Chapter

Reliability and Reproducibility of DNA Profiling from Degraded Samples in Forensic Genetics

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Abstract

Forensic DNA analysis is widely used to determine kinship and the identity of evidence from the crime scene and it is especially important in the identification of human remains after different types of exposure (water, heat, etc.). Currently, there are no official recommendations for forensic scientists as to which bones and tissues are the most reliable among degraded DNA samples. Since 2014 more than 350 fragments of unidentified corpses have been examined in the Forensic Biological Department (Republic Bureau of Forensic Medicine, Kazan, Russia). Based on our experience, the most reliable and reproducible DNA profiles are obtained from lower limber bones (in 90% cases), muscles (in 85% cases) and ribs (in 80% cases). However, we discovered a new source of DNA – the odontoid process of the 2nd cervical vertebra, which contains a high amount of DNA with a better state of preservation than many other bones. According to our results, when a complete skeleton or unidentified corpse is found, it is advisable to provide bones with soft tissue remnants in the absence of deeply embedded putrefactive changes. When working at the crime scene, special attention should be paid to separating small bones and fragments from skeletal remains.

Keywords: Forensic genetics, degraded DNA, DNA profiling, autosomal and Y-chromosome STRs, mitochondrial DNA

1. Introduction

Over the past 20 years, forensic genetic analysis has become the main method for kinship determination and human identification when working with human biological traces and unknown corpses.

DNA identity can be determined 99% of the time (9), but it depends, first of all, on the number and types of studied genetic markers. Traditional forensic DNA analysis is based on autosomal and sex chromosomes STRs polymorphism and sequencing of the non-coding region of mitochondrial DNA. The effectiveness of using these markers varies in different samples due to the state of DNA preservation, especially in samples that have undergone significant changes under the influence of external conditions, such as extreme temperatures (fire), prolonged water exposure or decay in the corpse.

Short Tandem Repeat (STR) analysis is based on DNA typing of specific microsatellite loci with 2 to 7 base pairs length of core units repeat both in autosomal and sex chromosomes.

Due to the high variability in alleles and genotype distribution among unrelated individuals, autosomal STRs DNA analysis serves as an effective human identification tool. The more STR markers are investigated, the more informative individual profiles can be obtained, and the less likelihood coincidence that can be observed. What is more, constant location at specific loci in the genome promotes their stability for a long time even under unfavorable DNA preservation conditions.

Y-chromosomal STRs (Y-STR) are used to determine the presence of male DNA in mixed biological traces. However, the degree of Y-STR preservation is much lower than for autosomal STR and this fact is especially noticeable in the analysis of highly degraded DNA samples. However, Y-STR can be very useful in the determination of paternal lineage relationships and these data can be used as supplementary information to autosomal profiles.

Mitochondrial DNA (mtDNA) markers are significantly better preserved in biological traces compared to autosomal loci (especially in bones) due to their smaller size and multicopy per cell. That is why the study of mtDNA in degraded samples is more often successful. However, there is a significant disadvantage of only mtDNA analysis because of its maternal inheritance. Therefore in order to obtain a complete genetic profile, both types of markers (autosomal and mitochondrial) should be used.

Beside the different stability of described genetic markers, the degree of DNA preservation in biological traces plays a key role in the effectiveness of forensic genotyping and this fact is especially important in DNA profiling of bones and corpse's fragments after putrefactive changes during personal identification analysis [1].

2. DNA profiling efficiency in human remains

Identification of skeletal human remains rightfully belongs to the category of most complicated molecular genetic analysis. This is due, first of all, to the difficulties in DNA extraction from bones with various degradation degree as well as different DNA amount in specific bone cell types [2, 3].

When there is only part of a body, even the smallest bone fragments can be very useful to determine:

1. The fact of the death;

2. The nature of the death; and

3. Relationships with living relatives and so on.

Special traces analysis will assist to answer two questions, but genetic analysis is the only method that allows determination of the kinship between unidentified human remains and probable relatives.

There are various methods and kits for genetic identification that depend on each laboratory's capacity but it is important to obtain all possible genetic marker profiles from the each DNA sample.

For example, obtaining only mitochondrial DNA profile cannot be an objective proof in personal identification because the same mtDNA profile will be found in all maternal relatives, as well as in persons who have a very distant relationship with the deceased. The same applies to the Y-chromosome profile since all male relatives

will have the same Y-chromosome haplotype, as well as men who are very distantly related to the deceased [4].

Therefore, only a complex of molecular genetic profiles will give valuable evidence in skeleton fragments or the whole human body identification, especially when working with degraded DNA. It will allow a significant reduction in the probability error, which in turn, can minimize errors in the probability assessment in identification results [5, 6].

In international expert practice, there are no official recommendations as to what types of bones should be analyzed by preference. It is known that long tubular bones both from lower and upper limbs as well as ribs and teeth more often provided for forensic genetic analysis. But there are several assumptions: first of all, it is not always possible to collect all bone remains from the crime scene and secondly excavated bones may belong to different persons.

Over 350 fragments from unidentified corpses were analyzed in our laboratory. Our data show that ribs and their fragments were more often sent for genetic analysis (114 cases (32%)); in 83 cases (23%) lower limbs (mainly femurs) were analyzed. The bones of the upper limbs (29 cases/8%), teeth (21 cases/6%) and skull fragments (15 cases/4%) were also found suitable for genetic analysis. Only a few cases of sternum, clavicle, vertebrae, phalanges, heel bone, a spongy bone fragment, pelvic bone and liver (total 8% of all cases) were analyzed.

Soft tissues can also be an additional source of DNA. According to our data, muscles usually go along with bones and in very rare cases as independent objects because of its quick destruction due to putrefactive changes (**Figure 1**).

Muscles were examined in 69 cases (19%), and what is more, in 49 cases, tissues were chosen by an expert-geneticist. However, it is noteworthy that in all these cases a stable genetic profile was obtained.

The reliability of DNA profiles in human remains varies significantly. According to Jakubowska et al., DNA is better preserved in the sternum (90–100% recovery), in lower extremities bones (80–90%), in the lower jaw and teeth, worst - in pelvis, ribs and shoulder girdle bones [7]. These data are somewhat contradictory because different laboratories use their own equipment and reagents for DNA extractions that can affect the final DNA profiling results. Besides, the degree of DNA preservation in bones is influenced by many physical and chemical factors both of exogenous and endogenous nature [8]. In addition, bone cells - osteocytes – carrying basic genetic information, are located unevenly along the length of the tubular bone. That is why the amount of DNA and its state of preservation would not correlate with the bone type [9].

