DNA EXAMINATION OF SUPERIMPOSED TRACES OF BLOOD FROM A VICTIM AND EPITHELIAL CELLS FROM PERPETRATOR ON TRACE EVIDENCE

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Abstract

Introduction

Examination of biological traces on physical evidence by the method of DNA profiling is an extremely important and reliable way to identify the perpetrators of criminal offenses. In expert practice, it is not uncommon for biological material to be deposited by the victim and the physical perpetrator of the crime.

Materials and methods

A case of murder of a 29-year-old woman with multiple traumas to her body is presented. In the course of the investigation, several objects with blood traces on them have been obtained. The purpose of the examination conducted by the method of DNA profiling has been to look for the presence of deposited biological material on the physical evidence from the perpetrator of the crime, applying Real-Time PCR; Quantifiler™ Trio DNA Quantification Kit; NGM Detect™ PCR Amplification Kit; Yfiler™ Plus PCR Amplification Kit.

Results

After the initial genotyping of the traces of blood on the objects, a mixed genetic profile of the victim and low added alleles of another male person has been established, without the possibility of deriving his genetic profile.

Discussion

In the presented case, due to the prevailing amount of genetic material from the victim in a mixed sample, is analyzed the possibility to use the approach for genotyping the left cellular material by the man holding the knives and golf club, using sex-defining genetic markers contained in his Y-chromosome.

Conclusion

The use of sex-defining Y-chromosome sets of genetic markers to differentiate genetic profiles in mixed female-male biological traces allows ignoring the female component, with its predominant amount masking the deposited cellular material by the perpetrator.

Keywords: *material evidence, mixed biological traces, DNA identification, Y chromosome.*

Introduction

The examination of biological traces on physical evidence by the method of DNA profiling is an extremely important and reliable way to identify the perpetrators of criminal offenses. Y chromosome analysis is an excellent method for detecting the presence of specific male DNA in mixed traces or excluding a subject as a male cell donor (1). A number of researchers recommend the sequential use of a primary autosomal STR´s assay and a secondary Y-STR´s assay (2, 3, 4).

In expert practice, it is not uncommon for biological material to be deposited and superimposed by the victim and the physical perpetrator of the crime. A case from Germany, which arose in 2015, proves the importance of Y-STR´s testing. In a failed robbery attempt, a woman had been killed in her apartment. A suspect was detained. On a noose used to

embrace the woman, together with a large amount of female saliva, scarce epithelial material from a man was found. In the course of the study, the male autosomal profile was not delimited, but the Y-STR´s analysis generated a complete profile for 23 loci. The profile corresponded to the suspect who had been arrested. Such cases are a real challenge to choose the most appropriate expert approach for delimiting and determining the individual DNA characteristics and deriving the DNA profile of the criminal. The options are related to the correct selection and use of the possibilities of the autosomal and sexual X- and Y-chromosomal genetic loci. Currently, different kits and additional multiplexes can be combined to amplify more than 40 Y-STR´s sequences that are used in forensic expertise. All of these Y-STR´s were carefully selected from the male-specific region of the Y chromosome (5, 6).

In order to identify and isolate the less represented male component in female / male mixed samples, the Capillary Electrophoresis method (7, 8) and the use of online accessible population databases consisting of Y-STR´s profiles, introduced into reference populations, are currently most appropriate. (9, 10, 11, 12).

As already noted, Y chromosome analysis can detect hidden male DNA against a background of predominant female DNA. Therefore, Y-STR´s screening is recommended in all cases where male DNA left by the perpetrator is expected but is not detected in regular autosomal STR screening. In severe crime cases, Y chromosome analysis may be considered the only way to determine the DNA profile of an unknown perpetrator and to reduce the number of suspects (13, 14).

Materials and methods

1. DNA extraction

The extraction of DNA from the traces of biological material - human blood and possibly epithelial cells by Objects NoNo 2.1, 2.2.1- handle, 2.2.2- handle, 2.3 and 2.4- handle and the samples comparative material from the persons BBN / Object No 17- injured woman / and SNS / Object No 18- suspect man / was performed by organic phenol extraction (phenol: chloroform: isoamyl alcohol = 25: 24: 1). DNA precipitation was performed with chilled to minus 20 ° C absolute alcohol. The extracted DNA was dissolved in TE-4 Buffer in a volume of 50 microliters when stored at minus 20 ° C.

