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DNA Fingerprinting: A Tool in Forensic Analysis

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Abstract: ***DNA (Deoxyribonucleic acid) fingerprinting methods have revolutionized the field of forensic science. We can create a person's unique genetic profile by using this method to examine the DNA region. Nowadays, DNA fingerprinting is a daily test. The DNA fingerprinting method has been in use for the last 26 years. During all these years of this method numerous changes have been made. New techniques were developed during all these years, which gives more accurate results then the previous one. The steps involved to perform this method are DNA extraction, DNA quantification, amplification of up to 23 humans short Tandem repeat and then splitting them on capillary column and the last stage is interpretation of data which is followed by reporting the evidence in the court. This study looks at how different DNA fingerprinting techniques may be used to distinguish between blood samples collected from different people. To accomplish a complete approach to person identification, the study combines standard blood grouping, RBC and WBC cell count analysis, and sophisticated Short Tandem Repeat (STR) and Restriction Fragment Length Polymorphism (RFLP) approaches. The study's goal is to show how successful these strategies are in forensic to differentiate between individuals.***

Keywords: genetic profile, DNA extraction, amplification, STR, DNA fingerprinting

1. Introduction

DNA fingerprinting (also known as DNA profiling or forensic genetics) is an approach implemented by forensic scientists that helps in the identification of persons or materials based on their DNA profiles (1). DNA fingerprinting got its name because this technique uses DNA for the identification rather than using the latent physical fingerprints and this technique generates the unique profile which is unique for every individual except identical twins. Throughout these years this technique underwent various developments which made it possible to identify the source of biological samples found at the crime scene and also help in solving the disputes of paternity and other criminal cases, this technique is still in the development phase.DNA is a sub-field of forensic science which focuses on the use of genetic evidence in criminal investigation and aids in cases which involves human crimes such as rape. murder. DNA is also referred to as the “blueprint of life” since it contains all the data which are needed by an organism for healthy development and reproduction.{2) Each creature is made up of different quantities of DNA, which give them characteristics like their hair and eye hues. The “ultimate method of biological individualization” is usually regarded as DNA analysis. Every cell in an organism has DNA, which makes every individual except from identical twins. Only 1.5% of the DNA in the human genome or “junk DNA” is said to code for proteins according to the HGP. [3] DNA profiling can be used to identify human remains or to investigate ancient or unsolved crimes.(4). When other methods failed, DNA fingerprinting was used as a last resort, and it played a supportive role when strong proof was required. In both small and large scale disasters.(5). Minisatellite and Microsatellite , also known as VNTR and STR are repeating sequences that produce a lot of junk DNA. Sir Alec Jeffreys created a special inheritable pattern known as DNA fingerprint using these unusual tandem repetitions and the process is known as DNA finger printing [6]. In 1980, sir Alec Jeffreys developed a novel technique for DNA fingerprinting at the university of Leicester [7]. Karl Landsteiner developed ABO blood grouping in humans in 1901. Blood type markers were employed to identify victims and suspects in criminal investigations. The second most important blood group system is the Rh system, a person can either be Rh positive or Rh negative(8).The introduction of DNA-based markers has suddenly transformed the area of forensic research, since it can assess an infinite number of polymorphisms necessary to distinguish one individual from another(9). Prof. Alec Jeffreys proposed employing restriction fragment-length polymorphism (RFLP) to apply DNA fingerprinting in forensic research in the early 1980s. The method employed to examine hyper variable sequences makes use of the fact that the DNA molecule may be cut at certain recognition sites by proteins known as restriction enzymes, resulting in millions of little DNA pieces(10).PCR another technique which is useful in DNA fingerprinting is developed by Dr. Kary Mullis in 1985. The polymerase chain reaction has transformed molecular biology. It can amplify a specific DNA sequence into millions of copies in a relatively short amount of time (typically less than 3000 bp). The polymerase chain reaction feature has allowed the analysis of many forensic materials, particularly those that have deteriorated (11). STR also known as microsatellites are 2-6 base pair long and highly polymorphic between the individuals because this technique examines the specific loci present on the DNA strands the possibility of 2 people sharing the same profile is 1 in 30 million except identical twins. Some widely used commercial kits are also available for STR markers.(12).

