

Available online at <http://www.ijims.com>

ISSN - (Print): 2519 – 7908 ; ISSN - (Electronic): 2348 – 0343

IF:4.335; Index Copernicus (IC) Value: 60.59; Peer-reviewed Journal

Advancements in DNA Fingerprinting technology, methods and its environmental applications

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Abstract

Fingerprinting is the most unique and specific method for physical evidence identification. It is used in various fields like forensics, paternity testing, and population genetics, relationships. Recently a lot of developments have taken place in this field and in this review paper we highlight various techniques used for DNA fingerprinting, like DGGE, AFLP, TGGE, TRFLP, SSCP, MUST, etc. For example, TGGE can be used in forensic investigations to analyze DNA samples from crime scenes. By analyzing the melting behavior of PCR-amplified DNA fragments from different samples, it is possible to identify unique patterns that can be used to identify suspects or determine biological samples. The multidimensional data and their classification and various pattern recognitions are also emphasized in this review.

Keywords: DNA fingerprinting, forensics, gel electrophoresis, techniques

Introduction

A fingerprint is an impression made by a human finger's friction ridges. Fingerprints are generated with unique individual patterns composed of elevated friction ridges and recessed and valley-like furrows. These patterns appear on the fingers and thumbs, specifically on the pads (Cothron, Gretchen R.]. DNA Fingerprinting also known as Genetic Profiling was developed by Sir Alec Jeffrey in 1984. It is used to determine the individuality of a person based on the nucleotide sequence of specific regions of human DNA (Schmidtke, J. (1999))

Fingerprint analysis plays a significant role in the analysis as well as the identification of suspects dating from 100 years, and remains a helpful method for law enforcement. Since every person's fingerprints are different, they aid detectives in connecting related crime scenes. Each person has a distinctive pattern, a specific pattern that makes them different from another person [Radin, P. (Ed.). (1952)]. There are three types of patterns:

Loops:



Arch:



Whorl:



They also help in Biometric security to provide access control to buildings, and smartphones. It is a method of authentication that provides high-level security. Personal identification was also done especially in rural areas, often used to sign legal documents or to verify an individual's identity. In case of natural disasters such as earthquakes, floods, or accidents where the body is damaged or unrecognized, using fingerprints they can be identified.[Younhee Gil et al., 2003]

Mostly studied in medical research particularly in genetics for any genetic or hereditary conditions. In most of the research, it is mentioned that hereditary diseases are linked to fingerprints [Champine M, Kohlmann W et al., 2012].

It is also used to determine biological relationships between individuals for maternity or paternity testing. Also plays an important role in legal cases or disputes for inheritance claims.

Conservation and Wildlife management used as evidence to combat illegal wildlife trade and poaching, helps in identifying the origin of confiscated animal products and endangered species. Mostly in genetic genealogy to trace familial relationships or ancestry. Helps individuals discover family heritage. DNA fingerprinting is based on variability and polymorphism.

Finding of evolutionary history of any organism. Many medical significance such as:

1. Matching of tissue with organ donors for transplant
2. Identification of hereditary diseases
3. Finding cures for diseases

DNA Fingerprinting Techniques

DNA Fingerprinting

Every human being on earth has DNA as genetic material which makes each one of us different in one or the other aspects. Even twins have differences in their DNA sequence. Apart from this several patterns such as fingerprints, lip prints, etc. are unique to an individual. This uniqueness caters to many sectors as discussed above (Birkenbihl, R. P. (1998). Endonuclease VII has...). DNA fingerprinting is one such laboratory method used to recognize people by their distinctive DNA sequences. However, for carrying out forensic studies, the major problem faced is the recovery of DNA from samples and fingerprints. Various methods have been adopted to simplify this issue.

Forensic experts started using sterilized cotton plugs for swabbing on suspicious objects at the crime scene and also dead bodies which comparatively yielded higher amounts of DNA. A study found that the DNA content of pens, vehicle keys, and leather briefcase handles ranged from $n = 1$, 1.1 ng to $n = 3$, with a mean of 75 ng for each. Also, hands swabbed after shaking hands showed a transfer of DNA. DNA extraction techniques that are usually carried include QIAamp DNA mini kit & DNeasy® (kits developed by QIAGEN®, UK).

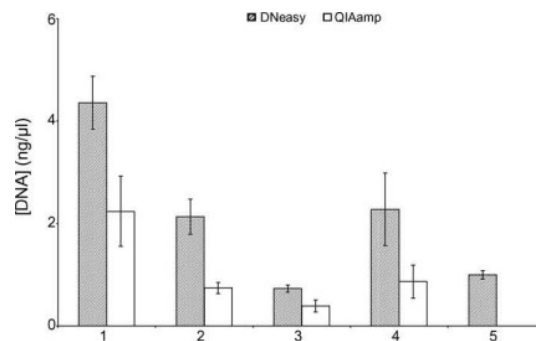


Fig. 1. Comparison of DNA recovery (ng/μl) from various amounts of saliva on two paper types, and five fingerprints using DNeasy® vs. QIAamp®. Error bars indicate standard error, $N = 4$. Treatments: (1) 20 μl saliva (Whatman®); (2) 10 μl saliva (Whatman®); (3) 5 μl saliva (Whatman®); (4) 5 fingerprints (Whatman®); (5) 20 μl saliva (office paper).