Herein, we describe the results of our experience for genetic profiling of different bone types and tissues.

2.1 Ribs

Stable genetic profiles were obtained in more than 80% of analyses of ribs and their fragments. Moreover reliable results were obtained in DNA samples extracted

Figure 1.
Femur with the fat wax indicating muscle tissue degradation.

from rib fragments after fire and in exhumed corpse bones. In 2% of cases a partial profile was obtained, which made it possible to compare the unidentified corpses with close relatives. In 18% of studied cases DNA profile was not determined.

2.2 Lower limb bones

As noted above, femurs were the most frequent type of bones for DNA analysis; rare cases were presented by the tibia. In three cases, bone fragments (cuts) from lower limbs were provided without specifying their localization. Stable genetic profiles were obtained in 90% of cases of examined bones.

It i's significant tooworth noting that in 30% of cases of lower limb bones genotyping, STR profiles were obtained only after repeated DNA extraction using the same or different extraction methods.

2.3 Upper limb bones

In most cases, bone fragments were presented by the humerus as well as clavicles, radial bones and ulna. Positive results were obtained in 65% of cases.

2.4 Teeth

A stable genetic profile was obtained in 61% of cases and 39% of cases were not successful. Moreover, in seven of the positive cases, the profile was obtained cumulatively while studying other bones from the same skeleton. Several types of research show that teeth are the preferred source for DNA extraction [10, 11]. However, the methods proposed differ from those used in our laboratory which may affect the low percentage of obtained stable profiles.

2.5 Skull fragments

Skull bone fragments and whole skulls were provided in only 15 cases. Moreover, the whole skull was presented for analysis in only five cases. A stable genetic profile was obtained in 54% of cases, mainly when analyzing the mastoid process and mandible.

2.6 Other fragments

Out of 7 cases of sternum analysis, only three cases gave positive results in determination of stable autosomal STR profile. There have been several cases of examination of fragments of the lung, cancellous bone, calcaneus, vertebrae (without specifying localization). The profile was obtained in all indicated cases. When examining the bones of the pelvis and phalanges of the fingers, the genetic profile has not been established.

Thus, a stable genetic profile was often obtained in long bones of upper and lower limbs and ribs compared to the other bone fragments (**Table 1**).

2.7 Muscles and tissues

A stable genetic profile was obtained in more than 85% of cases in tissue analysis. However, negative results in most of the unsuccessful cases were associated with the circumstances of the accident (heating or chemical exposures) which led to DNA damage. In such cases, bones with/without soft tissues remnants were provided for analysis to obtain possible DNA profiles.

Storage conditions, Death duration	2nd cervical vertebrae (odontoid process)	Femur	Ribs	Teeth	Elbow	Brachial bone	Skull bones
Forest, 2 years	100	100	0	90	—	—	—
Forest, 4 years	—	80	—	—	30	—	30
Forest, around 10 years	80	80	0	50	—	—	40
Exhumation after 10 years burial	—	90	20	—	—	50	—
World War II victims (over 78 years), 1–2 m under the ground	80	—	30	50	—	—	—
Water, 2 years	100	80	—	80	—	60	—
Water, 12 years	100	—	—	80	—	—	—
Field, 1 year	100	100	100	—	—	—	—
Heating track ($+80^0$C), 2 years	0	0	0	—	—	—	—

Table 1.
Effectiveness of autosomal STR loci analysis (%) in different bones (based on AmpFLSTR® Identifiler® Plus PCR Amplification Kit (Applied Biosystems, USA)).

In two cases it was not possible to obtain a genetic profile of almost fresh muscles without any signs of putrefactive changes even after using different DNA extraction methods (classical phenol-chloroform extraction; magnetic particles extraction (PrepFiler Forensic DNA Extraction Kit® (Applied Biosystems, USA) and ion-exchange columns (QIAamp DNA Investigator Kit, Qiagen, USA)). These cases were not related to each other and they did not coincide in time. However, common factors that may influence the effectiveness of genetic analysis were hectic lifestyle and abuse of low-quality alcoholic beverages and alcohol substitutes (as it was determined later by bone identification).

It is also difficult to obtain genetic profiles from muscles after a fire but our practice shows that good results can be obtained when examining different tissue types. For example, we determined a stable DNA profile in 5 cases of genetic analysis of liver fragments. Therefore, forensic scientists should be very careful when choosing muscles for DNA analysis and, if it is possible, to duplicate results DNA profiles from bones and other tissues.

A stable genetic profile can be obtained in unexpected for DNA analysis samples. And in contrary, bones or muscles that seem to be quite appropriate for DNA extractions at first glance may turn out to be unsuitable for getting DNA profiles as further described in several practical cases.

For example, a fragment of a tubular bone with soft tissues remnants (**Figure 2**) and a second cervical vertebra (**Figure 3**) from the corpse of an unidentified woman were provided for analysis. The duration of death was no more than 5 days and no putrefactive changes were noted.

At first glance, the objects seemed to be quite suitable for research. After DNA extraction with PrepFiler Forensic DNA Extraction Kit®, DNA concentration was measured by qPCR with Quantifiler Human DNA Quantification Kit® (Applied Biosystems, USA).

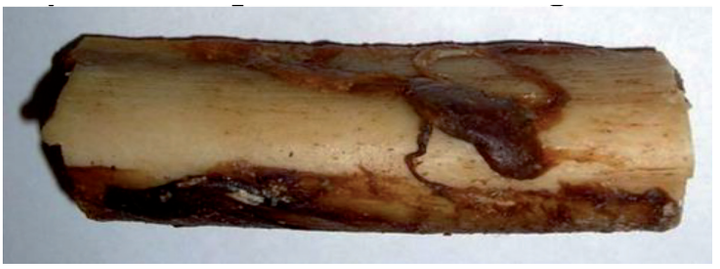

Figure 2.
Tubular bone fragment with soft tissue remnants.

