNA profile utilizing one of several methodologies, elaborated further. The DNA profile then undergoes a comparison process with another sample to find out whether there is a genetic match or not.

DNA Fingerprinting Techniques

Restriction Fragment Length Polymorphism (RFLP)

The RFLP technique involves the treatment of genomic DNA with one or more restriction enzymes that cleave the DNA at specific base sequences, known as restriction sites. Each restriction enzyme has a unique site at which it cleaves the DNA. This process results in the production of multiple DNA fragments with different lengths. In certain individuals, stochastic alterations in the DNA sequence can result in the loss of one or more sites or introduce variability in the fragment lengths among individuals. The DNA fragments undergo separation through gel electrophoresis. Shorter DNA fragments exhibit higher mobility in the gel matrix compared to longer fragments. The gel is blotted onto a nylon membrane through a blotting process, allowing for the absorption of DNA fragments while maintaining their original positioning. The membrane is treated to a radioactive probe which selectively binds to particular sites on the fragments. The membrane is brought into contact with a radiation-sensitive X-ray film. After a brief processing duration, the film is developed, resulting in the formation of an photograph also known as an autoradiograph. This technique is highly delicate and possesses a precision rate of 1 in 100,000 + 100 million.[18] In order to perform this procedure, it is necessary to have a substantial amount of DNA, typically ranging from 1 to 5 micrograms. [19]

Polymerase Chain Reaction (PCR)

PCR is a highly effective method for amplifying a particular DNA segment in vitro.[20] The Polymerase Chain Reaction (PCR) is conducted using a thermocycler and consists of three primary stages: The first step involves denaturing the double-stranded DNA template at a temperature range of 92-95°C. The second step is the annealing of primers to the template at a temperature range of 50-70°C. Finally, the third step involves the extension double-stranded DNA molecules at approximately 72°C. The amplification process continues for 30-40 cycles. The chemical components of PCR, including $MgCl_2$, buffer with a pH range of 8.3-8.8, Deoxynucleoside triphosphates (dNTPs), PCR primers, target DNA, and thermostable DNA polymerase.[21]

Target sequence: is the sequence inside the DNA template, that is amplified by PCR.[21]

PCR primers are short, single-stranded DNA molecules that typically range from 18 to 25 nucleotides in length. They are designed to specifically match the sequences located at the ends or within the target DNA region. The primers serve as the starting point for DNA synthesis in PCR.[21]

Various types of PCR

Multiplex PCR: This technique enables the amplification of multiple targets in a single polymerase chain reaction, facilitating their simultaneously analysis.

Nested PCR: It is a modified PCR designed to reduce nonspecific product binding caused by amplification of unanticipated primer-binding sites. It involves two polymerase chain reaction steps. in the primary step the primer (one pair) is utilised in the first PCR reaction to generate DNA products that serve as a target for the second PCR reaction. It contributes to the precision of DNA amplification. [22,23]

Reverse transcriptase PCR: The RT-PCR technique involves mRNA as the initial template and it involves the use of reverse transcriptase enzyme to synthesise the complementary DNA (cDNA) strand. The complementary DNA (cDNA) is subsequently amplified using standard polymerase chain reaction (PCR) methodology.

Quantitative PCR: This technique is used to quantify the target DNA (or RNA) present in a specific sample.

Hot-start PCR: The major benefit of hot-start PCR is that it reduces non-specific DNA amplification during PCR stages at lower temperatures. Before adding Taq polymerase, reaction components are manually heated to the 95°C melting temperature of DNA**.**[23]

Touchdown PCR: During the first two cycles of amplification, the annealing temperature is set at around 3–10°C above the expected Tm, and it gradually drops throughout the following cycles. Higher annealing temperatures during the first two cycles increase the specificity of primer binding, whereas lower temperatures enable more effective amplification later on. [23]

Assembly PCR: Assembly PCR helps in formation of lengthy DNA segments achieved by performing PCR on a pool of long oligonucleotides contain short overlapping segments. As a result, multiple DNA segments are assembled into a single segment.

Methylation specific PCR: To determine patterns of DNA methylation at cytosine guanine regions in genomic DNA, this PCR uses sodium bisulfite treatment.

LAMP assay (loop-mediated isothermal amplification): It is a variant of PCR that utilises 3:6 primer sets, one of which is a loop-shaped primer. This technique uses Bst-polymerase.

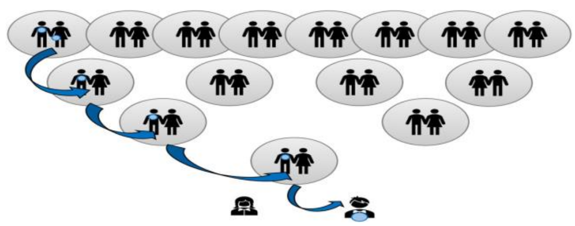
Real-time PCR: It enables a quantitative assessment of PCR product as amplification continues. It involves a non-specific dye, such as SYBR® green I or fluorescence resonance energy transfer.

