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DNA EXAMINATION OF MIXED TRACES ON PHYSICAL EVIDENCE, IN KINSHIP AND RECOGNITION DETERMINATION BY YFILER™ PLUS PCR AMPLIFICATION KIT FOR Y CHROMOSOME IDENTIFICATION

Plamen Iliev[2], Bogdan Mirchev[8], Ekaterina Angelova[2], Vera Djeliova[3], Atanas Hristov[1,2], Yanko Kolev[4], Milka Mileva[5], Dimo Krastev[6,7], Aleksandar Apostolov[1,2,*]

[1]Medical University, Faculty of Medicine, Department of Forensic Medicine and Deontology, Sofia, [2]University Hospital "Alexandrovska", Clinic of Forensic Medicine and Deontology, Sofia, [3]Institute of Molecular Biology "Roumen Tsanev", Department of the Molecular Biology of the Cell Cycle, Bulgarian Academy of Sciences, Sofia, [4]Medical University, Department of General Medicine, Forensic Medicine and Deontology, Pleven, [5]Laboratory of Biological Response Modifiers and Pathogenesis of Viral Infections, Department of Virology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, [6]South-West University "NeofitRilski", Department of Anatomy and Physiology, Blagoevgrad, [7]College of Medicine "Yordanka Filaretova", Medical University, Sofia, [8]University Hospital "Burgas", Departament of Forensic Medicine, Burgas, Bulgaria

Abstract: In expert practice, the Y chromosome analysis is an excellent method for detecting the presence of male DNA in mixed traces as well as for identifying males, or for establishing kinship. The goals of the research were to identify a minimal male component in mixed biological samples, as well as to identify unknown deceased men or to determine genealogical kinship. In the course of the research for identifying males with unconfirmed identity, with the help of Y chromosome markers, the rare allel 20.2 was detected at locus DYS627, embedded in the Yfiler™ Plus PCR Amplification Kit.

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

INTRODUCTION

The Y-chromosome analysis is an extremely successful method for detecting the presence of male DNA in mixed traces or excluding a subject as a male cell donor [1, 2]. Following numerous studies, the Working Group on Forensic Expertise on Sexual Assault (SAFER), established by the National Institute of Justice (USA), has published a paper recommending the consistent use of primary autosomal STRs analysis and a secondary Y-STRs test [3] as this approach has been developed by other researchers aswell [4-6].

Deposition and superimposition of biological material by the victim and the physical perpetrator of a crime are common findings in the process of examining biological traces on physical evidence. A case from Germany reported in 2015 proves the importance of Y-STR testing. A suspect had been detained following an unsuccessful robbery attempt that resulted in the murder of a woman in her apartment. On the noose that was used to strangle the woman, on the background of massive female saliva, scarce male epithelial material was found. Due to the well-known effect of preferential amplification in conventional autosomal analysis, the male autosomal profile was not established, but a Y-STR analysis generated a complete profile for 23 loci. The profile corresponded to the suspect who had already been arrested. This case strongly influences the process of drafting guidelines for the interpretation of Y-STRs in Germany [7-9].

It is in such cases that it is a real challenge to choose the most appropriate expert approach to delineate and determine the individual DNA characteristics and derive the offender's DNA profile. The options are related to the correct selection and use of the possibilities of the autosomal and sexual X-

*Correspondence to: Assoc. Prof. Aleksandar Apostolov, MD, PhD. Department of Forensic Medicine and Deontology, Medical University, Faculty of Medicine, 2 Zdrave St., Sofia 1431, Bulgaria, E-mail: alexa2000@mail.bg

and Y-chromosomal genetic loci. Currently, various kits and additional multiplexes can be combined to amplify more than 40 Y-STR sequences, and are used in forensic expertise. All of these Y-STRs have been carefully selected from the male-specific region of the Y chromosome [10,11].

In order to identify and isolate the less represented male component in female/male mixed samples, the Capillary Electrophoresis method [12] is currently the most appropriate [13] and the use of online accessible population databases consisting of Y-STR profiles introduced into reference populations [14-17]. As already noted, Y chromosome analysis can detect hidden male DNA against a background of predominant female DNA. Therefore, Y-STR screening is recommended in all cases where male DNA left by the perpetrator is expected but is not detected in regular autosomal STR screening.

