Robbery with Murder Solved by DNA profiling.

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Abstract :- DNA profiling has become an established part of criminal justice process, and the admissibility of the test results in the court has become routine. It has ability to eliminate the suspect in the cases where DNA profile of suspect failed to match with the evidence sample. In most cases, the best it can ever do is to place a suspect at the scene of the crime. The spots of blood give forensic scientists a chance to track down a least one or two suspects in mass crimes like murder, robbery, gang rape etc. In past decade, DNA profiling has been used widely to link suspects to the blood, hair, tissue found at crime scenes as well as on clothes or weapons recovered from suspects. With the use of short tandem repeat DNA profiling technique, it is possible to find out the involvement of suspects in the crime. In the present case, four suspects, in the parking area of the building, while robbing a bag full of about 6,00,000/- Rs., killed a businessman with knife as he opposed them. While running after the act, one of the suspect got injured with the broken pieces of glass adhered on the wall compound. Police submitted clothes of accused, deceased along with knife and crime scene articles. DNA profile obtained from blood found on clothes of accused, weapon, on the bag containing money and crime scene articles matched with DNA profile of deceased. Thus, the DNA profiling technique not only proved involvement of suspects in the murder but also in robbery.

Key words: Polymerase Chain Reaction, DNA, Short Tandem Repeat, Genotype, Allele.

Introduction: DNA profiling came into use around three decades ago, in the late 1980s. It was first developed in England in 1985 by Sir Alec Jeffreys [1, 2]. It has been a useful tool in law enforcement as it works both ways, securing correct convictions and also exonerating the innocent. Furthermore, DNA profiling unlike other forensic evidence, can be collected easily and sustains for long thereby increasing chances of accurate analysis by manifold. It has become important method for human identification by introducing the study of microsatellite regions – Short Tandem Repeats (STR) Loci in criminal and civil cases [3, 4, 5]. The STR fragments are separated and detected by using capillary electrophoresis. Certain regions of DNA contained repeated DNA sequences. The regions with repeat units that are 2-6 base pair in length are called Short Tandem Repeats. The first STR multiplexes developed was quadraplex created by Forensic Science Services (FSS) that comprises four STR Loci [6]. Short tandem repeat (STR) analysis is regularly performed for generating DNA profiles from blood, saliva and hair samples encountered as evidence to solve the crime [7].

While alternatives exist, most DNA typing laboratories use commercially available kits to amplify and label STR alleles associated with evidence and reference samples that are the size fractioned with Capillary electrophoresis instruments such as ABI 310 or 3130 Genetic Analyzer and latest 3500 Genetic Analyzers[8,9,10,11]. Software GeneMapper® is used to determine the presence or absence of STR alleles associated with the sample.

Traditional workflow for generating DNA profiles includes below steps:

1. Extraction of DNA from body samples found on different substrates
2. Quantification of extracted DNA
3. Amplification of DNA using polymerase chain reaction (PCR) based STR reactions
4. Denaturation of amplified DNA
5. Genotyping using short tandem repeat (STR) technique
6. Analysis and comparison of generated DNA profiles.
7. Now-a-days, advanced instruments such as AutoMate Express and EZ1 Advanced which work on robotic principle are helpful to minimize the analysis time. Commercially available kits for amplification of DNA allow the faster turn-around time [12, 13].

Our forensic science laboratory had received a case of robbery with murder. The deceased was a businessman. In the evening, while returning to home from the shop, he carried a cash of about 6,00,000/- in his bag. His son was with him. Four persons who came to know about money in his bag, chased them and reached to building parking where deceased used to live. There they tried to pull his money bag and as he opposed, they killed him with knife and ran away after robbing the bag. While running, one of the four persons, jumped from the wall compound on which glass pieces were adhered. His hand got injured. The complaint was lodged to the police station under IPC 394, 396, 397, 302 Arms Act 4/25. Such type of robbery with attempt to murder or murder cases are very common in Maharashtra now a days. So, to control the crimes, police has to face lot of challenges. They need to produce proper evidences in the court to convict the accused. They submitted blood stained exhibits from crime scene, clothes of all the four accused, clothes of deceased and postmortem blood of deceased, reference blood samples of all the accused in DNA kit. Our laboratory analyzed the samples by DNA profiling technique and found that two out of four accused connected with the crime. According provided the report to police authority for further court procedure.