The analysis of DNA after collection is further carried out using techniques like -

Denaturing Gradient Gel Electrophoresis (DDGE)

Amplified Fragment Length Polymorphism (AFLP)

Temperature Gradient Gel Electrophoresis (TGGE)

Terminal Restriction Length Polymorphism (TRFLP)

Single-stranded Conformation Polymorphism (SSCP)

Denaturing Gradient Gel Electrophoresis(DGGE)

PCR-amplified DNA fragments are separated based on their sequence using the more recent fingerprinting technique known as denaturing gradient gel electrophoresis (DGGE). This method

divides DNA fragments of the same size but with distinct base pair sequences. DGGE reduces the electrophoretic mobility of partially denatured DNA molecules in a polyacrylamide gel compared to the helical form of the molecule in this separation. DGGE has been used in environmental microbiology, food microbiology, and microbial community analysis in the human body.(Eyers, L., Agathos, S. N., & Fantroussi...)

The foundation of DGGE is the hypothesis that the nucleotide content of the DNA sequence affects the denaturation rates of double-stranded DNA molecules. When exposed to a denaturing environment, DNA molecules denature in discrete regions known as melting domains. The melting temperature is the temperature at which the melting domain denatures (T_m). Through an acrylamide gel, DNA molecules can be electrophoresed to detect differences in denaturation within the melting domain. To denature the DNA molecules, acrylamide gels are used. To create a denaturing environment, urea and formamide are added to an acrylamide gel. Furthermore, by varying the concentrations of urea and formamide, a gradient former can produce a wide range of denaturing gradients. Electrophoresis at a constant temperature of 60 ° C helps in denaturation of DNA. When the DNA molecule begins to denature, its mobility through the acrylamide gel changes. The migration of molecules depends on the amount of denaturation that occurs. Because of the differences in mobility, the DNA molecules will appear in different positions on a gradient gel. Each DNA molecule will appear as a distinct band that can be visualized using ethidium bromide or SYBR-Gold (Merck Biosciences, Nottingham, UK) in conjunction with ultraviolet illumination or silver staining. This method can be performed either perpendicular or parallel in which the gel runs horizontally or vertically respectively (Fig. 2).

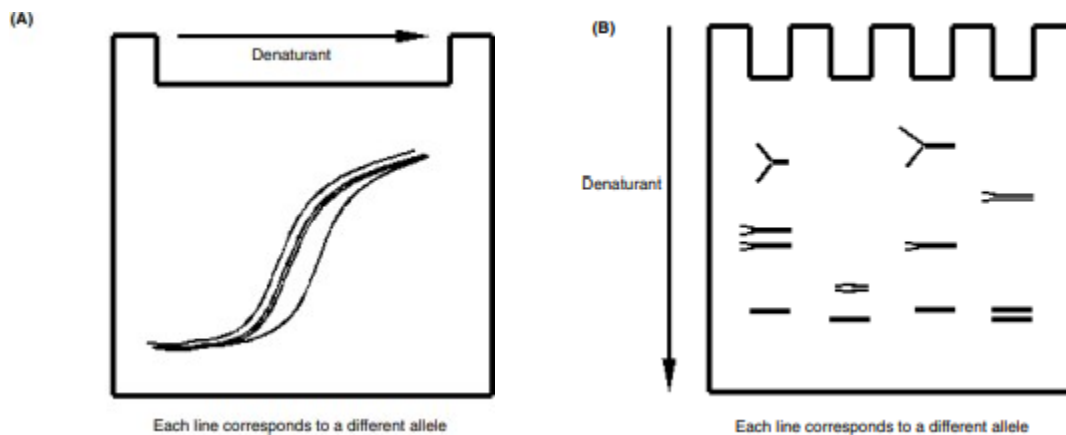


Fig. 2. Denaturing gradient gel electrophoresis (DGGE) can be used to separate MHC alleles that are of the same size, but different sequences, with a horizontal (i.e., perpendicular) or vertical (i.e., parallel) denaturing gradient. (A) The optimal denaturing gradient for allele separation is determined by using a perpendicular DGGE. (B) Multiple alleles, from various species, are separated on the appropriate parallel denaturing gradient. Alleles with DNA sequences that denature rapidly will move through the gel at a slower rate than alleles that denature more slowly. The benefit of parallel gels is the simultaneous electrophoresis of numerous samples and a narrower gradient, which should increase mobility differences between DNA molecules. For the separation of various sequences of time, studies should be done to know the optimal time to obtain a better yield. Under ideal conditions, samples will only be partially denatured to maximize mobility differences. Because fully denatured samples have equivalent mobilities, complete denaturation usually results in poor resolution. A long string of Gs and Cs, known as a GC-clamp, can be used to ensure that DNA molecules do not fully denature under DGGE conditions. GC-clamps are typically composed of 30-40 Gs and Cs which have a higher T_m than DNA sequences with a more homogeneous nucleotide distribution.