As examples of determining Y-based DNA profiles in the DNA laboratory at the Forensic Medicine and Deontology Clinic at University Hospital "Alexandrovska" Sofia, we present the following two cases. The first case concerns the determaining the Y-based DNA profile of a male suspect from a mixture of biological material from two individuals, a female and a male. In the analyzed mixture, the biological material from the female victim was in much greater quantity. This makes it difficult to differentiate the DNA profile of the male after appling autosomal STR DNA profiling.

In the second case, Y-based DNA profiling was used to establish kinship, and so the identity, of an unknown male subject. A biological sample had been obtained from his suspected male relative. In this case a rare allele 20.2 in Y chromosome locus DYS627, embedded in the Yfiler ™ Plus PCR Amplification Kit (Applied Biosystems) had been detected. This rare allele is positioned between allele bins 20 and 21 in the Allelic Ladder, and up to this moment has not been detected in the Bulgarian population. The mutation rate of the chromosome locus DYS627 is 1.39e-02 (103 in 7404) (18-21). This result, despite the relatively high mutation frequency in the DYS627 locus, added an additional uniqueness to the profile, which aided the identification of the deceased man. No record for the DYS627 locus was found when referring to the website https://strbase.nist.gov/ of the NIST [23].

The isolation of DNA was performed with Auto Mate Express Forensic DNA Extraction System and ion-exchange resin Chelex100. DNA profiles for Y chromosome STR's markers with Yfiler ™ Plus PCR Amplification Kit and for autosomal STR's with NGM Detect ™ PCR Amplification Kit (Applied Biosystems).

MATERIALS AND METHODS

In our expert practice for DNA isolation and determining genetic profiles, we use the established biological traces in the physical evidence analysis, which are most often epithelial cells, blood, semen, saliva. To derive the individual DNA profiles of suspects, victims or alleged relatives, we use swabs of cellular material from buccal mucosa, blood samples, bone material, etc. in order to conduct a comparative identification analysis.

The described procedures were performed in the Forensic DNA Laboratory at the Clinic of Forensic Medicine and Deontology at the University Hospital "Alexandrovska" - Sofia, Bulgaria. After collecting the described samples, their initial assessment and careful selection, a specific research approach was determined for each individual case.

The analysis were performed following a sequence of basic steps:

1. DNA extraction – The extraction of DNA from biological material - epithelial cells, blood, semen, etc. established on the basis of the examined material evidence and the comparative cellular material from the compared persons we performed on a DNA extractor model AutoMate Express Forensic DNA Extraction System with PrepFilter Express ™ Forensic DNA Extraction Kit /Thermo Fisher Scientific/ or with the help of ion exchange resin Chelex100 / SIGMA ALDRICH 95577 - 100G-F /.

2. PCR (polymerase chain reaction) that consists of three main steps - thermal denaturation, hybridization and extension.

The polymerase chain reaction for the samples was initially performed in two stages:

- 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 using HID Real-Time PCR Analysis Software v1.2;

- PCR device SimpliAmp ™ Thermal Cycler, 96 x 0.2 ml (Life Technologies) in volume 25 μl with NGM Detect ™ PCR Amplification Kit (Applied Biosystems) for the studied autosomal and sex-defining STR´s markers for biological material - template (extracted DNA).

The 16 autosomal STR and 2 sex-defining genetic markers contained in the NGM Detect ™ PCR Amplification Kit applied and analyzed by us 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 discussed approach, the polymerase chain reaction of the samples containing human biological material of suspicious origin from a malewas carried out in two stages:

- 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 using 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 Yfiler ™ Plus PCR Amplification Kit (Applied Biosystems) for the analyzed Y chromosome STR markers for biological material on the objects in question and sample comparative material from the analyzed persons - template (extracted DNA).

For our analyzes we used 25 Y chromosome 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 of the samples is 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 detection of fragments and computer analysis by Gene MapperTM v1.2 Full Software Life Technologies) for HID analysis [17].