2. PCR polymerase chain reaction for the samples was initially carried out in two stages on:

- Real-Time PCR system 7500 (Life Technologies) with PC Notebook, for HID analysis - with Quantifiler ™ Trio DNA Quantification Kit and qualitative and quantitative assessment of the available DNA in the samples through HID Real-Time PCR Analysis Software v1.2;

- PCR device SimpliAmp ™ Thermal Cycler, 96 x 0.2 ml (Life Technologies) in a volume of 25 μl with NGM Detect ™ PCR Amplification Kit (Applied Biosystems) for the studied autosomal and sex-defining STR´s markers for biological material - blood and possibly epithelial cells by Objects NoNo 2.1, 2.2.1- handle, 2.2.2- handle, 2.3 and 2.4- handle and samples comparative material from the persons BBH / Object No 17- woman / and SNS / Object No 18- male / - template (extracted DNA).

The analyzed 16 autosomal STR and 2 sex-defining genetic markers contained in the NGM Detect ™ PCR Amplification Kit are the following: D2S1338, SE33, D16S539, D18S51, TH01, D12S391, D3S1358, FGA, Y indel, Amelogenin, vWA, D21S11, D1S1656, D2S441, D8S1179, D19S433, D22S1045, D10S1248.

In the second stage of the present study, the polymerase chain reaction for the samples from Objects NoNo 2.1, 2.2.1-handle, 2.2.2-handle, 2.3 and 2.4-handle was performed in two stages on:

- Real-Time PCR system 7500 (Life Technologies) with PC Notebook, for HID analysis - with Quantifiler ™ Trio DNA Quantification Kit and qualitative and quantitative assessment of the available DNA in the samples through HID Real-Time PCR Analysis Software v1.2;

- SimpliAmp ™ Thermal Cycler PCR, 96 x 0.2 ml (Life Technologies) in a volume of 25 µl with Yfiler ™ Plus PCR Amplification Kit (Applied Biosystems) for the studied Y chromosome STR´s markers for the biological material - epithelial cells by Objects (No samples- "2.1", "2.2.1- handle", "2.2.2- handle", "2.4- handle" and the sample comparative material from the person SNS / Object No 18 / - template (extracted DNA).

The analyzed 25 Y chromosomal STR´s genetic markers contained in Yfiler ™ Plus PCR Amplification Kit are the following: DYS 576, DYS 389 I, DYS 635, DYS 389 II, DYS 627, DYS 460, DYS 458, DYS 19, YGATAH 4, DYS 448, DYS 391, DYS 456, DYS 390, DYS 438, DYS 392, DYS 518, DYS 570, DYS 437, DYS 385 a/b, DYS 449, DYS 393, DYS 439, DYS 481, DYS 387 S1, DYS 533.

3. Fragment analysis:

Fragment analysis was performed on a Genetic Analyzer model 3500 Series Genetic Analyzers for Human Identification (Life Technologies) by 8 capillary electrophoresis (with 3500 POP-4 ™ Polymer) with laser fragment detection and computer analysis by Gene MapperTM v1.2 Full Software Technologies) for HID analysis.

The control and standardization of the analyzes were performed by:

- positive control - DNA Control 007;
- negative control - NS;
- Matrix Standard Kit DS- 37 (6- FAM ™, VIC ™, TED ™, TAZ ™, SID ™, LIZ ™ dyes) - for NGM Detect ™ Kit (Applied Biosystems);
- Matrix Standard Kit - 36 (6- FAM ™, VIC ™, NED ™, TAZ ™, SID ™, LIZ ™ dyes) - for Yfiler ™ Plus Kit (Applied Biosystems);
- internal standard - GeneScan ™ 600 LIZ ™ Size Standart v2.0;
- internal quality control markers - IQCS and IQCL.
- allele witness (NGM Detect ™ Allelic Ladder) for the respective STR markers validated and embedded in the NGM Detect ™ Kit (Applied Biosystems);
- allelic witness (Yfiler ™ Plus Allelic Ladder) for the respective Y chromosome STR markers validated and embedded in the Yfiler ™ Plus PCR Amplification Kit (Applied Biosystems).

