

# Salivary DNA Analysis: A Proof of Evidence

Bhale NP<sup>\*</sup>, Khan SG, Mali RS, More BP, Shinde SA, Kulkarni KV

Directorate of Forensic Science Laboratories, DNA division, Mumbai, India

## Abstract

A number of evidences are found on the scene of crime, of which some prove to be the sole proof. In the present case, a steel cup found at the scene of murder proved to be the only evidence that could link the murder to the scene of crime. The steel cups were swabbed using the 'Double-swab technique' and profiles were generated. DNA profiles obtained from reference blood samples of suspect matched with DNA on the evidence found at the scene of crime.

**Keywords:** STR DNA profiling; steel cup; Crime Scene Investigation; Saliva

## Introduction

The importance of DNA in criminal investigations has increased in recent years. Law enforcement investigators heavily rely on DNA profiling of evidences to identify and solve routine as well as difficult cases. Short tandem repeat (STR) DNA Profiling technology has emerged as the most accurate and trusted scientific method to help prove the crime and the victims to achieve justice. When solving cases like murder, rape, theft, burglary etc, the presence of any evidence that can act as a source of DNA, at the crime scene helps the investigators. The presence of a DNA source helps to confirm the presence of the perpetrator at the scene of crime. It becomes the major work of the Crime Scene Investigation (CSI) Team to thoroughly check and collect all the evidences that can be used as a source for DNA.

Researchers have found 5 sources of DNA on a crime scene, arranged in order of DNA recovery and ability to obtain DNA profile i.e. processing rates; such as blood, cigarette ends or butts, saliva, chewing gum and cellular DNA. They identified that saliva and cigarette butts as the main source of DNA [1]. Proper collection, preservation and timely submission of crime scene evidence are of utmost importance, while solving forensic cases. Failure to follow these parameters may cause loss of important DNA evidence. Saliva as DNA evidence has been previously recovered and analyzed from inorganic substrates, such as cigarette butts [2,3], postage stamps [4], envelopes [5] and other objects [6]. Also, it has been shown that saliva can be recovered and typed from bite marks, sucks, fingerprints, etc. on DNA rich substrates, such as human skin [7]. Saliva has been recovered and profiled from bite marks on cheese [8]. Saliva is present in miniscule quantities on any substrate, hence proper sampling before extraction is important. Sweet et al. [9] used the 'Double-swab technique' to sample DNA from human skin. In this case study, we present a case where the only source of DNA were the steel tea cups left on the scene of crime by the perpetrators, after committing murder.

## History

A 29 year old woman was found brutally strangled at her residence, by her husband when he returned from work after completing his nightshift. During the course of investigation, it was found that the suspects knew the household and that the woman would be alone at the time. She was sleeping with her children wherein the culprit knocked the door, called her by maternal nick name which caused her to open the door. Both the culprits entered the house and drank tea that the woman had prepared. The culprits tried to force her for sexual intercourse which she denied. Hence, the culprits strangled her to death. The body was sent for autopsy, where cause of death was confirmed to be strangulation. On reaching the scene of crime, crime

AmPF/STR PCR Reaction Mix	10 µl
AmPF/STR Primer set	5 µl
DNA Sample	10µl

**Table 1:** Quantity Requirement for PCR Technique

Instrument	Operating Parameters
Capacity	96 wellx0.2ml PCR tubes/one 96 well plate
Heating/cooling	Peltier based
Capable of testing temperatures	Denaturation, Annealing & Extension steps
Block ramp rate	5.0°C/Sec.
Sample ramp rate	4.4°C/S
Temperature range	4-99°C/S
Temperature accuracy	±0.2°C
Customized programming	Allows a maximum of 20 steps and 99 cycles
Display	LCD touch screen, about 8.5 in

**Table 2:** Parameters for PCR Thermal Cycler Machine

scene investigation unit collected various evidences, among which two steel cups were collected. These cups were used by the accused to drink tea before commission of crime. The two steel cups along with reference blood samples of accused were submitted for DNA analysis to our laboratory [10].

