forensic sciences

MDPI

Case Report

Application of X-STRs for Forensic Identification in Mixed DNA Profile: A Case Report

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Abstract: Autosomal polymorphisms (STRs) or Y-Chromosome polymorphisms (Y-STRs) are usually used for the study and deconvolution of mixed DNA profiles in forensic genetics, accompanying data interpretation with biostatistical evaluations (e.g., RMP, RMNE, LR). Sometimes, however, some mixed DNA profiles are so complex that autosomal and Y markers are not sufficient for correct discrimination and identification. In this work is reported a robbery case in which the analysis of the polymorphic markers of the X Chromosome (X-STRs) was applied to the mixed profiles obtained from the traces. This falls outside the classic use of the X-STRs. Indeed, the aim of the authors is to encourage the usage of X-STRs not only in parental relationships, but also in pure forensic cases for interpreting complex mixed DNA profiles, since their application in case resolution could be more decisive than autosomal STRs and Y-STRs.

Keywords: X-chromosomal markers; X-STRs; DNA; mixture; forensic genetics; case report

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1. Introduction

In cases of mixed traces found on crime scenes, sometimes, DNA profiles have more than two alleles per locus: these are defined as "mixed", i.e., made up of material from more than one biological source, or rather by multiple individuals [1,2].

The polymorphic genetic markers of autosomal DNA (STRs) and Y Chromosome (Y-STRs) have been widely used in such cases to reach a correct interpretation and identification of the contributors [3,4]. Nevertheless, in a few complex situations, the interpretation of the analytical data can be harder or even impossible, thus preventing the complete and correct deconvolution of the mixed profile and the consequent identification of the contributors who participated in the trace formation [5].

In the case of autosomal genetic markers, the evaluation of the electropherogram allows us to establish whether a genetic profile is mixed, and whether it is in a complex asset [6]. In fact, the number of contributors that may have generated a trace is assessed through the maximum number of alleles per locus (Maximum Allele Count, MAC) [7] and their quantitative relationship through the calculation of the Mixture Ratio (MR) and the Peak High Ratio (PHR) [1,8]. The degree of complexity is directly proportional to the number of alleles for each locus, and to what extent stochastic effects occur. These are mainly related to the quality and quantity of the DNA extracted from the trace [9]. Furthermore, if the contributors are related, the genotypic combinations make the interpretation even more difficult [10].

The study of Y-Chromosome genetic markers in a mixed DNA profile, always in combination with autosomal data, allows us to determine the minimum number of male contributors, but not their identification [11]. Indeed, the Y-Chromosome does not undergo

recombination during meiosis, and this means that it is virtually inherited across generations in the original haploid form. Therefore, the Y-Chromosome haplotype is common to a paternal line of a family. As a consequence, in a mixture formed by two individuals belonging to the same paternal line, Y-Chromosome typing is not useful for discriminatory purposes [12].

When the mixture is made up of several contributors, male and female, related or not to each other, their discrimination can be performed by typing the genetic markers of the X-Chromosome [10,13]. The molecular typing of X-STRs is usually applied to population studies and parental relationships between relatives in complex cases (to complement the analysis of autosomal STRs and the Y-Chromosome), such as investigations of deficient paternities in which female are involved [14]. X-STRs can also be applied in cases of missing people, incest, and in the identification of victims of attacks and mass disasters (DVI) [14–17].

Currently, there are a lot of publications regarding the development and validation of new X-STR markers for forensic purposes [18–22], especially for kinship determination and complex kinship discrimination [23,24]. However, their use in criminal cases, especially in the study of mixed traces, is still rather rare [25].

The typing of the X-Chromosome in a male subject (XY) allows us to directly obtain the individual haplotype, since the individual inherits one of the two X-Chromosomes from the mother and the same Y-Chromosome from the father. Thus, for a single male individual, the X-Chromosome haplotype is represented by only one allele per typed locus [26,27], while in the case of mixed profiles formed by two male contributors, the profile is represented by a maximum of two alleles for each typed locus [28].