This is why the DNA fragment of interest is allowed to denature while the GC clamp remains annealed. Also, the GC-rich string at the 3' ends of one of the PCR primers allows for the addition of a GC clamp. This technique is very efficient and has widespread use such as -

Microbial community analysis: DGGE can be used to study the diversity of microbial communities by analyzing the 16S rRNA gene. This gene is highly conserved in bacteria but also contains variable regions that can be used to differentiate between different species. By analyzing the DGGE banding pattern, researchers can identify the different species present in a microbial community.

Mutation detection: DGGE can be used to detect mutations in genes by analyzing PCR products containing the mutated sequence. The presence of a mutation can cause the DNA fragment to denature at a different position on the gel, allowing for easy detection.

Genetic fingerprinting: DGGE can be used to generate genetic fingerprints of organisms by analyzing PCR products from multiple loci. The banding pattern generated by DGGE can be used to differentiate between different strains or species.

Analysis of gene expression: DGGE can be used to analyze changes in gene expression by comparing the banding pattern of PCR products from different samples. Differences in the banding pattern can indicate changes in gene expression levels.

Environmental monitoring: DGGE can be used to monitor environmental samples for the presence of specific microbial species or genes. For example, it can be used to detect the presence of pathogenic bacteria in food or water samples.

Amplified Fragment Length Polymorphism (AFLP)-

AFLP stands for "Amplified Fragment Length Polymorphism," which is a molecular biology technique used to analyze genetic variation in organisms. AFLP involves the selective amplification of specific DNA fragments from a sample using PCR (polymerase chain reaction) and then separating those fragments based on their size using gel electrophoresis. By comparing the resulting DNA fragment patterns between different individuals or populations, AFLP can be used to study genetic diversity, population structure, and evolutionary relationships between organisms. It has been used in genetic studies and even in forensic studies.

This technique involves the following methods -

DNA extraction: The first step is to extract DNA from the sample of interest. This can be done using a variety of methods depending on the sample type. **Restriction enzyme digestion:** Next, the DNA is digested using two restriction enzymes. One enzyme cuts the DNA at a specific sequence, while the other enzyme cuts at a different sequence. This creates DNA fragments with sticky ends that can be ligated to adapters. **Adapter ligation:** The sticky ends of the DNA fragments are ligated to adapters that contain specific sequences for PCR amplification. These adapters are designed to allow for selective amplification of specific fragments. **Pre-amplification:** The ligated DNA fragments are pre-amplified using PCR with primers that anneal to the adapters. This results in a pool of DNA fragments that can be selectively amplified in the next step. **Selective amplification:** The pre-amplified DNA fragments are selectively amplified using PCR with a combination of selective primers. The selective primers are designed to amplify only a subset of the fragments in the pool. This generates a set of DNA fragments that are specific to the sample being analyzed.

Visualization: The amplified DNA fragments are separated by size using gel electrophoresis and visualized by staining with a DNA-specific dye or by autoradiography. Analysis: The resulting AFLP profile can be analyzed to determine genetic variation within the population, such as the presence or absence of specific DNA fragments.



Fig AFLP fingerprints of genomic DNAs of various complexities: A, DNA (panel I), AcNPV DNA (panel II), Acinetobacter DNA (panels IUa and IIIb), and yeast DNA (panels IVa and IVb). Letters A, B, C, D, and E refer to none, one, two, three, and four selective bases in the AFLP primers respectively. The primer combinations used were from left to right: I. EcoRI+O/AfeI+O, II. EcoRI+O/Afj«I+O, IIIa. EcoRI+O/Msel+A, EcoRI+C/Msel+A, EcoRI+C/Afs«- I+AT, IIIb. EcoRI+G/Msel+T, EcoRI+C/AfreI+T, EcoRI+CMtoI+TA, IVa. EcoRI+CMfrd+C, EcoRIhC/Msel+GC, EcoRI+CA/Afsel+GC IVb. EcoRI+O Afsel+T, EcoRI+CMfsel+TA, EcoRI+CA/Msel+TA. (+0 indicates no selective nucleotides, +A indicates selective nucleotide = A, etc). The molecular weight size range of the fingerprints is 45-500 nucleotides.

It is important to note that AFLP can be a complex and time-consuming technique that requires specialized equipment and expertise. It is also important to carefully control for experimental variables, such as the amount of DNA template and the PCR conditions, to ensure reproducibility of the results.

