

# Can lip prints provide biologic evidence?

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## Abstract

**Background:** Lip prints are unique and can be used in personal identification. Very few studies are available which establish them as biological evidence in the court of law. Thus, the objective of this study was to attempt to isolate DNA and obtain full short tandem repeat (STR) loci of the individual from the lip prints on different surfaces.

**Materials and Methods:** Twelve lip prints were procured on different surfaces such as tissue paper, cotton cloth, ceramic tile, and glass surface. Latent lip prints were developed using fingerprint black powder. Lipstick-coated lip prints were also collected on the same supporting items. DNA was isolated, quantified, and amplified using Identifiler™ kit to type 15 STR loci. **Results:** Ample quantity of DNA was extracted from all the lip print impressions and 15 loci were successfully located in seven samples. Fourteen loci were successfully typed in 3 lip impressions while 13 loci were typed in 2 samples.

**Conclusion:** This study emphasizes the relevance of lip prints at the scene of crime. Extraction of DNA followed by typing of STR loci establishes the lip prints as biological evidence too. Tissue papers, napkins, cups, and glasses may have imprints of the suspect's lips. Thus, the full genetic profile is extremely useful for the forensic team.

**Key words:** Biological evidence, DNA isolation, forensic odontology, forensic sciences, latent lip prints, short tandem repeat loci

## Introduction

Recent studies have emphasized the study of wrinkles and fine grooves on the lips; the study of which is now popularly known as cheiloscopy.<sup>[1,2]</sup> It is well accepted by the forensic fraternity that lip prints are unique analogous to fingerprints and can be used for personal identification of a criminal in the court of law when no other evidence is available.<sup>[3-6]</sup> Forensic experts analyze the physical remains of the criminal actions, for example, in case of house burglaries, where the specific modus operandi of the offender can be conveniently recognized by the investigating police officer. Although fingerprints are the most accepted physical evidence at the crime scene, it is also not infrequent to find

latent lip prints on glasses, cups, paper, clothes, cigarette butts, window panes, etc., These different surfaces also contribute to the viability of their identification. However, though the wrinkles and grooves on labial mucosa can be instrumental in identifying a suspect thus verifying the presence or absence of a suspect at the scene of crime, still biologic evidence is of greater weightage in such cases. Unfortunately, frequently, the quality of lip print is very poor; either it is very vague or blurred, which makes for an impossible cheiloscopy identification, even under a magnifying lens. In recent years, lipsticks have been developed that do not leave any visible trace after contact with surfaces such as clothing, tissue paper, ceramic, and glass. These are the latent lip prints which can be developed

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with fingerprint powders such as black powder, gray powder and even lysochrome dyes such as Sudan III and Sudan black.<sup>[7]</sup> Research studies have proved that DNA can not only be successfully isolated but also can be typed for short tandem repeat (STR) loci from the fingerprints.<sup>[8-10]</sup> Thus, if DNA is extracted and analyzed for the full genetic profile of the suspect, it is considered as the final proof in the court of law.<sup>[11]</sup> Methods of extracting DNA from different biological materials such as bloodstains and semen include separation and purification steps utilizing phenol-chloroform extractions and/or ethanol precipitation.<sup>[12,13]</sup> The polymerase chain reaction (PCR) is a method of amplifying small quantities of relatively short target sequences of DNA using sequence-specific oligonucleotide primers and thermostable *Taq* DNA polymerase. PCR requires only the target sequence to be intact, therefore amplifying even partially degraded and/or denatured DNA. Hence, PCR is extremely successful for the analysis of forensic casework samples, in which the quantity of DNA may be less than optimal for genetic profile analysis.<sup>[14]</sup> Therefore, the purpose of this study was to ascertain whether DNA can be extracted from the chemically processed latent lip prints as well as lipstick-coated lip prints. In addition, this research aimed to confirm whether DNA was of sufficient quality and quantity that can be amplified for genetic profiling of the suspect. In such instances, lip prints can be used as a biological evidence in the criminal courts. The objective of the study was to extract DNA and then to amplify the isolated DNA for typing of full STR loci (15 loci) to establish genetic profile of the individual on various surfaces using buccal sample as the control.

