International journal of basic and applied research
www.pragatipublication.com
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.86

Murder or suicide – crime scene reconstruction through DNA profiling to solve mystery surrounding double murder

Rajeev Kawatra* & G. Pandu
Forensic Science Laboratory, Madhuban, Karnal, Haryana, India - 132037

Abstract: DNA profiling is proving very useful to recreate the crime scene in homicide cases. For example a double murder shocked the Karnal city of Haryana State in India and mystery surrounded on it. The case involved Sham Lal, Navin (his son), Suman and Rohit (her brother). One day after returning back from parental house along with her brother Rohit, Suman had altercation with her husband. Upset with the insult of the sister, Rohit fired on Navin with revolver who died on the spot due to lethal injury. Rohit also died later due to gun fire in mysterious circumstances. The police heard two versions about the murder of Rohit. As per testimony of Sham Lal, he escaped from the incident immediately after the death of his son Navin and told that Rohit have committed suicide after murdering Naveen as told to him by his wife. On the contrary Suman stated that Sham Lal has infact snatched the revolver from Rohit after murder of Naveen and fired Rohit with the same revolver to take revenge of his murdered son. Forensic examination was carried out and a minute greasy only 2mm size, blood stain was detected on the pajamas of Sham Lal. DNA profiling test of blood stain on the cloths of accused and deceased's was conducted. However, only unclear mixed profile of Autosomal STR using Identifile kit was obtained in the blood stain on the pyjama of Sham Lal. The DNA extracted was further processed for Y-STR markers by PCR amplification. Full profile of all the 16 Y-STR markers was obtained on Genetic Analyzer which was compared with DNA profile of the blood stains on the cloth of both the deceased persons. After comparison of DNA profile it was observed that DNA profile of the blood stain on the pajama of accused Sham Lal was matching with Rohit. It proved that Rohit did not committed suicide and was murdered by Sham Lal who was refusing his presence at the time of death of Rohit. Sham Lal was held guilty of murder in the Court of Law and convicted.

Keywords: Minute blood stain, Crime Scene, Y-STR, Homicide, DNA profiling

INTRODUCTION

Ever since the discovery of DNA fingerprinting by Sir Alec Jeffrey in 1985 and its use in a paternity issue, it has been widely used and developed. Human Genome Project acted as catalyst in new sequencing techniques and instrumentation. It is now best technique for human identification and comparison in several types of crime cases like murder, rape, paternity etc. Due to its extreme power to differentiate DNA of two individuals, it is accepted in judiciary beyond doubt. The witness may turn hostile in the court but DNA profiling report is irrefutable.

In India Dr. Lalji Singh first introduced this technique. He established in 1980s that a *Bkm*-derived probe can be used for generating unique DNA Fingerprints of humans specifically for using in forensic investigations. He solved his first case of paternity dispute in 1988 by using this probe in India[1]. It gained its popularity because of its use in many famous controversial and high profile cases such as identifying remains of Beant Singh, Rajiv Gandhi, ND Tiwari case, tandoor murder case of Delhi. DNA fingerprinting is genotyping of a set of DNA loci such as STR, SNP etc out of individuals genome to generate his DNA profile. Kirby refers it as portrayal of unusual features of an individual's hereditary

Received: 5 March Revised: 13 March Accepted: 22 March
Index in Cosmos
April 2018 Volume 8 Number 4
UGC APPROVED

International journal of basic and applied research
www.pragatipublication.com
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.86

sequences[2]. It is like signatures that resemble the bar codes on market goods. Unique genetic structure of each individual can be detected by recombinant DNA techniques.

Short tandem repeats (STR) are frequently analyzed in forensic science laboratories due to its short size and easiness to amplify through PCR based method[3]. Short tandem repeat contain tandemly repetitive sequences of 1-6 bp core units ranging in size from 50-300bp[4]. Because of its uniqueness even the low and degraded DNA can be amplified with great sensitivity[5]. STRs have the ability to be amplified in a multiplex reaction simultaneously. Due its additional benefits of requirement of less DNA template, automation and less human intervention ample evidence can be obtained in a single investigation[6].