## Materials and methods

### Evidence: Steel Cups

The surface edge of the steel cup with tea stains was swabbed with a sterilized wet cotton swab followed by a second sterilized dry cotton swab using sterile forceps. Each swab was placed in different mini-centrifuge tube for analysis and 300 µL of Lysis buffer (1M Tris HCl, pH 8.6, 0.5M EDTA, 5M NaCl) was added with 25 µL of Proteinase K (10 mg/ml). The samples were incubated overnight at 56°C. Each sample was submitted to organic extraction in Phenol Chloroform:Isoamyl alcohol extraction (25:24:1) (Amerosco, Biotechnology Grade) [11]. The aqueous phase was washed with 70% ethanol and DNA saturated

**\*Corresponding author:** Bhale NP, Assistant Chemical Analyser, Directorate of Forensic Science Laboratories, DNA division, Mumbai, India, Tel: +919096440910; E-mail: [nehabh2010@gmail.com](mailto:nehabh2010@gmail.com)

**Received** October 09, 2018; **Accepted** January 22, 2019; **Published** January 29, 2019

**Citation:** Bhale NP, Khan SG, Mali RS, More BP, Shinde SA, et al. (2019) Salivary DNA Analysis: A Proof of Evidence. J Forensic Res 10: 433. doi:10.4172/2157-7145.1000433

**Copyright:** © 2019 Bhale NP, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Instrument	Operating Parameters
Fragment Size (bp)	500bp
No. of Markers	16
Polymer	POP4
Detector	CCD
Oven Temp	60°C
Column Size	36cm
Software	GeneMapper® ID-X

Table 3: Parameters for Genetic Analyser-3500

STR Loci	GENOTYPE		
	Accused 1	Accused 2	Steel Cup
D8S1179	10,12	10,13	10,12
D21S11	31.2,33.2	29,30	31.2,33.2
D7S820	12,12	10,11	12,12
CSF1PO	11,12	11,12	11,12
D3S1358	15,17	17,17	15,17
TH01	6,6	6,9	6,6
D13S317	11,12	11,11	11,12
D16S539	11,11	10,12	11,11
D2S1338	19,26	19,21	19,26
D19S433	13,16.2	13,14	13,16.2
vWA	16,18	18,20	16,18
TPOX	8,11	11,11	8,11
D18S51	12,15	17,17	12,15
AMELOGENIN	X,Y	X,Y	X,Y
D5S818	11,12	11,13	11,12
FGA	21,25	23,25	21,25

Table 4: Comparative Analysis of Autosomal STR Results of Accused 1, Accused 2 and Steel Cup

in TE buffer (pH 8.0).

### Reference Samples

An indigenous inexpensive blood collection kit devised in our laboratory which contains 4% EDTA in a 2ml mini-centrifuge tubes is used by medical officers to collect blood, as reference samples. Reference blood sample of both the suspect were received in a blood collection kits with their consent. DNA extraction was performed using HiPurA Mammalian Genomic DNA Purification Kit (HiMedia Laboratories, India) wherein 200 µL of blood was treated and saturated in 100 µL Elution Buffer [12].

### Polymerase Chain Reaction (PCR) & Genotyping

Extracted DNA from evidence as well as reference blood samples was subjected to Polymerase Chain Reaction (PCR) using Amp/STR Identifier Amplification Kit (Applied Biosystems, Foster City, CA). PCR products were separated and detected using Capillary Electrophoresis (ABI-3500 Genetic Analyzer, Applied Biosystems, Foster City, California) and DNA profiles were generated. PCR products were separated and detected using Capillary Electrophoresis (ABI-3500 Genetic Analyzer, Applied Biosystems, Foster City, California) and DNA profiles were generated.