## Materials and Methods

Our research was an institutional prospective study completed within a short span of 1 month. Individuals having lesions, swelling, or any pathoses on the lips were excluded from the study. An agreement for the usage of lip prints for DNA extraction and genetic profile analysis was approved by the Ethics Committee for Research Involving Human Subjects of Swami Vivekanand Subharti University, Meerut, and the study was conducted in accordance with its ethical standards. Consent forms were duly signed by the volunteers participating in the study, which emphasized the educational and research purposes of our project.

For the chemical processing of the invisible or latent lip prints, the same technique and armamentarium are used as for the fingerprints development in the field of forensic and legal medicine. In the present study, we used fingerprint feather brushes and fingerprint black powder (both from JK Consultancy Forensic Products Pvt. Ltd. ISO 9001: 2008 Government Certified Organisation) to develop the lip prints on different surfaces. The purpose was to use both porous surfaces (tissue paper and cloth fabric) and nonporous ones (ceramic tile and glass surface) for lip

print impressions, thus assessing the quantity of DNA extracted from a variety of surfaces. For the lipstick-coated lip impressions, dark-brown-colored lipstick (extra-lasting lipstick, Avon Cosmetics India Limited, India) was used. All through the chemical processing of the latent lip prints, protective equipment such as gloves and facemask were used.

Latent lip prints were imprinted by the subject on paper handkerchiefs; cloth surface, transparent glass, and ceramic tile which were later (within 24 h) developed using fingerprint black powder. Long-lasting lipstick was applied on the volunteer with a lip brush, and then after waiting for 5 min, the volunteer was asked to make lip impressions by making sustained pressure for about 3 seconds on different supporting items. On each supporting surface, at least three impressions were made by the same individual. These lip prints were made visible using fingerprint black powder by carefully applying a small quantity of powder on the surface to locate the latent lip print. The best lip prints were selected to be processed for DNA extraction.

Finally, the tissue paper and cloth piece carrying the lip impressions were cut into very small pieces. Approximately, 3-mm side squares were cut out from all prints to attempt DNA extraction using the routine organic method (phenol-chloroform method)<sup>[12,13]</sup> using DNA extraction kit from Identifiler™ kit (AmpFLSTR® Identifiler® PCR Amplification Kit, Applied Biosystems, USA).<sup>[15]</sup> Buccal sample was collected from the subject using a cotton swab, which was kept as a control. The buccal swab was selected as the control which helped in confirming the genetic profiling of the lip prints. In case of lip prints on ceramic and glass surfaces, two methods were used to procure lip prints so as to enable them to be subjected for DNA analysis. In the first method, a cotton swab was used to lift the DNA remains directly from the surface and then it was processed for DNA analysis. Furthermore, adhesive surface of the cello tape was pressed firmly on the ceramic and glass surfaces several times, thus imprinting lip impression on the adhesive side. This cello tape was then cut into very small pieces which were processed to attempt DNA isolation. Thus, in all, 12 samples were used to type the STR loci.

The amount and quality of DNA obtained were determined (Eppendorf Biophotometer® D30 Eppendorf AG, Hamburg, Germany) to ascertain whether sufficient quantity of DNA was extracted which could be subjected for genetic profiling or STR loci of the individual.

