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DNA FINGERPRINTING OF DIFFERENT BIOLOGICAL SAMPLES OBTAINED FROM CRIME SCENE USING AMELOGENIN GENE AMPLIFICATION BY PCR, VNTR AND RFLP ANALYSIS

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Abstract: DNA is the material within every cell of the body and represents the blueprint of life. Although the majority of the human genome (the complete set of genes for an individual) is the same across all ethnic populations, people differ in their genetic makeup by a minuscule amount, and thus have their own unique DNA pattern. In forensic science, DNA profiling is used to identify those who have committed a crime and used to find suspects involved in crimes of unsolved cases. Here in our study we have done Amelogenin gene amplification by PCR to gender identity in DNA typing. The polymerase chain reaction, a technique that can amplify large amounts of specific small sequences of DNA from the human genome. Additionally, in VNTR analysis, genomic DNA is digested with restriction enzymes and then run on a gel. The fragments produced are transferred to a membrane and probed with a radiolabeled sequence of DNA that matches the VNTR sequence. The migration of the VNTR fragment on the gel determines their size and generates a pattern. The radiolabeled probe produces dark bands on x-ray film. This analysis has been done for different biological samples like buccal cells, blood, nail, hair and was compared using PCR, RFLP and VNTR.

Key Words: Amelogenin gene, PCR: polymerise chain reaction, VNTR: Variable Number Tandem Repeats, RFLP: Restriction Fragment Length Polymorphism.

INTRODUCTION

DNA Forensics is a branch of forensic science that concentrates on the employment of genetic component in crime investigation. Besides the helping with human crimes like rape and murder, DNA Forensics could be used to track food borne epidemics, determine endangered species in shipments of black-market material, and to trace the history of humans over the world, among other things. DNA is the Nucleic Acid molecule that contains genetic information. All organisms carry various amounts of DNA which introduces many characters of organisms like hair and eye colors, and so on. Laboratory techniques could be used to recognize and isolate DNA and then to sequence it. DNA Sequencing includes figuring out the order of the four nucleotides in a strand of DNA (Chambers *et al*. 2014).

DNA Fingerprinting is a method to identify an individual, rather than determining a species or some particular traits. It is also known as DNA profiling. As a technology which has been around since 1985, when it was announced by its inventor, Sir Alec Jeffrey's (1985). DNA Fingerprinting is recently used for both paternity/maternity and for identifying criminals or victims. DNA fingerprinting employs a specific type of DNA sequence, called a micro satellite, to make identification pretty easier. Micro satellites are short segments of DNA which repeat over and over in an exceedingly given person's DNA. In a given area Micro satellites tend to be extremely variable, providing them ideal for DNA finger printing. By comparing a variety of microsatellites in a given area, identification of a person would be relatively simple (Abdul *et al.,* 2014). The sections of DNA employed in DNA fingerprinting, though are highly variable, no child has pairs that their parents do not have. This means that by comparing large number of these sections, paternity, maternity, or even both could be found out. DNA fingerprinting has a very high successful and a low false rate, making it an extremely popular way of verifying paternity and maternity (Yuki *et al.,* 2014 and Meyer *et al.,* 2013).

In Forensics, DNA Fingerprinting is extremely important because it doesn't require typical fingerprints, which may not be left behind, could also be obscured. Because all of the DNA components are contained in every cell, any piece of a person's body, from strand of hair to a skin follicle to a blood drop, can be utilized to identify them using DNA fingerprinting (Ramilo *et al.,* 2002). It is useful in terms of investigating a criminal, because just a drop of blood or a flake of skin left at the crime scene may be enough to determine guilt or innocence, and it is virtually impossible to remove all physical trace of one's presence. According to LOCARD'S exchange principle, every time when someone enters an environment, something is added to and removed from the scene. Every contact between individuals and physical environment leaves a trace.

In spite of many problems, such as the probability of detecting the precise criminal or parentage becomes obscured once dealing with direct descendents, who might share a large portion of the examined places of DNA with a parent. DNA

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fingerprinting is becoming more common in the world of criminal forensics. Although a few legal queries exist, like the decisiveness of DNA fingerprinting and the extent to which it is legal by national laws to collect databases of people's DNA and use their DNA samples for comparison, the pros currently seem to outweigh the cons (Chisum & Turvey 2000).

Gender identity in DNA typing

Amelogenin gene located on both X and Y chromosomes and X chromosome Amelogenin gene is shorter by 6 nucleotides. Sex determination by Polymerase Chain Reaction (PCR) analysis of the X-Y homologous amelogenin gene is highly reliable since the detection of an X-specific amplified fragment validates the procedure. This is a powerful technique for the analysis of trace forensic samples (PRIMORAC *et al.,* 2014).