A female subject, on the other hand, inherits one of the two X-Chromosomes from the mother (or rather a combination of them), and the other X-Chromosome from the father. Therefore, for a single female subject, the profile of the X-Chromosome haplotype is represented by a maximum of two alleles for locus [27,29,30]. When mixed DNA profiles consist of two individuals, one male and one female, the X-Chromosome haplotype is represented by a maximum of three alleles for each locus analyzed, two of which belong to the female contributor and one to the male [29,31].

Few studies have been conducted on criminal cases [13,25], in which, however, the use of X-STRs has been decisive. For example, in cases of mixtures taken from vaginal swabs of female victims of rape, the suspects were related and the analyses of both autosomal and Y-Chromosome STRs were not conclusive [13].

Hence, X-STRs could be a very useful tool when used to reach a correct interpretation of DNA mixtures, especially in cases of mixtures formed from male and female cells, even if they must always support autosomal and Y-Chromosome data [32].

In order to highlight the decisive importance of the usage of X-STRs in the context of mixed genetic profiles, this work reports a judicial case of robbery. The judge's request was to understand whether there were traces of one or more contributors on the evidence (balaclava) found at the crime scene, and if those contributors were all males, all females, or both. Moreover, the reference profile of only one male suspect was available.

The first analyses of autosomal markers and Y-Chromosomes were not useful to determining how many and which contributors had participated in the formation of the trace; so X-Chromosome markers were used, and this analysis was resolutive.

Before now, the study of the X-Chromosome profiles obtained from mixed traces or from reference samples could not be supported by biostatistical evaluations because of the rare application of X-STRs in criminal cases, and the availability of X-STR population frequencies for only a few individuals [33]. Nowadays, the scientific community offer guidelines on the usage of X-STRs in kinship analyses alone [34], and their biostatistical evaluation using FamLinkX software [35]. Thus, there are no tools that provide a statistical weight to the application of X-STRs in the interpretation of mixed traces [33,34].

2. Materials and Methods

2.1. Sampling Procedures

Two double swabs (Copan Italia Spa, Brescia, Italy) were performed in the internal portion of the balaclava, the object of the technical assessment, at the level of the central nose–mouth and in the apical forehead area.

The reference saliva sample was obtained using two Whatman FTA Cards.

2.2. DNA Extraction and Quantification

DNA extraction was performed with the innuPREP Forensic DNA Kit-IPC16 (AnalytikJena, Jena, Germany), following the manufacturer's standard saliva swab protocol.

The extracted DNA were quantified with a Quantus Fluorometer (QuantiFluor™-ST and QuantiFluor™ dsDNA System kits (Promega, Madison, WI, USA)).

2.3. DNA Amplification, Electrophoresis and Data Analysis Software

PCR was performed with the following kits: PowerPlex Fusion System kit (Promega) for autosomal markers, PowerPlex Y23 System kit (Promega) for Y-Chromosome markers and Investigator Argus-12X kit (Qiagen, Hilden, Germany) for X-Chromosome markers on "VeritiTM Dx Thermal Cycler" (Thermo Fisher Scientific, Waltham, MA, USA).

Capillary electrophoresis was performed using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Waltham, MA, USA). STRs were subsequently typed with the GeneMapper ID v3.2 (Applied Biosystems) software.

The analytic threshold (AT) and the stochastic threshold (ST) for the interpretation of autosomal STRs and Y-STRs were set at 40 RFU (Relative Fluorescence Unit) and 150 RFU, respectively, while the stochastic threshold for X-STRs was set at 120 RFU as the internal validation.