CASE HISTORY

DNA profiling is becoming most powerful technique to solve various types of crime cases by reconstructing the crime scene to find the actual culprit. In the present case double murder happed in Karnal of Haryana state in India. By changing to assumed name, the case involved an old man Sham Lal, Navin (his son), Suman (wife of Naveen) and Rohit (brother of Suman) were persons involved in the case. Sham Lal stated the police that due to dispute of Suman with her husband Naveen, her brother Rohit came to the house and shot his son dead. Fearing his life he escaped from the crime scene to save his life. Sham Lal later learned from his wife that Rohit had committed suicide after murdering Naveen. But during interrogation Suman who was also injured gave opposite version of the crime. She told the police that her brother Rohit shot her husband in anger but after this he dropped the revolver in the feeling of guilt. Then hes father-in-law Sham Lal picked up the revolver and shot Rohit dead. Holding her responsible of the episode he also shot her and left injured. During forensic investigation a very minute and greasy blood stain (approximately 2mm) was detected on the Pajamas of Sham Lal (Fig. 1). DNA profiling was performed to reach the conclusion.

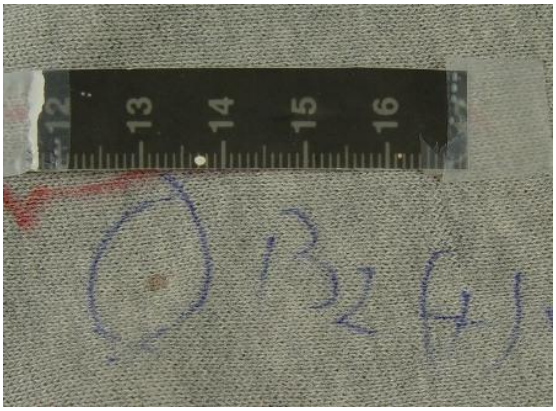

Figure 1: Blood stain detected on Pajamas of Sham Lal.

MATERIALS AND METHODS

3ml of Blood sample was collected through sterilized syringe by venipuncture in EDTA coated vacutainer to prevent coagulation. The sample was preserved at -80°C

DNA Extraction from bloodstains: For extraction of DNA from blood stains of clothes of accused and deceased persons, a portion of stained clothes was cut off (1×1cm) and soaked into lysis buffer-III

Received: 5 March Revised: 13 March Accepted: 22 March
Index in Cosmos
April 2018 Volume 8 Number 4
UGC APPROVED

International journal of basic and applied research
www.pragatipublication.com
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.86

consisting of 10 mM Tris-HCl, 10 mM EDTA, 100 mM NaCl (pH 8.0). 1% sodium dodecyl sulfate and 100 µg/ml Proteinase K were also added for proteolytic digestion[7]. Overnight incubation was done in lysis buffer at 37°C. After incubation the processed cloth portions were taken out and squeezed in a syringe without needle to recover elute thus discarding unnecessary fibers and undigested biological material. It was further centrifuged for 5 min at 18 500xg[8]. DNA was separated with phenolic extraction. DNA was then precipitated from aqueous fraction by adding 3M sodium acetate (pH 5.3) and iso-propanol. The DNA thus pelleted was washed in first 70% ethanol followed by absolute alcohol. The DNA was dried through vacuum evaporator and resuspended in Tris-EDTA buffer.

Quantification: DNA was quantitated in Nano-Drop-1000 UV Visible spectrophotometer. The ratio of absorbance at 260 to 280 nm indicates the purity of the sample. The A260/280 ratio of DNA in the range of 1.8 to 2.0 ensures absence of impurities like proteins or phenols. Quantity and quality of DNA was also tested in 0.8% Agarose gel electrophoresis.

