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# Forensic DNA Analysis

**Suchismeeta Behera[1]*, Amulyaratna Behera[2], Shruti Rajwar[1] and Varsha Singh[1]**

[1]*Department of Forensic Scinece, State Forensic Sciences Laboratory, Rasulgarh, Odisha, India*
[2]*School of Forensic Sciences, Centurion University of Technology and Management, R. Sitapur, Odisha, India*

## Abstract

The application of DNA (deoxyribonucleic acid) in criminal justice testing is known as forensic DNA analysis. When a culprit commits a crime, then evidence is left at the scene of crime which may contain biological materials with DNA. If the evidence and the latent print match, it may be possible to infer that the person was committed to the crime based on the match. The DNA of a suspect can also be linked to DNA found in blood, semen, saliva, or materials like hair, bone, or skin. Fingerprints can even be used to recover DNA. In the criminal justice system, forensic DNA analysis and DNA profiling are crucial components. Every year, new methods and tools for DNA profiling are developed. Recent developments in nearly all areas of DNA analysis, including sample collection, storage, pre-treatment, DNA extraction, DNA quantification, quality control of DNA testing, and DNA databases, are reviewed in this overview of the literature.

**Keywords:** Forensic DNA analysis • Polymerase chain reaction • Deoxyribonucleic acid • DNA profiling • Criminal justice testing

## Introduction

Since its initial introduction in 1981, forensic DNA analysis, also known as DNA profiling, has advanced and solidified itself as a potent tool for criminal justice practitioners to solve cases at crime scenes. In order to address legal issues, such as those involving civil and criminal proceedings, forensic science focuses on the use of genetic information in the criminal justice system. DNA's individuality is what gives it its tremendous strength and usefulness. Even though 99.9% of human DNA sequences are the same for every person, forensic scientists only require 0.1% of the DNA's unique sequences. This investigation is crucial to the criminal justice system. Since the advent of DNA analysis techniques in crime laboratories, even minute quantities of blood, saliva, semen, skin cells, or other biological materials can be used to generate leads for an investigation, connect a suspect or victim to a crime scene, or support or refute a witness' account of what happened. The accuracy and reliability of forensic DNA analysis have made this evidence an essential tool for countless people who have been wrongfully condemned [1].

The majority of the other forensic disciplines lack some of the features that DNA analysis offers. DNA retrieved from the crime scene has the ability to identify the offender in violent crimes like murder and rape where biological material is transmitted from the attacker to the victim. Theoretically, even in the case of identical twins, probabilistic 'individualization' of a DNA profile is statistically possible when testing enough genetic markers; in some cases, even twins can be distinguished from one another using additional genetic information. Because of the way DNA is inherited—with half of a person's genetic code coming from their mother and the other half from their father—close biological relatives can be used as benchmarks. This is another crucial ability of DNA. In other words, because of the capabilities of known genetic trait transmission principles, it is possible to go beyond the information contained in a sample itself. If no direct reference sample is available for comparison, missing people and disaster victims can be found through familial relationships.

Typically, forensic DNA analysis involves the following steps:

- DNA is collected and prepared for study before being transferred to a DNA laboratory.
- DNA extraction: DNA extraction is a crucial step that reveals the amount of DNA present in an unknown material. It is the method by which scientists are able to separate DNA. The sample can be kept for additional study by successfully using this data to provide improved findings.
- DNA amplification: Increasing the quantity of a certain DNA segment artificially.
- Calculating the average DNA concentration in a mixture is known as DNA quantification.
- DNA profile matching: This is the procedure used to identify a person's DNA traits.
- In this study, current advancements are outlined with a focus on novel DNA manipulation and analysis methods.

### Goals of forensic DNA analysis

- To connect a person to a crime scene or to criminal act: For instance, bloodstains at a burglary crime scene.
- To keep out suspects, people of interest, or cohabiting couples.
- Examples include sexual assault and acquiring the husband's DNA for the purpose of exclusion.
- Other applications include locating missing people and identifying human remains from catastrophic disasters.
- Testing for paternity.
- To aid research on recent developments in forensic DNA analysis.

To calculate the likelihood that a particular person is the real source of the sample being examined (Figure 1).

### Challenges in forensic genetics

Biological evidence obtained at crime scenes is frequently not in the best condition for a molecular study. The DNA molecule is impacted by a number of circumstances, which presents a variety of difficulties for the forensic scientist

***Address for Correspondence:** Suchismeeta Behera, Department of Forensic Scinece, State Forensic Sciences Laboratory, Rasulgarh, Odisha, India; E-mail: suchismeeta.behera@gmail.com*



