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# Advantages of Salivary DNA in Human Identification

*Raluca Dumache, Veronica Ciocan, Camelia Muresan,  
Ramona Parvanescu and Alexandra Enache*

## Abstract

Since two and a half decades, in human identification, the short tandem repeat (STR) markers represent the “gold standard.” Besides them, haploid markers such as X-STR and Y-STR are also used to complement the autosomal markers. In human identification, DNA from body fluids, especially saliva, represents an important tool. The aim of this chapter is to present the importance of analyzing X-STR markers in a relatedness case between a sister and her presumptive brother, a carbonized victim using body fluids for their DNA identification. Our laboratory had to establish the relatedness between a woman and her presumptive brother (PB), who was the victim of a car accident explosion. In this case, as reference sample we used saliva collected on swabs from the woman and blood sample from the deceased victim. For the DNA extraction, DNA IQ Casework (Promega, USA) was used. DNA quantification was done with PowerQuant System kit (Promega, USA). Furthermore, the DNA samples were amplified with Investigator 24plex QS (Qiagen, Germany) for the STR markers and Investigator Argus 12-X QS kit (Qiagen, Germany) for the X-STR markers. The amplified DNA products were separated by capillary electrophoresis on a 3500 Genetic Analyzer. In this case, full genetic profiles were obtained for the woman and her presumptive brother on both STR and X-STR markers. Thus, we could confirm a full sibling relationship between them. Since the introduction of DNA in human identification, it represents a useful tool in establishing sibling relationship from different biological samples.

**Keywords:** saliva, deoxyribonucleic acid (DNA), short tandem repeat (STR) markers, X-STR markers, PCR (polymerase chain reaction), capillary electrophoresis (CE)

## 1. Introduction

A few years ago, the FBI Laboratory announced the expansion of the common core of 13 STR markers included in the National DNA Index System (NDIS) since 1997. Starting with 1st of January 2017, FBI required the CODIS (Combined DNA Index System) laboratories to implement additional STR markers [1]. The new markers added to the common core of 13 STR markers aid in human identification and in kinship analysis of missing persons due to a better power of discrimination [2]. In order to confirm the identity of a person, it is necessary to perform a comparative study of DNA profiles belonging to that person and a first-degree relative (mother/father; brother-sister), in which the respecting of the classical

laws for transmitting the hereditary characters is followed, so that a maternal allele and a paternal allele are found on a DNA locus [3, 4]. Within a paternity investigation, the child’s mother being presumed as being certain, the child’s DNA profile presents a lot of alleles that are found in the presumptive father profile besides the maternal alleles; we can speak about a confirmation of paternity that is always expressed in probability terms. In cases where the biological parents do not exist anymore, a sibling or cousin is recommended to participate in the DNA identification process [5–7]. In DNA human identification, body fluids such as saliva, blood, semen, and urine represent important components. Regarding saliva, it can be obtained easily, by non-invasive techniques through the buccal swabbing. Herein, we present the importance of salivary DNA and STR markers and haploid markers in establishing the identity of a carbonized corpse through DNA sibling analysis.

2. Materials and methods

2.1 DNA extraction from the biological samples

For the reference samples of the victim’s sister, we collected epithelial cells from the inner cheeks on three buccal swabs (Copan, Italy) [8]. In case of the carbonized victim, during the autopsy procedure, we collected blood samples from the heart chambers in collection tubes with EDTA. In this case of kinship analysis, the saliva was collected by swabbing from the inner cheeks of the woman. In cases of carbonized corpses, depending on their grade of carbonization, the biological samples used for the identification by DNA analysis can be saliva obtained from the mouth and blood obtained from the heart chambers or different parts of the internal organs.

The swabs from the victim’s sister were left to dry for 3–4 h in a laminar PCR flow hood. Following the recommendations of the manufacturer, we proceeded to the DNA extraction from the saliva and blood. The DNA extraction was performed by the automated Maxwell® 16 RSC instrument (Promega, USA) using the Maxwell® RSC Whole Blood DNA kit (Promega, USA) for the blood samples and the Maxwell RSC Buccal Swab DNA kit (Promega, USA) for the swabs [9].