Case presentation

We present a case of the murder of a 29-year-old woman with multiple impacts to her body with blunt and blunt-edged objects, as well as objects with the characteristics of piercing and cutting weapons. A forensic autopsy of the body was performed. In the course of the investigation, material evidence was seized - a golf club, two knives and parts of broken knives with abundant traces of blood on them. The purpose of the research conducted by the method of DNA profiling was to look for the presence of deposited biological cellular material on the material evidence from the perpetrator. In the case there is a man suspected of committing the crime, from whom comparative cellular material was taken from the buccal mucosa.

OBJECTS AND CONDUCTED EXAMINATIONS

Object No 1- black knife handle with a rough surface, with metal fittings to the stop. Along its entire length, circularly, and along the metal fittings of the handle, there are pronounced reddish stains of a substance resembling blood. From the area of the middle part of the handle, from an area with less pronounced staining of reddish matter, which is also a pre-selection place for deposition of cellular material by the person holding the handle of the knife, a cotton swab was rubbed to perform a test by the method of fragment DNA analysis. The sample is conditionally designated as: "2.1".

Object No 2- two knives, type "kitchen", with black handles with rough surfaces and metal fittings to the stops.

One of the knives has a machine wavy serrated cutting edge on the blade and is painted with black paint applied longitudinally to the back of the knife. The object is conditionally designated as: "2.2.1". Along its entire length, circularly, and along the metal fittings of the handle, there are pronounced reddish stains of a substance resembling blood. From the area of the casing and the upper part of the handle, from an area with less pronounced staining of reddish matter, a cotton swab was obtained for examination by the method of fragmentary DNA analysis. The sample is conditionally designated as: "2.2.1 - handle".

The second knife has a normal cutting edge on the blade and is painted with black paint applied longitudinally to the back of the knife. The object is conditionally designated as: "2.2.2". Scattered pale red stains appeared on the blade and handle of the knife. To perform the test, a sample was taken by swab from the area of the knife handle, which is a pre-selection place for the deposition of cellular material by the person holding the knife. The sample is conditionally designated as: "2.2.2 - handle".

Object No 3- top part of a broken metal stick for golf brand “WILLSON” № 47336, with a bent tubular part. Pale reddish stains were found on the massive metal part of the stick. From the area of the middle part of the stick, from the area with a stain of reddish matter, a cotton swab was obtained for examination by the method of fragmentary DNA analysis. The sample is conditionally designated as: "2.3".

Object No 4-handle with brown leather cover with slightly bent tubular part of a broken metal stick for golf brand "WILLSON" PAT. No 1974875. On the handle of the stick, in the areas of the many linear inlays, reddish traces with the character of seeps, stains and single flows were found. From the area of the middle part of the handle, from the area with staining of pale reddish material and next to it, which is also a pre-selection place for deposition of cellular material from the person holding the handle of the golf club, a cotton swab was rubbed to perform the method of fragment DNA analysis. The sample is conditionally designated as: "2.4 - handle".

Object No 17- comparative material / blood / on a white gauze swab, seized from the body of VVN / female victim /. Gauze strands were seized from a section of reddish-brown material for analysis by fragmentary DNA analysis. The sample is conditionally designated as: "2.17".

Object No 18 - comparative sample of a buccal mucosa swab, seized by the SNA person / suspected male perpetrator of the crime /, of Sterile Foam Tipped Applicator with LOT 4698 / 2021-07-01. Material from the described applicator was taken to perform a fragment DNA analysis assay. The sample is conditionally designated as: "2.18".

Results and Discussion

The initial genotyping of the traces of blood on the golf club and knives revealed the presence of a mixed genetic profile of the victim and low added alleles by another male person, without the possibility of deriving his genetic profile.