Materials and Methods:

Detection of blood on the clothes:

Blood stains on the clothes of the deceased, accused and crime scene were confirmed by testing with Kastle-Meyer solution (Phenolphthalein solution) and 3% Hydrogen peroxide. Hemoglobin in the blood catalytically decomposes Hydrogen peroxide to release nascent oxygen which reacts with Phenolphthalein to give pink color. Blood was detected on car cover, Full shirt of deceased, Money bag seized from accused 1, Full shirt of accused 1, Full pant of accused 2, Full shirt and Full pant of accused 3 and Knife.

After confirming the presence of blood, DNA analysis of blood stains was performed.

Instruments and chemicals used for DNA analysis were as follows-

PrepFiler Express DNA extraction kit. Lot No. 1807201.

AmpFlSTR® Identifiler kit. Lot No. 1807261

HiDi Formamide.

Liz 600 Size standard.

Quantifiler Duo DNA kit. Lot No. 1710101.

AutoMate Express™ Forensic DNA Extraction System. Catlog number: 4441763

PCR thermal cycler GeneAmp 9700. Catlog number: 4375786

3500 Genetic Analyzer. Catlog number: 4406017

Isolation of DNA: For extraction of DNA the PrepFiler™ Forensic DNA extraction Kit (Applied Biosystems) was used. It enables the isolation of DNA from biological samples that contain small quantities of biological material in such a way that it removes the substances interfering with PCR. Additionally, the extracted DNA is having sufficiently high concentration due to which the volume of extract for downstream analysis is minimal [(14)].

Blood stains on all the positive articles were cut into small 1 x 1 mm pieces and were placed in 2ml micro centrifuge tube. For the reference profile, 40 µl blood samples of all the four accused and deceased were taken into another micro centrifuge tubes. 500 µl Lysis buffer from PrepFiler Express F DNA extraction kit [(15)] was added to all the sample tubes. The sample tubes were kept on thermo shaker at 750 rpm at 70˚C for 40 min. The tubes were then centrifuged at 10,000 rpm for 2 min. Cartridges from PrepFiler Express F DNA extraction kit were loaded to the cartridge rack in AutoMate Express DNA extraction system [(16)], Sample tubes, elution tubes and tips were loaded as per machine guidelines and the machine program was run as per the recommended machine protocol. After completion of program, elution tubes containing extracted DNA in highly pure form was stored at 4˚C till the next PCR amplification process.

Quantification of the extracted DNA:

Extracted DNA was quantified using Quantifiler® Duo DNA Quantification Kit[(17)] on an Applied Biosystems 7500 Real-Time PCR System according to manufacturer recommended protocols. Quantified DNA was taken for downstream application.

PCR based STR Analysis: The quantified DNA extracted from all the blood stains and reference blood samples of all the accused and deceased was processed for Polymerase Chain Reaction using the AmpFlSTR® Identifiler PCR Amplification Kit (Applied Biosystems) [(18)] with the help of PCR thermal cycler GeneAmp 9700[(19)] following the protocols recommended by the manufacturer. This kit contains Reaction mixture, Primer set and Taq Gold Polymerase enzyme. Primer Set contains locus-specific 6-FAM™, VIC™, NED™ and PET™ dye-labeled and unlabeled primers in the buffer. The primers amplify the STR loci CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, vWA and gender marker Amelogenin.