2.2 Quantification of the extracted DNA samples

For the quantification of the DNA samples from the woman and her presumptive brother, PowerQuant System kit (Promega, USA) [10] was used. In this case, following the manufacturer’s recommendations, we prepared a mix solution for each sample with a final volume of 18 µL consisting of 10 µL of PowerQuant 2× Master Mix; 7 µL of amplification grade (water), and 1 µL of Power Quant 20× Primer Mix. The quantification was made on a 7500 real time PCR (Applied Biosystems, USA), using the HID Real-Time PCR Analysis Software v 2.0.6. The concentrations of saliva and blood are presented in **Table 1**.

Person’s identity	Concentrations (ng/µL)
Sister	1.08
Presumptive brother	3.25

**Table 1.**  
*DNA concentrations of the woman and her presumptive brother.*

### 3. Amplification of the DNA samples

The DNA samples from the woman and her alleged brother were amplified for the STR markers using the Investigator 24plex QS kit (Qiagen, Germany) [11]. Also, in this case, the X-STR markers have been analyzed using the Investigator Argus 12-X-QS kit (Qiagen, Germany) [12]. All these genetic markers are contained in a single reaction, known as multiplex. For the STR markers, a number of 23 STR markers were investigated, and for the X-STR markers, a number of 12 markers were analyzed. The analysis was done after the recommendations of the manufacturers.

#### 3.1 Amplification of the autosomal STR markers

In this step, the DNA sample's amplification was performed on a ProFlex PCR System (Applied Biosystems, USA). We used the Investigator 24plex QS Kit (Qiagen, Germany). Furthermore, the PCR reactions of the salivary DNA samples were carried out in a total volume of 25  $\mu$ L. The final volume of the reaction contained Fast Reaction Mix 2.0: 7.5  $\mu$ L, Primer Mix: 2.5  $\mu$ L, nuclease-free water: 12.5  $\mu$ L, and template DNA: 2.5  $\mu$ L.

The Investigator 24plex QS Kit (Qiagen, Germany) contains 23 autosomal markers, as follows: D1S1656, D2S441, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, CSF1PO, FGA, TH01, TPOX, Vwa, SE33, DYS391, and amelogenin. All these genetic markers are contained in a single reaction, known as multiplex. The conditions for the PCR amplification were as follows:

- 3 PCR cycles: temperature (T) = 98°C for 30 s; T = 64°C for 55 s; T = 72°C for 5 s;
- 27 PCR cycles: T = 96°C for 10 s; T = 61°C for 55 s; T = 72°C for 5 s.

After 30 PCR cycles were completed, a final extension (hold) at T = 68°C for 2 min followed by final hold indefinite at T = 10°C.

#### 3.2 Amplification of the X-STR markers

In this case, because we have a brother-sister relationship, we amplified the X-STR markers using the Investigator Argus 12-X QS kit (Qiagen, Germany). The kit contains 12 X-STR markers as follows: DXS8378, DXS10135, DXS10148, DXS7132, DXS10074, DXS10079, XPRTB, DXS10101, DXS10103, DXS7423, DXS10134, and DXS10146.

The final reaction volume contained: Fast Reaction Mix 2.0: 7.5  $\mu$ L; Primer Mix: 2.5  $\mu$ L; nuclease-free water: 12.5  $\mu$ L, and template DNA: 2.5  $\mu$ L.

The amplification conditions for X-STRs were as follows in the cycling protocol:

- 3 PCR cycles as follows: T = 98°C for 60 s; T = 61°C for 100 s; T = 72°C for 5 s.
- 27 PCR cycles as follows: T = 96°C for 10 s; T = 61°C for 100 s and T = 72°C for 5 s.