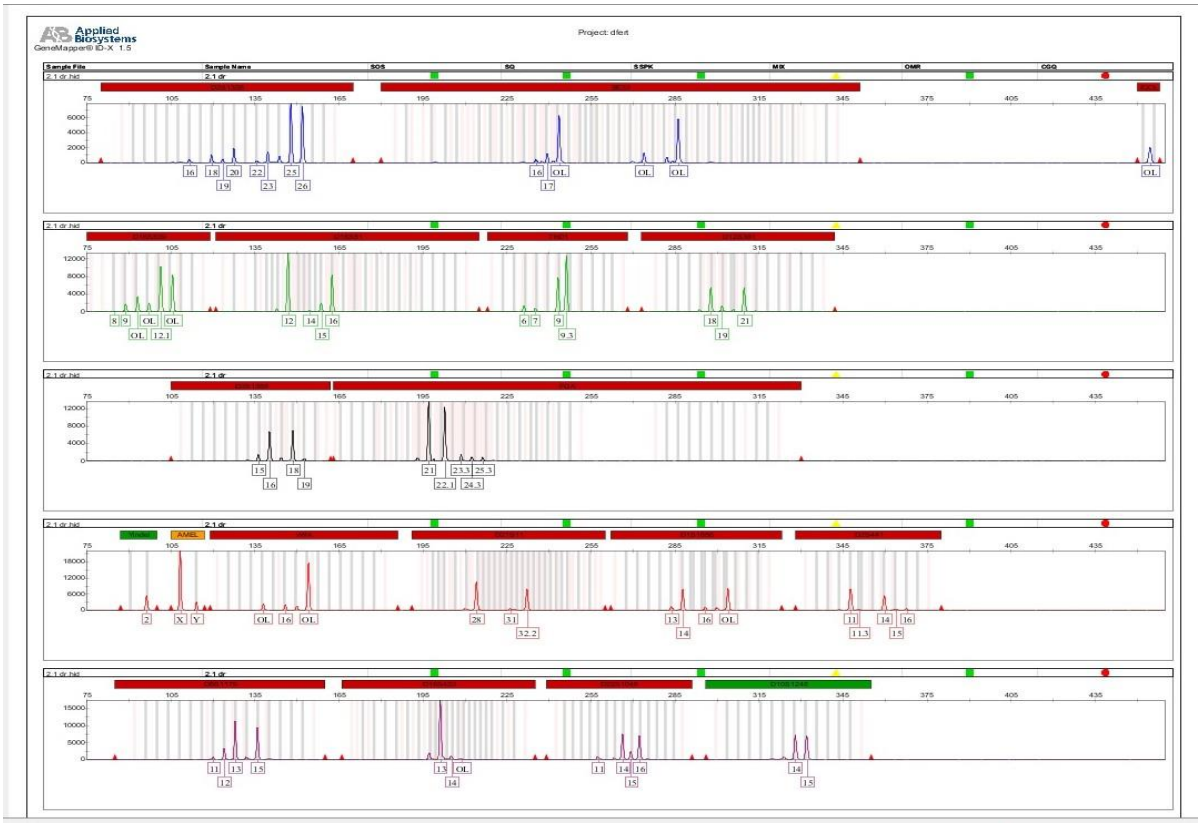

Fig. 1. Electrophoregram of a certain autosomal STR genetic profile / 16 autosomal STR and 2 sex-defining genetic markers contained in the NGM Detect ™ PCR Amplification Kit / of the blood on the handle of a knife - Object No 2.1., which is identical to the established blood profiles by Objects NoNo 2.2.1- handle, 2.2.2- handle, 2.3 and 2.4- handle, and is identical to the genetic profile of VVN / female victim- Object No 17 /. The presence of mixed genetic material in the examined samples, mainly from the victim, and low added alleles from another male person / incl. and a peak in the sex-defining marker Yindel /, without the possibility of deriving its genetic profile.

For the examined biological traces on Objects NoNo 2.1, 2.2.1- handle, 2.2.2- handle, 2.3 и 2.4- handle, identical autosomal STR´s genetic profiles with the determined autosomal STR profile of the female victim /Object No 17/ have been isolated, comprising the following characteristics:

Objects NoNo 2.1, 2.2.1- handle, 2.2.2- handle, 2.3 и 2.4- handle- D2S1338- 25/26, SE33- 18/28.2, D16S539- 12/13, D18S51- 12/16, TH01- 9/9.3, D12S391- 18/21, D3S1358- 16/18, FGA- 21/22, Yindel- /-/, Amelogenin- X/X, vWA- 18/18, D21S11- 28/32.2, D1S1656- 14/18, D2S441- 11/14, D8S1179- 13/15, D19S433- 13/13, D22S1045- 14/16, D10S1248- 14/15.

Object No 17 /sample 2.17/-D2S1338- 25/26, SE33- 18/28.2, D16S539- 12/13, D18S51- 12/16, TH01- 9/9.3, D12S391- 18/21, D3S1358- 16/18, FGA- 21/22, Yindel- /-/, Amelogenin- X/X, vWA- 18/18, D21S11- 28/32.2, D1S1656- 14/18, D2S441- 11/14, D8S1179- 13/15, D19S433- 13/13, D22S1045- 14/16, D10S1248- 14/15.