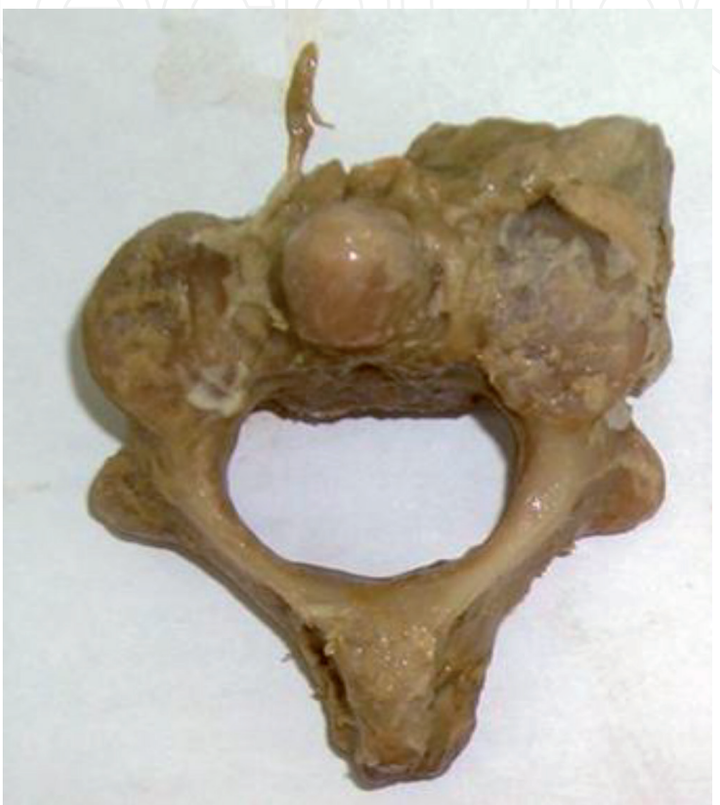

Figure 3.
2nd cervical vertebrae.

According to the PCR results, no DNA was detected in muscle fragments, but DNA concentration in samples extracted from both tubular bone and the odontoid process of the 2nd cervical vertebrae was sufficient for further autosomal STR analysis (0.054 ng/μl and 1.5 ng/μl respectively). However, in spite of sufficient DNA concentration, additional amplification peaks for more than 2 alleles were obtained in some short length loci due to probable stutter effects (**Figure 4**) and normal one and two alleles genotypes were detected for other markers.

Stutter effect can be due to the DNA degradation and the predominance of amplification of shorter fragments that was proved by another qPCR method. We determined DNA quantification and degradation degree estimation by the Quantum DNA-Set kit (Eurogen, Russia) by measuring the concentration of different DNA fragment lengths (91 bp, 156 bp and 211 bp) and their ratio. It turned out that the studied DNA was predominantly presented by the short fragments up to 156 bp length, and their content exceeded the content of long fragments (up to 211 bp) at least three times. After that a number of new DNA extraction experiments were carried out and finally a robust genetic profile was obtained.

In contrast to what was said above, a stable result can be obtained in objects that at first glance are unsuitable for research. Thus, the remains of an unidentified

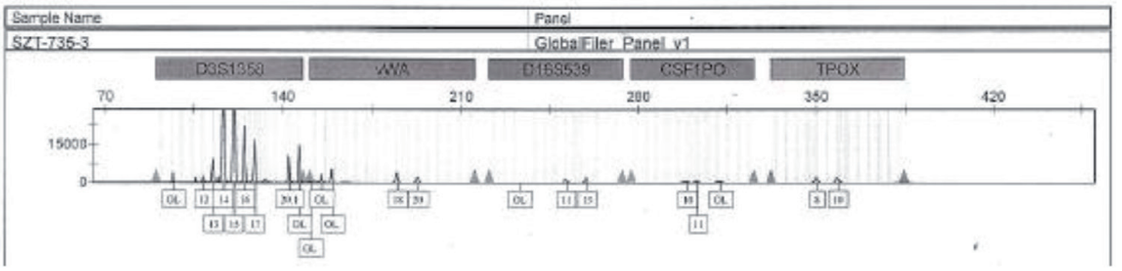

Figure 4.
Partial electrophoregram of multiple alleles genotypes for autosomal STR-typing the DNA extracted from 2nd cervical vertebrae.

Figure 5.
Fragments of blood vessels after a fire.

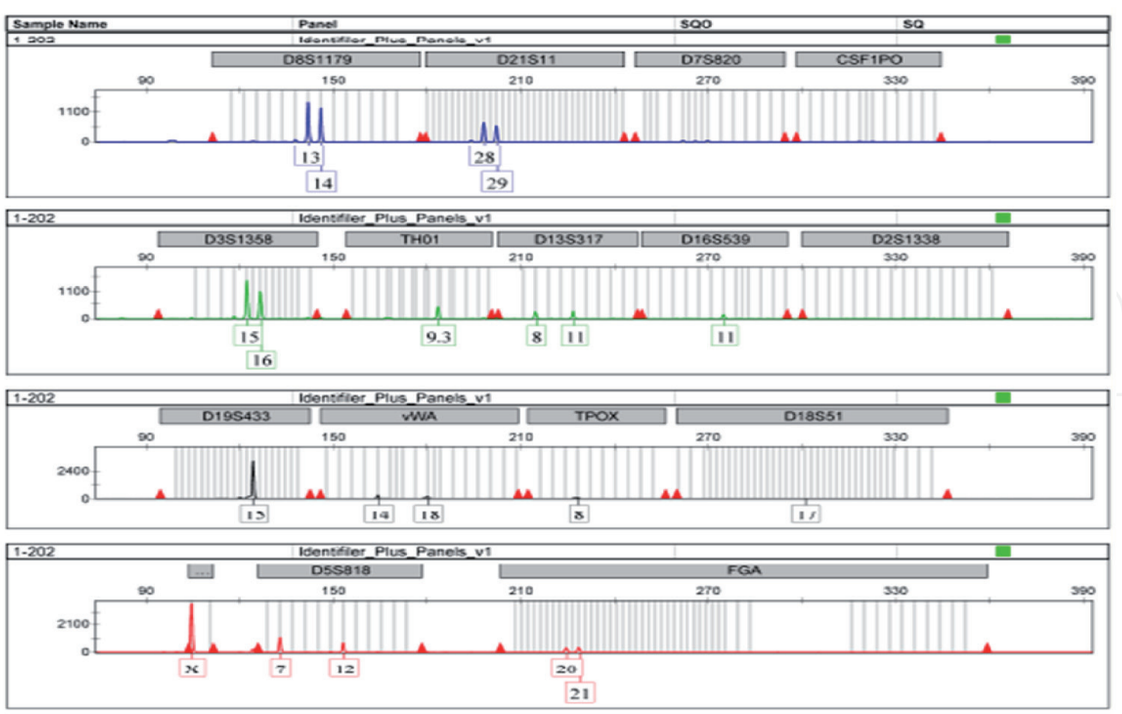

Figure 6.
The DNA profile of autosomal STR loci in DNA sample extracted from burnt blood vessel.

body, discovered after a fire, were delivered to the laboratory. Most of the body was burned out completely (bones were burned out to the point of white heat; the skull was destroyed). In addition, body fragments were exposed by water and

special agents during extinguishment of the fire. However, in the abdominal and chest cavities, a fragment of a large blood vessel with elements of clotted blood was discovered (**Figure 5**).