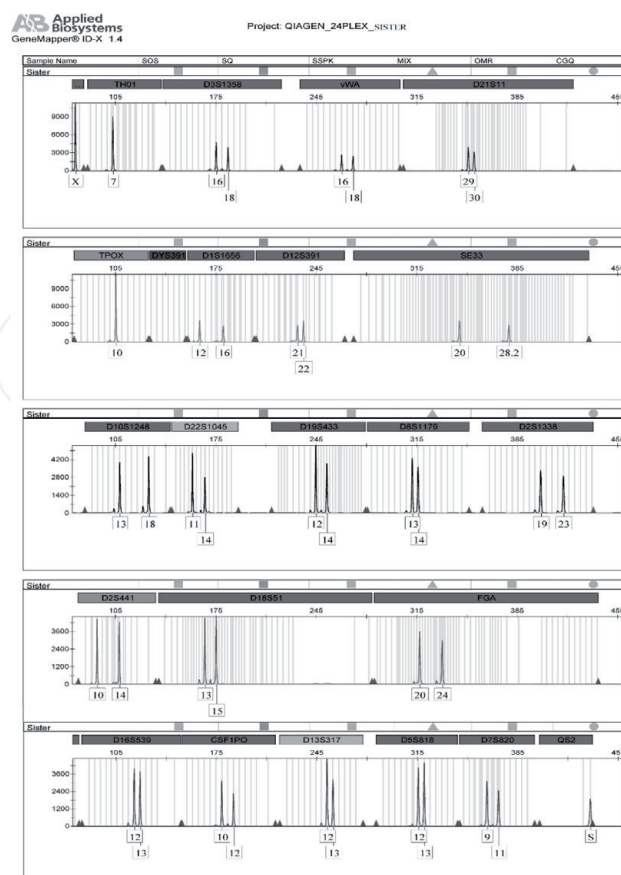
After 30 PCR cycles were completed, a final extension (hold) at T = 68°C for 2 min followed by final hold indefinite at T = 10°C.

#### 4. Capillary electrophoresis of the amplified DNA samples

For the capillary electrophoresis, the samples were analyzed on a 3500 Genetic Analyzer, following the manufacturer's recommendations. For the STR markers, we used 1  $\mu\text{L}$  of the amplified PCR product (DNA sample) and the allelic ladder (AL). They were added into the mix containing: 12.5  $\mu\text{L}$  of Hi-Di formamide (Applied Biosystems, USA) and 0.5  $\mu\text{L}$  DNA size standard BTO (Qiagen, Germany). To analyze the X-STR markers, we added 1  $\mu\text{L}$  of the amplified PCR product (DNA sample) and the allelic ladder (AL) into the mix containing: 12.5  $\mu\text{L}$  of Hi-Di formamide (Qiagen, Germany) and 0.5  $\mu\text{L}$  DNA size standard BTO (Qiagen, Germany). Gene Mapper ID-X Software version 1.4 (Applied Biosystems, USA) was used to analyze the obtained data [13].

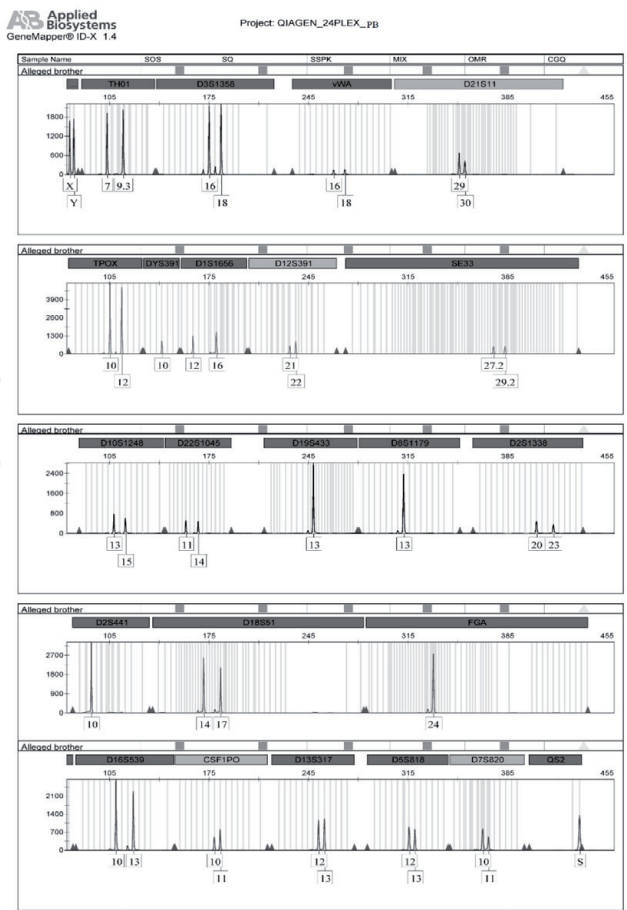
## 5. Results

After the DNA separation, we obtained the electropherograms and the genotypes of the unidentified person and his alleged brothers using the Gene Mapper ID-X software version 1.4 (Applied Biosystems, USA). GenoProof 3 Software-Kinship Examination (Qualitytype GmbH, Dresden, Germany) [14] was used for the statistical part. In this case, a full sibling probability of 99.999972% was obtained between the woman and her presumptive brother. Also, because they had a sibling relationship as brother-sister, we analyzed the X-STR markers. The genetic profiles on STR markers between the woman and her presumptive brother are presented in **Figures 1** and **2**, respectively. X-STR haplotypes on the woman and her presumptive brother are presented in **Figures 3** and **4**, respectively.