The control and standardization of the analyzes were carried out by:

- positive control - DNA Control 007;

- negative control - HC;

- Matrix Standard Kit DS-37 (6- FAM ™, VIC ™, TED ™, TAZ ™, SID ™, LIZ ™ dyes) - for NGM Detect ™ Kit (Applied Biosystems);

- Matrix Standard Kit DS-36 (6- FAM ™, VIC ™, NED ™, TAZ ™, SID ™, LIZ ™ dyes) - for Yfiler ™ Plus Kit

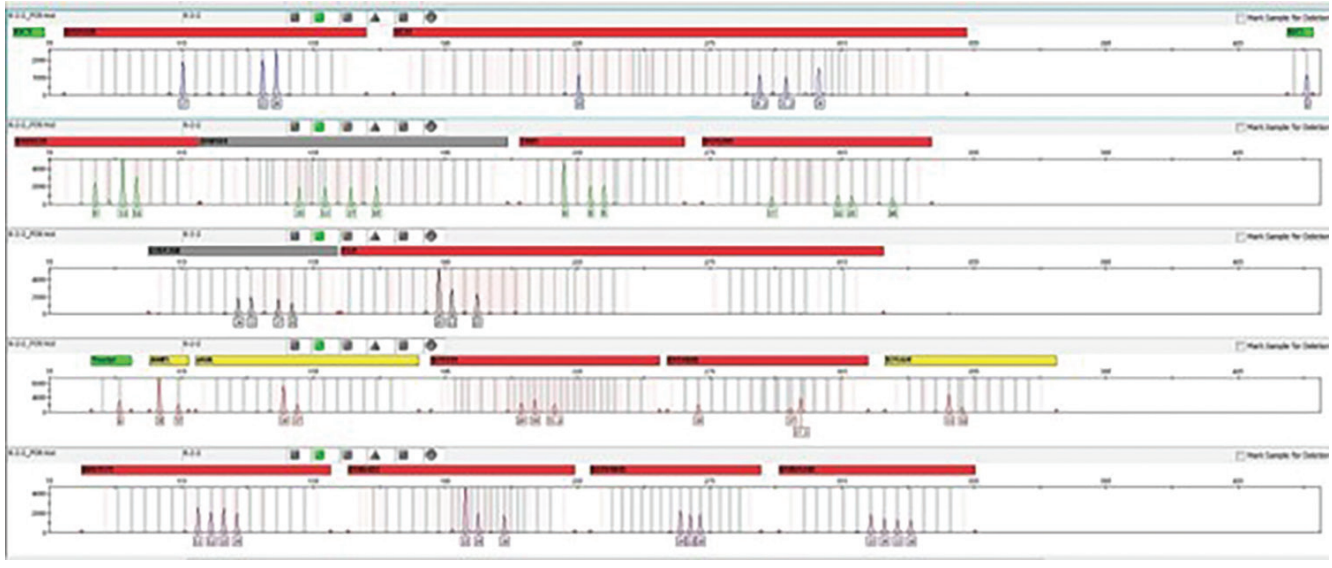

Figure 1. Electrophoregram - mixed genetic material from the "bite mark" in the abdominal area of a female victim in a case of fornication - a male and female mix is established.

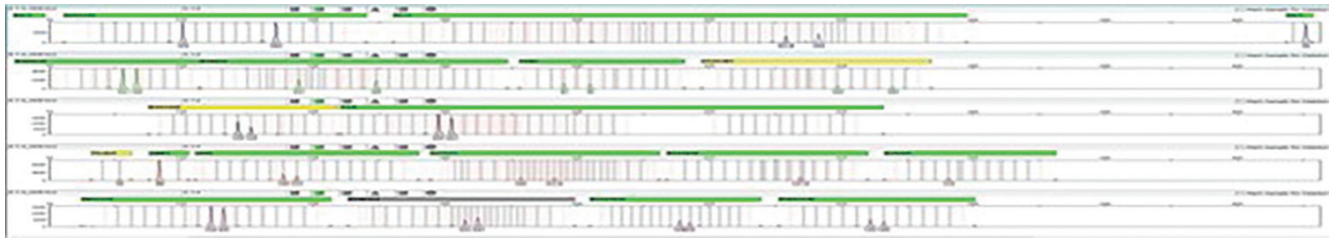

Figure 2. Electrophoregram- a part of autosomal DNA profile of the injured woman.

(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);

- allele witness (Yfiler ™ Plus Allelic Ladder) for the respective Y chromosome STR markers validated and embedded in the Yfiler ™ Plus PCR Amplification Kit (Applied Biosystems).

RESULTS

In the practice of the DNA laboratory of the Clinic of Forensic Medicine and Deontology at the University Hospital "Alexandrovska" - Sofia in analyses requiring the use of methodological approaches to the so-called "male identification" using the Y-chromosome markers, some major areas of interest were highlighted.