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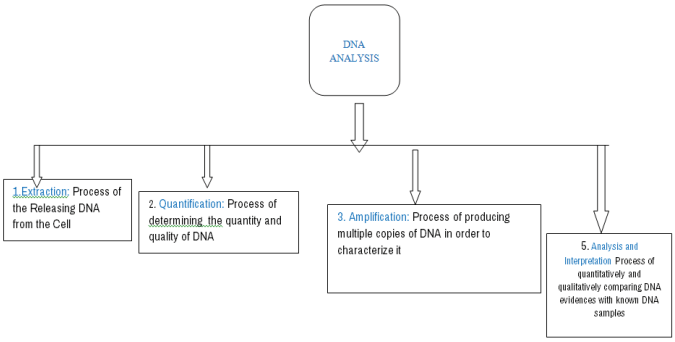


**Figure 1.** Steps of forensic DNA analysis.

to solve. Degradation and chemical changes: Evidence material is frequently subjected to a harsh environment where UV radiation, high temperatures, and bacteria can fragment and degrade DNA. The material may also undergo chemical alterations in addition to these environmental conditions that physically influence the DNA. Nucleases attack DNA during the death process of an organism, fragmenting the DNA molecule as a result. Additionally, the two main causes of DNA damage—hydrolytic and oxidative—are what can block the process of amplification and result in base alterations. In samples with degraded DNA, smaller PCR fragments, about 300 Base Pairs (bp), are more likely to be amplified, demonstrating an inverse association between fragment length and effective amplification [2].

**Inhibitors:** The presence of inhibitors in the samples can make the amplification of DNA in evidence samples from different crime scenes more difficult. Inhibitors can be found in the soil, blood, or colours used in textiles, and they interfere with the cell lysis step of the DNA extraction process as well as the activity of the polymerase in the PCR reaction. Equipment and benches should be exposed to bleach and UV light on a regular basis. Disposable gloves should be replaced frequently, and safety gear including face masks, hats, and shoe covers should always be worn. Negative controls must be used at every step of the analysis process and pre- and post-PCR laboratories must be physically separated. The collecting of the biological evidence prior to the DNA analysis still carries a significant danger of contamination, even with all safety safeguards in place. It is crucial that everyone involved in handling crime scene evidence is aware of the danger of contamination and takes precautions to reduce it [3].

**Mixtures:** The possibility that the samples of biological evidence from a crime scene contain DNA from various people is another problem. In cases of sexual assault, where DNA from both male and female people may be present, mixtures are very common. Often, there is a significant excess of the female's DNA.

**DNA quantity:** Low DNA molecule counts in forensic evidence samples might cause drop-outs and incomplete DNA profiles during typing. The PCR primers struggle to correctly hybridize to all of the DNA molecules present in samples with few DNA templates (100 pg, or around 17 diploid cells or 34 genome equivalents), which leads to uneven amplification of the alleles. Due to replication slippage, these stochastic effects can cause allele drop-out, heterozygote peak imbalances, and enhanced stutter, or signals that are one repeat unit longer than the original allele. In order to boost sensitivity when examining samples with limited amounts of template DNA, more PCR cycles (than the typical 28 cycles to 34 cycles) can be used. However, utilizing LCN analysis increases the possibility of contamination while not completely eliminating the issues with amplifying low template DNA samples.

### Current and previous techniques

Forensic analysis was developing slowly, but since 1985, the use of more potent procedures has expanded dramatically. The ABO blood types, the first practical marker system, were found in 1900. A quarter century later, the MN groups emerged as the second. There were 17 recognised blood group systems by the 1960s, although not all of them were helpful for forensics. A few serum proteins and enzymes were added in the 1970s. About 100 protein polymorphisms were known by the 1980s, however the majority were not particularly helpful for forensics. The year 1985 saw a significant development. Varying Number of Tandem Repeats (VNTRs) rapidly started to be employed for forensic studies since they demonstrated far greater individual variability than earlier systems. Although they are still in use, Short Tandem Repeats (STRs) are quickly replacing them [4].

Due to the wide range of DNA polymorphisms, it is now possible to provide compelling evidence that the DNA found at a crime scene and in a suspect is from the same individual. Before this time, it was feasible to rule out a suspect, but the evidence for inclusion was weaker than it is now because there was a higher chance that the two cases were coincidental. DNA polymorphisms caused a significant change. The likelihood that two DNA samples come from the same person can still only be inferred from the data. But with the array of genetic markers available today, it is almost probable that two similar profiles come from the same person. Although statistics support the notion that two samples came from the same person, the conclusion that they did not is statistically supported. There is a great deal of heterogeneity in the exact number of repeats, which varies greatly from person to person [5].

Typically, 20 to 30 different length-types can be successfully differentiated at each chromosomal site. With five or six loci, there are a huge number of possible combinations, and there is a 1 in 100 billion or lower chance that a random person's profile may resemble that of a suspect. The 13 STR loci selected by the Federal Bureau of Investigation (FBI) as the core loci for the Combined DNA Index System (CODIS) are intended to be handled by all forensic laboratories. Laboratories may be able to handle additional loci as well, and they typically do. There are further systems. SNPs (single nucleotide polymorphisms) identify alterations in a single DNA base. Each person has millions of them, thus the potential for further exploitation is practically limitless. They are frequently employed in the research on human evolution and medical genetics. HLA-DQA1 is a forensic case in point. This is still available and has been in use for a while. It is widely used and applied right away.