AFLP-based fingerprinting relies on the fact that DNA sequences differ between individuals due to genetic variation. By selectively amplifying and visualizing specific DNA fragments, AFLP can generate unique patterns that can be used to identify individuals or distinguish between populations. AFLP fingerprinting has been used in a variety of applications, such as forensic investigations, genetic mapping, and plant breeding. In forensic investigations, AFLP fingerprinting has been used to link suspects to crime scenes or to identify human remains. In genetic mapping, AFLP fingerprinting has been used to identify genetic markers associated with specific traits or diseases. In plant breeding, AFLP fingerprinting has been used to identify genetic markers associated with desirable traits, such as disease resistance. This can be used in a wide range of sectors viz.,

Evolutionary biology: AFLP can be used to study the evolution of populations by comparing genetic variation among individuals or groups. This can help researchers understand how species have evolved and how they have adapted to changing environments.

Forensic analysis: AFLP can be used in forensic investigations to link suspects to crime scenes or to identify human remains. The technique can be used to generate unique DNA profiles that can be used for identification purposes.

Conservation biology: AFLP can be used to study genetic diversity within and among populations of endangered species. This can help conservationists develop strategies to protect these species and their habitats.

Plant breeding: AFLP can be used to identify genetic markers associated with desirable traits in plants, such as disease resistance, improved yield, or drought tolerance. This can help plant breeders develop new varieties of crops that are better suited to specific environments.

Microbial ecology: AFLP can be used to study microbial communities in various environments, such as soil, water, and the human microbiome. The technique can be used to identify microbial species and to study their interactions with each other and with their environment.

Temperature Gradient Gel Electrophoresis (TGGE)-

Temperature Gradient Gel Electrophoresis (TGGE) is a powerful molecular biology technique that allows the separation and analysis of DNA fragments based on their temperature-dependent melting behavior. It is an electrophoretic technique that is used to separate double-stranded DNA fragments based on their different melting temperatures. TGGE has a wide range of applications in molecular biology research, including DNA fingerprinting and analysis of genetic variation. In this article, we will discuss the principle of TGGE, how to perform the technique and its application in DNA fingerprinting. The principle of TGGE is based on the differential melting behavior of DNA fragments. The double-stranded DNA fragments have a specific melting temperature, which is the temperature at which the two strands of DNA separate. The melting temperature of a DNA fragment depends on several factors, including the length and base composition of the fragment. The higher the GC content of the DNA fragment, the higher its melting temperature. TGGE exploits this principle by subjecting a mixture of double-stranded DNA fragments to a temperature gradient during electrophoresis. The gel is heated from one end to the other, creating a temperature gradient across the gel. As the DNA fragments migrate through the gel, they encounter regions of the gel with different temperatures. When a DNA fragment reaches a region of the gel with a temperature equal to or higher than its melting temperature, the two strands of DNA separate, and the fragment becomes single-stranded. Single-stranded DNA fragments migrate more slowly through the gel than double-stranded

fragments, resulting in the separation of the fragments based on their melting behavior. TGGE is typically performed using a denaturing gel, which contains a denaturant such as urea or formamide. The denaturant disrupts the hydrogen bonds between the two strands of DNA, making it easier for the DNA to separate. The denaturing gel is usually run at a constant voltage and temperature, and the temperature gradient is created by varying the concentration of a chemical denaturant such as urea or formamide from one end of the gel to the other. (Harwood, 2008)

Applications of Temperature Gradient Gel Electrophoresis (TGGE) is a versatile technique that can be used in a variety of applications, from genetic research to clinical diagnostics. Here are some of the main applications of TGGE:

DNA fingerprinting: As mentioned previously, TGGE can be used in DNA fingerprinting to analyze highly variable regions such as STRs and VNTRs. By comparing the melting behavior of these regions in different DNA samples, it is possible to identify unique patterns that can be used for DNA profiling.

Genetic variation analysis: TGGE can be used to analyze genetic variation in a variety of organisms, from bacteria to humans. By analyzing the melting behavior of DNA fragments containing specific genes or regions of interest, researchers can identify genetic variation and study its effects on gene expression, protein function, and disease susceptibility.

Mutation detection: TGGE can be used to detect mutations in specific genes or regions of the genome. By comparing the melting behavior of PCR-amplified DNA fragments from healthy and diseased individuals, it is possible to identify mutations that may be responsible for disease.

Genotyping: TGGE can be used for genotyping, or the analysis of an individual's genetic makeup.

By analyzing the melting behavior of DNA fragments containing specific genes or regions, researchers can determine an individual's genotype and identify potential disease risk factors.

Microbial identification: TGGE can be used to identify different strains of bacteria based on their DNA profiles. By analyzing the melting behavior of PCR-amplified DNA fragments from different bacterial strains, it is possible to identify unique patterns that can be used for bacterial identification.

Environmental monitoring: TGGE can be used to monitor microbial populations in the environment. By analyzing the melting behavior of PCR-amplified DNA fragments from environmental samples such as soil or water, researchers can identify the presence and abundance of different microbial species.

Quality control: TGGE can be used in quality control applications to ensure the consistency and purity of biological products such as vaccines or recombinant proteins. By analyzing the melting behavior of PCR-amplified DNA fragments from these products, it is possible to identify potential contaminants or impurities.