Usually in the analysis of mixed traces containing female and male genetic material, the female component predominates quantitatively (Fig.1). This is expressed by the dominant visualization of alleles of female genetic profile (Fig.2) in autosomal genetic analysis of markers contained in NGM Detect ™ PCR Amplification Kit.

Also, additional low-intensity alleles, slightly above the baseline (Fig. 1), have been jointly reported, which may be an obstacle to determining the overlapping male genetic profile (Fig. 3).

In such situations, we took a direct amplification approach using a set of 25 Y chromosome markers contained in the Yfiler ™ Plus PCR Amplification Kit (Fig. 4), thus directly eliminating the female component, whether or not vaginal swabs or on traces seized by swabs from the area of "bite marks" evidence or traces on various objects.

The obtained result of DNA profiling with the Yfiler ™ Plus PCR Amplification Kit of the biological material from the victim's skin swab in the area of the bite mark is presented in fig. 4. After the direct comparison of the Y-based DNA profile obtained for the mixed biological sample with the Y-based DNA profile of the suspect, a match was detected /Case 1/.

Another area for male identification is by the use of Y chromosome markers to identify deceased persons when first-degree direct relatives (parents, children) are absent. In order to solve such genetic problems, referring to the genealogical determination of the transmission of the Y chromosomal information via the direct male line, we used genetic material from alleged uncles, biological brothers and cousins from the paternal line of the deceased men, the so-called silver line.

In most cases, when we explored comparative material from such men, we established their Y chromosome identity or derived male profiles, which identified them. At the same time, in the absence of comparative material from putative male relatives, the derived individual Y chromosome profiles of unidentified men could be compared with those from Y chromosome databases.

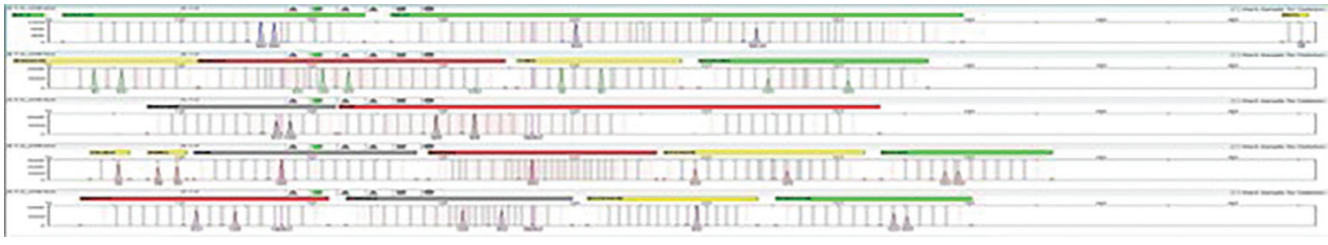

Figure 3. Electrophoregram - a part of autosomal DNA profile of the suspected man.

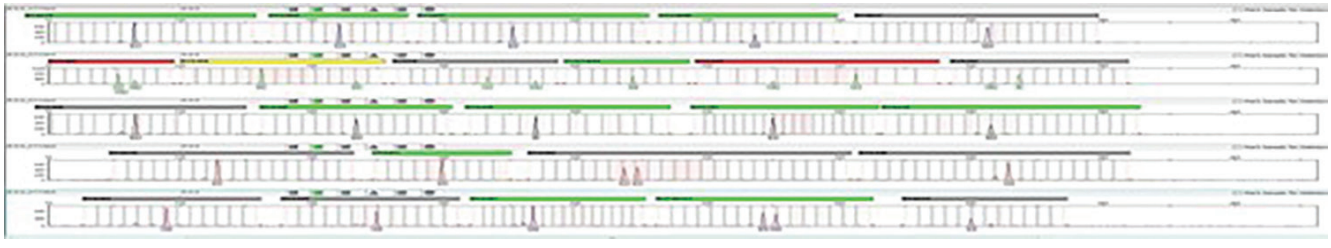

Figure 4. Electrophoregram - a part of Y based DNA profile found in a sample of mixed genetic material from a "bite mark" in the abdominal area of an the victim /woman/. The established Y based DNA profile in the sample is identical to the derived Y based DNA profile from the suspect.