Despite the obvious degradation, DNA was isolated by the method of magnetic particles and appropriate concentration was determined (0.18 ng/μl). What is more, the female gender was verified and a partial genetic profile was obtained (**Figure 6**). Further identity determination was continued by the comparison with the mtDNA sequence of the person suspected to be the biological son.

Considering the fact that only a partial profile was obtained for autosomal STRs, hypervariable regions of mtDNA were analyzed both in the blood vessel sample and person suspected to be the son, which showed complete a match in both samples so the identification of unknown corpse was determined by two types of DNA markers.

3. Examples of DNA-profiling in certain cases

3.1 Case #1

The skeletonized human remains from the burial of an unknown soldier from World War II (1941 was presumed to be the year of death) were delivered to the forensic biological department of the Republic Bureau of Forensic Medical Expertise. Skeletal remains were represented by a fragment of the humerus, clavicle, a fragment of the temporal bone, the second cervical vertebra and three molars (**Figure 7**).

Bone and teeth powder (300–400 mg and 100–150 respectively) was used for DNA extraction.

DNA was extracted with PrepFiler Forensic DNA Extraction Kit® (Applied Biosystems, USA) using the standard bone extraction protocol according to the

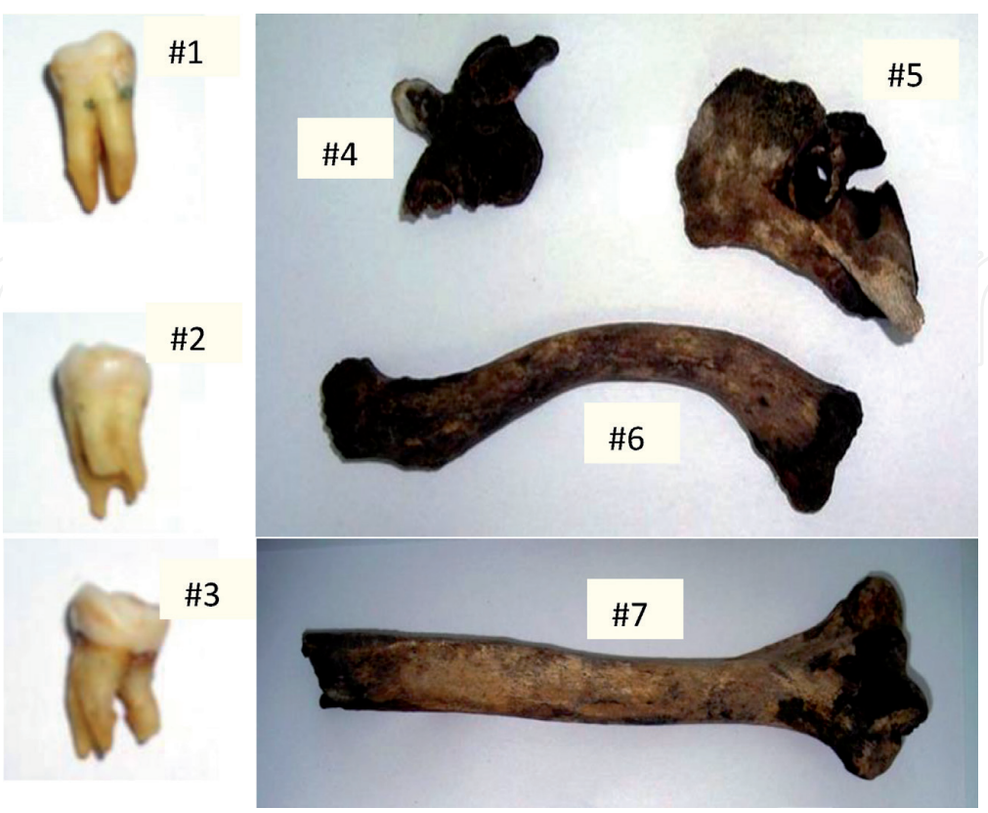

Figure 7.
Bone fragments and teeth from the victim burial were provided for DNA analysis. 1–3 –teeth, 4 – 2nd cervical vertebrae, 5 – Humorous bone fragment, 6 – Clavicle, 7 - temporal bone fragment.

manufacturer's instruction with several modifications. The bone and teeth powder was preincubated for 18 hours at 56°C in PrepFiler BTATM Lysis Buffer (Applied Biosystems, USA) with the addition of DTT and proteinase K before the main extraction procedures.

DNA concentration, measured by qPCR with Quantifiler Human DNA Quantification Kit® (Applied Biosystems, USA), was sufficient for further analysis in two teeth (objects 1, 2) and odontoid process of the 2nd cervical vertebrae (object #4) (0.03 ng/μl and 0.021 ng/μl respectively). DNA concentration extracted from 3rd tooth (object #3), humorous fragment (object #5), clavicle (object #6) and temporal bone fragment (object #7) turned out to be below the lower limit of 0.01 ng/μl. For this reason the samples were set aside for further DNA analysis.

DNA profiling was performed by both autosomal and Y-STR loci with AmpFLSTR® Identifiler® Plus PCR Amplification Kit and AmpFLSTR™ Yfiler™ PCR Amplification Kit (Applied Biosystems, USA) according to the manufacturer's instructions.

Individual DNA profiles on autosomal STRs were detected both from tooth #1 and tooth #2 (**Figure 8**) and were found to be identical to the genotypes determined in STR typing from the odontoid process. (**Figure 9**).

In addition, similar Y-chromosome DNA profiles were obtained from these DNA samples (**Figures 10** and **11**).

It is known that DNA is not well preserved in the vertebrae. However, the odontoid process of 2nd cerebral vertebrae is a newly discovered DNA source with a better state of preservation and higher effectiveness for further DNA analysis as already described in our previous work [12].