Variable Number Tandem Repeats (VNTRs)

VNTRs, a type of RFLP (Restriction Fragment Length Polymorphism), are regions of DNA comprising a thousand to several thousand base pairs. These are not genes, for they produce no product, which means that they are not responsible for any observable genetic traits. A typical VNTR is made up of a large number of tandem repeated units. The size of each repeat unit in different VNTRs used for forensic analysis varies from 8 to 80 base pairs (usually 15 to 35) (McClintock 2014).

Although the unit size is nearly constant at a given VNTR locus, the number of repeats is highly variable, so that there are sometimes a hundred or more different lengths observed in different persons. It is this great variability in length that makes VNTRs so useful for forensic analysis. The technique for determining the length of a particular VNTR is simple in principle. The DNA is first extracted from whatever material is to be analysed. It is then treated with an enzyme that cuts the DNA at every point where a specific base sequence occurs. The collection of fragments is then placed on a flat gel, which is exposed to an electric field. The fragments migrate at different rates, depending on their size, a process called electrophoresis. The separated fragments are then treated chemically to denature them, that is to separate each fragment into two single strands of complementary base sequence. Because gels are somewhat fragile and awkward to handle, the fragments are transferred by direct contact to a nylon membrane, to which they adhere (much as a blotter picks up ink from a paper).

Thus the fragments are located on the membrane in positions congruent with their positions in the gel. The membrane is then flooded with a probe, which is a short, single–stranded piece of DNA that is complementary to a particular fragment among the millions on the membrane. Because of the specific DNA base-pairing rules (A with T and G with C), the probe finds and attaches itself to the appropriate fragment, one in which the complementary bases match those of probe. Any excess probe that does not bind to a DNA fragment is washed off. Attached to the probe is a label used to signal its presence. This is detected by a photographic film placed in labels were radioactive contact with the nylon membrane.

Originally, labels were radioactive atoms, exposing the film at sites corresponding to the probe's position on the membrane. Although radioactive probes are still employed in some settings, luminescent detection is now more commonly employed. The membrane is coated with a material that is converted into light by an enzyme attached to the probe. The light output is captured on film over a period of a few hours. The position of bands visualized in this process is used to characterize the original DNA sample.

Restriction Fragment Length Polymorphism (RFLP)

A RFLP represents a stretch of DNA that serves as a marker for mapping a specified gene. RFLP's are located randomly throughout a person's chromosomes and no apparent function. A DNA molecule can be cut into different fragments by a group of enzymes called restriction Endonucleases. These fragments are called polymorphs. The basic principle of RFLP is separation of the desired repetitive sequences by cleaving them out from the genome using an appropriate restriction endonuclease enzyme, electrophoresis of the digested DNA and thereafter their detection by DNA probes. According to the nature of core sequence of the mini satellite, restriction enzyme and probe are selected. RFLP was adapted for forensic DNA analysis in human identification. This kind of analysis determines variation in the length of a defined DNA analysis in human identification. This kind of analysis determines variation in the length of a defined DNA fragment (McClintock 2014).

Restriction Endonucleases

Restriction Endonucleases identify and cleave DNA at specific sequences (recognition sequences or restriction sites) to come up with a group of smaller fragments. Restriction Endonucleases are found in a large number of bacterial species. Werner Arber (1960) discovered their biological function is to recognize and cleave foreign DNA (the DNA of an infecting virus, for example); such DNA is claimed to be restricted (Sunar 2014). There are three types of Restriction Endo nucleases, designated as I, II, III (Table. 1). Types I and II are usually large, multi-subunit complexes consisting both the Endo nuclease and methylase activities and

Type I Restriction Endonucleases cleave DNA sites haphazardly that could be more than 1,000 base pairs (bp) from the recognition sequence. Type III Restriction Endo nucleases cleave the DNA regarding 25 bp from the recognition sequence. Both types move toward the DNA in a process that needs the energy of ATP. Type II Restriction Endo nucleases, first isolated by Hamilton Smith in 1970, are simpler, require no ATP, and cleave the DNA within the recognition sequence itself. Daniel Nathans, who first utilized them to construct novel methods of mapping and analyzing genes and genomes, demonstrated the extraordinary utility of this group of Restriction Endo nucleases. The common size of the DNA fragments generated by cleaving genomic depends on the frequency with which a specific restriction site happens within the DNA molecule; this in turn depends mostly on the size of the recognition sequence (Sharma *et al.*, 2013)