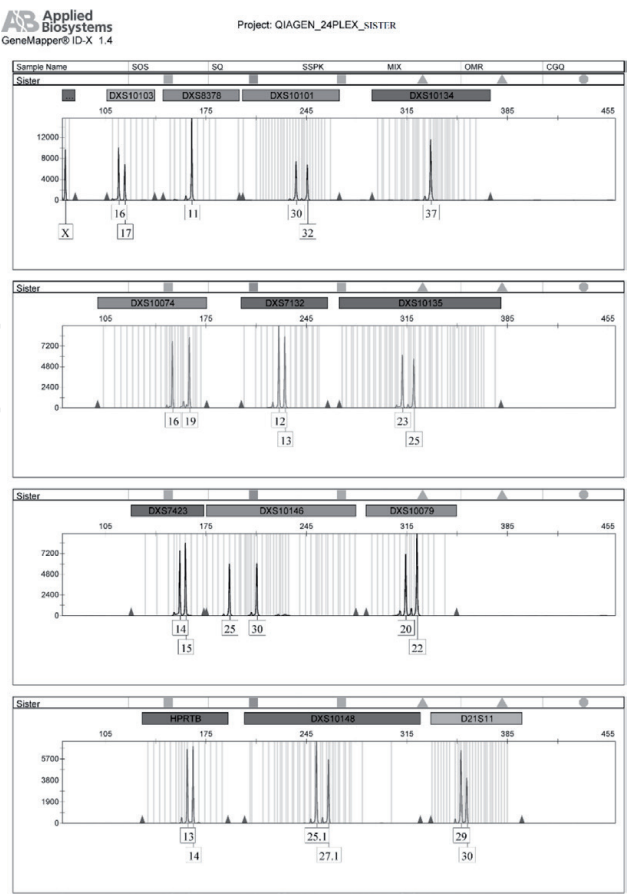


**Figure 1.**  
*Woman's genetic profile on 23 STR markers.*



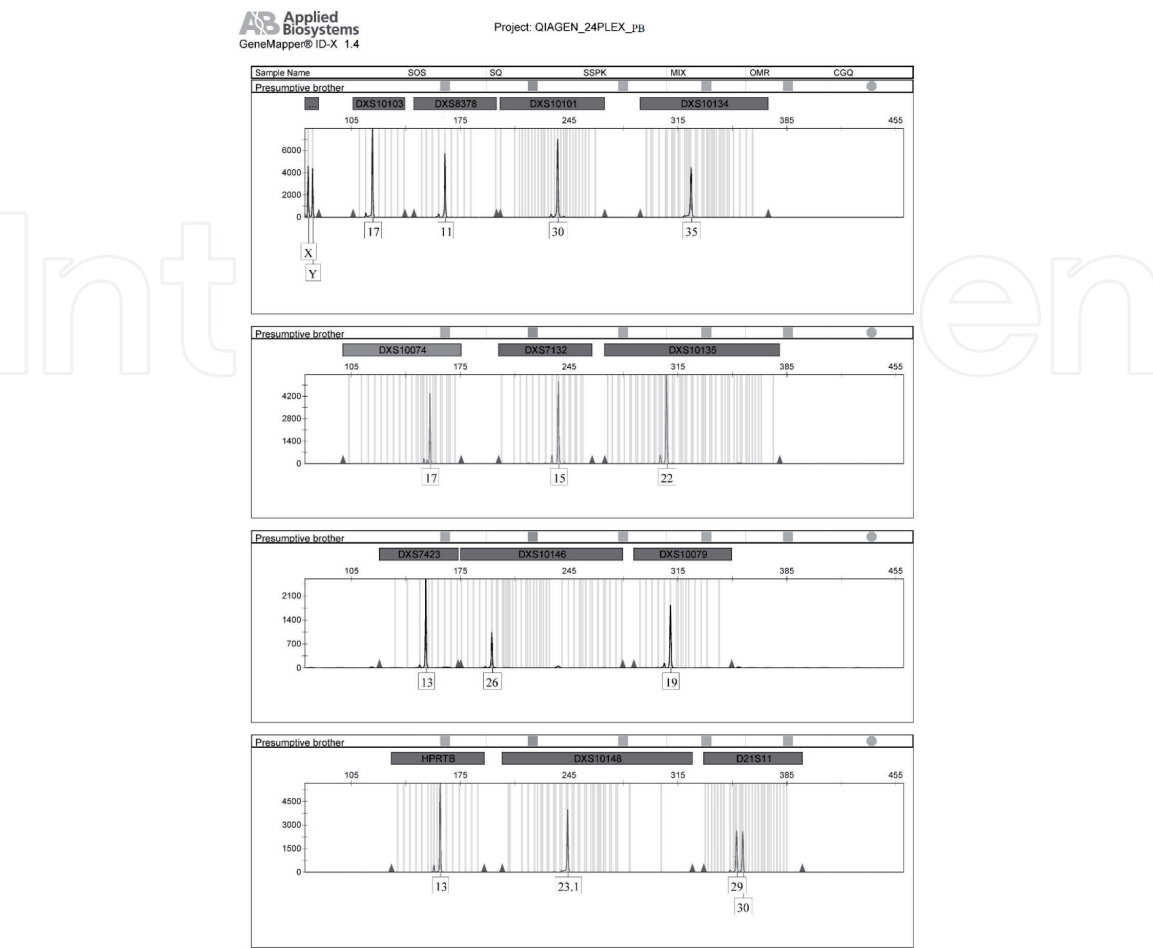


**Figure 2.**  
*Genetic profile of the presumptive brother on 23 STR markers.*



**Figure 3.**  
*X-STR haplotype of the woman.*

The genetic profiles of the woman and her presumptive brother on X-STR markers are presented in **Table 2**.



**Figure 4.**  
*X-STR haplotype of the presumptive brother.*

X MARKER	Sister	Common genes	Presumptive Brother
DXS8378	11	-	10
DXS10135	20;30	-	23.1
DXS10148	25.1	25.1	25.1
DXS7132	14	14	14
DXS10074	18;19		16
DXS10079	19	19	19
HPRTB	13;14	-	12
DXS10101	28.2;32	-	24;29.2
DXS10103	16;19	-	20
DXS7423	14;15	-	16
DXS10134	36;37	36	36
DXS10146	28	-	29
D21S11	30;33.2	-	30;33.2

*Note: The 4 colors represent the dyes. The markers are divided into 4 linkage groups (3 markers per group).*

**Table 2.**  
*Common X-STR markers between the woman and her presumptive brother.*

## 6. Discussions

Saliva as a body fluid is very often used in laboratory tests for diagnosis, prevention, and monitoring of different diseases. Compared to blood tests, saliva tests present some advantages like easy and non-invasive collection techniques; its collection does not present any risks for the technician who collects the samples.

In the future, salivary DNA diagnostic tests can eliminate the blood tests, because the DNA contains the same genetic information from all the biological samples.