For such identification of a deceased unknown man, we present an expert case conducted through a comparative analysis with a Y-based DNA profile with the presumed brother of the deceased male. The Y-based DNA profile of the deceased man (Fig. 5) was determined after extraction of genetic material isolated from the femur, with comparative buccal mucosa material from the male indicated as the brother (Fig. 6). The obtained result of the conducted DNA profiling with the Yfiler ™ Plus PCR Amplification Kit of the biological material isolated from the femur of the unknown male subject is presented in fig. 5. After the direct comparison of the Y-based DNA profile obtained from from a swab from the oral cavity of the presumed brother, a match was found between the two DNA profiles /Case 2/.

In the course of the research for identification of men with unconfirmed identity with the help of Y chromosome markers, was detected the rare allel 20.2 at locus DYS627 (Figs 5, 6), embedded in the Yfiler ™ Plus PCR Amplification Kit. The rare allele is positioned in the interstitial space between allele's bins 20 and 21 in the Allelic Ladder (Fig. 7), and up to this moment has not been detected in the Bulgarian population. This result, despite the relatively high mutation frequency in the DYS627 locus, determined an additional uniqueness of

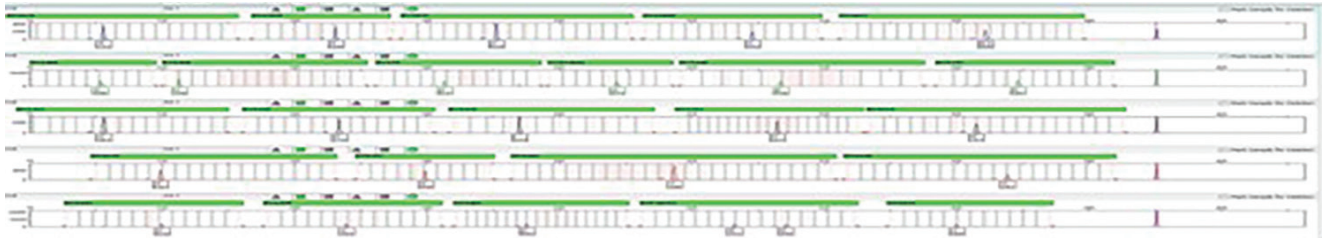

Figure 5. Electrophoregram - a part of Y based DNA profile of a deceased unidentified man, identified by Yfiler ™ Plus PCR Amplification Kit (Applied Biosystems).

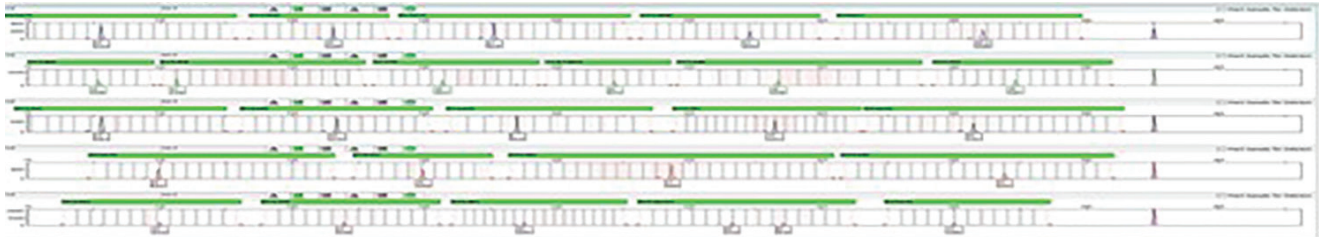

Figure 6. Electrophoregram of a part of Y based DNA profile of the presumed brother of the deceased unidetified man determined by Yfiler ™ Plus PCR Amplification Kit (Applied Biosystems).