3.2 Case #2

A hair sample was provided to our laboratory for comparison with a possible suspect's DNA profile. According to morphological examination, hair belongs to the group of regional human hairs from the upper extremities. The hair follicle was partially turned off but the vaginal membranes were preserved.

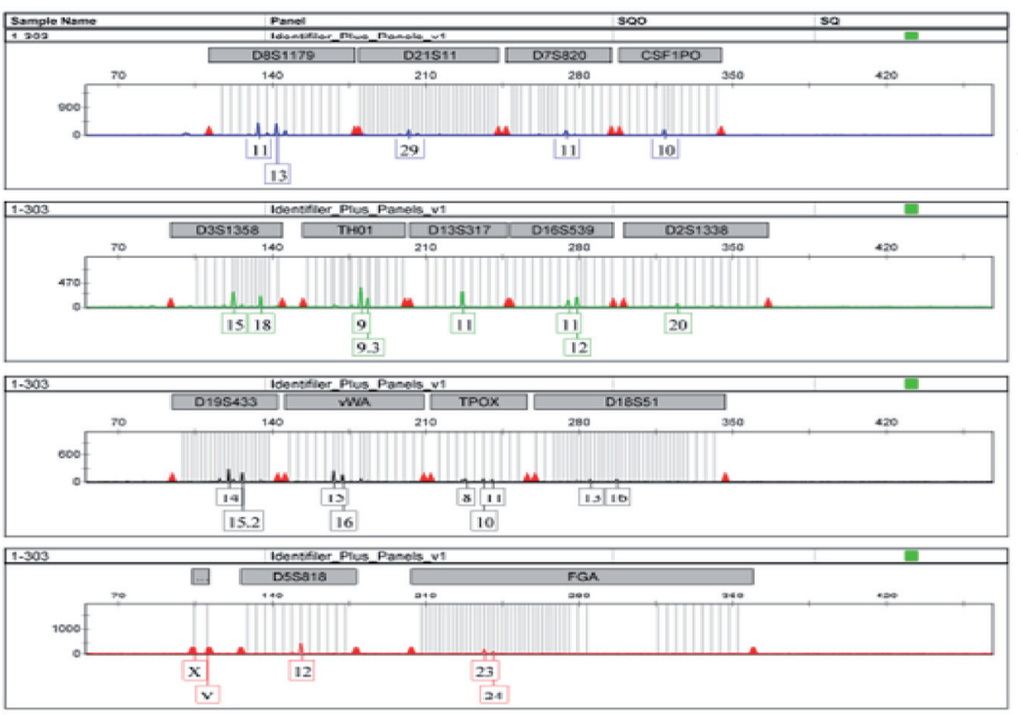

Figure 8.
Autosomal STR profile from object #1 (tooth).

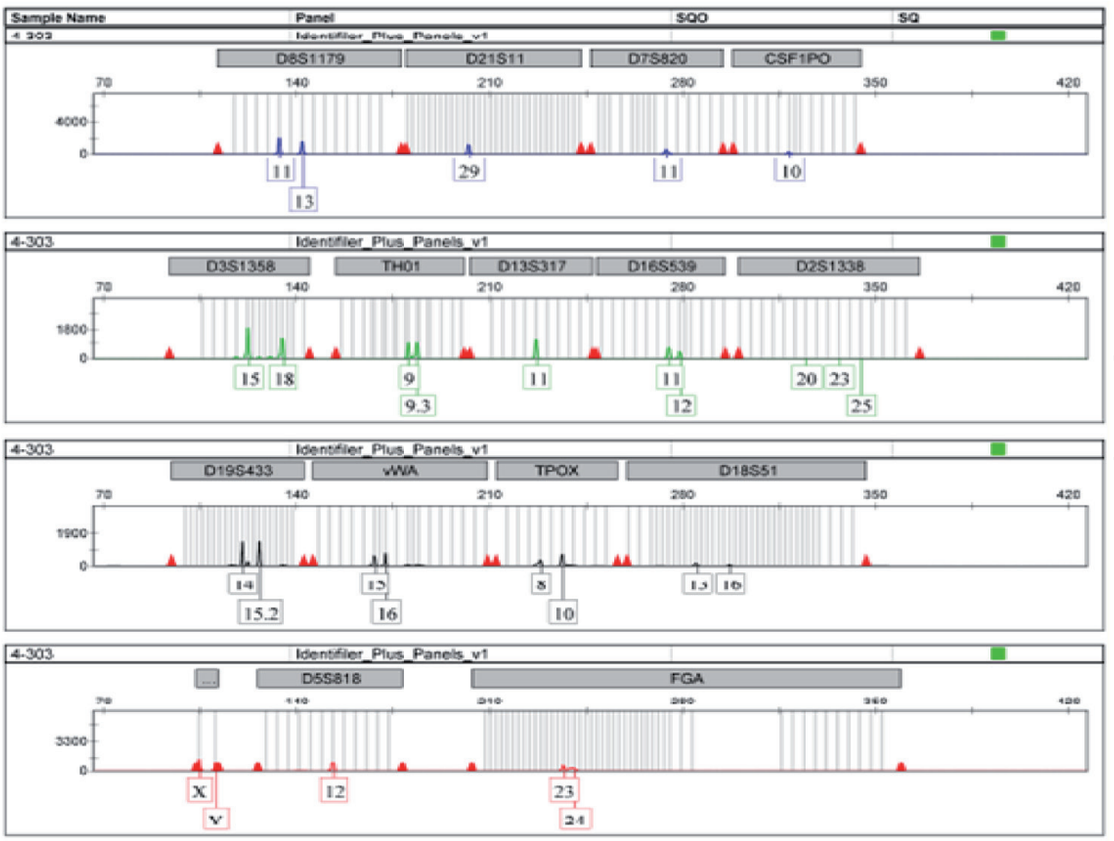

Figure 9.
Autosomal STR profile from object #4 (the odontoid process of 2nd cervical vertebrae).