It should be noted that in the course of the examination of Objects NoNo 2.1, 2.2.1-handle, 2.2.2-handle, 2.3 and 2.4-handle in the laser detection of fragments of the studied autosomal STR´s and sex-defining genetic markers contained in the NGM Detect ™ PCR Amplification Kit, additional alleles were visualized, with a critically low unreadable height,

around the baseline for reporting alleles other than those demonstrated in the genetic profile of the female victim / Object No 17 /.

These results indicate the presence of genetic material from another person with a predominant amount of biological material - blood from the injured female person (Object No 17) in mixed samples. Most likely, it is the presence of single epithelial cells left by the person holding these objects, whose genetic profile cannot be deduced from a comparative analysis of autosomal STR´s loci. With the results thus obtained, additional analysis by sex-specific Y chromosome markers could be applied.

Given the possibility of deposition of biological material - epithelial cells, the person holding the handles of Objects NoNo 2.1, 2.2.1 - handle, 2.2.2 - handle, 2.3 and 2.4 - handle, with a predominant amount of genetic material - blood from the victim from females / Object No 17 /,the study proceeded to using Y-STR chromosome analysis..

This type of analysis generally allows, with a minimal amount of male biological material in a mixed female-male sample, to ignore the female component with amplification only of genetic loci on the male Y chromosome. This possibility motivates the conduct of a comparative study of material from Objects NoNo 2.1, 2.2.1- handle, 2.2.2- handle, 2.3 and 2.4- handle and the comparative material from the person SNS / suspected perpetrator of the male crime - Site № 18 / using 25 Y chromosomal STR´s genetic loci contained in Yfiler ™ Plus PCR Amplification Kit.

In the course of the study it was established that the genetic Y chromosomal STR´s profiles of the epithelial cells by Objects NoNo 2.1, 2.2.1- handle, 2.2.2- handle, 2.3 and 2.4- handle and SNS / suspected male perpetrator of the crime - Object No 18 / are the same.

Identical Y-STR´s genetic profiles / 25 Y chromosomal STR´s genetic markers contained in Yfiler ™ Plus PCR Amplification Kit / were isolated for the studied biological traces by Objects №№ 2.1, 2.2.1- handle, 2.2.2- handle, 2.3 and 2.4- handle, identical to the defined DNA profile of the suspected perpetrator of the crime / Object No 18 / with the following characteristics:

Objects NoNo 2.1, 2.2.1- handle, 2.2.2- handle, 2.3 и 2.4- handle- DYS 576- 18, DYS 389 I- 13, DYS 635- 24, DYS 389 II- 32, DYS 627- 21, DYS 460- 10, DYS 458- 17, DYS 19- 15, YGATAH 4- 11, DYS 448- 19, DYS 391- 11, DYS 456- 15, DYS 390- 24, DYS 438- 10, DYS 392- 11, DYS 518- 39, DYS 570- 18, DYS 437- 15, DYS 385 a/b- 14/15, DYS 449- 32, DYS 393- 13, DYS 439- 13, DYS 481- 29, DYS 387 S1- 38/39, DYS 533- 13.