Y-Chromosome Analysis

The Y chromosome in humans carries genetic traits that serve as a marker for lineage. This marker is in the form of a single haplotype that is passed down exclusively from father to son. [24] A haplotype refers to the specific combination of short-tandem repeat (STR) alleles that have been identified and analyzed on a single Y chromosome. The Y chromosome has been ignored for some time in forensic casework due to its inability to provide the same level of identification certainty as autosomal DNA. The haploid characteristic of this genetic marker can prove advantageous in specific scenarios such as cases of sexual assault, missing individuals, identification of disaster victims, complicated kinship analysis, and population inference.[25]

The Y chromosome is a sex chromosome that occurs in a pair with the X chromosome. It is located in the nucleus of human cells, along with 22 pairs of autosomal chromosomes. Individuals possessing an X and Y chromosome typically exhibit male phenotype, whereas those with two X chromosomes exhibit female phenotype. However, there are uncommon changes in the amount of these chromosomes or other specific mutations that might affect that phenotype. [26]

Figure 1 shows the inheritance pattern typical of men in the population, in which the single Y chromosome is inherited down from father to son nearly intact (barring mutations).



The diagram of Y chromosome inheritance across four generations shows ancestral men with the same Y chromosome haplotype as the offspring at the bottom.

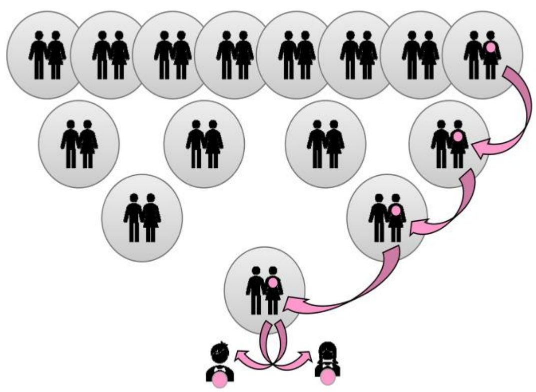
Y-chromosome DNA testing is useful for a variety of human genetics applications, such as forensic evidence assessment, paternity testing, historical investigations, examining human migration patterns throughout history, and genealogical research. In terms of forensic applications, Y-chromosome testing has both benefits and drawbacks; the fundamental importance of the Y-chromosome in forensic DNA testing is that it is only found in males. The likelihood of finding trace amounts of the perpetrator's DNA in the presence of a large background of the female victim's DNA may be increased by using ChrY-specific polymerase chain reaction (PCR) primers. Amelogenin Y-deficient men have also been confirmed using Y-chromosome assays. The Y-chromosome's male-specific nature, which makes it advantageous for forensic analysis, is also its main limitation.To shuffle their DNA and provide future generations more genetic variability, the bulk of the Y-chromosomes are passed from father to son straight and without recombination.

The non-recombining nature of the majority of the Y chromosome is a characteristic that has evolved over time.[27] The sequenced chromosome contains numerous small repeats, inversions, and palindromic sequences (when nucleotide sequences match those on the complementary strand exactly).[28] The latter, referring to specific factors, can potentially enhance gene conversion and promote autonomous deletion and mutation occurrences in particular regions.[29,30] When there is an impact on genes involved in spermatogenesis, it can lead to a potential impact on male potency. However, it is important to point out that the chromosome can tolerate significant deletions without any consequences. The likelihood of mutation in STRs on the Y chromosome is similar to those selected for forensic examination on autosomes. However, the selection of STRs on the Y chromosome is based on significant diversity measures and the regular occurrence of mutations between father and son is due to the high number of mitotic events in spermatogenesis.[31] Due to the nature of Y-STR haplotypes, their variability is much higher compared to that of a single autosomal STR. Therefore, it is necessary to have larger Y-STR haplotype reference databases than autosomal STR allele reference databases to ensure accurate frequency estimates.

Mitochondrial DNA (mtDNA) Analysis

Mitochondrial DNA is present in the mitochondria, which are membrane-bound organelles located in the cytoplasm of nucleated human cells. These organelles are responsible for producing the majority of the cell's ATP. Mitochondria exhibit several similarities with bacteria, leading to the hypothesis that they were initially prokaryotic organisms that later became endosymbionts within eukaryotic cells.[32] The quantity of mitochondria present in a cell is varying and depends on cell function. The count can range from zero in a fully mature red blood cell to numerous hundreds of thousands in an oocyte. A human liver cell typically contains approximately 2000 mitochondria [33], making them a main focus in forensic Analysis when nuclear DNA is absent, restricted, or deteriorated.