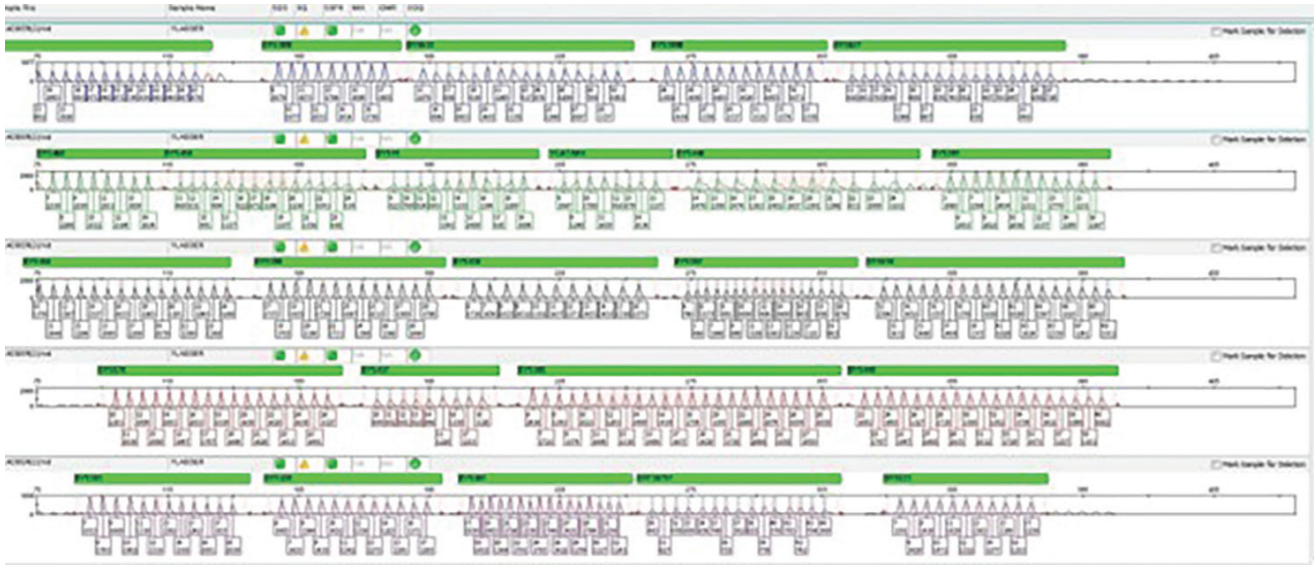

Figure 7. Electrophoregram of the Allelic Ladder for the corresponding Y chromosome STR markers validated and embedded in Y filer ™ Plus PCR Amplification Kit (Applied Biosystems).

the profile, respectively the result for the identification of the deceased man.

In general, allelic witnesses in the multiplex STR and Y analysis are divided into three coloured longitudinal stripes: grey, pink, and white (Fig. 7). Gray indicates an established bin, pink for a virtual bin, and the white stripe is out of the range of the allele witness. Peaks within grey or pink stripes are automatically marked by STR analysis software (Gene Mapper™ v1.2 Full Software) [22]. The set of bins in the allele witness provides reference allele sizes for: alleles physically present in it (physical bins – grey stripes) and alleles that are not present in the witness (virtual bins – pink stripes), which are either reported in the STR base (the website https://strbase.nist.gov/) [23] or detected during validation. To compensate for the virtual bins, the software uses the offset from the nearest physical bin or virtual bin to the left of the bin [22].

DISCUSSION

The Y-chromosome analysis is a successful method for detecting the presence of male DNA in mixed traces, excluding a subject as a male cell donor, or identifying a male. After numerous studies, we confirmed the benefits of sequential use of primary autosomal STRs analysis and secondary Y-STRs testing, and in some cases direct Y-chromosome determination is justified. Achieving such a good result is achievable with a good initial assessment of the potential of the biological trace in question. Typically, when mixed traces containing female and male genetic material are being analyzed, the female component prevailed quantitatively in studies of autosomal genetic markers. Additional alleles suggesting a superimposed male genetic profile have also been reported. We took a direct amplification approach using the 25 Y-chromosome markers contained in the Yfiler™ Plus PCR Amplification Kit, thus directly eliminating the female component by displaying Y chromosome profiles in the mixed sample.

In severe crime cases, Y chromosome analysis may be considered the only way to determine the DNA profile of the unknown perpetrator and to reduce the number of suspects [24, 25]. From what has been said so far, it is clear that Y chromosome markers can be successfully used to support forensic research in a wide range of cases, be it for rapid separation of traces, for identification and profiling of masked male DNA components in mixtures with excess female DNA or to identify unknown individuals who left biological traces.

To identify deceased individuals, referring to the genealogical determination for the transmission of Y chromosomal information in the direct male line, we used comparative samples from presumed fathers, uncles and cousins.

Cases related to detection of rare alleles in allele distribution loci are also possible, as is the case when we identified a 20.2 allele in the Y chromosome locus DYS627, set in the Yfiler™ Plus PCR Amplification Kit in a case of identifying an unknown man. Recently, the same variant allele 20.2 at the DYS627 locus was detected between eight intermediate alleles in four Y chromosme loci in the study of genetic polymorphisms of 44 Y chromosomal genetic markers in the Inner Mongolia Han population (26).