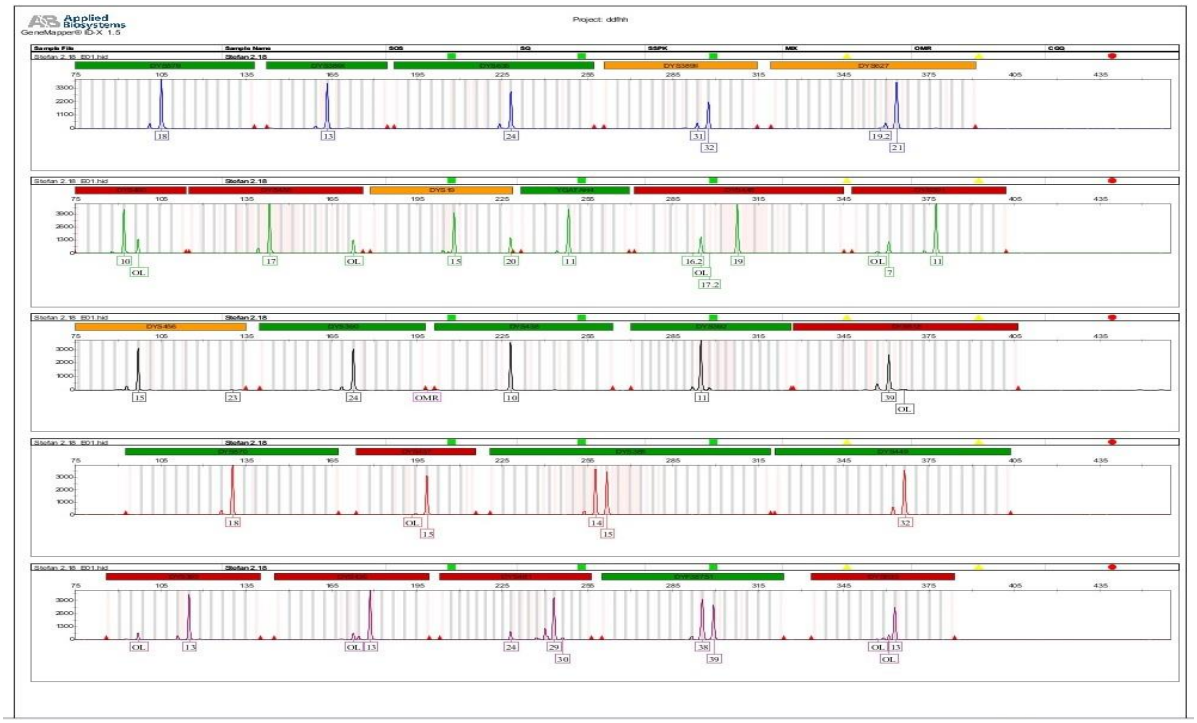

Fig. 2. Electrophoregram of a specific Y-chromosomal STR genetic profile / 25 Y-STR´s genetic markers contained in Yfiler ™ Plus PCR Amplification Kit / of the cellular material on the handle of a knife-Object No 2.1., which is identical to the established profiles of the cellular material by objects NoNo 2.2.1- handle, 2.2.2- handle, 2.3 and 2.4- handle.

Object No 18 /sample 2.18- SNS- male suspect perpetrator of the crime/- DYS 576- 18, DYS 389 I- 13, DYS 635- 24, DYS 389 II- 32, DYS 627- 21, DYS 460- 10, DYS 458- 17, DYS 19- 15, YGATAH 4- 11, DYS 448- 19, DYS 391- 11, DYS 456- 15, DYS 390- 24, DYS 438- 10, DYS 392- 11, DYS 518- 39, DYS 570- 18, DYS 437- 15, DYS 385 a/b- 14/15, DYS 449- 32, DYS 393- 13, DYS 439- 13, DYS 481- 29, DYS 387 S1- 38/39, DYS 533- 13.