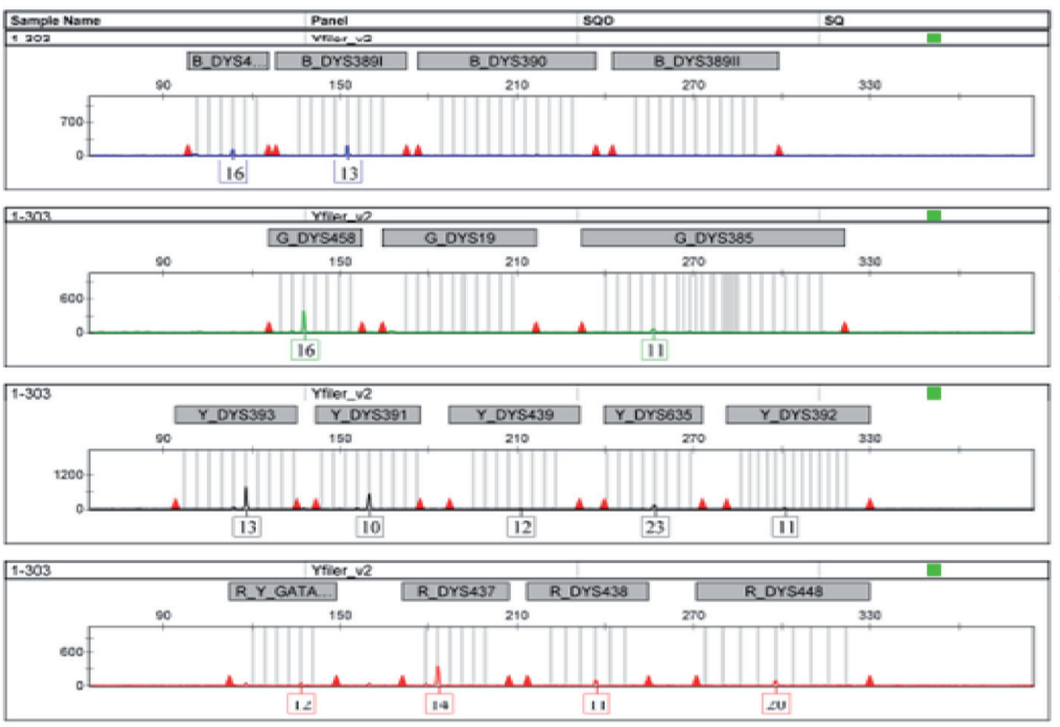

Figure 10.
Y-STR profile in DNA sample extracted from the tooth (object #1).

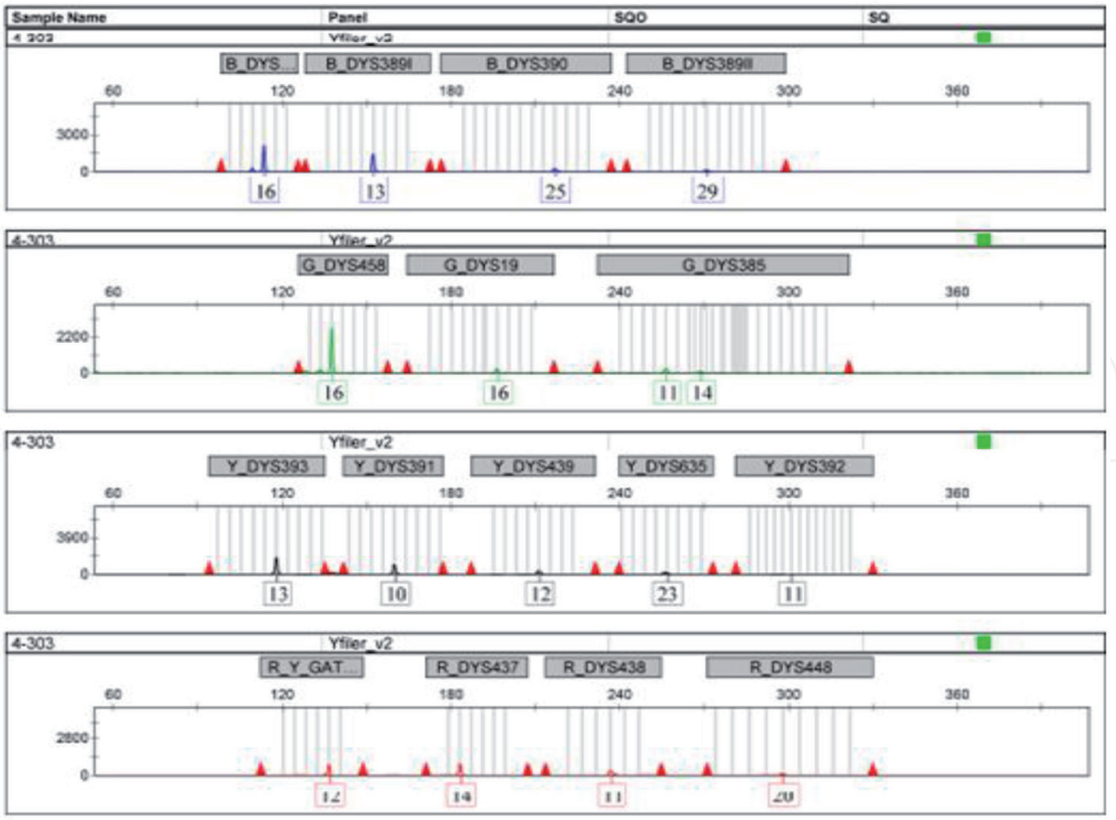

Figure 11.
Y-STR profile in DNA sample extracted from the odontoid process of 2nd cervical vertebrae (object #4).

DNA extraction was performed from bulb using Chelex-100 in order to avoid DNA loss and DNA concentration was measured by qPCR using the Quantifiler Human DNA Quantification Kit® (Applied Biosystems, USA) which was showed to be outside the lower detectable limit (0.009 ng/μl) for autosomal STR amplification. However, we attempted to establish an autosomal genetic profile. A profile was partially obtained and the male gender identity was verified (**Figure 12**).

The identified loci were found to be matched with one of the suspects; however, the data obtained were not sufficient for the identification process. Accordingly, we analyzed hypervariable regions I and II of the mtDNA D-loop (HVS-1 and HVS-2) with MitoPlex system (Gordiz, Russia).

Individual sequences from hair were obtained (**Figure 13**) which that was it made it possible fr there to be a comparison to compare with the suspect's mtDNA sequence (**Figure 14**).

According to these results even one hair with a partially preserved bulb can be sufficient to obtain an incomplete autosomal STR profile which can be supplemented with a profile of mitochondrial DNA to enable a comparison with the DNA of suspects.