Rare allelic variants significantly increase discrimination strength when comparing DNA profiles. In this regard, for Forensic Medicine and Criminalistics, it is important to report any new information about the emergence of new allele variants, detected in a particular population group. The National Institute of Standards and Technology (NIST) maintains a DNA Internet database (https://strbase.nist.gov) since 1997 – STRBase, which registers all new allele variations [23].

In conclusion, the goal of the present study was to detect a male DNA from a background of predominant female DNA with the direct amplification approach using a set of 25 Y-chromosome markers. It was confirmed that Y-STR screening is a suitable approach in all cases where male DNA deposted by the perpetrator is expected, but is not detected with regular autosomal STR screening. During the process for identification of men with unconfirmed identity with the help of Y-chromosome markers, the rare allele 20.2 at the Y chromosome locus DYS627 was detected. This rare allele is positioned between allele's bins 20 and 21 in the Allelic Ladder, and up to this moment has not been detected in the Bulgarian population.

Data Availability Statement (DAS). The data that supports the findings in this article are available in the Experimental protocols of the last author, as well as in the Archives of DNA laboratory of the Department of Forensic Medicine and Deontology of University Hospital "Alexandrovska"– 1, "St. Georg Sofiiski" blvd., Sofia 1431, Bulgaria.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Schiermeier Q. Y-chromosome analysis urged for sex crimes. Nature. 2001;413(6851):6.
2. Syndercombe Court D. The Y chromosome and its use in forensic DNA analysis. Emerg Top Life Sci. 2021;5(3): 427-441.
3. National Institute of Justice. National best practices for sexual assault kits: A multidisciplinary approach. 2017, Retrieved from https://nij.gov/safer-report
4. Hanson E, Ballantyne J. A Y-short tandem repeat specific DNA enhancement strategy to aid the analysis of late reported (≥ 6 days) sexual assault cases. Med Sci Law. 2014;54(4):209-218.
5. Hanson EK, Ballantyne J. Enhanced DNA profiling of the semen donor in late reported sexual assaults: Use of Y-chromosome-targeted pre-amplification and next generation Y-STR amplification systems. In W. Goodwin (Ed.), Forensic DNA typing protocols Methods in molecular biology series. New York, NY: Humana Press. 2016; 1420: 185-200.
6. Purps J, Geppert M, Nagy M, Roewer L. Validation of a combined autosomal/Y-chromosomal STR approach for analyzing typical biological stains in sexual-assault cases. Forensic Sci Int Genet. 2015;19:238-242.
7. Roewer L, Willuweit S. Y-chromosomal STR analysis in forensic practice. Rechtsmedizin. 2018; 28(2): 149-164. German.
8. Willuweit S, Anslinger K, Bäßler G, Eckert M, Fimmers R, Hohoff C, Kraft M, Leuker C, Molsberger G, Pich U, Razbin S, Rothämel T, Schneider H, Schneider PM, Templin M, Vennemann M, Wächter A, Weirich V, Zierdt H, Roewer L. Joint recommendations of the project group "Statistical analysis of DNA" and the German Stain Commission on the statistical analysis of Y-chromosomal DNA typing results. Rechtsmedizin. 2018; 28(2)138-q42. German.
9. Willuweit S, Weirich V, Schneider PM, & Roewer L. Gemeinsame Empfehlungen der Projektgruppe "Biostatistische DNA-Berechnungen" und der Spurenkommission zur biostatistischen Bewertung von Y-chromosomalen DNA-Befunden. Neue Zeitschrift für Strafrecht. 2018; 8: 437-440. German.
10. Ballantyne KN, Goedbloed M, Fang R, Schaap O, Lao O, Wollstein A, Choi Y, van Duijn K, Vermeulen M, Brauer S, Decorte R, Poetsch M, von Wurmb-Schwark N, de Knijff P, Labuda D, Vézina H, Knoblauch H, Lessig R, Roewer L, Ploski R, Dobosz T, Henke L, Henke J, Furtado MR, Kayser M. Mutability of Y-chromosomal microsatellites: rates, characteristics, molecular bases, and forensic implications. Am J Hum Genet. 2010;87(3):341-353.