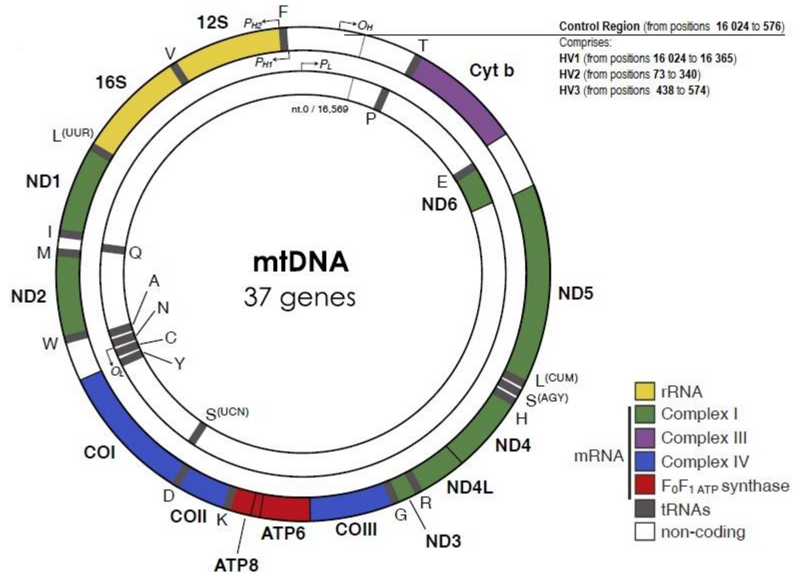
The process of identifying humans for forensic purposes involves analysing a specific set of autosomal genetic markers known as short tandem repeats (STRs) that provide an almost unique combo. These short tandem repeats (STRs) consisting of a core sequence of four nucleotides that are repeated in a specific pattern. The allelic type is determined by the number of repeats. The mitochondrial DNA sequence, in contrast, is related to a lineage of individuals who share a common maternal ancestry, inherited exclusively from the mother to her offspring, as depicted in the Figure.



The illustration of MtDNA inheritance across four generations depicts progenitor females who share the same MtDNA haplotype as the offspring at the bottom.

The mitochondrial genome was sequenced for the first time in 1981[34]; the Cambridge Reference Sequence, also known as the Anderson sequence, was subsequently reanalysed to confirm and rectify the sequence [35], with certain conventions maintained to preserve historic nomenclature. Sequences are described as variations from the revised Cambridge Reference Sequence (rCRS) as published in GenBank NC 012920 [36] for convenience.

MtDNA is composed of both heavy (H) and light (L) strands, with the density varying according to the relative nucleotide distribution in each area. These regions constitute the majority of the mtDNA molecule and are comprised of 37 genes encoding for rRNA and tRNA as well as thirteen polypeptides, with majority of the products support oxidative phosphorylation. The presence of intron-free genes in the mtDNA genome is a hallmark of prokaryotes, which supports the theory of bacterial origin; human nuclear DNA cannot regenerate the mitochondria if it is removed from the cell.



The human mitochondrial DNA genome with genes and control regions labeled.

The 1121 base pairs long non-coding stretch that includes the D-loop and a transcription promoter region is referred to as the 'control region', which accomplishes the molecule. The displacement (D) loop is formed by three strands of DNA, where one of the strands is complementary to one of the other strands, holding it apart forming the displacement (D) loop.[37] The International Society for Forensic Genetics (ISFG) has generated guidelines for forensic mitochondrial DNA (mtDNA) typing.[38] and the Scientific Working Group on DNA Analysis Methods (SWGDAM).[39]

Short Tandem Repeat (STR) Analysis

Short tandem repeats (STRs) exhibit a high degree of polymorphism, and the alleles of the STR loci are distinguished by the varying number of copies of the repeat sequence present within each of the STR loci.

The greater the number of STR loci used for typing, the higher the discrimination value, because the likelihood of a single individual having an identical STR profile, with the exact same number of repeat units for all the STR being analysed, with another individual chosen at random in the population becomes extremely unlikely.

The current DNA profiling system relies on PCR and utilises short tandem repeats (STRs). This approach employs highly polymorphic regions containing short tandem repeats (STRs) of DNA, with the most prevalent is 4-base repeats, although other lengths such as 3 and 5 bases are also utilised. Due to the high variability of repeat units among unrelated individuals, STRs are a reliable technique for distinguishing between unrelated individuals . The sequence-specific primers are used to target the STRs loci on the chromosome and amplify them through PCR. The resulting DNA fragments are subsequently separated and identified through electrophoresis. Capillary electrophoresis (CE) and gel electrophoresis are two widely used techniques for separation and detection DNA

Each of the short tandem repeat (STR) exhibits polymorphism, however, the quantity of alleles is confined. The efficacy of STR analysis arises from the concurrent examination of numerous STR loci. The allelic pattern has the potential to precisely distinguish an individual's identity. The analysis of Short Tandem Repeats (STRs) is a highly effective method for identification purposes. The higher the number of STR regions analyzed in an individual, the greater the level of discrimination achieved by the test.