3. Results

At first, the two samples collected inside the balaclava (in the nose–mouth area and in the forehead area) were analyzed for autosomal and Y-Chromosome STRs. The results of DNA quantification were 7.254 ng/uL and 1.237 ng/uL, respectively. The profile obtained from sampling in the nose–mouth area of the balaclava was an indistinguishable mixed DNA profile belonging to at least two contributors, of which at least one was male, thanks to the presence of the Y-Chromosome signal in the Amelogenin locus. An important aspect of this type of mixture is the presence of a strong allelic imbalance in the Amelogenin locus; here, the peak corresponding to the X-Chromosome was almost three times higher than the peak of the Y-Chromosome (X-Chromosome: 2306 RFU; Y-Chromosome: 880 RFU) (Figure 1A,B).

In order to estimate the contributions of the subjects in this unbalanced mixture, only the heights of the peaks in loci with four alleles were taken into account, assuming that the height of the peaks is proportional to the amount of DNA template. The average Mixture Ratio (MR) evaluation was equal to 2.64:1. Therefore, it was not sufficient to genetically discriminate the numbers of contributors within the mixture [1].

On the other hand, the profile of the Y-Chromosome provided a single haplotype; this, however, does not allow us to infer if the male subject present in the trace numbers only one, or if there are more male contributors related to each other through the paternal line (Figure 2).

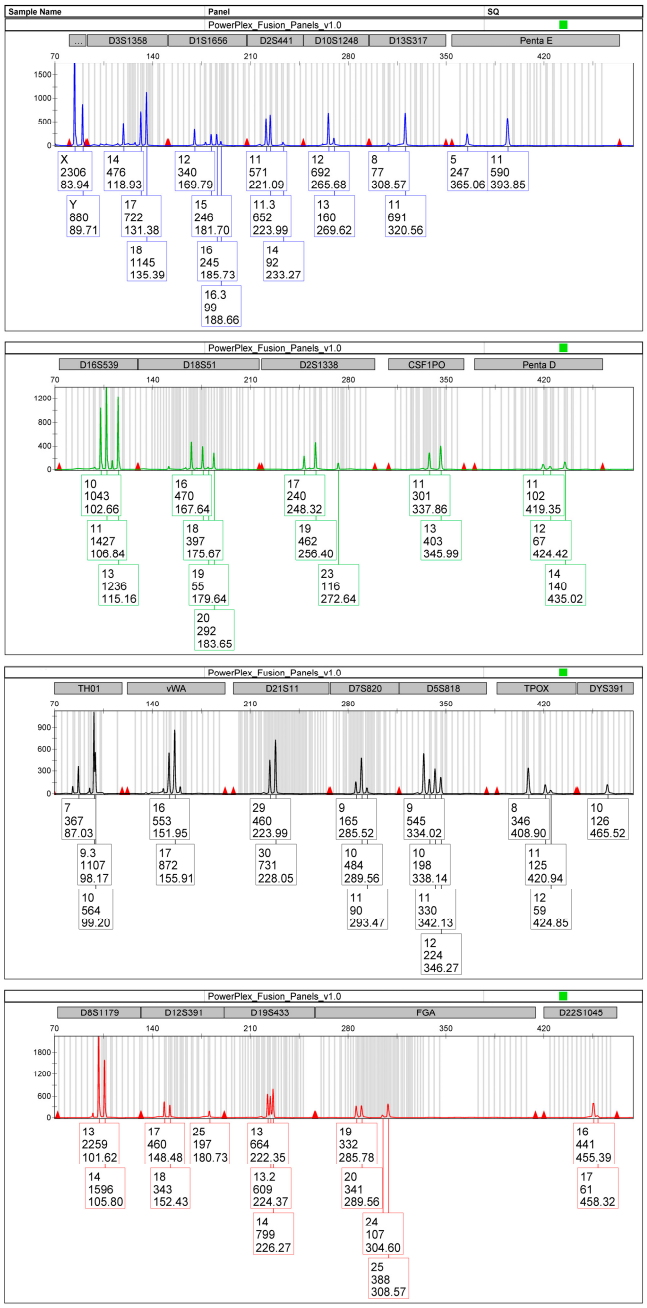
The profile obtained from sampling in the forehead area of the balaclava was identical to and indistinguishable from the mixture from the first sampling. Nevertheless, in this second profile, the allelic imbalance was absent at the Amelogenin locus, probably because the male subject contributed the most to the formation of the trace.