11. Redd AJ, Agellon AB, Kearney VA, Contreras VA, Karafet T, Park H, de Knijff P, Butler JM, Hammer MF. Forensic value of 14 novel STRs on the human Y chromosome. Forensic Sci Int. 2002;130(2-3):97-111.
12. Köcher S, Müller P, Berger B, Bodner M, Parson W, Roewer L, Willuweit S; DNASeqEx Consortium. Inter-laboratory validation study of the ForenSeq™ DNA Signature Prep Kit. Forensic Sci Int Genet. 2018;36:77-85.
13. Just RS, Moreno LI, Smerick JB, Irwin JA. Performance and concordance of the ForenSeq™ system for autosomal and Y chromosome short tandem repeat sequencing of reference-type specimens. Forensic Sci Int Genet. 2017;28:1-9.
14. Roewer L, Krawczak M, Willuweit S, Nagy M, Alves C, Amorim A, Anslinger K, Augustin C, Betz A, Bosch E, Cagliá A, Carracedo A, Corach D, Dekairelle AF, Dobosz T, Dupuy BM, Füredi S, Gehrig C, Gusmaõ L, Henke J, Henke L, Hidding M, Hohoff C, Hoste B, Jobling MA, Kärgel HJ, de Knijff P, Lessig R, Liebeherr E, Lorente M, Martínez-Jarreta B, Nievas P, Nowak M, Parson W, Pascali VL, Penacino G, Ploski R, Rolf B, Sala A, Schmidt U, Schmitt C, Schneider PM, Szibor R, Teifel-Greding J, Kayser M. Online reference database of European Y-chromosomal short tandem repeat (STR) haplotypes. Forensic Sci Int. 2001;118(2-3):106-113.
15. Redd AJ, Chamberlain VF, Kearney VF, Stover D, Karafet T, Calderon K, Walsh B, Hammer MF. Genetic structure among 38 populations from the United States based on 11 U.S. core Y chromosome STRs. J Forensic Sci. 2006;51(3):580-585.
16. Roewer L. The Y-chromosome haplotype reference database (YHRD)-Publicly available reference and research datasets for the forensic interpretation of Y-chromosome STR profiles. In Handbook of forensic genetics (2016, pp. 231– 248). London, England: World Scientific Publishing Europe Lda.
17. Willuweit S, Roewer L. The new Y Chromosome Haplotype Reference Database. Forensic Sci Int Genet. 2015;15:43-48.
18. Lin H, Ye Q, Tang P, Mo T, Yu X, Tang J. Analyzing genetic polymorphism and mutation of 44 Y-STRs in a Chinese Han population of Southern China. Leg Med (Tokyo). 2020;42:101643.
19. Fan G, Pan L, Tang P, Zhou Y, Liu M, Luo X. Technical note: developmental validation of a novel 41-plex Y-STR system for the direct amplification of reference samples. Int J Legal Med. 2021;135(2):409-419.
20. Otagiri T, Sato N, Asamura H, Parvanova E, Kayser M, Ralf A. RMplex reveals population differences in RM Y-STR mutation rates and provides improved father-son differentiation in Japanese. Forensic Sci Int Genet. 2022;61:102766.
21. Liu Z, Long G, Lang Y, Liu D, Zhang B, Yu S, Guo F. Sequence-based mutation patterns at 41 Y chromosomal STRs in 2 548 father-son pairs. Forensic Sci Res. 2023;8(2):152-162.
22. GeneMapper® ID-X Software, v1.5, Reference Guide, https://assets.thermofisher.com/TFSAssets/LSG/manuals/100031707_GeneMapIDX_ver1_5_ReferenceGuide.pdf. (Last access date 21 March 2023).
23. Ruitberg CM, Reeder DJ, Butler JM. STRBase: a short tandem repeat DNA database for the human identity testing community. Nucleic Acids Res. 2001;29(1):320-322.
24. Qian X, Hou J, Wang Z, Ye Y, Lang M, Gao T, Liu J, Hou Y. Next Generation Sequencing Plus (NGS+) with Y-chromosomal Markers for Forensic Pedigree Searches. Sci Rep. 2017, 7(1), 11324.
25. Yao HB, Wen SQ, Tong X, Zhou B, Du P, Shi M, Li J, Li H. Y chromosomal clue successfully facilitated the arrest of Baiyin serial killer. Sci. Bull. 2016, 61, 1715-1717.
26. Jin X, Xing G, Yang C, Zhang X, Cui W, Chen C, Zhu B. Genetic polymorphisms of 44 Y chromosomal genetic markers in the Inner Mongolia Han population and its genetic relationship analysis with other reference populations. Forensic Sci Res. 2021;7(3):510-517.