In practical terms, the probability of contaminated-matching is far higher than the risk of matching a distant relative. For example, a sample might be contaminated by surrounding items or by cells that passed over from a previous test. For that reason, numerous control samples are routinely checked to make sure they remained clean, when prepared during the same interval as the actual test samples. There is a considerable likelihood that the test samples will be contaminated if there are unexpected matches (or variances) in numerous control samples. In order to demonstrate that a person was not genuinely matched as being linked to their own DNA in another sample, the entire DNA profiles should vary (except from twins)

Variable Number of Tandem Repeat Sequences (VNTR) Analysis

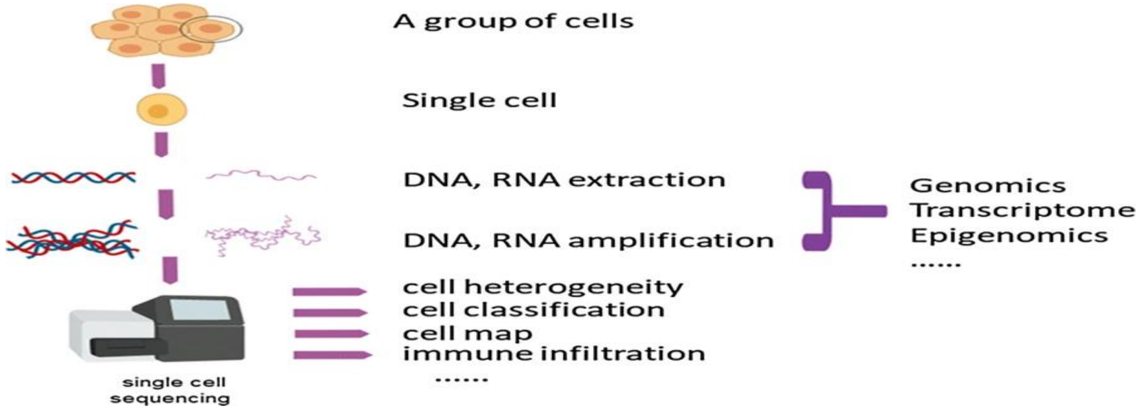
Tandemly repeated short DNA sequences are present in certain regions of the human genome. The variability of the number of short tandem repeats in a specific locus is highly variable among unrelated individuals . The VNTR sequences are commonly referred to as variable number of tandem repeat sequences. VNTRs can be classified into mini- and micro-satellites according to the length of the repeated units. Micro-satellites are characterized by a sequence repeat unit that typically ranges from 2 to 9 base pairs, whereas mini-satellites are defined by a sequence repeat unit that typically ranges from 9 to 100 base pairs. Microsatellites or short tandem repeats (STRs) are commonly preferred for individualization purposes due to their practicality. The RFLP technique for DNA fingerprinting, as explained earlier, has been substituted with the more easy STR typing method, which is combined with the highly sensitive polymerase chain reaction (PCR) technique.[40-42]

FUTURE DIRECTIONS AND EMERGING TECHNOLOGIES

Single-Cell DNA Analysis

Single-cell sequencing technologies involve the sequencing of a single cell's genome or transcriptome to gather multi-omics info. This information is used for discovering variations between cell populations and trace cellular evolutionary relationships. Conventional sequencing techniques are confined to obtaining an average of multiple cells, and are incapable of analysing a limited number of cells while also losing valuable cellular heterogeneity data. In contrast to conventional sequencing methods, single-cell technologies offer the benefits of identifying diversity among individual cells [43], discriminating a limited number of cells, and mapping out cellular landscapes. The publication was designated as "Nature Methods" in 2013, as part of its yearly technological update.[44] However, the early single-cell sequencing technique was restricted in its widespread application owing to its expensive cost. As the research advanced, various novel single-cell sequencing techniques had been developed to lower the cost threshold for single-cell sequencing. Currently, the utilisation of single-cell sequencing technology is progressively prevalent across diverse domains such as tumours, microbiology, neurology, reproduction, immunity, digestion, and urinary systems. It emphasises the crucial role of single-cell sequencing technologies in both basic and clinical research.

As the field of research progresses, the efficacy of single-cell sequencing techniques (depicted in Fig. 1) is constantly improving and becoming more cost-effective, enabling scientists to get into deeper into the molecular mechanisms at the single-cell level.