Table 1: Restriction Endonucleases

Source Organism	*Hind III*	*Eco RI*	*Bam III*
	H. influenza	***E. coli***	***C. albidum***
Restriction site sequence	...AAGCTT... ...TTCGAA...	..GAATTC.... ..CTTAAG....	..GGATCC.. ..CCTAGG..
Restriction site sequence after cut	..A AGCTT..... ..T TCGAA.....	.G AATTC.. ..CTTAA G.	..G GATCC.... ...CCTAG G..
Restriction enzyme reaction condition	37°C	16°C	37°C
Cut end	Blunt Ends	Sticky Ends	Sticky Ends
Heat Denaturation	At 65°C for 15 min.	At 65°C for 20 min.	At 65°C for 15 min.

13 CODIS region

DNA is present in almost every cell of our bodies, and we leave DNA traces wherever we go unknowingly without even noticing it. Drops of blood, skin flakes, hair, and saliva all possess DNA that would be employed to identify us. In fact, the study of forensic sciences, usually utilized by police departments and prosecutors all over the world, frequently depends upon these small segments of DNA to connect criminals to their committed crimes. This enchanting science is often pictured on famous television shows as an easy, precise, and inerrant method of finding a delinquent and bringing him or her to justice.

In truth, however, testing out a DNA fingerprint and determining the likelihood of a match between a suspect and a crime scene is a complicated process that depends on the probability to a larger extent than most of the people notice. Government-administered DNA databases, like the Combined DNA Index System (CODIS), do facilitate to make the process fast, but also they bring to light complex moral problems including the rights of victims and suspects alike. Hence, understanding the ways in which DNA evidence is obtained and analyzed, what this evidence will prove investigators, and how this proof is employed through the legal system is critical to appreciating the actual ethical and legal impact for Making an STR Match**.**

In order to match, for example, crime scene evidence to a suspect, a lab would determine the allele profile of the 13 core STRs for both the evidence sample and the suspect's sample. If the STR alleles do not match between the two samples, the individual would be excluded as the source of the crime scene evidence. However, if the two samples have matching alleles at all 13 STRs, a statistical calculation would be made to determine the frequency with which this genotype is observed in the population. Such a probability calculation takes into account the frequency with which each STR allele occurs in the individual's ethnic group. Given the population frequency of each STR allele, a simple Hardy-Weinberg calculation gives the frequency of the observed genotype for each STR. Multiplying together the frequencies of the individual STR genotypes then gives the overall profile frequency.

Sample Collection from Crime Scene

Any dead or living matter, small or even minute can give us a lot of information. Samples like soil, glass paint, metal scraping, fabric or leaving matter like Blood, Saliva stains, Hair, and wood can be useful. Various biological samples like Blood, Hair, Buccal, Nail were taken in the present study. Many types of kits are available for sample collection, mostly used once are mentioned below:

Whole Blood Collection

The Blood Collection Kit would require qualified medical staff to perform the collection of the blood sample. The kit includes all essentials for collecting a small blood sample from the individuals. It contains a syringe to draw blood, sterile gauze pad, a glass vial to hold the sample, security and biohazard labels and a padded envelope to protect the blood sample while it is in transition. The kit comes in a small box which could be utilized to post or submit the blood sample to a laboratory. It is considerable that the

officer involved be present and witnesses the collection process.

Buccal Swab

The Buccal Swab Collection Kit is in the form of a medium size envelope which can be mailed or hand submitted to the laboratory. This kind of collection could be done by law enforcement personnel and does not require medical staff to perform the collection. The simple direction in the kit is followed as to the collection of the sample by rubbing the cotton swabs in the individual's mouth or inside of the cheek. Gloves are provided in the kit, along with security labels and documentation forms. Once the collection is finished, the swab is placed within the smaller internal envelope, seal it with one of the enclosed evidence seals and ship the sample to the desired laboratory.

Hair

The examination of hair or fibers found at the scene of a crime, on weapons, clothes, etc, is however of much complicated investigation of crime itself. Samples of hair removed from different parts of the motorcycle and car are often sent by authorities for examination and comparision with hair on the head of the deceased person who has been the victim of the road accident. In sexual offence, especially in the rape and bestiality, and examination of hair frequently becomes momentous. In chronic poisoning by m*etal*s, test of hair enables essential information for the investigation of crime (Walbank & McDonald 2012).