The extracted DNA from various surfaces was amplified using the kit from Identifiler™ kit (AmpFLSTR® Identifiler® PCR Amplification Kit, Applied Biosystems, USA).<sup>[15]</sup> PCR was carried out in an Eppendorf Mastercycler for the typing of the following STR markers: D8S1179, D21S11, D7S820, CSFIPO, D3S1358, TH01, D13S317, D16S539,

D251338, D195433, vWA, TPOX, D18S51, D5S818, and FGA. The PCR-amplified products obtained were subjected to electrophoresis separation and amplified alleles were run on 4% denatured polyacrylamide gels and then visualized using silver staining.<sup>[16]</sup>

## Results

In the present study, ample quantity of DNA was obtained from all the lip print samples, i.e., on all the surfaces whether porous ones such as cloth and tissue paper or nonporous ones such as ceramic tile and glass surface. Both swab technique and cellotape method showed sufficient quantity of DNA, and of 15 STR loci, most of the alleles were located successfully on all the 12 samples taken on different surfaces and matched the profile of the buccal swab taken as the control [Tables 1 and 2]. Thus, the full genetic profile of

the individual was successfully established from the lip impressions. The average quantity of DNA from all the samples ranged between 15 and 21 ng/ $\mu$ L. The minimum DNA for amplification recommended is 5 ng.<sup>[17]</sup>

## Discussion

Diligent research work on cheiloscopy has ascertained that lip prints can be established as a means of evidence in the court of law and it is certainly possible to analyze these lip prints analogous to fingerprints.<sup>[1-3,18]</sup> Tsuchihashi<sup>[19]</sup> has classified them into six types for convenience, namely, vertical, incomplete vertical, branched, intersected, reticular, and indeterminate. Furthermore, the lip prints are unique, thus can be used as a potential source of investigation. However, sometimes, substantial forensic evidence is required to prove the individual identity in

**Table 1: Alleles for short tandem repeat loci on porous surfaces (tissue paper and cloth fabric) of the subject**

STR loci	Buccal swab sample	Cloth LC	Cloth LP	Tissue paper LC	Tissue paper LP
D8S1179	11, 14	11, 14	11, 14	11, 14	11, 14
D21S11	28, 33.2	28, 33.2	28, 33.2	28, 33.2	28, 33.2
D7S820	10, 12	10, 12	10, 12	10, 12	10, 12
CSF1PO	11, 12	11, 12	11, 12	11, 12	11, 12
D3S1358	15, 16	15, 16	15, 16	15, 16	15, 16
TH01	7, 9	7, 9	7, 9	7, 9	7, 9
D13S317	9, 11	9, 11	9, 11	9, 11	9, 11
D16S539	9, 11	9, 11	9, 11	9, 11	9, 11
D2S1338	17, 19	17, 19	17, 19	17, 19	17, 19
D19S433	12, 13	12, 13	12, 13	12, 13	12, 13
vWA	17, 18	17, 18	17, 18	17, 18	17, 18
TPOX	8, 12	8, 12	-	8, 12	8, 12
D18S51	12, 17	12, 17	12, 17	12, 17	12, 17
D5S818	10, 12	10, 12	10, 12	10, 12	-
FGA	22, 23	22, 23	22, 23	22, 23	-

LC: Lipstick-coated lip prints, LP: Latent lip prints, Swab: Cotton swab method, STR: Short tandem repeat