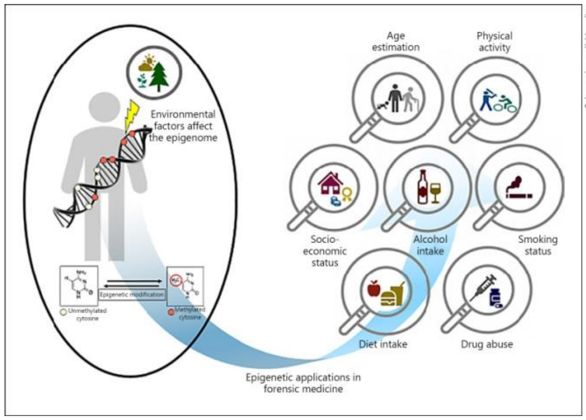
Single-cell sequencing. It isolates a cell for sequencing and investigating cell heterogeneity, molecular mapping, immunological infiltration, and epigenetic alterations.

Epigenetic Profiling

Since the pioneering work of Jeffreys et al.[45] who uses human myoglobin gene-based probes for DNA fingerprinting, the technique has been widely applied to various living organisms, including economically important plants, extinct animals, human pathogens, etc. [46,47,48,49,50] Recently, there has been a significant surge in the attention to forensic epigenetics due to the increasing recognition of the importance of epigenetic changes in addressing forensic inquiries.[51] Epigenetic modifications are significant heritable variations in DNA. The epigenetic process is a non-genetic alteration that does not affect the DNA sequence. It includes modifications of DNA bases like cytosine 5-CpG-3 methylation and posttranslational modifications of histones such as histone H3 methylation or acetylation.[52] It plays a role in regulating gene expression in response to short-term as well as long-term exposure to different environmental factors.[53] The presence of methylated CpG islands in close vicinity to gene promoters results in gene inactivation due to the compact and inaccessible nature of the chromatin structure, which prevents transcription factors from binding and initiating transcription.[54]

Epigenetics is also important in the area of forensics. When DNA methylation levels change across the genome in reaction to different short-term or long-term external triggers, results in individual epigenomic variation which is referred to as epigenetic fingerprints.[55]

When it comes to forensics, DNA methylation is the preferred epigenetic modification due to its high sensitivity and in vitro stability, as compared to other modifications like histone modifications or changes in chromatin structure. Additionally, DNA methylation requires smaller amounts of DNA. Differential DNA methylation patterns have been examined in forensic investigations for the identification of tissue/cell types [56] , the determination of sex [57], the assessment of age [58], and also to distinguish monozygotic twins.[59] DNA methylation is a process that can have diverse impacts on an individual's lifestyle, health, physical appearance, and more.**60** The analysis of DNA can provide information about an individual's socioeconomic status, dietary habits, intensity of physical activity, alcohol consumption habit [61], drug usage, and nicotine consumption.[62] In forensic investigations, DNA methylation levels are a valuable tool for determining the origin of a biological sample recovered from a crime scene. They can be used to estimate the age of an individual who left the trace [58] and predict certain visible traits, such as hair, skin, and eye colour.[63,64] This information can help to narrow down the list of potential suspects related to the crime (see Fig. 2). Forensic epigeneticists have developed innovative methods for profiling and analysing DNA methylation. However, there are challenges presented by the quantitative character of the epigenetic analysis.



Various environmental factors can influence the methylation status of the genome, leading to distinct epigenetic variations that are commonly known as the epigenetic fingerprint. Epigenetic markers exhibit diverse phenotypic correlations, including age, personal habits, well-being, physical traits, alcohol consumption, and more. These associations are valuable in addressing forensic inquiries.

Artificial Intelligence and Machine Learning

Digital forensics is described by the Digital Forensic Research Workshop (DFRWS) as "Using scientifically proven methods to maintain, collect, validate, analyse, and interpret document digital evidence from digital sources, to support the reconstruction of events found to be critical, and to promote them." It is a sophisticated profession that requires sharper analytical techniques to address its difficult problems by examining several data sets.[65] The main goal of forensic analysis is to gather reliable and accurate evidence using methods that have proven reliable and consistent across the board. The Prosecutor must convince the Court of Justice that the evidence is reliable, credible, and admissible before it may be used.[66] The safety of justice is compromised by inaccurate or flawed information because forensic evidence may be very sensitive and destructive to law enforcement. This discipline has gotten more and more important as forensic data volume, variety, and speed have increased. Due to the growing number of cases and the complexity of investigations, DF investigators are now dealing with enormous obstacles.[66]

Forensic Science with Artificial Intelligence (AI) : The rapidly developing field of artificial intelligence (AI) has many advantages in its many diverse fields, including public image and video analysis, image recognition, gunshot detection, armed firearms, 3D scene crime reconstruction, massive digital data analysis, building statistical evidence, handwriting identification, estimation time since death or dental age, and personal data.[67,68]