### Result and Discussion

Autosomal STRs have high power of discrimination and are useful in forensic cases involving identification of the victim, the perpetrator and missing persons. STRs have become very popular in forensic science laboratories in India as the DNA profile can be obtained even if the sample is in very little amount. Double swab technique can be used not only for recovery of saliva from bite marks [1] and human skin [5]

but also for swabbing saliva from steel cups. The autosomal STR profiles obtained were compared to the alleged accused 1 and accused 2. On interpretation of the DNA profiles obtained from one of the steel cups, it was inferred that, DNA profile obtained from steel cup matched with DNA profile of one of the perpetrators, proving his presence at the scene of crime. For the 15 STR loci, profile obtained from steel cup matched with the DNA profile of Accused 1 completely. Profiles generated are given in tabular form. Profiles generated are given in tabular form.

### Conclusion

Due to backlog of cases at our laboratory this particular case was analyzed after about a period of 2 years. The evidence sample was stored at room temperature, at a secure and dry place. Successful recovery of DNA from the evidence, reiterates the fact that proper handling of evidences collected from the crime scene and further preservation, help in preservation of DNA even after long periods of time. While drinking tea, at time of crime, the perpetrator left the presence of saliva, on surface edge of steel cup, through contact of his lips, which yielded DNA profile of one of the perpetrator. DNA profiling successfully linked the perpetrator to the crime scene. The sampling method -double swabbing technique- used during the analysis of this case, proved to be a crucial factor. The initial use of wet cotton helps in collection of dried saliva from the surface and the later use of dry cotton swab efficiently lifts the trace amounts of saliva from the now wet surface. Use of two cotton swabs- wet and dry- assures that the surface of interest has been thoroughly swabbed for traces of saliva. Using the double swabbing technique for collection of saliva has yielded successful results on human skin and bite marks. In this paper, the authors present the successful use of double swabbing technique for collection of saliva from steel cups.

### References

- Bond JW, Hammond C (2008) The Value of DNA Material Recovered from Crime Scene. J Forensic Sci 53: 797-801.
- Hochmeister MN, Rudin O, Ambach E (1998) PCR analysis from cigarette butts, postage stamps, envelope sealing flaps, and other saliva-stained material. In: Lincoln PJ, Thomson J, editors. Forensic DNA profiling protocols. Methods in molecular biology, Totowa: Humana Press, pp 27-32.
- Thirunavakarasa T, Ahmada U, Khanb H, Rahmat Z (2016) DNA Analysis of Saliva Traces on Cigarette Butts Exposed to Indoor and Outdoor Environmental Conditions. Malaysian J Forensic Sci 7: 10-16.
- Hopkins B, Williams NJ, Webb MBT, Debenham PG, Jeffreys AJ (1994) The use of minisatellite variant repeat-polymerase chain reaction (MVR-PCR) to determine the source of saliva on a used postage stamp. J Forensic Sci 39: 526-531.
- Allen M, Saldeen T, Gyllensten U (1994) PCR based DNA typing of saliva on stamps and envelopes. Biotechniques 17: 546-552.
- Sweet D, Shutler GG (1999) Analysis of salivary DNA evidence from a bite mark on a submerged body. J Forensic Sci 44: 1069-1072.
- Kanto E, Hirata M, Hirata R, Nunes F, Melani R, Oliveira R (2005) DNA extraction from human saliva deposited on skin and its use in forensic identification procedures. Braz Oral Res 19: 216-222.
- Sweet D, Hildebrand D (1999) Saliva from cheese bite yields DNA profile of burglar: a case report. Int J Legal Med 112: 201-203.
- Sweet D, Lorente JA, Lorente M, Valenzuela A, Villanueva E (1997) An improved method to recover saliva from human skin: the double swab technique. J Forensic Science 1997; 42: 320-322.
- Sweet D, Lorente JA, Lorente M, Valenzuela A, Villanueva E (1997) PCR-based typing of DNA from saliva recovered from human skin. J Forensic Sci 42: 447-451.
- Köchl S, Niederstätter H, Parson W. Methods in Molecular Biology, vol. 297: Forensic DNA Typing Protocols (PCI protocol).
- Mago J, Sidhu L, Kaur R, Anurag T (2016) Saliva in Forensics. World J Pharm Med Res 2016; 2: 196-198.