The comparison of the autosomal STR and Y-STR profiles of both traces with the reference sample for the only suspect revealed a complete match; this confirms his certain contribution to the mixtures. The compatibility was further supported by a biostatistical evaluation of the Likelihood Ratio (LR) performed with LRMix Studio, v.2.1.5, which for

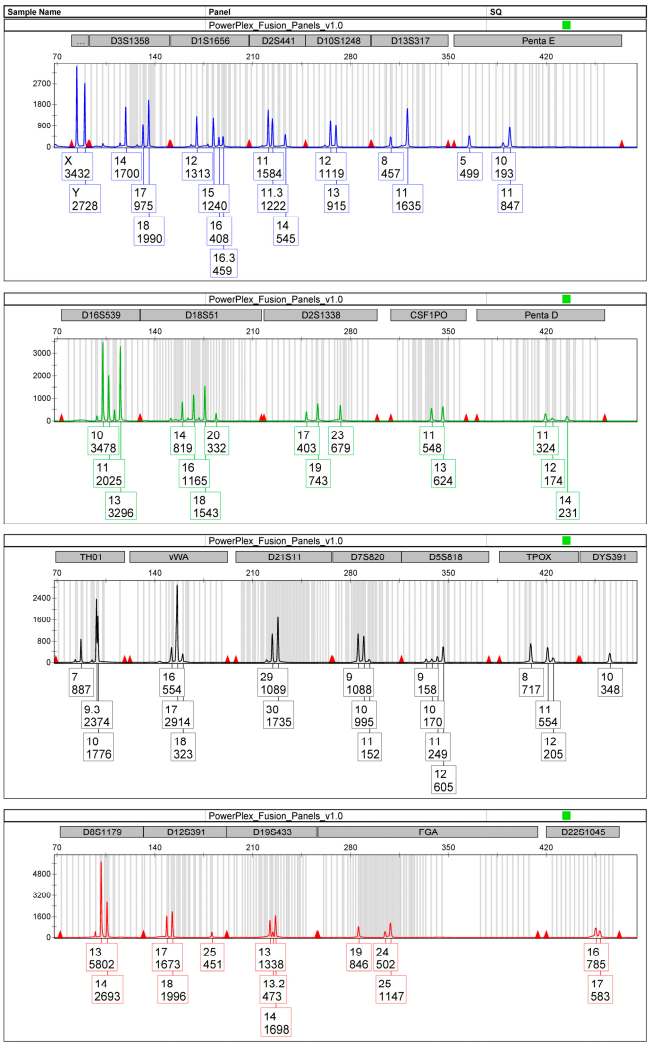
the autosomal profiles, provided LR values equal to 9.5888×10^{12} and 1.8814×10^{12}, respectively [36]. The haplotype profile frequency of the Y-Chromosome in the reference database YHRD (http://www.yhrd.org/ (accessed on 17 December 2019) [37,38]) was equal to 4.7749×10^{-5}, and the LR was equal to 2.0943×10^{4}.

Given the presence of genetic mixtures, the Judge's request was also to understand how many other contributors could have been present in the traces, and their sex, in order to focus the investigations. However, the results obtained from the analyses of autosomal and Y-Chromosome STRs were not sufficient to completely clarify this issue.

For this reason, the STRs of the X-Chromosome were also investigated so as to better understand the distribution of the contributors in the mixtures.



(**A**)



(**B**)

Figure 1. (**A**) Autosomal electropherogram from the first trace (balaclava mouth–nose area); (**B**) autosomal electropherogram form the second trace (balaclava forehead area).