Forensic analysis: TGGE can be used in forensic investigations to analyze DNA samples from crime scenes. By analyzing the melting behavior of PCR-amplified DNA fragments from different samples, it is possible to identify unique patterns that can be used to identify suspects or determine biological relationships. Overall, TGGE is a valuable tool in a wide range of applications that require the analysis of genetic variation. Its ability to rapidly and efficiently analyze large numbers of DNA samples makes it a valuable tool in many fields, from genetic research to clinical diagnostics to forensic analysis.

Terminal Restriction Length Polymorphism (TRFLP)-

Terminal restriction fragment length polymorphism (TRFLP) analysis is a technique used to analyze changes in microbial community structure and composition. This is a molecular biology technique used to identify microbial communities based on the location of restriction sites closest to the end of a gene sequence. The method relies on digesting a mixture of PCR-amplified variants of a gene using one or more restriction enzymes and using DNA sequencing to determine the size of each end. This technology is based on PCR amplification of targets. It is based on the principle that small differences in DNA sequences can change the restriction patterns of restriction enzymes (Osborn et al., 2000), and the types used must be produced based on the digestion of PCR amplicons with restriction enzymes. Some of the key applications of RFLP are:

Determine a person's genetic disease, such as cystic fibrosis.

To determine or confirm the source of DNA, for example in paternity tests or criminal investigations.

In genetic mapping, recombination rates are determined, which indicates the genetic distance between loci.

Identifies the disease-causing mutation in the carrier's family.

A study of inter-individual discrimination using forensic bacterial terminal restriction fragment length polymorphism profiling was performed. Evidence found at a crime scene may contain DNA from naturally occurring bacteria on the skin. A profiling method was established using T-RFLP of the amplified bacterial 16S ribosomal RNA gene. These profiles were compared to 12 individuals. Out of 12, one sample was similar to the others. Thus, a method for reproducible T-RFLP profiling of bacteria from handprints was established.

This method helped us narrow down the list of suspects. The t-RFLP method is also used in the statistical analysis of bacterial t-RFLP profiles in forensic soil comparisons. Soil plays an important role as evidence in the case. It shows us the geographical location where the crime took place where the criminal came from or where the vehicle came from. T-RFLP is used here to visualize bacterial populations. T-RFLP is also used to assess fungal diversity. Here fungal DNA is extracted, amplified, and purified from rotting leaves or pure cultures. Isolation of fragments is performed using T-RFLP. The extracted DNA is fluorescently amplified using primers, and the PCR products are digested with a restriction enzyme, and then the labeled terminal fragments are isolated and detected by a DNA sequencer. The number of different DNA fragments estimates the minimum number of spots present. Here, it does not provide information that it is produced by the same species, but provides information about the number of phlotypes present, which is related to the abundance of fungal species. These have been the few applications where T-RFLP has been used so far (Nishi et al., 2014). This technique was used a long time ago but was not modernized with more scientific theories. Now this technique is considered more convenient in some cases, but it is still not widely used. Today, autosomal STR short tandem repeat (STR) markers are used for forensic DNA typing. Essentially, it establishes a family connection to the suspect and creates a link to the crime scene. It checks if the suspect's family has a criminal record. In addition, forensic experts have used DNA typing techniques to re-examine old cases that were previously closed due to lack of evidence. The DNA of all existing prisoners will be taken for future reference.

Single-stranded Conformation Polymorphism (SSCP)-

Single-strand conformation polymorphism (SSCP) is a method for detecting variations in DNA sequences based on the differences in the way single-stranded DNA molecules fold and migrate

through a gel matrix under an electrical field. It is based on the fact that a single-stranded DNA molecule will have a unique three-dimensional structure that is influenced by its nucleotide sequence. This structure affects the mobility of the molecule in a gel matrix during electrophoresis, allowing for the detection of differences in DNA sequence (Gasser et al., 2006) To perform SSCP, DNA samples are denatured into single-stranded fragments and then subjected to electrophoresis in a polyacrylamide gel. The gel matrix causes the single-stranded DNA fragments to fold into distinct conformations based on their sequence and structure. The resulting banding pattern is then visualized using staining or autoradiography, and compared between samples to identify any differences in banding pattern that may indicate genetic variations.(Calabrese et al., 2020) SSCP is a relatively simple and inexpensive method for detecting genetic variations and has been used in a variety of applications, including medical genetics, population genetics, and molecular ecology.SSCP analysis can be used to detect a wide range of DNA sequence variations, including single nucleotide polymorphisms (SNPs), insertions, deletions, and other small-scale mutations. It is a relatively simple and inexpensive technique, but it does have some limitations, including the difficulty of distinguishing between different types of mutations and the potential for false positives and negatives.SSCP can be used to detect these variations in different types of DNA samples, including genomic DNA, PCR products, and cDNA

Multi-Surface Multi Technique(MUST) Latent Fingerprint Database.

