

## Comparitive Study of DNA Fingerprinting of Biological samplies obtained from Crime Scene using AFLP Methods

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### Abstract:

The use of DNA finger printing technology in the area of crime investigation and individual identification has gained tremendous importance. These are several techniques like AFLP, RFLP, RAPD, VNTR and others which will help to analyze the variations among the test samples based upon the type of fragmentation seen and the band pattern observed. The current work is related to the application of the techniques like AFLP an RFLP in the field of Forensic investigation by individual identification. The current work involves the collection of the various biological samples from the assumed crime scene followed by the use of AFLP technology to digest the samples. This step can be preceded by the separation of the fragmented DNA of both test and the standard samples on the Agarose gel thereby observing the band pattern after agarose gel electrophoresis. The main objective of the study is to compare DNA fingerprints of different DNA samples extracted from various biological sources like hair follicle, blood cell, tooth using the polymorphism in RFLP regions and AFLP to finally establish if they are from the same individual.

**Keywords:** DNA Finger printing, AFLP, RFLP, VNTR and RAPD

### Introduction:

DNA Finger printing is a method of identifying the uniqueness in the samples based on the band patterns obtained after the process of restriction digestion of the samples. The DNA of each individual differs from the other with respect to the junk DNA or heterochromatin. It is this DNA that makes a unique Finger print pattern on gel electrophoresis.

There are certain repeats like STR's, VNTR's etc that would be present at different sites in different numbers among different individuals. Based upon the presence of these patters each individual differs from the other in their DNA Fingerprinting.

DNA fingerprinting has been accepted in most courts in the United States, and has in several notable instances been used to exonerate or free persons convicted of crimes, but the Supreme Court has ruled (2009) that convicted criminals do not have a constitutional right to DNA testing <sup>[1]</sup> There are several techniques within the DNA Fingerprinting method like VNTR, AFLP, RFLP etc. VNTR infers variable number of tandem repeats which are the repeats that are present at variable regions within the DNA. These regions have specific sites that can be digested by specific restriction enzymes. These sites can be used in the individualization of the DNA samples based on their restriction pattern. The probability of matching VNTR pattern between two individual is very much rare as it may occur in one person within twenty billion of people <sup>[2]</sup>. VNTRs are the result of genetic heritability. AFLP'S refers to the polymorphism observed in the band size of the amplified DNA fragments which is caused due to the presence of SNP's Single Nucleotide Polymorphisms. The AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA <sup>[3]</sup> DNA Finger printing van be inferred as a process used in the identification of the individual when an attacker or assailant has left some kind of bodily fluid or blood at the scene of a crime and when no visual identification is possible <sup>[4]</sup>. Not only in the Forensic identification but DNA Fingerprinting is also employed in describing the populations of several phytonematodes, including the pinewood, burrowing, root-knot, and cyst nematodes <sup>[5]</sup>.

### Materials and Methods:

#### *Isolation of DNA from Hair Samples*

The biological samples like Hair, Nail, Blood and buccal cells are generally used for the extraction of DNA so as to identify the individuals based on their DNA Profiling. The current work involves the

collection of Hair follicles from the selected source and processing them for the extraction of DNA. The hair follicles containing the dna has been subjected for DNA Extraction using CTAB Method.

**Isolation of DNA from Blood samples**

The Blood samples were collected from the source and were subjected for the extraction of DNA using Buncee method for the standard operations. This is also called as the rapid non enzymatic method.

**Isolation of DNA from Buccal cells:**

The buccal samples were collected from the selected individuals. The samples were subjected for DNA extraction using Saline method. The samples were processed and observed for the presence of DNA threads.

**Restriction Digestion of Eco RI (GAATTC) region by AFLP method:**

All the DNA samples extracted were subjected for restriction digestion based on AFLP regions focused to Eco R1 sites. The samples obtained were than observed by Agarose Gel Electrophoresis. The band pattern observed was different for different samples which indicate that the DNA collected was of different individuals.

**PCR amplification of AFLP regions using specific primers:**

The above DNA samples were further amplified at the AFLP regions using specific primers that were designed using the PRIMER 3 tool. Primer 3 is a web based tool used to design the primers for a given set of DNA fragments. Once the primer id designed using this tool these primers are further used for the amplification of the selected DNA samples.