Reaction mixture used for PCR was prepared by adding Reaction mix 10.5 µl, Primer set 5.5 µl and Taq Gold DNA Polymerase 0.55 µl. The extracted DNA sample 10 µl was added to it. The DNA was amplified in 28

cycles using PCR machine selecting 94.0 ºC, 59.0 ºC and 72.0 ºC as temperatures of denaturing, annealing and extension respectively (Figure 1) (Table 1).

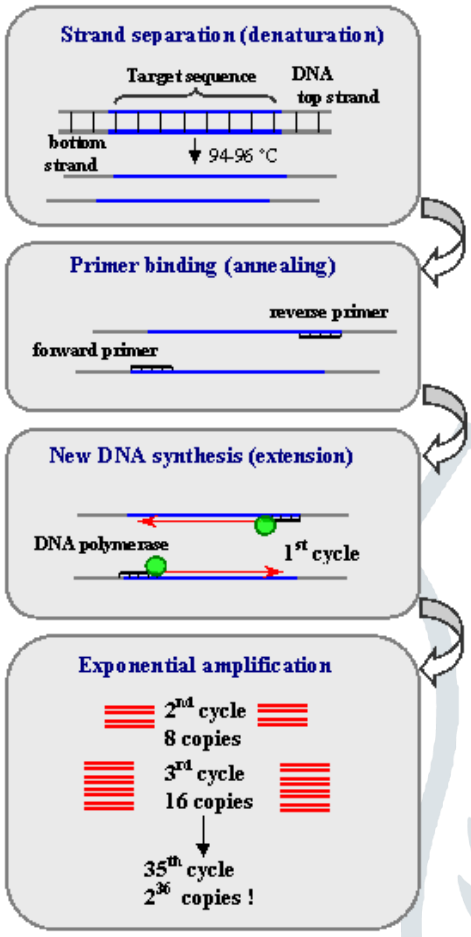

Figure 1

Step	AmpliTaq Gold Enzyme Activation	PCR			PCR Final Step	PCR product till separation of STRs
	Hold	CYCLE (28 cycles)			Hold	
		Denaturation	Anneal	Extend		
Temp	95 ˚C	94 ˚C	59 ˚C	72 ˚C	60 ˚C	4 ˚C
Time	11 min	1 min	1 min	1 min	60 min	∞

Table 1: PCR Protocol used for amplification of DNA

The amplified DNA samples were kept at 60.0 ºC for an hour and then at 4.0 ºC till the separation of STRs. PCR produces millions of DNA fragments of different sizes. Amplified products were separated and detected using 3500 Genetic Analyzer [20] and analyzed using GeneMapper® ID-X Software V 1.5. The separation of different fragments of DNA molecules on the basis of their sizes was achieved by capillary electrophoresis. Simultaneous amplification of 16 STR Loci was completed and analyzed [21], [22]. DNA profiles obtained were interpreted by comparing with each other.

Results and Discussion: The extracted DNA was typed at 15 STR Loci and gender specific Amelogenin locus using PCR amplification technique. The DNA profiles obtained from blood detected on Car cover collected from crime scene, Full shirt of deceased, Money bag recovered and seized from accused 1, Full pant of accused 2, Knife recovered and seized from accused 3, Full pant and Full shirt of accused 3 were found to be identical and from one and the same source of male origin and matched with DNA profile of deceased (Table 2).