The Multi-Surface Multi-Technique (MUST) latent fingerprint database helps in the development and testing of fingerprint identification systems, which are very important for solving criminal cases, identifying suspects, and also in improving forensic capabilities.

By utilizing this database, researchers and developers have an opportunity to evaluate the efficiency and precision of their algorithms across various conditions. This, in turn, paves the way for advancements in the field of fingerprint analysis and identification. The phrase "Multi-Surface" implies that the fingerprints in this database originate from a wide array of surfaces, encompassing materials such as paper, glass, plastic, or metal. As each surface presents distinct challenges for fingerprint recovery and analysis, a diverse database becomes crucial for the development of robust fingerprint identification algorithms. The phrase "Multi-Surface" implies that the fingerprints in this database originate from a wide array of surfaces, encompassing materials such as paper, glass, plastic, or metal. As each surface presents distinct challenges for fingerprint recovery and analysis, a diverse database becomes crucial for the development of robust fingerprint identification algorithms. A latent fingerprint database is a database where fingerprint images are collected from real-world scenarios such as crime scenes, where criminals leave their fingerprint impressions while committing crimes also it has fingerprints of known individuals, which help in crucial forensic investigations to find out the suspects. As latent prints are not visible to our naked eyes, we shall require various techniques to develop them and visualize them for identification purposes (Malhotra et al., 2023,). Recent advances in enhancement techniques for blood fingerprints. There are mainly two forms of fingerprint in forensic investigation: latent and patent ones. As most prints at crime scenes are latent or invisible, more focus has been paid to latent fingerprint development by forensic investigators and academics in chemistry, materials, or optical science. On the contrary, fingerprints in or by blood are sometimes patent marks, which means that no specific treatment is needed for their visualization. Blood serves as one of the most common contaminants of fingerprints detected in criminal investigations, particularly in vicious crimes.

Especially in the latest decades, there has been a swell of nanotechnology combined with fluorescence imaging, which leads to higher sensitivity, higher contrast, and higher selectivity in latent fingerprint detection. Blood has more proteins than any other ingredients, so techniques based on PDR are generally more sensitive than those of HCR innately, although they are not only reactive for blood. Nonetheless, for developing older blood prints, HCR may be equivalently sensitive as PDR or ARR, due to the higher stability of hemoglobin compared with other proteins in the human body. Most PDRs for forensic use are acid dyes, usually represented by the presence of sulfonate groups and sodium salt. Their development effect relies on two steps: first, they provide for solubility in water or alcohol, the favored major solvents from which to apply these dyes; Second, they assist the reaction in the way of their negative charge. If acidic conditions are allowed, the blood protein becomes positively charged and adsorbs the acid dye anions through ionic bonding. The main trouble of the HCR-based method for blood tests is that it may cause false positive outcomes in the presence of oxidants and catalysts, or heavy metal ions, including copper, iron, lead et al., or plant peroxidase-contained substances. Besides, the toxicity of some reduced reagents has long been denounced by the forensic community. The aqueous solvent is widely involved, so there must be a special fixing procedure to prevent the blood from diffusion before staining, otherwise, it will lead to a faint ridge pattern. This procedure enables proteins in the blood to be denatured and become insoluble in water so that they can be “fixed” onto the substrate surfaces the presence of short-chain alcohol in the developing solution helps to prevent blood spread during the staining stage, and ethanol is recommended as it brings lower toxicity and flammability than methanol. On nonporous surfaces, it only rinses unreacted reagents; however, on porous surfaces, it poses as a detainer to remove reagents that are absorbed by the background surface.

To maintain the integrity of the developed fingerprint, the same solvent is used in the staining and washing process. In the following sections of the review, some improvements both in chemical reagents and fixing agents are under discussion. Luminol is such a popular chemical to decipher hidden bloodstain in consequence of its high sensitivity, simple preparation, and low cost for decades. Luminol does not contain a fixing agent, and it may cause the ridge details to become diffuse when repeated applications take place on non-porous surfaces. Their investigation demonstrated that luminol had no destructive effect on species tests and elution methods for the detection of blood group antigens, and also showed little impact on subsequent PCR typing. Lac dye (*Laccifer lacca*) was used to enhance bloody finger marks on various types of non-porous and porous materials. Bloody finger marks were deposited using a depletion series technique on eleven different surfaces. To assess the efficiency of Lac dye stain, comparisons were performed with Amido black stain as a reference method.

Amido black on non-porous materials, in terms of both fingermark grades and color intensity. However, Lac dye showed relatively low performance for enhancing and developing bloody fingermarks on porous materials. This indicates that Lac dye can be beneficially used as an alternative to chemicals such as Amido black on a non-porous surface. (Chingthongkham et al., n.d.)

Applications of Fingerprint

1. Fingerprint recognition serves as a means of personal identification and authentication across diverse sectors, including unlocking smartphones, accessing secure buildings, and verifying identities at border control points.
2. Law enforcement relies on fingerprints to identify suspects and cross-reference them with prior criminal records, constituting a foundational tool in crime resolution.