3.3 Case #3

The laboratory received biological materials from a crime scene after a quarrel between two men who had been drinking alcohol. One of them committed the murder of the other. The offender tried to hide the traces of the crime by the dismemberment of the body and attempted to destroy the traces by burning them. However, he did not take into account that the human body cannot be burned

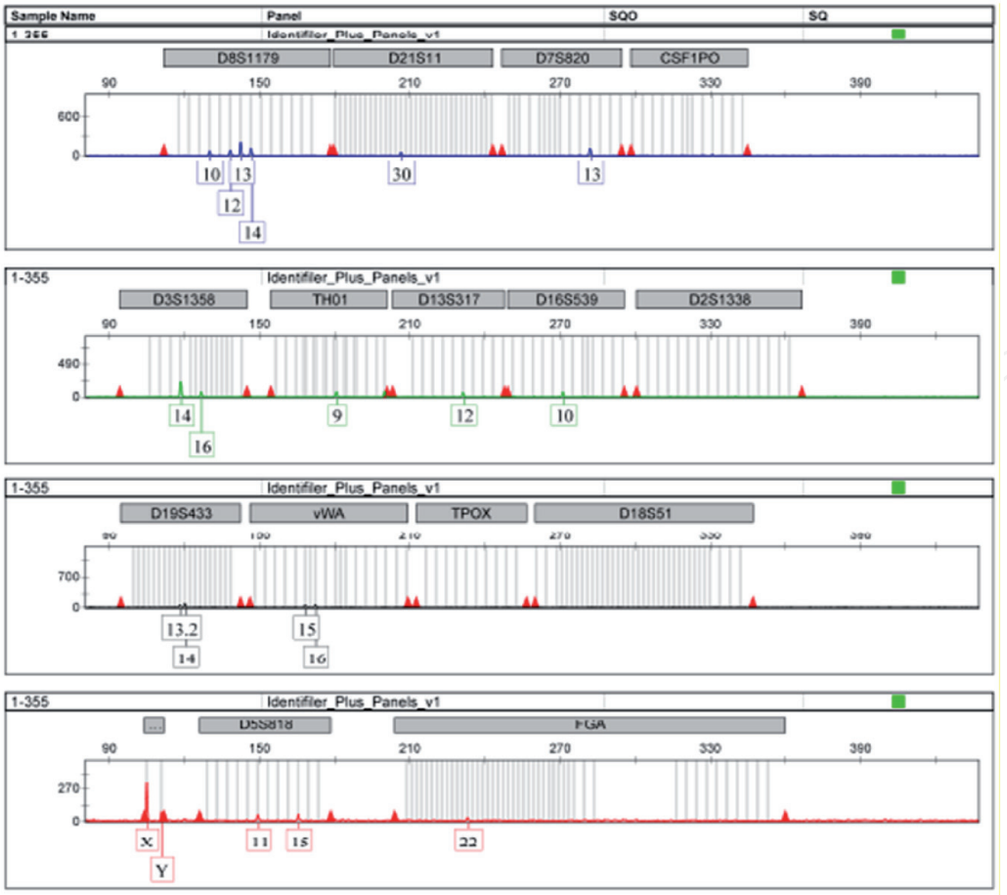

Figure 12.
Partial autosomal STR profile obtained from the hair bulb.

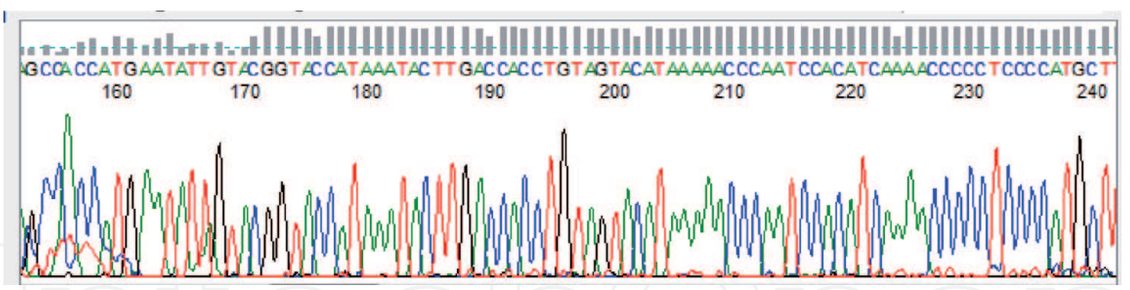

Figure 13.
Part of HVS1 sequence for DNA sample extracted from hair bulb.

quickly. He tried to pull the body out of the fire by the lower limbs, but the upper limbs and head remained in the pit and continued to burn. Then the suspect threw the lower limbs with a part of the pelvic girdle into the river where they were found a day after. The victim and the criminal were identified.

To identify the deceased person, it was necessary to clarify several circumstances based on the morphological and molecular genetic analysis:

1. whether objects discovered at the fireplace were tho0se of a human;

2. to identify the anatomical origins of the remains;

3. to establish the identity of the remains discovered in the river;

4. to prove the identity of the missing person's genetic profile using the profile obtained from the items of clothing

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CLUSTAL format alignment by MAFFT (v7.475)

hair_HVS1       acggtaccataaatacttgaccacctgtagtacataaaaacccaatccacatcaaaaccc
suspect_HVS1    acggtaccataaatacttgaccacctgtagtacataaaaacccaatccacatcaaaaccc
                ************************************************************

hair_HVS1       cctccccatgcttacaagcaagtacagcaatcaaccttcaactatcacacatcaactgca
suspect_HVS1    cctccccatgcttacaagcaagtacagcaatcaaccttcaactatcacacatcaactgca
                ************************************************************

hair_HVS1       actccaaagccacccctcacccactaggataccaacaaacctacccacccttaacagtac
suspect_HVS1    actccaaagccacccctcacccactaggataccaacaaacctacccacccttaacagtac
                ************************************************************

hair_HVS1       atagtacataaagccatttaccgtacatagcacattacagtcaaatcccttctcgtcccc
suspect_HVS1    atagtacataaagccatttaccgtacatagcacattacagtcaaatcccttctcgtcccc
                ************************************************************

hair_HVS1       atggatgacccccctcagataggggtcccttgaccaccatcctccgtgaaatcaatatcc
suspect_HVS1    atggatgacccccctcagataggggtcccttgaccaccatcctccgtgaaatcaatatcc
                ************************************************************

hair_HVS1       cgcacaagagtgctactctcctc
suspect_HVS1    cgcacaagagtgctactctcctc
                ***********************
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Figure 14.
Multiple alignment of mtDNA HVS1 sequences in DNA samples extracted from hair and suspect's blood using MAFFT program (https://mafft.cbrc.jp/).