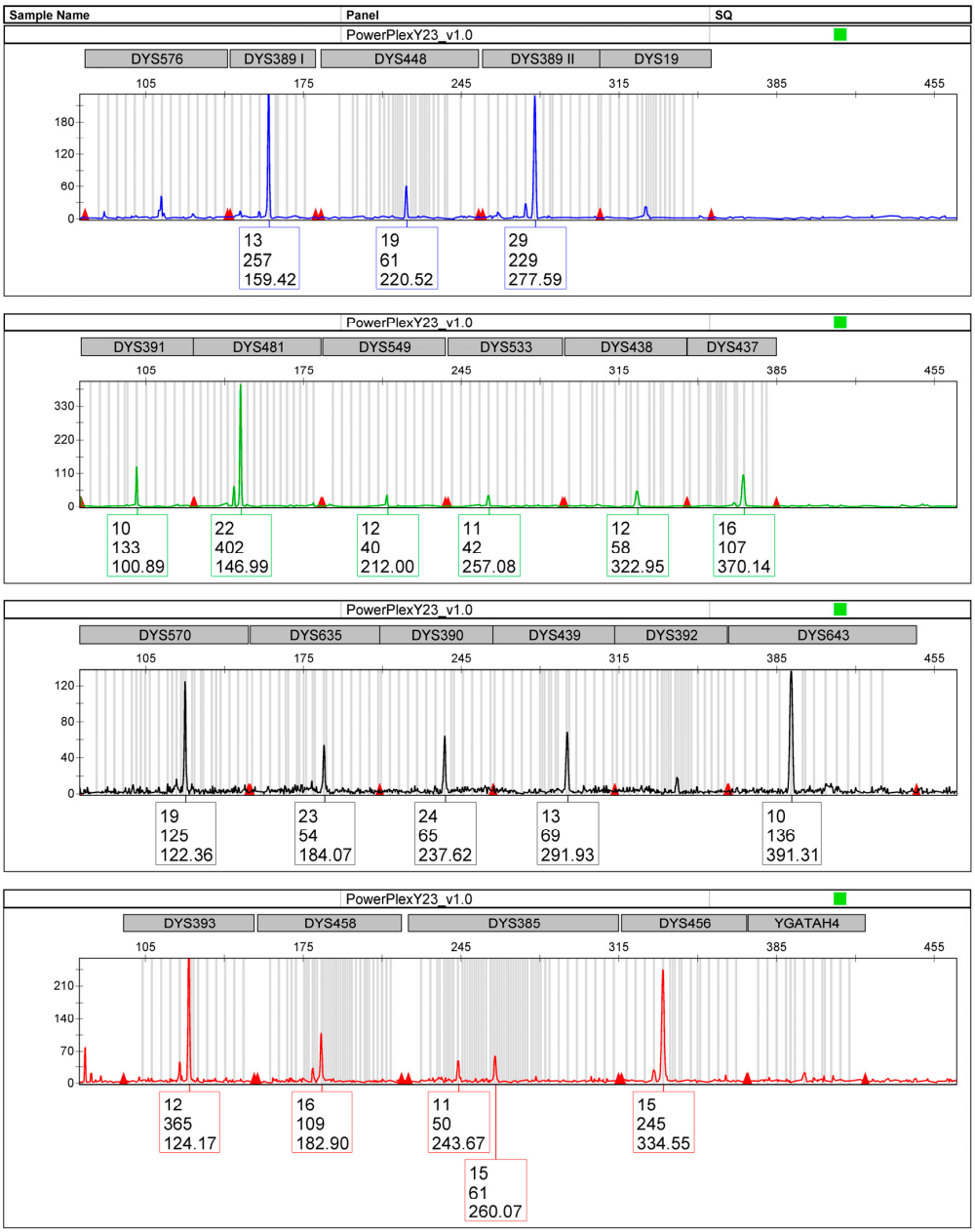


Figure 2. Y-STRs electropherogram from the first trace (balaclava mouth–nose area).