TABLE 1. Some widely used commercial kits for autosomal STR markers

Kit name	Source	STR loci included (amel = sex-typing marker amelogenin)	Power of discrimination[a]
PowerPlex 16	Promega	CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, amel, D16S539, D18S51, D21S11, Penta D, Penta E	1 in 3.4×10^{17}
Profiler Plus	Applied Biosystems	FGA, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D18S51, D21S11, amel	1 in 9.4×10^{10}
COfiler	Applied Biosystems	CSF1PO, TH01, TPOX, D3S1358, D7S820, D16S539, amel	1 in 1.3×10^{6}
SGM Plus	Applied Biosystems	FGA, TH01, VWA, D3S1358, D8S1179, D16S539, D18S51, D21S11, D2S1338, D19S433, amel	1 in 4.8×10^{12}
Identifiler	Applied Biosystems	CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, D2S1338, D19S433, amel	1 in 2.5×10^{17}

TABLE 2. Characteristics of common autosomal STR markers

STR marker	Chromosomal location	Repeat motif	Allele range[a]	PCR product size (kit)	RMP[b]
CSF1PO	5q33.1	TAGA	6–15	305–342 bp (Identifiler)	0.112
FGA	4q31.3	CTTT	17–51.2	215–355 bp (Identifiler)	0.036
TH01	11p15.5	TCAT	4–13.3	163–202 bp (Identifiler)	0.081
TPOX	2p25.3	GAAT	6–13	222–250 bp (Identifiler)	0.195
VWA	12p13.31	[TCTG] [TCTA]	11–24	155–207 bp (Identifiler)	0.062
D3S1358	3p21.31	[TCTG] [TCTA]	12–19	112–140 bp (Identifiler)	0.075
D5S818	5q23.2	AGAT	7–16	134–172 bp (Identifiler)	0.158
D7S820	7q21.11	GATA	6–15	255–291 bp (Identifiler)	0.065
D8S1179	8q24.13	[TCTA] [TCTG]	8–19	123–170 bp (Identifiler)	0.067
D13S317	13q31.1	TATC	8–15	217–245 bp (Identifiler)	0.085
D16S539	16q24.1	GATA	5–15	252–292 bp (Identifiler)	0.089
D18S51	18q21.33	AGAA	7–27	262–345 bp (Identifiler)	0.028
D21S11	21q21.1	[TCTA] [TCTG]	24–38	185–239 bp (Identifiler)	0.039
D2S1338	2q35	[TGCC] [TTCC]	15–28	307–359 bp (Identifiler)	0.027
D19S433	19q12	AAGG	9–17.2	102–135 bp (Identifiler)	0.087
Penta D	21q22.3	AAAGA	2.2–17	376–449 bp (PP16)	0.059
Penta E	15q26.2	AAAGA	5–24	379–474 bp (PP16)	0.030
Amelogenin (sex-typing)	Xp22.22 Yp11.2	not applicable		X = 107 bp Y = 113 bp	

Other techniques which can be used in DNA fingerprinting are Y chromosome and Mitochondrial DNA analysis. Y-chromosome analysis helps in gender identification because Y chromosome is only transferred to the son from the father and Mitochondrial DNA is used in finding maternal lineage because it is transferred from the mother to their children's. Various techniques are available for isolating the DNA from the different biological samples but nowadays the most preferred method for isolation in the laboratory are FTA (Flinders Technology Associates) cards14)

2. Methodology Sample Collection

Fresh blood samples (n=3) were obtained from the blood bank in heparinized vacutainers (stored at 4℃) and processed for DNA extraction (salting-out) within 72 hours.

Salting- out method is used for the DNA extraction, this method uses 2 lysis buffers to breakdown the cells and the TE buffer is used for storing of DNA for a longer period and can store in distilled water for immediate use. This method is easy, quick, safe, and inexpensive (15). RBC and WBC cell test was done by using neuber's chamber. This test uses 2 fluid Hames fluid and Turk's fluid respectively. Blood grouping is a traditional technique for differentiating blood samples based on the presence or lack of particular antigens (A, B, O, and Rh) on the surface of red blood cells (RBCs). All samples were classified according to their blood type by using the normal slide method. In RFLP analysis, genomic DNA is digested using restriction enzymes and the resultant fragments are separated on a gel. Variations in the number and size of restriction pieces are used in this strategy. It was utilized to distinguish blood samples further.STR analysis is a very sensitive method of DNA fingerprinting. In this work, DNA was taken from each blood sample, and polymerase chain reaction (PCR) was used to amplify particular loci with highly polymorphic STR regions. STR profiles were generated for different 3 STR loci (D18S51 (Repeat - AGAA), D5S818 (Repetition - AGAT), D8S1179 (TCTA Repeat)).To find discrepancies, the obtained DNA profiles were compared.

3. Result

The blood grouping findings gave a preliminary classification of the samples (A, B, AB, and O). In situations where samples had the same blood type, further analysis was required to differentiate between people.