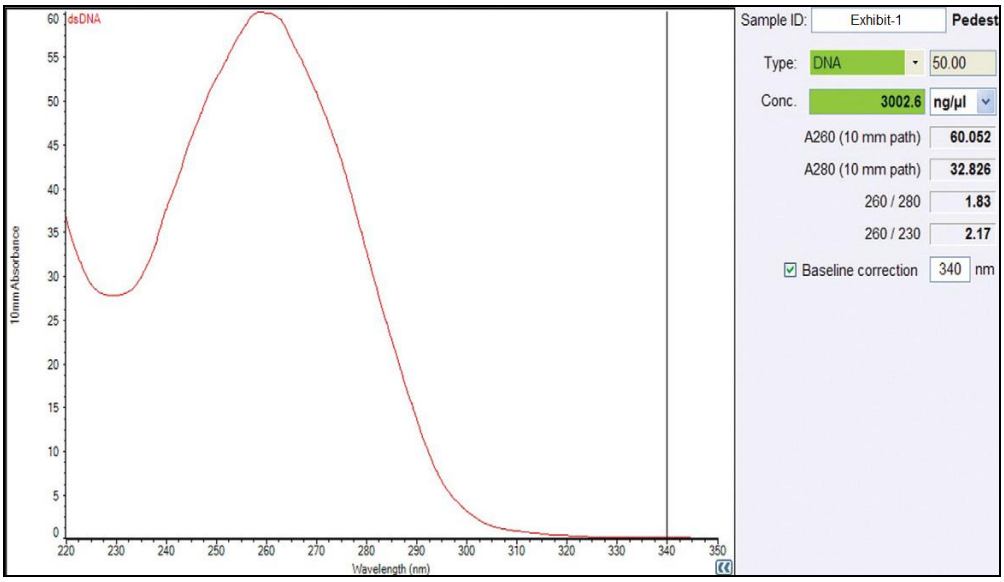

Figure 2: Absorption spectra of DNA on ND-1000 spectrophotometer

Amplification of Autosomal STRs: Purified DNA was amplified at 16 Autosomal STRs in Genamp9700 thermal cycler of Themo-fisher by using AmpF*l*STR identifiler kit. The chemistry of the kit has 5-dye technology, for better separation and analysis of amplified 15 Autosomal STR markers and amelogenin during genotyping[9]. Each sample was mixed in PCR reaction mix prepared from the reagent contained in the kit by pipetting PCR Master mix (9.5 □l), PCR Primer mix (5.0 □l), Taq Polymerase (0.5 □l), Template DNA (10 □l at concentration of 0.125ng/□l) in a single micro centrifuge tube.
PCR was done in thermal cycler by setting following program:

Received: 5 March Revised: 13 March Accepted: 22 March
Index in Cosmos
April 2018 Volume 8 Number 4
UGC APPROVED

International journal of basic and applied research
www.pragatipublication.com
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.86

Table 1: PCR thermal cycle for Autosomal-STRs set in ABI 9700 thermal cycler.

INCUBATION	30 CYCLES			INCUBATION	INCUBATION
95^0C 11 minutes	94^0C 1 minute	59^0C 1 minute	72^0C 1 minute	60^0C 30 minutes	4^0C∞

These tubes after PCR were stored at -15°C to -25°C till further analysis.

Amplification of Y-STRs: Purified DNA was amplified at 16 Y-STRs in Genamp9700 thermal cycler of Themo-fisher by using AmpF*l*Y-filer identifiler kit. The chemistry of the kit has 5-dye technology, for better separation and analysis of amplified 16 Y-STR markers during genotyping[10]. Each sample was mixed in PCR reaction mix prepared from the reagent contained in the kit by pipetting PCR Master mix (9.2 □l), PCR Primer mix (5.0 □l), Taq Polymerase (0.8 □l), Template DNA (10 □l at concentration of 0.125ng/□l) in a single micro centrifuge tube.

PCR was done in thermal cycler by setting following program:

Table 2: PCR thermal cycle for Y-STRs set in ABI 9700 thermal cycler.

INCUBATION	30 CYCLES			INCUBATION	INCUBATION
95^0C 11 minutes	94^0C 1 minute	61^0C 1 minute	72^0C 1 minute	60^0C 80 minute	4^0C∞

Genotyping: The PCR amplicon was run in capillary electrophoresis in ABI 3130 Genetic Analyzer by using 36cm Capillary Array filled with POP4 polymer. Reaction chemistry include HIDI Formamide (8.7 µl) and Liz (0.3) taken into the well of reaction plate. Positive control, negative control and allelic ladder were also run alongside 1µl of PCR amplicon of DNA samples. Reaction plate was capped with a septum. Septa was checked prior to capping wells. Sample amplicons were denatured at 95°C in a PCR cycler for 3 minutes and quick chilled at 4°C for 2 minutes. Reaction plate was loaded in autosampler. Electrophoresis was then started in ABI3130 Genetic Analyzer. The results obtained were analyzed in Genemapper IDv3.0 software[11].

RESULTS

There was only unclear mixed profile obtained in autosomal STR markers in minute blood stain on Pajamas of Sham Lal using AmpF*l*STR identifiler kit. Amplification of amelogenin showed XY genotype in the source of blood stain thus proving that stain was of a male. Hence DNA sample was amplified on 16 Y-STR loci using AmpF*l*STR Y-filer kit. Full profile was obtained from blood stain in all the 16 Y-STR loci from the blood stain on the clothes of accused and deceased persons (Table-3). Y-STR DNA profile generated from the minute blood stain of Pajamas of Sham Lal was compared with the DNA profile generated from the blood stains on the cloths of Naveen and Rohit.