Nail

Nails can be compared with claws in other animals. Fingernails and toenails are made up of a hard protein called keratin, as is present in animals' hooves and horns. The mammalian nail, hoof, claw and horn are all examples of *unguis* [plural *ungues*]. (Vass *et al.,* 2013)

Isolation of DNA from blood sample by Bunce method

The blood consists of proteins, fats, WBC and RBC. The small amount of DNA is present in the white blood cells. Whereas the RBC do not contain any DNA in it. The solution A is an anionic detergent used to remove the RBC present the blood cells. The solution A consist of sucrose in it form that a protective layer around the WBC which protect the WBC from breakage. $MgCl_2$ present in the solution A helps in the lysis of RBC. Centrifugation at 5000rpm helps in the precipitation of WBC cells followed by the removal of supernatant containing RBC. The addition of solution B which consists of Nacl will act on the cell wall of WBC and helps in the breakdown of WBC. DNA is collected after centrifugation and precipitated by iso-propanol. Finally centrifugation at maximum speed helps the DNA to settle down as pellet which is collected and stored in TE buffer.

In brief, 1ml of anti-coagulated blood (EDTA) was added to 3ml of solution A (10mM Tris, 10mM KCl, 10mM $MgCl_2$ and 2mM EDTA) vortexed it slowly for 10min. Incubated for 5 min at 37°C. At the end of the incubation centrifuge at 5,000rpm for 5min. To the pellet added 2 vol. of solution B10mM Tris, 10mM KCl, 10mM $MgCl_2$, 0.4M NaCl and 2mM EDTA) and vortexed. To the pellet 2 vol. of solution B added, mixed and incubated at 65° C for 30 min. To the above mixture 650µl of ice cold chloroform was added mixed vigorously for 60 min. Centrifuged at 4000rpm for 10 min. Equal vol. of cold Isopropanol was added to the supernatant and incubated at -20° C for 30 min to precipitate DNA. Washed the DNA pellet with 70% ethanol and air dried the DNA pellet. Dissolve the DNA pellet in 100 µl of TE buffer.

Isolation of DNA:

DNA isolation carried by sambrook method and buccal sample DNA by saline method

Isolation of DNA from Hair Follicle by Proteinase K Method: We isolated 3-4 Hairs containing follicles, and placed the hairs in the bottom of the eppendorf tube. We added 500µl of lysis buffer solution and incubate at 50°C for 15mins, and cooled it for 30sec. Then we mixed by vortex for 15sec, and incubated in boiling water bath for 10mins, and cooled in ice for 2mins. Again we mixed by vortex for 10sec and centrifuged at 10,000rpm for 5mins. We took the supernatant and added equal volume of ice cold iso-propanol. Then we incubated at -20°C for 30mins, and centrifuged at 12,000rpm for 5mins. Finally we took the pellet and washed in 70% ethanol, centrifuged at 12,000rpm for 5mins and removed the supernatant. The pellet was dried by the air and then dissolved in TE buffer.

Isolation of DNA from Nail Sample

Sample preparation: Nail clippings were collected and each of them was cut and sliced with the help of forceps and knives and ground to fine powder with the pestle.50mg of the powdered sample was weighed and added to each 1.5ml eppendorf tube. The sample was then washed with 1ml absolute ethanol, followed by washing with 1ml distilled water twice. These washes remove off traces of blood and surface contaminants. Distilled water wash was done to ensure complete removal of ethanol. We added lysis buffer solution and proteinase K to the sample, and incubated at 60°C for 4hrs. Then we mixed by vortex for 30sec. It was centrifuged at 10,000rpm for 10mins. We collected the supernatant into the different fresh eppendorfs and discarded the pellet, and added equal volumes of

chloroform and isoamyl alcohol (24:1) in one eppendorf tube, 50µl of 3M sodium acetate to one tube and other was kept on ice for 10mins. Then we centrifuged at 10,000rpm for 10mins, and collected the upper aqueous solution (in case of chloroform and isoamyl alcohol extraction) into fresh tubes. For remaining tubes we collected the supernatant into fresh tubes. To the tubes containing solution, we added equal volume of ice cold isopropanol. Then we incubated at 4°C for 24hrs / -20°C for 30mins, and centrifuged the tubes at 12,000rpm for 5mins. Finally we discarded the supernatant immediately. The pellet was dried by the air and added 50ml of TE buffer to the tubes.

Restriction Digestion of DNA Sample Using Restriction Endonucleases

We took 7µl of standard DNA sample in a fresh clean eppendorf. We added 2µl of restriction enzyme (EcoRI), and then added 2-5µl of 10X assay buffer to the sample and made the volume upto 25µl by adding distilled water. We incubated the tubes at 37°C for 1hour (to accelerate the reaction) and stored the tubes at 4°C for further analysis. Finally by running agarose gel electrophoresis we examined the gel under uv transilluminator.