It has been especially helpful for swiftly clearing defendants whose DNA does not match the evidence sample, saving time, money, and undue suffering. 95% likelihood exists that an innocent person who has been falsely accused would be vindicated. This probability is increased to 99.9% when combined with the polymarker system's other five loci. SNPs often only have two alleles. The mitochondria, which are small organelles in the cell and not connected to the nuclear chromosomes, contain Mitochondrial DNA (mtDNA). They are not passed on by sperm but rather by the egg. Consequently, the study of individuals connected through the female line benefits greatly from the use of mtDNA. It is also particularly helpful because much less DNA can be examined than if it were chromosomal DNA, such as DNA from a shed hair, because there are many mitochondria per cell. An alternative is to use DNA on the Y chromosome, which is passed from the father to all of his kids, to determine the male lineage. When separating DNA from various guys, as with sexual assault mixtures, Y markers are especially helpful. CODIS is a national database and search engine that now makes use of the 13 essential STR loci. Finding prospective suspects is the aim. The FBI supports laboratory collaboration and comparison to achieve this. The CODIS system has now been installed in more than 100 laboratories. There will be on file some 300,000 STR profiles from convicted criminals by the end of 2000. Although the STR database is significantly smaller, it is growing quickly. However, we stress that the present approaches are trustworthy and reliable even though improvements are certain to occur.

## Materials and Methods

### Collection of DNA sample and its quantification

Various factors may affect the molecular size of DNA isolated from biological evidentiary materials. One of the most prevalent sources of DNA erasure is the common endonucleases and exonucleases found in nature. While the analyst has little control over nucleases' destructive behaviour prior to specimen collection, there are several things that can be done to inhibit their activity once the specimen environment can be controlled. Specimens

should be maintained cool and dry before starting DNA recovery techniques. Forensic experts continue to assess the efficiency of sample collection methods, the sincerity of DNA samples, and sample preservation to ensure accurate and dependable DNA collection for future analysis. The cotton swab is a fundamental and essential tool for obtaining DNA evidence for forensic examination.

However, this study has been hampered by the small sample size available from verified items for Short Tandem Repeat (STR) analysis using the polymerase chain reaction. However, a number of factors, including the type of sample—whether it is a sample of body fluids or epithelial cells—and the kind of evidence being examined—such as skin, sexual assault kits, fingernails, and improvised explosive devices—can impact the recovery of a DNA sample. Mass spectrometry is one method for figuring out whether a sample's biological aetiology is semen, vaginal fluid, blood, saliva, faeces, or urine. For the detection, characterization, and quantification of proteins, mass spectrometry has emerged as the preferred technique. New applications in biological research, biopharmaceutical characterization, and diagnostic detection are now possible thanks to the precision, sensitivity, and flexibility of MS devices. The sample can be triedpsin digested to extract the peptides present, and the peptides can then be fed into a mass spectrometer. The type of sample being analysed can be determined using biomarkers [6].

This method is distinct from other biochemical tests and is not sample-type-specific. Because it might vary and take time, proper sample preparation for MS-based analysis is a crucial stage in the proteomics workflow. Results from MS are substantially influenced by the efficacy and repeatability of sample extraction and processing. The mass spectrometer test can also be included in the DNA extraction process's chelating or chelexing stage, which makes it simple to incorporate into an already established process. Using Fourier Transform Infrared (FTIR) spectroscopy is another option for identifying a sample's biological source.

On a macroscopic scale, Fourier Transform Infrared Spectroscopy (FT-IR) has shown to be a useful technique for forensic scientists. By enabling rapid, nondestructive investigation of samples close to 10 microns, that kind of micro spectroscopy expands the application of conventional FT-IR. When working with bone materials, FTIR can be employed as an indirect screening method for DNA integrity. Since extreme heat can harm the DNA in bones, FTIR spectroscopy can assess the collagen's bonds, which correspond to DNA's hydrogen and covalent bonds. Many biological media, including blood, saliva, semen, and urine, have been shown to contain extracellular or cell-free DNA, which is easily detected in the supernatant of DNA samples during the DNA analysis process [7].

## PCR (Polymerase Chain Reaction)

The 1983 invention of the PCR method made it possible to sequence DNA and determine the nucleotide sequence of specific genes. Polymerase chain reaction, or PCR, is a molecular biology technique for amplifying DNA segments by making many copies of them under carefully regulated circumstances using DNA polymerase enzymes. A DNA segment or gene can be cloned into millions of copies from as little as one, enabling detection using dyes and other visualisation methods. Protein engineering, cloning, forensics (DNA fingerprinting), paternity testing, the diagnosis of genetic and/or infectious disorders, and the investigation of environmental samples are all fields of biotechnology where PCR techniques are used.