3. Essential to forensic investigations, fingerprints aid in linking suspects to crime scenes, identifying victims, and establishing timelines of events.
4. Several nations integrate fingerprints into their national ID cards and passports to bolster security and thwart identity theft.
5. Fingerprint-based access control systems are deployed in businesses, government facilities, and residences to limit entry to authorized individuals.
6. Fingerprint scanners find application in companies for tracking employee attendance, ensuring accurate records of work hours.
7. Healthcare settings employ fingerprint verification to guarantee patient identity, minimize medical errors, and secure access to electronic health records.
8. Some banks and financial institutions leverage fingerprint recognition for secure transactions, heightening the security of mobile banking apps and payment systems.
9. At border crossings and airports, fingerprint scans are utilized to verify travelers' identities, ensuring alignment with their travel documents.
10. In certain countries, fingerprint recognition is integrated into voting systems to thwart fraud and uphold the integrity of elections.
11. Fingerprint sensors are commonplace on smartphones, tablets, and laptops, serving for user authentication, safeguarding personal data, and enabling mobile payment systems.
12. Educational institutions employ fingerprint scanners for attendance tracking, securing access to sensitive areas, and ensuring student safety.
13. Employers and organizations commonly mandate fingerprint-based background checks as part of their hiring or membership procedures.

Future Perspectives

A new fingerprint technique that has been developed by Sheffield Hallam University, in partnership with West Yorkshire Police, could revolutionize how the justice system identifies potential suspects and how admissible evidence can be given in court – particularly in murder and rape cases. The technique is based on mass spectrometry, an analytical technique that ionizes chemicals and sorts the ions based on their mass-to-charge ratio. In practical terms, this means that authorities will be able to detect, via a fingerprint sample, whether that individual has, for example, handled a condom, consumed alcohol or drugs, whether the subject is male or female, and even the brand of hair gel they use. Mass spectrometry is an analytical technique that can find traces of a substance within the ridges of a fingerprint. It then evaporates the sample and fires it through an electric and magnetic field inside a vacuum – this causes the particles to behave differently meaning that different molecules can be identified. The project team, who have been working on the project since 2012, have also stated that by using mass spectrometry, blood could be detected in a fingerprint that is up to 30 years old, meaning the technology could be used in cold case reviews. Project leader, Dr Simona Francese, highlighted that a fingerprint not only includes molecules from within the body but also ones that have contaminated the body, so the amount of information that can potentially be harvested using mass spectrometry is enormous.

How fingerprints have been taken and used in criminal cases has stayed pretty much the same for 80 or 90 years, but West Yorkshire Police are hopeful that this new technique will enable the authorities to glean far more useful information that will help to prevent and detect crime. Unlike the development of most ground-breaking technologies, this technique could be put into action sooner rather than later, with the Home Office predicting that mass spectrometry could be used in

casework in a matter of months. The technique also has the potential for use in several other applications in pharmaceutical, agrochemical, forensic, food, and biomedical research. It can also be used to investigate changes in metabolism that may occur in the body, primarily about therapeutic interventions or the progression of disease. While significant fingerprint detection research has taken place over the last 25 years, surfaces remain where fingermark development is problematic. In addition, while current capabilities provide a reasonable level of detection sensitivity on most common surfaces, they may be ineffective for the detection of weak fingermarks, or may not reveal sufficient ridge detail in such marks for identification purposes. For these reasons, latent fingermark detection remains an area where there is significant ongoing research around the world. A meeting of the International Fingerprint Research Group (IFRG) was recently held in Canberra, Australia, and was hosted by the Australian Federal Police. The purpose of this article is to summarize many areas of fingerprint detection research that were discussed at this IFRG meeting. This provides insight into prospects in this field and priority areas for research within the fingerprint detection discipline. Although fingerprints have been used for evidential purposes in forensic science for over 100 years, research into their use continues. We can expect to see further developments in enhancement techniques, including the use of functionalized nanoparticles. Imaging fingerprints using spectroscopic techniques is a relatively new field but can be expected to expand as technology improves. Spectroscopic techniques probably offer the most promising approach for a 'universal' technique to simultaneously detect and image latent marks at crime scenes; a holy grail for forensic science. Research into the chemistry of fingermarks has offered a tantalizing glimpse into possible information that may be available about the lever of a print but much remains to be discovered in this area including whether the chemistry of a print offers clues to individual identity.

Mass spectrometric approaches currently lead the field in revealing this information but if more sensitive spectroscopic techniques can be developed they may offer comparative information much more rapidly, cheaply, and conveniently.