Southern Blotting

DNA sample is digested with restriction enzymes. Digested sample is run by electrophoresis on agarose gel. Gel is placed on top of the buffer-soaked filters and a piece of transfer medium (nylon membrane) is placed on top of the gel completing the gel sandwich. Dry filter papers are then piled on top of the filter and a weight is put on it. Capillary action pulls the buffer from the bottom of the filter through the gel, to the transfer medium and up through the paper towel stack. This arrangement is left undisturbed for few hours or overnight and then top filters and weight is removed. SS DNA fragments are trapped on the transfer medium (nylon membrane) the nylon Membrane is baked at 80°c and cross linked the strands with UV light. DNA fragments on membrane are further used for probing of desired sequence by hybridisation. Membrane is washed to remove unbound DNA. The membrane was tagged with specific probe and observed (Figure 1D).

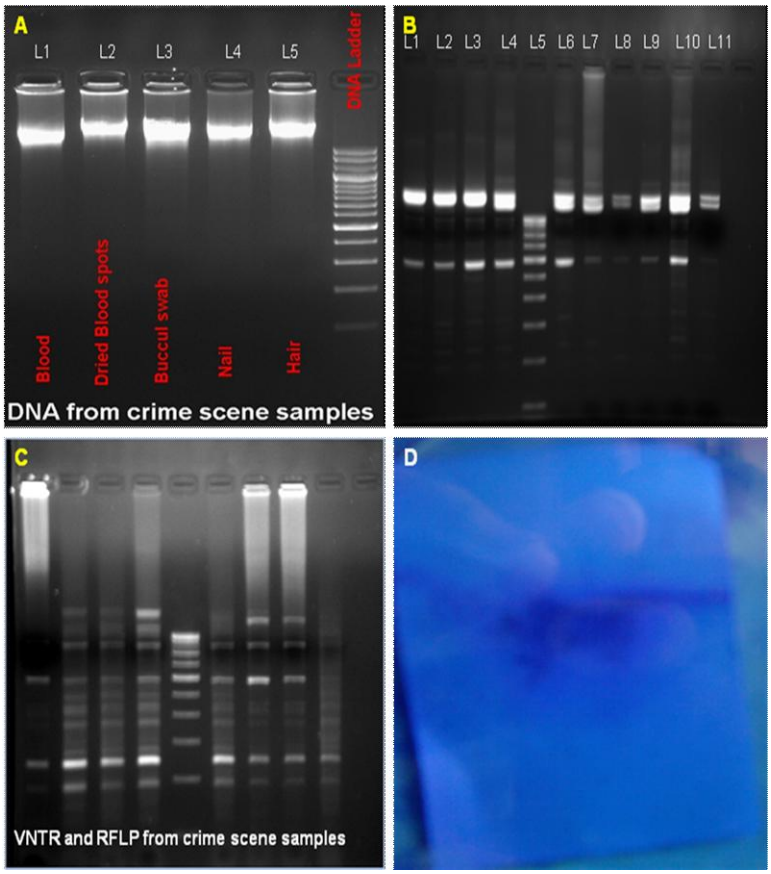

Figure 1.
A. DNA from different biological samples of crime scene.
B. Amplification of amelogenin gene,
C .VNTR and RFLP,
D. Southern Blotting

RESULTS AND DISCUSSION

DNA from 10 different biological samples was isolated by various isolation methods, amplified amelogenin gene by PCR and separated on agarose gel electrophoresis. Using restriction enzymes the DNA was digested and specific VNTR fragments were again amplified and were separated on agarose gel by performing agarose gel electrophoresis, and were finally observed under UV trans-illuminator (Figure 1). Bands of hair sample matched with blood sample. The gel was blotted on nylon membrane and blot was dry.

In-vitro amplification of DNA via PCR is one of the technical achievements of molecular biology that will have a profound impact on forensic science. The first reports of PCR performed on forensic samples were presented by Bugawan *et al.*, (1989) and Higuchi (1963). Since PCR has some indisputable advantages over the existing serological and RFLP DNA methodology used recently in forensic caseworks, potential applications of PCR are being investigated intensively. One approach to analyze PCR products in forensic work is amelogenin gene to detect gender for DNA typing. As a second approach for applying PCR in forensic analysis, the RFLP technique has become increasingly studied.

CONCLUSION

In our study, we extracted DNA from 10 different samples and amplified amelogenin gene to identify gender for DNA typing, simultaneous performed RFLP and VNTR are the traditional genetic markers as well.

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