Repeated DNA sections, which are found outside of the DNA coding regions, are utilised in forensics to further analyse DNA. Each person has unique regions that can be used to identify an individual as well as a group of people, such as a group of family members. Specific nucleotide sequences can be replicated by PCR from low DNA concentrations or damaged DNA. Since the PCR primers are unique to human DNA, any bacterial DNA that may be present will not have an impact on the results. Thermostable DNA polymerase, nucleotides, two primers that surround the target sequence, and a small amount of template DNA are all used in PCR to amplify a specific section of DNA, producing a lot of DNA from a tiny amount. In that, PCR is incredibly sensitive. The procedure requires very little of the template DNA, which contains the sequence to be amplified.

To create the short, single-stranded, oligonucleotide DNA primers, however, some familiarity of the surrounding sequences is necessary. For PCR, two primers are required, one for each end of the sequence. The primers confer the specificity of PCR for a target sequence by being complementary to the template DNA. Which are subsequently subjected to several temperature fluctuations. Electrophoresis is then used to separate the amplified products, or amplicons. The ability to amplify a single sequence-specific region or a whole genome is one of the more sophisticated applications of the amplification process that are still being developed. Using fluorescent dyes that bind to PCR primers in the amplicons, fluorescence is frequently used to further assess the detection of DNA. Dimethyl sulfoxide, glycerol, formamide, single-stranded DNA binding proteins, and betaine are frequently employed as reagents in the PCR process. PCR inhibitors, which are easily obtained with a sample during DNA extraction, have a significant impact on the quantification of DNA. The process of amplification can then be stopped using PCR inhibitors. Hematin, indigo, melanin, collagen, tannic acid, humic acid, and calcium phosphate are examples of common inhibitors discovered in forensic materials [8].

## DNA extraction methods

The process of extracting DNA is crucial because it can reveal how much DNA is present in an unknown material. The sample can be kept for additional investigation while effectively using this data to provide results of higher quality. The process of extracting DNA entails isolating a cell's nucleic acids from its proteins and other biological components. Forensic DNA researchers frequently employ several procedures, such as organic or solid-phase extraction, post-extraction filtering, and Laser Capture Microdissection (LCM), among others. Low concentrations of retrieved DNA are sometimes concentrated using these techniques.

Organic extraction is currently one of the most widely utilised DNA extraction techniques. Although labor-intensive, organic extraction (variations of phenol/chloroform) uses a multistep liquid chemical procedure to create very pure, double-stranded extracted DNA. An all-purpose technique known as organic extraction is often used in the majority of the other circumstances. In comparison to chelex extraction, organic extraction procedures are more likely to keep the DNA in large fragments and clean it effectively. Which variant of the organic extraction process may be used depends on whether sperm are present or not, either alone or in combination with other types of cells. The cell membrane is broken down using SDS and proteinase K, and proteolytic digestion is used to quickly inactivate nucleases (such DNases and RNases) that may otherwise destroy DNA during extraction. The DNA is first lysed, then purified by combining it with a phenol-chloroform solution, centrifuged, precipitated with ethanol, and finally resuspended in a buffer with a low salt content. High molecular weight DNA can be extracted more successfully using the phenol-chloroform technique. There are additional alternatives to the precipitation process, such as switching to a filtration method that makes use of Centricon, Microcon, and Amicon filter devices to enhance DNA recovery and purification. When attempting to selectively separate female and male DNA in situations of sexual assault, a modified form of the organic approach can be utilised. Chelating resins based on ion-exchange chromatography are another frequently used technique for DNA extraction. Based on their affinity to the ion exchanger, ions and polar molecules can be separated using ion-exchange chromatography. Almost any charged molecule can be utilised, including large proteins, tiny nucleotides, and amino acids. The samples are typically mixed with a 5% solution of Chelex before being cooked for a number of minutes. The resins can attach to $Ca^{2+}$ and $Mg^{2+}$, which deactivates undesirable nucleases and stops them from cleaving DNA. Polar components bind to the polar resin whereas non-polar components denature and remain in solution. Once the sample has been centrifuged, DNA is added. The sample is then centrifuged, and the supernatant contains DNA. Boiling the sample denatures the DNA, leaving only single stranded DNA, necessitating the employment of a PCR-based technique to analyse the DNA.

Additionally, compared to conventional organic extraction or solid-phase methods, the DNA purity is not as high. A greater number of PCR inhibitory components are extracted along with the DNA using the manual Chelex approach. The Short Tandem Repeat (STR) reactions and subsequent

quantification issues brought on by the PCR inhibitors are used in DNA profiling. Solid Phase Extraction (SPE) is another technique that is used. A type of stepwise chromatography known as solid phase extraction is used to separate, extract, and adsorb one or more components from a liquid phase (the sample) onto a stationary phase (a sorbent or resin). In the last 20 years, SPE has developed into the most potent method for quickly and selectively prepping samples for analytical chromatography. SPE enhances qualitative and quantitative analysis while extending the lifespan of chromatographic systems. The strain placed on an analytical system is significantly reduced by converting sample matrices from the original matrix to a simpler matrix environment.