**Table 2: Alleles for short tandem repeat loci on nonporous surfaces (glass surface and ceramic tile) of the subject**

STR loci	Buccal swab sample	Glass LC (swab)	Glass LP (swab)	Glass LC (cello)	Glass LP (cello)	Ceramic LC (swab)	Ceramic LP (swab)	Ceramic LC (cello)	Ceramic LP (cello)
D8S1179	11, 14	11, 14	11, 14	11, 14	11, 14	11, 14	11, 14	11, 14	11, 14
D21S11	28, 33.2	28, 33.2	28, 33.2	28, 33.2	28, 33.2	28, 33.2	28, 33.2	28, 33.2	28, 33.2
D7S820	10, 12	10, 12	10, 12	10, 12	10, 12	10, 12	10, 12	10, 12	10, 12
CSF1PO	11, 12	11, 12	-	11, 12	11, 12	11, 12	11, 12	11, 12	11, 12
D3S1358	15, 16	15, 16	15, 16	15, 16	15, 16	15, 16	15, 16	15, 16	15, 16
TH01	7, 9	7, 9	7, 9	7, 9	7, 9	7, 9	7, 9	7, 9	7, 9
D13S317	9, 11	9, 11	9, 11	9, 11	9, 11	9, 11	9, 11	9, 11	9, 11
D16S539	9, 11	9, 11	9, 11	9, 11	9, 11	9, 11	9, 11	9, 11	9, 11
D2S1338	17, 19	17, 19	17, 19	17, 19	17, 19	17, 19	17, 19	17, 19	17, 19
D19S433	12, 13	-	12, 13	12, 13	12, 13	12, 13	-	12, 13	12, 13
vWA	17, 18	17, 18	17, 18	17, 18	17, 18	17, 18	-	17, 18	17, 18
TPOX	8, 12	8, 12	8, 12	8, 12	8, 12	8, 12	8, 12	8, 12	8, 12
D18S51	12, 17	12, 17	12, 17	12, 17	12, 17	12, 17	12, 17	12, 17	12, 17
D5S818	10, 12	10, 12	10, 12	10, 12	10, 12	10, 12	10, 12	10, 12	10, 12
FGA	22, 23	22, 23	22, 23	22, 23	22, 23	22, 23	22, 23	22, 23	22, 23

LC: Lipstick-coated lip prints, LP: Latent lip prints, Swab: Cotton swab method, Cello: Lip print on cellophane tape

criminal cases. Undoubtedly, the full genetic profile of a person is the most reliable and foolproof evidence in the criminal courts. The present research is innovative as it successfully proves that it is not only possible to extract ample amount of DNA from the lip prints similar to the extraction from the fingerprints, but also possible to obtain a full STR profile of the individual. Using chemicals to develop the latent lip prints does not hinder the isolation of DNA. Similarly, the application of lipstick also does not, in any way, obstruct the extraction of DNA. The study also establishes that it is certainly feasible to extract DNA from porous as well as nonporous surfaces such as cups, glasses, and tissue paper which are most likely to be imprinted by the suspects in offensive cases. As per our literature search, only one similar study has been done so far on lip prints. However, researchers only used tissue paper as the surface and only three STR loci were typed.<sup>[20]</sup> The present research successfully located 15 STR loci on different supporting surfaces. The source of DNA from the lip prints is the saliva and the shredded skin epithelial cells. A minimum amount of 5 ng/ $\mu$ L of DNA must be extracted to amplify it for obtaining a full genetic profile.

Only small amounts of even badly degraded DNA may be sufficient for forensic use. STR typing methods are widely used today for human identity testing applications including forensic DNA analysis. They express a high degree of polymorphism, making them of particular use to the forensic scientist. Thousands of STR markers, only a small subset, core set of loci have been selected for use in forensic DNA and human identity testing.<sup>[21,22]</sup>

Nevertheless, the limitations of the study are that the lip prints used were of fresh lip impressions with a maximum of 24 hours- time overlap. In real circumstances, the forensic investigator faces challenges such as environmental pollution and poor conservation. Previous studies have shown the efficacy of fingerprint powder in developing these prints even after a gap of 30 days without any protection under environmental conditions.<sup>[7,23]</sup> This means of investigation should be relied on only when the conventional methods such as fingerprint or lip print development are unsuccessful. Furthermore, this evidence becomes vital and significant when DNA databases are available, and at a later point in the investigation, a suspect is developed. This study was an attempt to extract DNA from the lip prints, which was not only successfully located but also typed for 15 STR loci.

## Conclusion

The possibility of extraction of DNA from lip impressions makes these lip traces more successful and useful to a forensic investigator. This proves that the lip prints are not only physical evidence but also biological evidence in the court of law and their significance certainly cannot be

underestimated. The forensic fraternity can certainly rely on it as a substantial evidence if traditional methods such as fingerprint impressions fail or no other sources of evidence such as blood and saliva are present at the crime scene. Such breakthroughs surely will help the criminal justice system in arriving at faster and trustworthy conclusion.

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## Conflicts of interest

There are no conflicts of interest.

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