Criminal Investigation with Artificial Intelligence (AI): Due to vast amounts of complex information, such as videos, images, text files, electronic messages, audio files, and fraud monitoring risks, the hybrid intelligence of humans and machinery also benefits modern criminal investigations by uncovering various hints, weapons, bombs, or explosives on the scene of the crime and criminal activity. The development of AI technology aids law enforcement and security workers in detecting, preventing, and forecasting crime. Some highly developed AI algorithms are intended for spotting crime trends and suspected anomalies, anticipating future crime scenes, evaluating, and locating criminal networks.[69,70]

APPLICATIONS OF DNA FINGERPRINTING IN CRIME DETECTION

In sexual offences: Prior to the implementation of DNA fingerprinting, instances of sexual assault were exclusively resolved through the utilisation of circumstantial evidence. In cases of sexual offences, the absence of an eyewitness can pose challenges for the victim in establishing evidence of the offence. The discovery of DNA has provided significant potential in addressing cases of sexual offences. Biological samples such as semen, saliva, and swabs are crucial in DNA profiling and assist in suspect identification. Saliva stains are commonly encountered in various types of criminal cases such as homicides, physical altercations, sexual assaults, particularly in bite mark evidence from either the victim or the perpetrator, as well as on items such as cigarette butts and drinking vessels. The utilisation of DNA fingerprinting on semen, swabs, and saliva collected from the crime scene can aid in the swift apprehension of suspects. The presence of bodily fluids such as vaginal secretions, rectal matter, saliva, or semen from multiple individuals in cases of gang rape can be distinguished and attributed to specific individuals through the use of DNA fingerprinting.[71]

In kinship analysis: The application of DNA fingerprinting may also be used to determine if two or more people belong to the same family. Kinship analysis, another name for this sort of study, is commonly used in paternity tests. A child's paternity may be determined before birth thanks to DNA fingerprinting. Victims of sexual abuse, women who have several sexual partners, and husbands who suspect their wives of infidelity may find this to be of great importance. The majority of forensic case studies involve autosomal STR markers. Maternal line inheritance patterns may be investigated using mitochondrial DNA, while paternal line inheritance patterns can be identified using Y-STRs.[72]

In determination of sex: The sex of a person may also be determined through DNA fingerprinting. Forensic scientists work with remains that have suffered such severe trauma that establishing the gender of the deceased may only be possible via DNA analysis. The Y chromosome is the genetic difference between the sexes, hence identifying the Y chromosome-specific DNA helps distinguish between men and females. If the material is from a female, a Y chromosome-specific PCR would provide a result containing male DNA but no bands. The amelogenin gene, which codes for the protein present in tooth enamel, may also be analysed using PCR. However, each method has its own drawbacks and is no longer essential.[73]

In identification of missing persons: When compared to other forensic methods of identifying humans, such as dactyloscopy, forensic odontology, and anthropology, DNA fingerprinting is very important in locating missing people. As a result of the fact that DNA may be retrieved from any tissue, including soft tissue, bones, teeth, and even damaged tissues. Countries like Spain and the US have put in place specific programmes to use DNA fingerprinting to find missing people. Two databases that comprise the STR profiles and mtDNA sequences of the questioned samples and the reference samples from relatives are part of the Spanish Phoenix Programme (Programa FENIX). This programme analyses DNA and creates a connection between questioned samples, such as unidentified corpses or skeletal remains from previously unresolved cases, and reference samples from the families of missing people. Utilising the NMPDD (National Missing Person DNA Database) Programme, the FBI (Federal Bureau of Investigation) provides assistance in the US. The US database has three categories where DNA profiles may be submitted, including biological relations of missing people, unexplained human remains, and missing people. DNA fingerprinting of genetic markers such autosomal STRs, Y chromosome STRs, and mtDNA is the approach used in this programme.[74]

In Identification of Individuals from skeletal remains: The analysis of skeletal remains is a matter of significant interest in the field of forensic science. This is due to the fact that such remains are often well-preserved for extended periods of time, and in situations such as mass disasters, they may be the sole source of information available for analysis. The extraction and analysis of DNA profiles from skeletal remains is a crucial step in the identification of missing individuals and casualties of mass disasters. The process of generating a DNA profile from bones and teeth is a crucial step in identifying unidentified remains.