Conclusion

DNA fingerprint techniques have significantly transformed the landscape of biological and forensic sciences. With their high precision and discriminatory power, these methods provide a means of uniquely identifying individuals, establishing familial relationships, and studying genetic variations within populations. It has revolutionized various fields, including forensic science, paternity testing, and population genetics. The accuracy, reliability, and widespread adoption of DNA fingerprinting have made it an invaluable tool in solving crimes, setting legal disputes, and understanding human genetic diversity. As technology continues to advance, DNA fingerprinting is likely to play an even more significant role in various applications. The technique has excellent utility in criminal investigation, crime scene investigation, and establishing relationships between individuals.

References

- 1) Schmidtke, J. (1999). Multilocus DNA Fingerprinting. In: Epplen, J.T., Lubjuhn, T. (eds) DNA Profiling and DNA Fingerprinting. Methods and Tools in Biosciences and Medicine. Birkhäuser, Basel. https://doi.org/10.1007/978-3-0348-7582-0_5
- 2) Gupta, S.K. (2018). Application of DNA Fingerprinting and Wildlife Forensics. In: Dash, H., Shrivastava, P., Mohapatra, B., Das, S. (eds) DNA Fingerprinting: Advancements and Future Endeavors. Springer, Singapore. https://doi.org/10.1007/978-981-13-1583-1_5
- 3) Cothron, Gretchen R., Fingerprint Evidence Part I: Tracing Friction Ridges Through History (May 12, 2012). Available at SSRN: <https://ssrn.com/abstract=2130808> or <http://dx.doi.org/10.2139/ssrn.2130808>
- 4) Radin, P. (Ed.). (1952). *African Folktales*. Princeton University Press. <https://doi.org/10.2307/j.ctt13x1991>
- 5) Younhee Gil, Dosung Ahn, Sungbum Pan, and Yongwha Chung, "Access control system with high-level security using fingerprints," 32nd Applied Imagery Pattern Recognition Workshop, 2003. Proceedings., Washington, DC, USA, 2003, pp. 238-243, doi: 10.1109/AIPR.2003.1284278.

- 6) Champine M, Kohlmann W, Leachman SA (2013) Genetic Counseling and Testing for Hereditary Melanoma: An Updated Guide for Dermatologists. *Genetics S2*: 004. doi:10.4172/2161-1041.S2-004
- 7) Malhotra, A., Vatsa, M., Singh, R., Morris, K. B., & Noore, A. (2023). Multi-Surface Multi-Technique (MUST) Latent Fingerprint Database. *IEEE Transactions on Information Forensics and Security*. <http://dx.doi.org/10.1109/TIFS.2023.3280742>
- 8) Birkenbihl, R. P. (1998). Endonuclease VII has two DNA-binding sites each composed of one N- and one C-terminus provided by different subunits of the protein dimer—the *EMBO Journal*.
- 9) Calabrese, C., Duran, A. G., Reyes, A., & Attimonelli, M. (2020). Methods for the Identification of mitochondrial DNA variants. *The Human Mitochondrial Genome*, 243-275. <https://doi.org/10.1016/B978-0-12-819656-4.00011-5>
- 10) Eyers, L., Agathos, S. N., & Fantroussi, S. (n.d.). Denaturing Gradient Gel Electrophoresis(DGGE) as a Fingerprinting Tool for Analyzing Microbial Communities in Contaminated Environments. *Environmental Microbiology*, 407-417. <http://dx.doi.org/10.1385/1-59259-765-3:407>
- 11) Gasser, R., Hu, & Chilton, M. (2006). Single-strand conformation polymorphism(SSCP) for the analysis of genetic variation. *Nat Protoc*, 1, 3121-3128. <http://doi.org/10.1038/nprot.2006.485>
- 12) Malhotra, A., Vatsa, M., Singh, R., Morris, K. B., & Noore, A. (2023). Multi-Surface Multi-Technique (MUST) Latent Fingerprint Database. *IEEE Transactions on Information Forensics and Security*. <http://dx.doi.org/10.1109/TIFS.2023.3280742>
- 13) Osborn, A. M., Moore, E. R.B., & Timmis, K. N. (2000). An evaluation of terminal-restriction fragment length polymorphism(T-RFLP) analysis for the study of microbial

community structure and dynamics. *Department of Environment Microbiology, GBF-National Research Centre for Biotechnology, Braunschweig, D-38124 Germany*, 2(1), 39-50.

14) Nishi, E., Tashiro, Y., & Sakai, K. (2014, October 22). Discrimination among individuals using terminal restriction fragment length polymorphism profiling of bacteria derived from forensic evidence. *Int J Legal Med*, 425-433(2015), 129.

<https://doi.org/10.1007/s00414-014-1092-z>

15) Harwood, A. J. (Ed.). (2008). *Protocols for Gene Analysis*. Humana Press.
<https://doi.org/10.1385/0-89603-258-2:211>

16) Chingthongkham, P., Chomean, S., Suppajariyawat, P., & Kaset, C. (n.d.). Enhancement of bloody fingerprints on non-porous surfaces using Lac dye (*Laccifer lacca*). *Forensic Science International*, 307(2020). <https://doi.org/10.1016/j.forsciint.2019.11011>