Received: 5 March Revised: 13 March Accepted: 22 March
Index in Cosmos
April 2018 Volume 8 Number 4
UGC APPROVED

International journal of basic and applied research
www.pragatipublication.com
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.86

Table 3: Y-STR DNA Profiling generating from the crime exhibits

Sr. No.	Y-FILER LOCUS/MARKER	Pajamas of Sham Lal (corresponding allele)	Cloth of Rohit corresponding allele)	Cloth of Rohit corresponding allele)
1.	**DYS456**	15	15	12
2.	**DYS3891**	14	14	14
3.	**DYS390**	23	23	11
4.	**DYS38911**	31	31	20
5.	**DYS458**	18	18	13
6.	**DYS19**	15	15	11
7.	**DYS385**	14, 18	14, 18	10
8.	**DYS393**	14	14	23
9.	**DYS391**	10	10	11
10.	**DYS439**	11	11	16
11.	**DYS635**	25	25	15
12.	**DYS392**	10	10	11
13.	**Y-GATA-H4**	12	12	15
14.	**DYS437**	16	16	13
15.	**DYS438**	11	11	24
16.	**DYS448**	19	19	30

DISCUSSION & CONCLUSION

The Y-STR analysis conclusively proved that the DNA profile obtained from blood stains on the pajamas of Sham Lal was matching with DNA profile of Rohit. Thus it proved that the statement made by the Sham Lal that he escaped from the scene of crime before the death of Rohit is wrong. If he would have escaped then the blood stain of Rohit could not be present on his pajamas.

In this case the minute blood stain could not be typed for all 15 autosomal STR loci. But all the 16-Y-STR loci were amplified in the reaction chemistry provided in AmpFlSTR Y-filer kit by the manufacturer. Therefore, Y-STR markers can also be used in cases where Autosomal STRs fail to give any conclusive information. Through DNA profiling the crime scene could be reconstructed and Hon'ble Court reached to a conclusion otherwise trial process could have been difficult to complete. Use of DNA profiling in criminal investigation has become deterrent for the criminals.

Acknowledgement:

The work was carried out by the support and motivation of Director, Forensic Science Laboratory, Haryana who provided all the necessary facilities for concluding this work.

Received: 5 March Revised: 13 March Accepted: 22 March
Index in Cosmos
April 2018 Volume 8 Number 4
UGC APPROVED

International journal of basic and applied research
www.pragatipublication.com
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.86

Note: The names denoted in the paper were changed to respect privacy and keep anonymity of the actual persons.

References:

1. Jayaraman, K.S. (1989). "Cut-price fingerprints". *Nature*. 340: 175.
2. Kirby, L.T. (1990). *DNA fingerprinting: An Introduction.* Stockton Press, New York.
3. Butler, J.M. & Becker, C.H. (2001) *Forensic DNA Typing: Biology and Technology Behind STR Markers.* Academic Press, New York.
4. Goodwin, W., Linacre, A. & Hadi, S. (2007) An introduction to forensic genetics, John Wiley & Sons Ltd., England. 6-14.
5. Tautz, D. (1989) Hyper-variability of simple sequences as general source for polymorphic DNA markers. *Nucleic Acids Research.* 17:6463-71.
6. Murphy, E. (2017) Forensic DNA typing. *Ann. Rev. Criminology.*
7. Yoshida, K., K. Sekiguchi, N. Mizuno, K. Kasai, I. Sakai, H. Sato, and S. Seta. 1995. The modified method of two-step differential extraction of sperm and vaginal epithelial cell DNA from vaginal fluid mixed with semen. Forensic Sci Int 72(1): 25–33.
8. Singer E., Kuenzle C.C., Thomann P.E. & Husbscher U. 1988. DNA fingerprinting: Improved DNA extraction from small blood samples. Nucleic Acids Res. 16:7738.
9. Applied Biosystems (2001) AmpF*l*STR Identifiler PCR Amplification Kit User's Manual. Foster City, CA.
10. Applied Biosystems (2006) AmpFLSTR® Yfiler™ PCR Amplification Kit: User's Manual: Rev C
11. Thangaraj, K., Reddy, A.G. & Singh, L. (2004) Mutation in STR locous D21S11 of father causing allele mismatch in the child. *J Forensic Sci*. 49(1); 99-103.

Correspondence Author: **rajeevkw@gmail.com**

Received: 5 March Revised: 13 March Accepted: 22 March
Index in Cosmos
April 2018 Volume 8 Number 4
UGC APPROVED