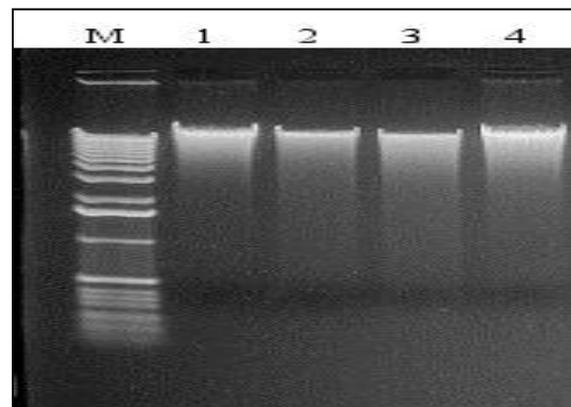
**Results and Discussion:**

DNA samples were collected from three suspects of a crime scene. The samples were from different sources like hair, buccal and blood. The sample collected from the crime scene was the blood. All the four samples were processed for DNA extraction using specific extraction methods. All the extractions resulted in the DNA fragments which were further confirmed by performing AGE so as to observe the bands.

**Restriction Digestion for GAATTC using Eco RI:**

The DNA samples obtained above were digested at the restriction site using the EcoR1 restriction enzyme. The digested samples were further observed by Gel Electrophoresis. It is this step which specifically produces different band patterns for different samples. Here the band is different with respect to the individual from whom the DNA is collected but it would be same for the different samples (hair, buccal, blood etc) of same individual.

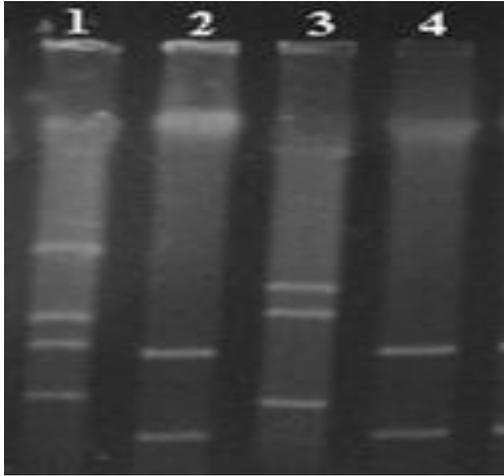
**Fig 1: Showing the DNA samples obtained from the 3 suspects and the sample from then crime scene along with the ladder DNA to prove that the DNA is from humans**



**Amplification of the specific AFLP fragment using PCR:**

To the above digested samples a specific primer is designed based on the site that has to be amplified. The **Primer 3 TOOL** [6] has given the specific primer that has been used for the amplification of the samples. The samples are now kept for amplification so as to process them for further analysis. The amplified fragments were further subjected to AGE to observe the band patterns.

**Fig 2: Showing the bands obtained after the AFLP analysis from the three suspects and one sample obtained from assumed crime scene**



In the above figure the sample 4 is the one obtained from the crime scene and the samples 1, 2 and 3 are the samples of the suspects.

From the above figure it can be clearly seen that the band pattern of sample 2 from the suspects is exactly matching the sample 4m (from crime scene). This can help to conclude that the DNA obtained at the crime scene was of the suspect 2 and the other two suspects did not match the DNA from the crime scene.

#### **Conclusion:**

From the results of restriction Digestion and AFLP analysis it can be concluded that the DNA fingerprints obtained from different individuals do vary in the number and size based on the AFLP sites present. This fact can be of extensive use in the identification of criminals from the DNA obtained at the crime scene. In the current work a total of 4 samples were collected one among which is from the assumed crime scene.

The other 3 samples correspond to the 3 suspects. Upon the analysis of AFLP band patterns it can be inferred that out of the three suspects the suspect sample 2 matches the band pattern of the sample obtained from the crime scene. This confirms that the DNA obtained at the crime scene is the sample of the suspect 2.

The main objective of the work is to highlight the importance of the AFLP methodology in the field of individual identification.

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