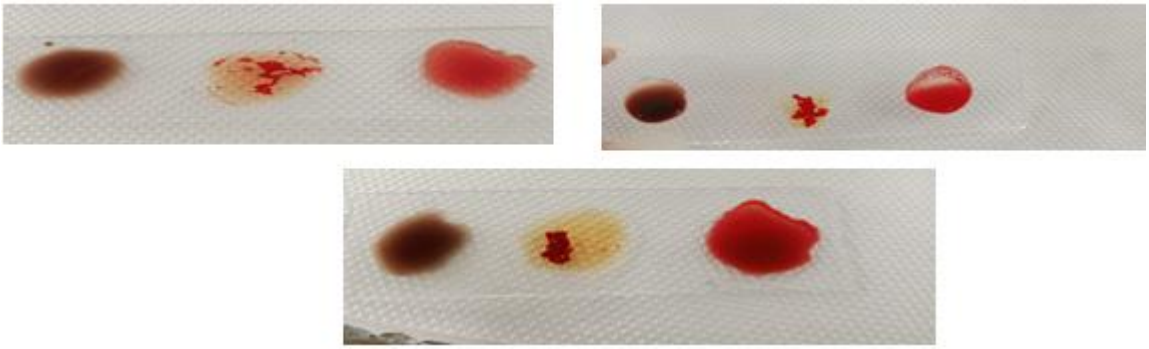

Figure 1: Makes it very clear that all three blood samples were determined to be B positive.

RBC and WBC counts were done to differ amongst people. The variance was slight in some situations, but it nonetheless contributed to the overall differentiation process.

Sample 1	4.6 million cell per microliter
Sample 2	4.9 million cell per microliter
Sample 3	5.5 million cell per microliter

For WBC Count
Sample 1-5000 µl
Sample 2-6550 µl
Sample 3-5690 µl

For each blood sample, STR analysis produced highly discriminating DNA profiles. When these profiles were compared, they revealed a significant amount of individual difference. Even when other strategies generated comparable findings, this strategy proved to be very successful in differentiating samples.

Figure 2: STR amplification with the help of 3 different STR primers clearly shows polymorphism pattern

By discovering variances in restriction fragment patterns, RFLP analysis enhanced the individual identification procedure. This approach was especially beneficial when STR analysis alone did not yield solid findings.

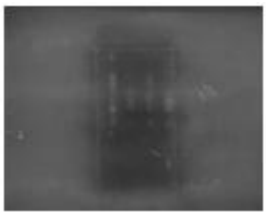

Figure 3: After RFLP different bands are observed under UV light

4. Discussion

The combination of blood grouping, cell count analysis, STR, and RFLP methods demonstrated to be an effective method for distinguishing blood samples from different persons. Each approach added to the overall precision of individual identification.

RBC and WBC cell count analysis revealed variances within the same blood type, whereas blood grouping offered a first classification. Individuals were distinguished using STR analysis, while RFLP analysis offered an extra layer of discrimination where needed.

This comprehensive strategy has substantial ramifications in practice. It can help solve crimes and identify victims in forensic science. It may be used in medical diagnostics to monitor patients' health state, identify illnesses, and test the compatibility of blood donors and receivers.

5. Conclusion

This study shows that combining numerous DNA fingerprinting approaches, such as blood grouping, cell count analysis, STR, and RFLP methods, may efficiently distinguish between blood samples from different persons. This complete technique can improve the accuracy and reliability of person identification from blood samples in forensic science, paternity testing, and medical diagnostics. All human cells have genetic material, and forensic scientists may harvest DNA from these cells. According to Lochard's concept, every interaction leaves a trace, and forensic genetics has progressed to the point where the miniscule quantity of DNA needed for analysis can be retrieved from skin cells, allowing investigators to compare the suspect's DNA to the evidence gathered at the murder scene. In conclusion, DNA fingerprinting is an effective method for figuring out a person's identity and deciphering genetic links. This method is now extensively used and has contributed to both significant medical advancements and the resolution of innumerable crimes. In Maryland v. King,

the Supreme Court ruled that police officers might legitimately collect a cheek swab sample of DNA from someone they had detained for a severe crime. Swabs can be obtained for DNA fingerprinting as part of routine investigative procedures in the USA, according to 28 states and the federal government. Once the unknown individual has been identified and relationships between unresolved instances have been established, these swabs may be compared to the CODIS. DNA fingerprinting is still a useful tool in many sectors, and it will be used for many years to come, despite its limitations and ethical issues, which scientists and law enforcement officials are attempting to address.

List of Abbreviations
HGP: Human Genome Project
VNTR: Variable Number Tandem Repeat
STR: Short Tandem Repeat
RFLP: Restricted Fragment Length Polymorphism
Rh blood group: Rhesus blood group
RBC and WBC: Red Blood Cell and White Blood Cell
CODIS: Combined DNA Index System
Conflict of Interest: None to declare.

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