This usually simplifies the analysis that follows. The difference in affinities between an analyte and interferents found in a liquid matrix is used in solid phase extraction. This affinity enables the target analyte to be separated from the contaminants. Solid-phase extractions, which use silica in the presence of chaotropic salts, are an extraction technique that keeps growing in popularity for DNA extraction. Thiocyanate, sodium iodide, and guanidinium hydrochloride are some of the salts. To facilitate the binding of DNA to silica, cells are frequently lysed with proteinase K first and then introduced to a chaotropic salt buffer. Impurities like proteins and other pollutants can be washed away once DNA has been linked to silica. After that, DNA can be eluted. Silica can be given as paramagnetic beads or in columns. There is a centrifugation stage necessary when using a silica column. Using robotic platforms and silica magnetic beads, a simple purification process can lead to high throughput extraction. Additionally, there is minimal cross contamination when using the magnetic beads with a variety of sample types, including blood, saliva, and sperm.

Laser Capture Micro Dissection (LCM) is another approach that is frequently employed with mixed materials. An essential technique for molecular analysis of extracted nucleic acids for molecular applications, such as PCR of tumour DNA, is laser capture micro dissection. LMD enables the separation of single cells or small groups of cells from cytologic materials that have undergone standard processing. These cells can now be isolated using a variety of commercial techniques. An operator there chooses the desired screen cells. The cells are chopped by the laser beam and then put in a microfuge tube, where extraction can be done immediately for later molecular analysis. This technique makes it possible to choose and gather cells according to the type of cell, which means less cellular material is required.

This technique typically performs better with mixed samples where there is a significant and minor contributor, such as when there are more female epithelial cells than sperm cells in the sample. It also reduces the likelihood of mixed DNA profile results and PCR inhibitor interference. UV cutting and IR capture systems are two different types of LCM techniques. By photo volatilizing them, the UV system can trap cells. After being isolated by laser energy to a thermolabile polymer, the cells can be seen under a microscope using the IR capture method.

Bone and teeth are frequently the sole accessible sources of DNA in situations like mass disasters or forensic identification where a deceased corpse or its remains are extensively burned or substantially degraded. DNA molecules in bone and teeth are mainly shielded from environmental hazards and/or biological attack because of their distinct makeup and structure. Bone is a type of connective tissue that is mostly made of the inorganic mineral hydroxyapatite and collagen. DNA has a great affinity for hydroxyapatite, and the amount of crystallinity loss in hydroxyapatite as well as the loss of collagen are related to the rate of DNA degradation. Another inherent component in bone material survival is bone density.

Men and women have significantly variable bone densities and skeletal element morphologies in various locations. Conversely, a tooth is made up of three main tissues: Enamel, dentin, and pulp. The toughest tissue in the human body, enamel, is 96% mineral and DNA-free. Except for a few mitochondrial DNAs that amass during the odontoblastic process, dentins typically lack nucleated cell bodies. Dental pulp is a highly vascularized connective tissue with a wide variety of cells and a significant amount of DNA. Since enamel covers the dentin and pulp, it acts as a physical barrier to shield the DNA inside the tooth from environmental factors such heat, sunshine, moisture, and microbial attack. The two basic processes in the extraction of DNA from bones and teeth are pulverisation in liquid nitrogen and incubation in EDTA. The majority of techniques for isolating genomic DNA involve the use of organic solvents like phenol and chloroform or the extraction of silica from materials like powdered bone or tooth debris. The mechanical grinding of complete teeth or bones necessitates a separate pre-amplification laboratory space and raises the possibility of dust contamination. There are even more DNA extraction techniques being developed to deal with time, storage, and extracting DNA from intractable sources.

## Quality assurance and validation of DNA analysis

To assist crime laboratories in complying with federal standards and utilising new DNA analysis technology, a variety of criteria are needed for the continual review and measurement of quality assurance and validation procedures. The creation of numerous commercial solutions for forensic DNA testing is also one of these elements, along with the pure ability of DNA analysis to generate extraordinarily high protection potential, advancements in technology and methodologies, widespread acceptance of DNA evidence in courtrooms, and others.

The prelaboratory, laboratory, and postlaboratory are the several components of the forensic DNA lab. However, case assessment is part of the pre-laboratory. Inspections, DNA quantification, DNA extraction, DNA amplification, electrophoresis, and typing all take place in the lab. Then, the databasing, statement reporting, and result interpretation are regarded as post-laboratory procedures. To prevent contamination and provide a seamless transition from one phase to the next, these processes are carried out in various locations. To encourage confidence in the results of DNA testing, quality assurance techniques have been established over time. Due to experience gained from groups like the European DNA Profiling Group (EDNAP) and the European Network of Forensic Science Institutes (ENFSI) in Europe and the Federal Bureau of Investigation's DNA Advisory Board (DAB) and Scientific Working Group on DNA Analysis Methods (SWGDAM) in the USA, the quality infrastructure for DNA testing is likely more advanced than that of many other forensic disciplines.