From the first trace (nose–mouth area of the balaclava), a partially mixed profile was obtained. This profile showed again the allelic imbalance at the Amelogenin locus (the peak corresponding to the X-Chromosome was almost three times higher than the peak of the Y-Chromosome). Since a maximum of three alleles were typed only in the DXS10101, DXS10135 and DXS10146 loci, it was possible to hypothesize that the trace was formed by two individuals, one male and one female (Figure 3A). The mixture was compared with the suspect's reference sample, and the haplotypic profile of his X-Chromosome was found to be present in the mixture. Therefore, it could be ruled out that the second subject that contributed to the formation of the mixture was a male, but it could still have been an unknown female. The allelic imbalance at the Amelogenin locus, both in the autosomal profile and in the X-Chromosome profile, supports this hypothesis.

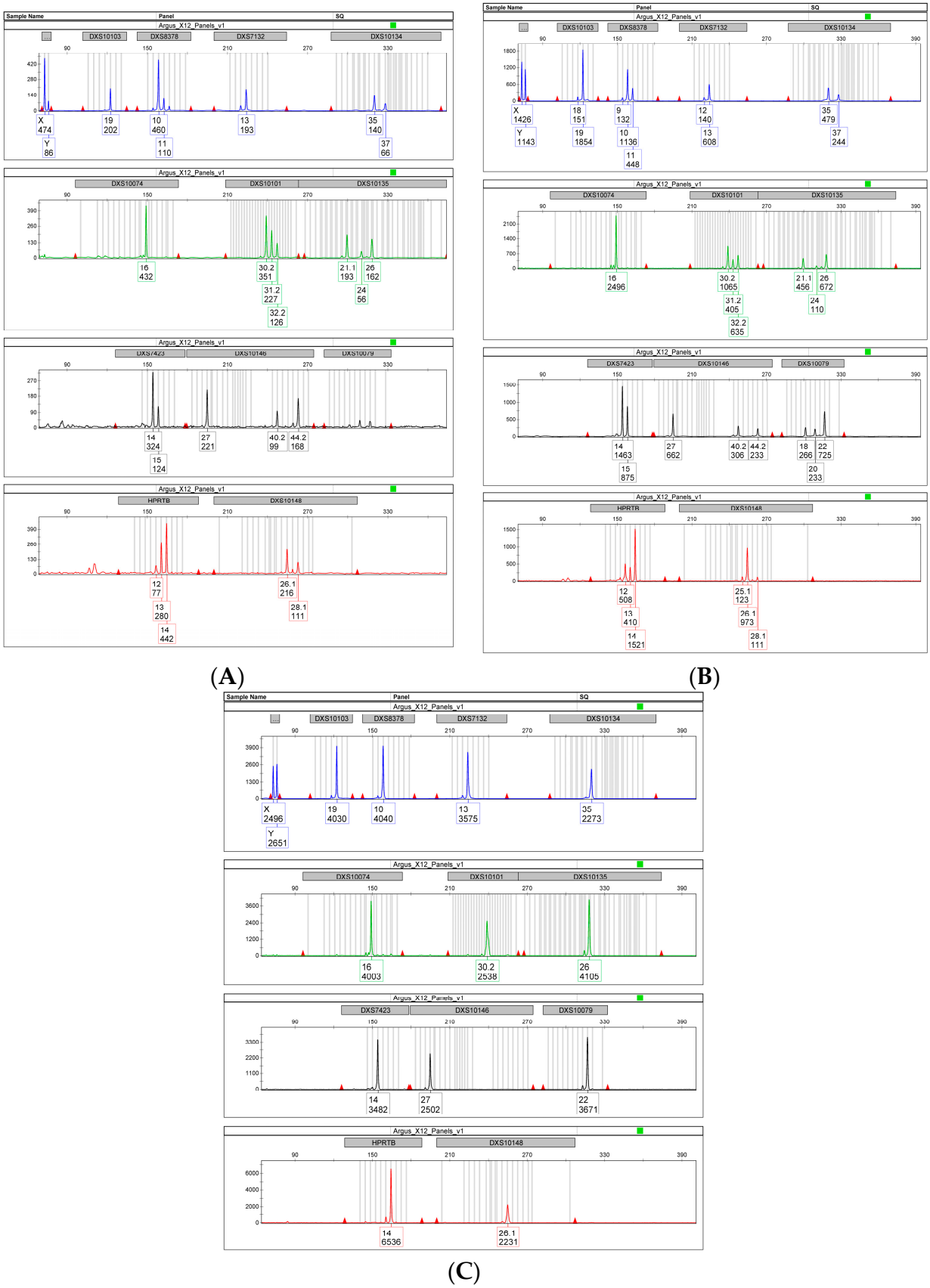


Figure 3. (**A**) X-STRs electropherogram from the first trace (balaclava mouth–nose area); (**B**) X-STRs electropherogram from the second trace (balaclava forehead area); (**C**) X-STRs electropherograms from the reference.

From the second trace (forehead area of the balaclava), a completely mixed profile was obtained. Also, in this case, since maximums of three alleles were typed only in the DXS8378, DXS10101, DXS10135, DXS10146 and DXS10079 loci, it is possible to assert the previous hypothesis (Figure 3B). The haplotypic X-Chromosome profile of the suspect is compatible even with this trace (Figure 3C). The only difference is that the first trace features the absence of allelic imbalance at the Amelogenin locus, probably because the male subject contributed the most. In conclusion, due to the haplotypic transmission of the X–Chromosome in males and given the presence of three alleles only in the DXS8378, DXS10101, DXS10135, DXS10146 and DXS10079 loci, it could be inferred that the second contributor to the formation of the mixture in the second trace was the same unknown female subject.

4. Discussion

This work reports on a robbery case. At the crime scene, a balaclava was found, and it was the target of technical investigations. The request of the judicial authority was to investigate the genetic profile and, in the event of a mixture, to determine the minimum number of contributors and their sex in a proportional relationship, with only the reference sample of a single male suspect being available.