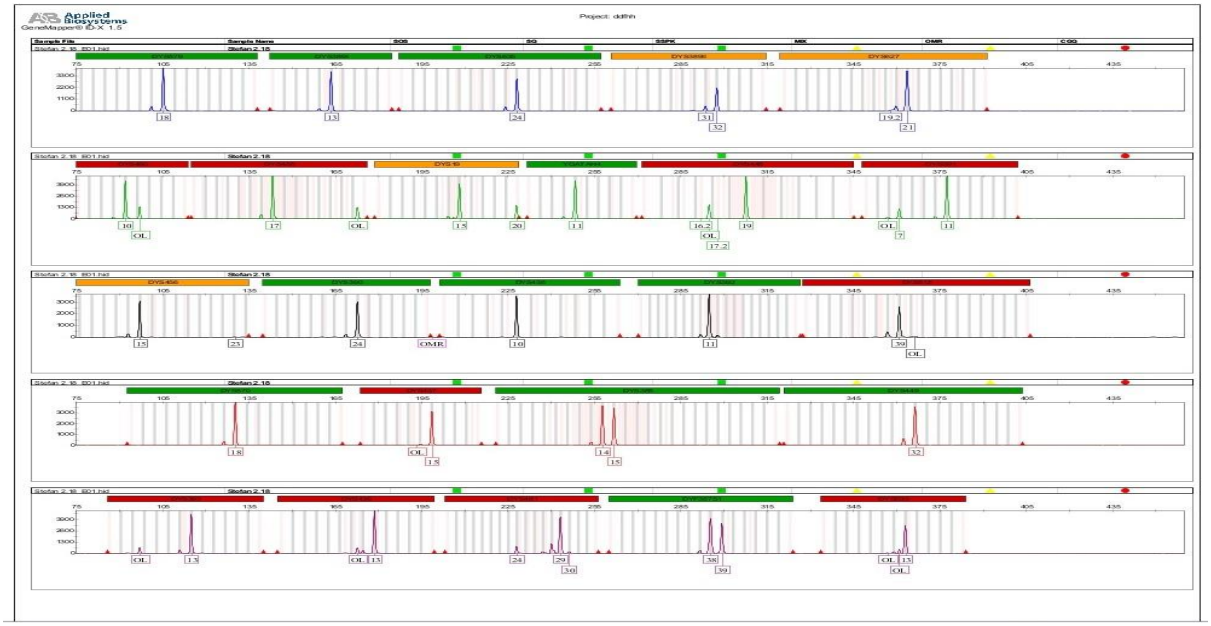

Fig. 3. Electrophoregram of a certain Y-chromosomal STR genetic profile / 25 Y - STR´s genetic markers contained in Yfiler ™ Plus PCR Amplification Kit / of the comparative cellular

material from SNS / suspected male perpetrator - Object № 18 /, which is identical to the established Y-STR´s profiles of the cellular material by Objects №№ 2.1, 2.2.1- handle, 2.2.2- handle, 2.3 and 2.4- handle.

The comparative analysis revealed a complete match between the alleles demonstrated in the respective studied genetic markers in the samples of biological material - the epithelial cells of Objects NoNo 2.1, 2.2.1- handle, 2.2.2- handle, 2.3 and 2.4- handle, building their combined DNA profiles with the characteristics of the combined genotype of the SNA / male suspected perpetrator of the crime - Object No 18 / (see the results presented above).

The indicated match of the DNA profiles (presence of the same alleles forming the same genotypes) in the epithelial cells by Objects №№ 2.1, 2.2.1- handle, 2.2.2- handle, 2.3 and 2.4- handle and in SNS / suspected male perpetrator of the crime - Object № 18 / gives grounds to assume that the biological material found at the objects may originate from it, whereby a biostatistical calculation was performed.

The combined genotypic frequency of profile occurrence was determined by the formula $SF = SF_1. SF_x .....SF_n$where SF_1 is the genotypic frequency for the respective locus and it is $SF_1 = 2pq$ for a heterozygous genotype (presence of two different alleles) and $SF_1 = p^2$for a homozygous genotype according to the respective STR marker, where p and q are the allelic frequencies for the population.

In the biostatistical calculation, allele frequencies of the applied markers from a population study were used.

The calculated combined genotypic frequency (SF) for the examined markers in SNA / suspected male perpetrator of the crime - Object № 18 / and respectively biological matter - epithelial cells by Objects-2.1, 2.2.1- handle, 2.2.2- handle, 2.3 and 2.4- handle is SF = 0.0000000018.

If genetic evidence is defined as a random relationship between two probabilities (LR), then LR = 1 / SF, which means the probability of establishing a specific DNA profile if the biological material left by an individual other than the SNS / suspected perpetrator of male sex - Object No 18 /, is:

1 in 1,776 000 000 000

The Probability distribution (PD), or the probability of two randomly selected males having different genotypes on specific markers, was determined by the formula PD = (1 - SF). 100%., as the calculation of the same gives a value for SNS / suspected male perpetrator of the crime - Object № 18 / and respectively the biological material - the epithelial cells in Objects NoNo 2.1, 2.2.1- handle, 2.2.2- handle , 2.3 and 2.4- handle for PD = 99.999999981%.

In the presented case is analyzed the possibility, due to the prevailing amount of genetic material - blood from the victim in a mixed sample, to use the approach for genotyping the cellular material left by the man holding knives and golf club using sex-defining genetic markers contained in his Y - chromosome.

Conclusion

The use of sex-defining Y-chromosome sets of genetic markers to differentiate genetic profiles in mixed female-male biological traces allows ignoring the female component, with its predominant amount masking the deposited cellular material by the perpetrator.

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