## Advanced and emerging techniques and methodologies of DNA analysis

Forensic DNA analysis has evolved through numerous stages since its inception in the 1980s. The forensic world no longer uses Restriction Fragment Length Polymorphism (RFLP) profiling since it needs a lot of DNA and can't accurately analyse degraded samples. A longer strand of DNA cannot be profiled using PCR techniques, which were utilised in the second generation of DNA profiling. The method of choice at the moment is short tandem repeat analysis, or STR analysis which is called as the third generation of DNA analysis.

**STR (Short Tandem Repeat) analysis:** Forensic DNA analysis and Short Tandem Repeat (STR) typing techniques are utilised for human identity testing. Thousands upon thousands of STR markers can be found in the human genome, but only a small core group of loci have been chosen for use in forensic DNA and identification testing. Universities, governments, and private laboratories conducting various types of identity testing for people, such as forensic casework, DNA databasing, missing persons victim identification, or paternity testing, generate thousands of STR analyses each year throughout the world. However, the small PCR product sizes produced by STR testing—roughly 200–400 bp—are typically consistent with degraded DNA that may be present as a result of environmental deterioration on biological material discovered at a crime scene.

**SNP (Single Nucleotide Polymorphism) analysis:** Due to a change in one nucleotide in the sequence, DNA might experience several kinds of single nucleotide polymorphisms. The DNA sequence will change as a result. However, the most prevalent sort of genetic variation among humans is due to these single nucleotide polymorphisms, also known as SNPs. Every SNP modifies a nucleotide, introducing a variation in the DNA sequence. A SNP might, for instance, swap out the nucleotide thymine for the nucleotide cytosine

in a particular DNA strand. On average, they occur every 200 nucleotides. The human genome typically contains 10 million SNPs, though. They can serve as biological markers, guiding researchers to genes linked to specific disorders. SNPs that impact the function of a gene when they appear within a gene or in a regulatory region close to a gene may contribute more directly to a disease.

**mtDNA (mitochondrial DNA) analysis:** Within the cells are mitochondria. However, mitochondria are found in countless numbers in every cell. The DNA found inside mitochondria is known as mitochondrial DNA. Forensic investigators now have a unique tool to identify the origins of DNA recovered from degraded, damaged, or minute biological components thanks to Mitochondrial DNA (mtDNA). The circular genome known as mtDNA is found outside of the cell's nucleus. The number of copies of mtDNA enhances the possibility of retrieving quantifiable DNA from compromised DNA samples; as a result, mtDNA plays a significant role in forensic analysis involving samples with little or no biological material, such as in the case of missing people inquiries, large-scale disasters, and other forensic studies. Aside from the improvements that SNPs have made for Low Template DNA (LT DNA), other sources and techniques, such as mtDNA and single cell analysis, are still being investigated. MtDNA continues to be a reliable source because to its abundance. Due of the higher proportion of mtDNA to nuclear DNA and its propensity to be less susceptible to degradation, mtDNA is frequently employed in LT DNA. Due to their polymorphism qualities, the Hypervariable (HV) sections of mtDNA are employed for study and are valuable sources for examination of damaged DNA, including bone samples. The most variances are seen in the hypervariable zones. The qPCR test can be utilised in the mtDNA study because it is very specific. Additionally, SNP-based screening techniques have been used with mtDNA sequencing to increase discrimination.

### DNA databases

One of the most significant advancements in combating crime since fingerprinting is the use of DNA to identify individuals who are suspected of committing a crime. However, when DNA analysis is applied correctly, technology can significantly advance society by aiding in the conviction of violent offenders like rapists and murderers. The National DNA Database is described as information on genetic material collected from a sample of tissues, as well as personal data, that is kept in a DNA database that can be used in forensic analysis (Schuster, 2005). There are worries that this information might be utilised inappropriately, endangering both our families' and our own rights. We need to have faith that the way the government and the police use DNA will support our fundamental right to privacy and safeguard our civil freedoms.

### The future of forensic DNA analysis

Up until recently, DNA profiling development was extremely modest, but after 1985, the quantity and calibre of more prevalent approaches have exploded. However, the blood groups were the first useful system to be established, and fifty years later there were found to be 17 blood group systems, albeit not all of them were useful for DNA profiling. Subsequently, a few enzymes and serum proteins were added. There are about 100 protein polymorphisms, although the majority are not particularly useful for DNA analysis. However, there was a widespread breakthrough in the 1980s. VNTRs (varying number of tandem repeats) instantly started to be employed for forensic studies since they demonstrated greater individual variability than earlier methods. They are still in use, although short tandem repeats are quickly replacing them. Nowadays, most people are aware that DNA techniques are being used. But what appears to be "a simple test" is actually the result of significant work by a relatively new subfield of the forensic sciences called forensic biology, which has integrated technical development, molecular genetics, statistics, and epidemiology into one discipline. By helping in the conviction of the guilty and the exoneration of the innocent, forensic DNA testing has played a significant part in the criminal justice system. By connecting reference samples to recovered remains, the remains of those who have gone missing and those who have perished in large-scale tragedies have been re-associated and identified. To increase the capabilities of laboratories working to recover DNA results with higher sensitivity and informativeness, new technologies are frequently presented and confirmed. Although different genetic markers are utilised for certain applications, Short Tandem Repeat (STR) typing remains the main workhorse in forensic DNA analysis. (Tables 1 and 2)