In Disaster victim identification: DNA analysis is a crucial technique for identifying victims of mass disasters. DNA profiling is a technique employed to match the genetic material obtained from mutilated and fragmented body parts to the victim's DNA profile. It is also utilised to match the DNA samples collected from crime scenes to the DNA profiles of suspects, thereby aiding in the identification of criminals. The application of DNA fingerprinting for disaster victim identification was first demonstrated in 1990, specifically in the identification of individuals who perished in a fire on the Scandinavian Star ferry. The process of identifying victims through DNA analysis involves the collection of both ante-mortem and post-mortem samples. Ante-mortem samples, including victim's personal belongings, buccal swabs, and pathology specimens, are commonly used in DNA analysis. Post-mortem samples typically comprise DNA samples extracted from bones, such as femur and metatarsal bones, as well as teeth, specifically molar teeth. In cases where the body is not in an advanced stage of decomposition, muscle tissues may also be utilised. The analysis of victim DNA involves the utilisation of Y-STR typing, mtDNA, autosomal STR markers, and Amelogenin markers to determine both the sex of the victim and their identification. Single nucleotide polymorphisms (SNPs) are highly advantageous in situations where DNA samples have undergone degradation.[75] The utilisation of commercially available kits, such as AmpFLSTRw Profiler Plusw ID and COfilerw PCR Amplification Kits, AmpFLSTRw Identifilerw PCR Amplification Kit, the AmpFLSTRw SGMw Plus PCR Amplification Kit (Applied Biosystems, Foster City, CA), and the PowerPlexw 16 System (Promega Corp., Madison, WI), facilitates the process of STR typing for mass disaster samples.[76]

In Differentiating Monozygotic Twins: It is not feasible to distinguish monozygotic twins as they possess indistinguishable autosomal STR profiles due to their identical genetic makeup. Through the recent identification of highly uncommon mutations using ultra-deep next-generation sequencing (NGS), it has become feasible to distinguish or individualise monozygotic twins. Paternity disputes involving monozygotic twins are infrequent. This methodology proves to be highly advantageous in instances where such occurrences are documented.[77]

In Immigration cases: Currently, numerous countries are implementing stringent regulations for individuals seeking entry into their respective nations. To establish one's identity, specific official documents such as birth certificates, marriage certificates, and passports are required. At times, the authorities may reject these documents due to doubts regarding their authenticity. In this context, it is recommended to conduct DNA analysis to address cases related to determining the parentage of children, illegal immigration, human trafficking, and other similar situations.[77]

In Bio-geographical ancestry analysis: DNA profiling can be utilised to determine the genetic lineage of an individual. The utilisation of DNA fingerprinting for bio-geographical ancestry analysis has proven to be a valuable tool in solving cold cases, particularly in situations where there is no eyewitness. This technique is also useful for refining DNA databases, conducting archaeological DNA analysis, and performing genetic studies with forensic sensitivity.[77]

In Microbial forensics: Microbial forensics is a rapidly developing field that involves examining the human microbiome and utilising microbial signatures to connect crime scene evidence with potential suspects. The bacterial population found on the surface of human skin can yield valuable insights for forensic analysts, revealing details about the host's identity and the geographic region they inhabit. The process of sequencing the microbiome present in soil samples aids in distinguishing the various species present in distinct geographical regions. A multiplex real-time PCR assay utilising oligonucleotide mixtures that target genomes is employed to specifically detect the bacterial signature of a selected group of bacteria. This is done to identify biological fluids in forensic case exhibits. However, extensive research is necessary to utilise this domain as a proficient instrument in forensic inquiry.[77]

FEW IMPORTANT CASES SOLVED BY DFSL BY DNA FINGERPRINTING TECHNIQUE

Case 1: South African Model Case: An individual from South Africa was under the influence of a substance and subjected to multiple instances of sexual assault by two perpetrators. The semen stains discovered on the victim's undergarment and car seat cushion were found to be a match with one of the suspects.

Case 2: Kidnapping and murder Case of a child: A juvenile individual of age 3 was abducted and killed in exchange for a sum of 1.5 million rupees. The remains were interred in an isolated location and subsequently retrieved in a severely disfigured condition. The DNA sample was extracted from the scalp tissue of the deceased individual and subsequently compared to the genetic profiles of the missing child's biological parents, resulting in a positive match.

Case 3: Murder of a senior citizen by his son: The paternal organism was deposited on the veranda of the house. The DNA samples obtained from the crime scenes scrapings, weapon scrapings, and control tissue of the deceased were subjected to analysis, and a match was observed.

Case 4: Rape and murder Case: An 11-year-old female victim was subjected to sexual assault and homicide in 2005. The DNA was extracted from the semen stains present on the victim's clothing. Upon conducting preliminary analyses, it was determined that the blood samples procured from the three suspects did not exhibit congruence with the semen profile extracted from the victim's attire. In the beginning of 2007, an analysis was conducted on the blood of a suspect which exhibited a complete match with the DNA profile of the semen stains found on the victim's clothing.

Case 5: Child Abduction Case: The initial report submitted by the Mumbai laboratory pertained to a case of juvenile kidnapping. At the Sir J.J. Group of Hospitals, a male infant who was 4 days old was reported as missing. During the course of investigation, a juvenile individual was discovered residing in a separate unit without parental supervision and under the care of a personal nurse. The complainant claimed parentage of the child, but DNA profiling conducted in our laboratory has ruled out these individuals as the biological parents of the child. These findings warrant additional scrutiny to unravel the scheme involving a private nursing home physician who unlawfully sold infants.