Also, in forensic human identification, salivary DNA has improved the laboratory workflow because it is easily collected and can be quickly analyzed by direct PCR method. After collecting saliva on buccal swab or FTA paper, the sample can be directly amplified by polymerase chain reaction (PCR) in less than an hour, followed by migration on capillary electrophoresis.

## 7. Conclusions

In human identification from DNA, establishing the paternity or maternity, sibling or kinship analysis can be done from body fluids, by non-invasive collection techniques. In this chapter, we presented the usefulness of both saliva and blood in a case of sibling relationship.

## Conflict of interest

The authors declare they have no conflict of interest.

## Author details

Raluca Dumache<sup>1,3\*</sup>, Veronica Ciocan<sup>1,2</sup>, Camelia Muresan<sup>1,2</sup>, Ramona Parvanescu<sup>3,4</sup> and Alexandra Enache<sup>1,2</sup>

<sup>1</sup> Faculty of Medicine, Victor Babes University of Medicine and Pharmacy, Timisoara, Romania

<sup>2</sup> Institute of Forensic Medicine, Timisoara, Romania

<sup>3</sup> Laboratory of Forensic Genetics, Victor Babes University of Medicine and Pharmacy, Timisoara, Romania

<sup>4</sup> Doctoral School of Victor Babes University of Medicine and Pharmacy, Timisoara, Romania

\*Address all correspondence to: [raluca.dumache@umft.ro](mailto:raluca.dumache@umft.ro)

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