STR Locus	GENOTYPE					
	Car cover (Scene)	Full shirt (Deceased)	Bag (Accused 1's home)	Full shirt (Accused 1)	Full pant (Accused 2)	Knife (Seized from accused 3)
D8S1179	14,15	14,15	14,15	11,12	14,15	14,15
D21S11	28,31.2	28,31.2	28,31.2	32.2,32.2	28,31.2	28,31.2
D7S820	11,11	11,11	11,11	11,11	11,11	11,11
CSF1PO	10,11	10,11	10,11	10,11	10,11	10,11
D3S1358	15,16	15,16	15,16	15,16	15,16	15,16
THO1	7,7	7,7	7,7	6,6	7,7	7,7
D13S317	8,9	8,9	8,9	8,12	8,9	8,9
D16S539	11,13	11,13	11,13	9,12	11,13	11,13
D2S1338	24,25	24,25	24,25	17,22	24,25	24,25
D19S433	14,15	14,15	14,15	13,13	14,15	14,15
vWA	17,18	17,18	17,18	15,17	17,18	17,18
TPOX	9,11	9,11	9,11	9,9	9,11	9,11
D18S51	13,18	13,18	13,18	13,14	13,18	13,18
AMELOGENIN	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D5S818	13,13	13,13	13,13	10,12	13,13	13,13
FGA	22,22	22,22	22,22	21,24	22,22	22,22

Table continue..

Table 2: DNA Profiles obtained from the blood stains found on exhibits

STR Locus	GENOTYPE						
	Full pant (Accused 3)	Full shirt (Accused 3)	Blood (Deceased.)	Blood (Accused 1)	Blood (Accused 2)	Blood (Accused 3)	Blood (Accused 4)
D8S1179	14,15	14,15	14,15	11,12	12,14	14,15	12,14
D21S11	28,31.2	28,31.2	28,31.2	32.2,32.2	29,32.2	30,31	32.2,32.2
D7S820	11,11	11,11	11,11	11,11	8,8	9,11	11,13
CSF1PO	10,11	10,11	10,11	10,11	10,11	11,12	11,12
D3S1358	15,16	15,16	15,16	15,16	15,15	16,17	17,18
THO1	7,7	7,7	7,7	6,6	8,9	9,9	7,8
D13S317	8,9	8,9	8,9	8,12	8,13	11,12	9,12

D16S539	11,13	11,13	11,13	9,12	11,13	9,11	11,12
D2S1338	24,25	24,25	24,25	17,22	18,24	19,25	24,25
D19S433	14,15	14,15	14,15	13,13	13,14	13,14.2	13,13
vWA	17,18	17,18	17,18	15,17	16,16	14,15	16,18
TPOX	9,11	9,11	9,11	9,9	11,11	11,11	8,11
D18S51	13,18	13,18	13,18	13,14	13,14	14,19	16,16
AMELOGENIN	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D5S818	13,13	13,13	13,13	10,12	11,12	10,11	12,12
FGA	22,22	22,22	22,22	21,24	21,24	22,23	20,22

Table continued..

Table 2: DNA Profiles obtained from the blood stains found on exhibits

As DNA profile of deceased obtained on clothes of accused 2 and accused 3, involvement of both of them proved in the murder. The DNA profile obtained from blood found on Full shirt of accused 1 matched with DNA profile of accused 1 himself. This might be due to injuries happened to him from glass pieces while climbing from the wall. But, DNA profile of deceased found on the bag recovered and seized from accused 1, proved his involvement in murder and robbery. DNA profile of deceased also found on Knife which was seized from accused 3 further proved his involvement in murder.

Conclusion: Robbery and theft along with murder and sometimes rape is increasing now a days. To control such crimes, it is necessary to provide strong evidence in the court so as to increase the conviction rate. It is skill of the analyst to get the DNA profile from the available biological samples without wasting it. Further, if the sample is received to the laboratory in proper condition, it becomes somewhat easy to perform the analysis. This is one of the most helpful techniques to solve the complicated heinous crimes. In the present case, as the three accused out of four connected with the crime by the DNA profile of deceased on their clothes and exhibits recovered from them, their involvement in the same crime has been proved. As the money bag was also stained by the blood of deceased and it was containing money, their purpose of robbery also proved.

Acknowledgement:

We are thankful to the Director General, Legal and Technical, Mantralaya, Mumbai, Home Department, Government of Maharashtra, India for his guidance and encouragement all the time extended to us.

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