Case 6: A blind girl rape Case: In this case, the victim is a 19-year-old female who is visually impaired. Based on the available information, it appears that the individual residing in close proximity to the victim exploited her physical disability and engaged in multiple instances of sexual assault. The suspect allegedly made a death threat towards the victim. The female individual underwent pregnancy and parturition resulting in the birth of an offspring. The neonate expired promptly following parturition. The grandmother exhibited her suspicion towards two individuals. The biological specimen obtained from the deceased juvenile's body and reference blood samples from the victim and two suspects were submitted for DNA testing. Based on the genetic analysis, it appears that one of the suspects shares the same paternal alleles that are necessarily inherited by the foetus.

Case 7 : Murder and Rape of I.T. Professional in I T hub of Maharastra-Pune: The recurrent incidents of rape and murder in Pune, Maharashtra's emerging IT centre, are a bane to a region with a rich cultural and civilised past. The operational methods have become nearly indistinguishable. Individuals with criminal records who operate tourist vehicles target unsuspecting young I.T. professionals, offering them transportation and subsequently committing atrocious acts.

The current case exhibits a similar history. The collected items include the victim's undergarments, the car's seat cover, and a duster cloth with visible stains. The semen stains on the seat cover and cloth were effectively subjected to DNA profiling. Upon analysis, it was determined that the control blood sample of one of the four individuals under suspicion exhibited a match with the DNA profile obtained from the semen stains.

Case 8: Murder and Rape of Innocent teenager in developing city Aurangabad: Urban areas such as Aurangabad are experiencing a rise in the incidence of severe criminal activity. The aforementioned criminal incident occurred in a highly developed area within the city of Aurangabad. The victim was an individual who possessed a DNA profile consistent with that of a young female. The suspect, who had a history of robbery, entered the flat where the victim resided with her sibling. As the sibling was on the nocturnal work schedule, the female individual was left unaccompanied. As the perpetrator was pilfering the precious items, the female individual aroused from slumber. The suspect became aware that the juvenile female was unaccompanied. The perpetrator exploited the circumstances and committed sexual assault. To conceal the initial offence, the perpetrator utilised a sharp-edged instrument to commit homicide against the victim. The suspect was apprehended by the investigating agency based on suspicion.

The vaginal aspirate of the victim, the bed sheet found at the crime scene, and the T-shirt worn by the accused were subjected to DNA analysis. Based on the DNA analysis, the DNA profile obtained from the semen stains on the bed sheet and the vaginal aspirate were found to be a match with the DNA profile of the accused individual. Additionally, the DNA profile obtained from the blood sample of the deceased individual was determined to be consistent with the DNA profile obtained from the blood stains identified on the T-shirt of the suspect.

Case 9: Opportunistic Rape by a famous movie star on innocent maid servant: In this high profile case, a perpetrator from the Hindi film industry allegedly committed sexual assault against his domestic worker and attempted to conceal the incident. The perpetrator coerced the victim into keeping the incident confidential. During the course of the interaction, he persuaded her to alter her attire. After a few hours, the female individual recounted the incident to her female colleague. With the assistance of other individuals, the victim filed a complaint. The forensic samples were collected by the doctors and sent for DNA analysis. The medical test was performed approximately 10 hours post-incident. The garments underwent alteration. However, the male DNA was effectively identified from her vaginal smear sample using the Y STR method. The DNA profile was determined to be a match with the DNA profile of the alleged celebrity suspect.

CONCLUSION

The utilisation of DNA fingerprinting has a significant influence on the criminal justice system. DNA analysis is a crucial tool in criminal identification. Irrespective of the scenario, it is apparent that DNA profiling has transformed the approach of global biological matching. The technique of DNA fingerprinting holds significant potential in the realm of forensic science, with a wide range of applications including identifying perpetrators in cases of sexual assault, determining paternity, and investigating wildlife-related incidents. DNA analysis is a highly reliable form of evidence that remains constant throughout an individual's lifetime, making it a valuable tool for accurate identification. The application of DNA analysis has played a crucial role in obtaining convictions for numerous cases of violent crimes, ranging from murder to physical assault. The utilisation of DNA analysis has proven to be beneficial in excluding potential suspects and has resulted in the absolution and liberation of formerly condemned persons. The utilisation of DNA can facilitate targeted investigations, potentially resulting in expedited trials and increased likelihood of plea bargains. It is possible that it may serve as a deterrent for certain perpetrators to engage in severe criminal acts. The utilisation of forensic DNA evidence will result in enduring cost-effectiveness for the criminal justice system.

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