## Discussion

### Challenges in DNA analysis

DNA testing has experienced substantial expansion as a result of its success, which has led to new difficulties, particularly in the areas of sample backlogs and data interpretation. Sample backlogs can be reduced through laboratory automation, expert system data assessment, and stringent case acceptance standards. Instead of carefully considering which materials could be the most probative, law enforcement investigators occasionally engage in "swab-athons" at crime scenes and submit numerous items in an effort to solve a case through a DNA database hit.

When DNA mixtures from three or more individuals are present, especially when low-template DNA 'touch' samples are used, data interpretation uncertainties are at their maximum and errors are most likely to occur. A rise in DNA detection sensitivity in recent years has made it possible to work with these kinds of materials. Greater responsibility in data interpretation is required as sensitivity increases. Unfortunately, handling DNA interpretation of complicated mixes inconsistently makes it more difficult to get consistent results from numerous analysts and/or forensic laboratories.

Improved detection sensitivity, more information content from larger sets of core STR loci and perhaps supplementary genetic markers, and deeper information from sequence analysis of alleles are the keys to higher volumes of information in forensic DNA in the future. Due to increased sensitivity in PCR assays and information content of produced profiles, more data are available from biological samples. The creation of increasingly complex DNA profiles for interpretation is a result of recent advancements in DNA test sensitivity. It's critical to remember that just because a DNA profile can be created from just one cell, it doesn't necessarily follow that the source of the profile is related to the crime event under investigation. Fundamental limitations in sensitivity are present with PCR amplification as a result of stochastic (random) variance in sampling each allele at a locus. During duplicate PCR amplification, these stochastic effects cause variations in peak heights and peak height ratios for heterozygous samples.

Despite the fact that both alleles of a STR locus are present in equal amounts in the DNA template, stochastic variation in the initial rounds of PCR can cause one allele to be amplified preferentially over the other, which can occasionally result in allele drop-out (i.e., inability to detect the allele). Studies using samples from a single source have demonstrated that stochastic effects, like higher stutter and allele drop-out, start to appear at about 15–20 cells, or 100–125 pg. When the number of PCR amplification cycles is increased to boost sensitivity, allele drop-in may also happen. It gets harder and harder to correctly identify specific DNA mixture contributors and couple alleles

**Table 1.** The 13 core set of STR loci included in CODIS with chromosomal location and repeat motif.

| Sl. No | Marker | Chromosomal Location | Repeat Motif |
|---|---|---|---|
| 1 | FGA | 4q31.3 | CTTT |
| 2 | CSF1PO | 5q33.1 | TAGA |
| 3 | TH01 | 11p15.5 | TCAT |
| 4 | TPOX | 2p25.3 | GAAT |
| 5 | vWA | 12p13.31 | [TCTG][TCTA] |
| 6 | D3S1358 | 3p21.31 | [TCTG][TCTA] |
| 7 | D5S818 | 5q23.2 | AGAT |
| 8 | D7S820 | 7q21.11 | GATA |
| 9 | D8S1179 | 8q24.13 | [TCTA][TCTG] |
| 10 | D13S317 | 13q31.1 | TATC |
| 11 | D16S539 | 16q24.1 | GATA |
| 12 | D18S51 | 18q21.33 | AGAA |
| 13 | D21S11 | 21q21.1 | [TCTA][TCTG] |

**Table 2.** Genetic markers utilized in forensic DNA analysis: Current usage and anticipated future applications.

| Sl. No | Marker | Current Practice | Future Potential |
|---|---|---|---|
| 1 | Mitochondrial DNA | Sanger sequencing of the control area combined with population database searches (such as EMPOP.org) to estimate the haplotype frequencies. | A full mt Genome by NGS will yield the best resolution achievable; more population databases will enhance estimates of haplotype frequency. |
| 2 | X-chromosome STRs | Population data for 12+ loci are usually collected, but kinship cases hardly ever used them. | X-STRs and X-SNP markers are frequently utilised in conjunction with autosomal STRs in order to answer difficult kinship concerns. |
| 3 | Y-chromosome STRs | Examination of 12 – 27 Y-STR loci with haplotype frequencies are searched in population databases (e.g. YHRD.org); familial searching candidate pool restricted with Y-STR screening | Larger population databases to improve haplotype frequency estimates; genetic genealogy database information combined with Y-STR casework data to help provide potential surname of perpetrator in some cases; rapidly mutating Y-STRs used to separate close male relatives |
| 4 | Autosomal STRs | core loci are used to create DNA profile databases and to perform casework; data generated in laboratories with CE systems | expanded core set of loci enabling more international comparisons; data generated by NGS |
| 5 | Bi-allelic markers (SNPs and InDels) | a few dozen SNPs are examined with multiple SNaPshot assays on CE platforms for simple phenotype or biogeographic ancestry prediction; some population data collected with insertion/ deletion (InDel) assays | hundreds of SNPs or InDels for biogeographic ancestry and phenotype predictions tested on NGS platform in parallel with STRs |