The study of the autosomal polymorphic markers (STRs) in the two sampled traces revealed indistinguishably mixed profiles, belonging to at least two contributors, one of which was certainly male. Moreover, only in the first trace was a strong allelic imbalance of the X-Chromosome present at the Amelogenin locus. On the other hand, the study of the polymorphic markers of the Y-Chromosome (Y-STRs) highlighted the presence of a single haplotypic profile. The comparison with the reference sample of the only suspect allowed the testers to identify him as the male contributor to the traces (thanks also to the biostatistical values of LR obtained). Nevertheless, the analysis of the autosomal STRs and Y-STRs did not allow us to establish with certainty the total number of contributors present in the mixture and their sex.

Therefore, the polymorphic markers of the X-Chromosome (X-STRs) were analyzed; mixed DNA profiles with a maximum of three alleles per locus were observed. This evidence can be compared to the biological status for which, in a mixed genetic profile represented by a maximum of three alleles per locus, two would belong to a female contributor and one to the X-Chromosome haplotype of a male subject. Furthermore, as concerns the first trace, the allelic imbalance observed at the Amelogenin locus, both in the autosomal profile and in the X-Chromosome profile, supports this hypothesis. Thus, thanks to the usage of X-STRs, it was possible to answer the judge's query, stating that the mixed traces found on the evidence were related to a male subject (fully compatible with the suspect) and to an unknown female subject.

This case report should be defined as a pilot study on the use of X-STRs for the discrimination and interpretation of mixed DNA profiles, beyond their classic application on deficient parental relationships. Indeed, the analysis of X-STRs markers could lead to excellent results when neither autosomal nor Y-Chromosome STRs are conclusive.

Considering the lack of other studies on the application of X-STRs in criminal cases in the presence of biological mixtures created by contributors of an unknown sex, this work aims to encourage the scientific forensic community to use X-STRs for discriminatory purposes. Their application could be determinant of the correct deconvolution of mixtures and the consequent identification of contributors, even if they are of different sexes [25].

Future work, in vitro and in the field, will aim to increase the number of cases of the application of X-STRs in mixtures, as well as trying to set up biostatistical evaluations of the likelihood ratio in order to provide scientifically and statistically relevant data.

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