into genotypes when stochastic variation occurs. Therefore, allele drop-out and probable allele sharing from numerous contributors contribute to higher uncertainty in the precise genotype combinations that may be securely assumed since stochastic effects are constantly present in low-level DNA PCR amplifications. Replicate amplification results can be combined to identify the real alleles in a profile. Future laboratories might elect to set a complexity threshold to stop working with low-quality data. The forensic DNA community must be diligent in its attempts to accurately interpret difficult evidence without going too far since sensitive DNA detection technology has the potential to outstrip credible interpretation.

To prevent the erroneous use of DNA, it is essential that investigators and court officers clearly communicate the constraints of their interpretation strategies. Future DNA profiles with a larger number of the core loci needed for inclusion in national DNA databases will have more information content. Future DNA profiles will have more information since they will include a greater number of the core loci that are necessary for inclusion in national DNA databases. When a K reference profile cannot be found in a DNA database, the search can be expanded by letting go of some of the criteria and using the principles of genetic inheritance to create a "familial" search in an effort to find the source of the unknown Q profile's close biological relatives. Due to the dearth of the genuine perpetrator's close relatives in the database, familial searches often have low success rates. Additionally, due to the search approach employed or specific inheritance patterns that result in an actual sibling (in the database) with different alleles compared to the Q search profile, true relatives may be overlooked. Long candidate lists may be the result of false positives when the Q profile contains common alleles that are shared by unrelated individuals. Male samples from a ranked candidate list can be subjected to Y-STR testing to help weed out false positives. Future DNA capabilities may enable prediction of exterior observable traits like eye colour or hair colour when no K reference sample is available for comparison after a database search.

In some circumstances, biogeographic ancestry estimation may be useful. Future research may also use microbial DNA to enable scenarios where microbial transfer occurs when using a computer keyboard or engaging in sexual activity. One of the biggest issues facing forensic DNA analysis in the future is dealing with complex DNA mixtures that contain genetic information from more than two persons, especially if any of the individuals are related. When dealing with difficult DNA mixture results that may otherwise be deemed inconclusive under a binary approach to interpretation, probabilistic genotyping provides a technique to reinforce conclusions.

### DNA in the courtroom

In the early years of forensic DNA analysis, two distinct technologies—Polymerase Chain Reaction (PCR) amplification of DNA sequence polymorphisms and Restriction Fragment Length Polymorphism (RFLP) analysis of VNTR loci—were used in criminal investigations. The analysis of length polymorphism, as in the STR loci, or sequence polymorphism, as in mitochondrial DNA, has since been done using PCR, which has been the foundation for all forensic tests. Using essentially the same DNA procedures—DNA extraction, PCR amplification, and analysis of the DNA sequence by a variety of techniques—millions of forensic samples have been examined since the initial work applying PCR to the analysis of a few autopsy samples in 1986. However, the analytical technology has continued to advance, making it possible, with each new advancement, to analyse increasingly more difficult samples, such as those that have trace DNA, degraded DNA, and complicated mixes that reflect numerous contributors.

## Conclusion

It is crucial to verify the real identification of the forensic materials even before DNA can be obtained. Fourier-Transform Infrared (FTIR) spectroscopy, mass spectrometry, and PCR are currently used methods to identify the biological source of samples. However, the precise type of sample (such as urine, blood, sweat, semen, saliva, and bone, etc.) determines the efficacy of various DNA extraction techniques. For DNA extraction procedures, there is a trade-off between purity and volume. One strategy for high-result DNA extraction, as an illustration, was made achievable using silica magnetic beads integrated with an automated robotic stage. The technique has been successfully used to a variety of samples, including blood, saliva, and sperm, however it is not the best option for samples with low DNA quantities. It has been demonstrated that Fast Technologies for DNA Analysis can speed up DNA extraction, particularly for blood or saliva. Nucleic acid can be extracted directly from whole blood, swabs, and blood found on cotton or denim using an enzyme-based microfluidic technique. Under forensic DNA analysis, continuity is a disadvantage since DNA begins to deteriorate under typical environmental circumstances. Thus, storing DNA and maintaining its integrity over a long length of time both become difficult. DNA can be stored for a long time by being either dehydrated or preserved in a medium that has been specifically created for the purpose. In addition to all currently used methods for speeding up DNA analysis, which may also be economical, researchers are always looking at novel methods.

## Conflict of Interest

The authors have no competing interests to declare that are relevant to the content of this article.

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