However, there were difficulties in identification of the victim because of the absence of a whole body and in addition, the alleged deceased did not have any relatives other than a sister. By way of biological material relating to the deceased man, items of his clothing with his traces were removed from his house (panties, T-shirt, hat, gloves).

Forensic scientists recovered several charred objects of biological (human) origin from the fireplace such as liver fragments (object 1) and finger phalanges (object 2) for further DNA analysis (**Figure 15**).

DNA was isolated from these objects, as well as from the lower limbs recovered from the river and clothes of the missing man using the PrepFiler Forensic DNA Extraction Kit® (Applied Biosystems, USA).

The DNA concentration was measured by qPCR using Quantifiler DUO DNA Quantification Kit® (Applied Biosystems, USA). By using Quantifiler DUO it was possible to prove that the lower limbs, burned liver fragments and finger's phalange belonged to a man.

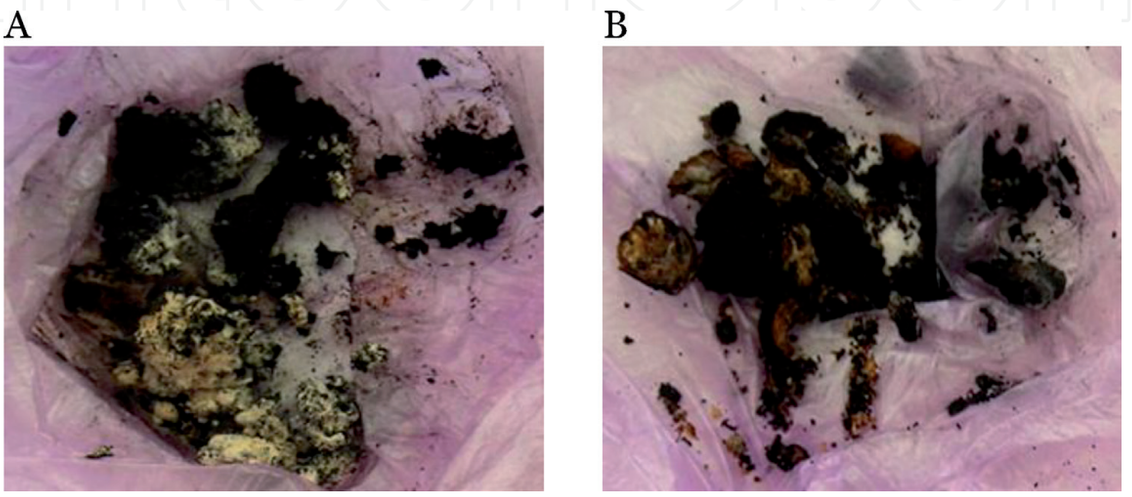

Figure 15.
A. Burnt liver fragments; B. burnt finger phalanges.

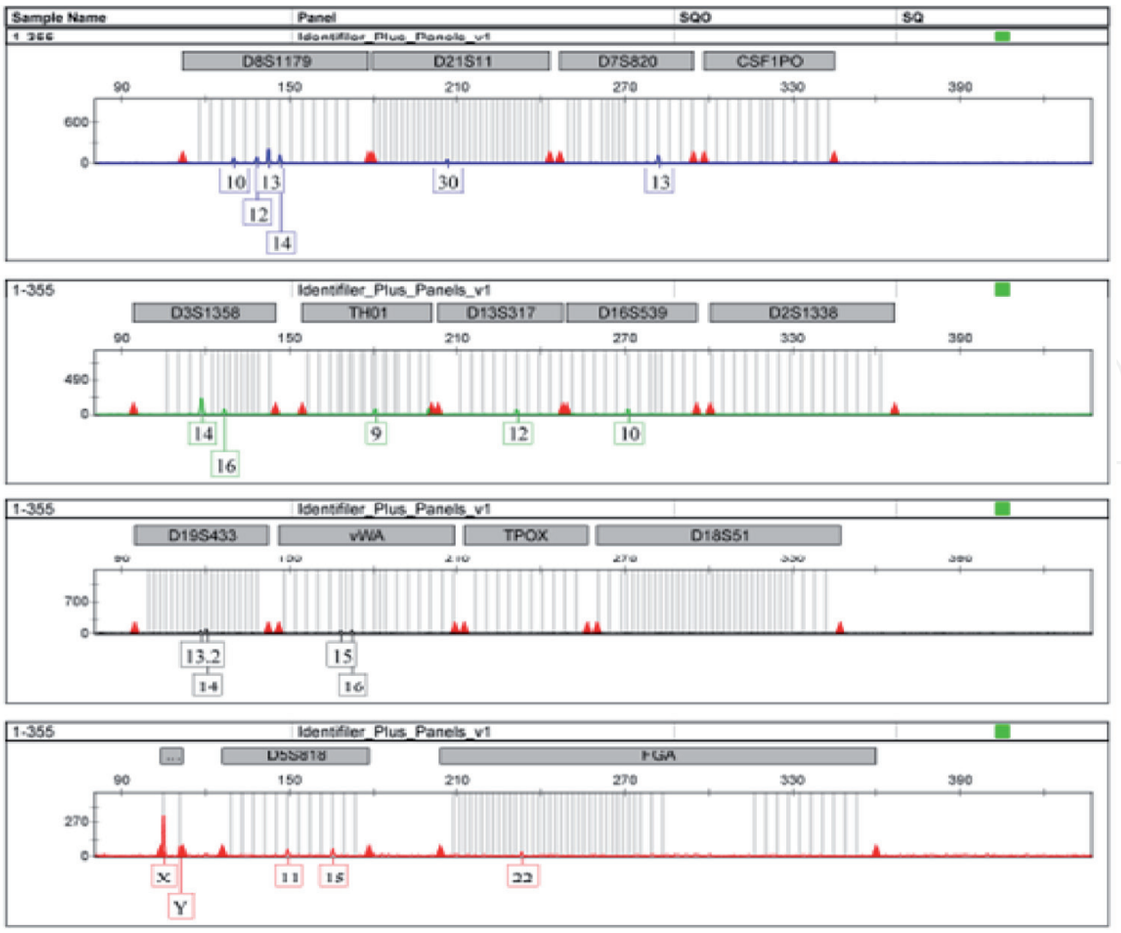

Figure 16.
Autosomal STR profiling from the burnt liver fragment.

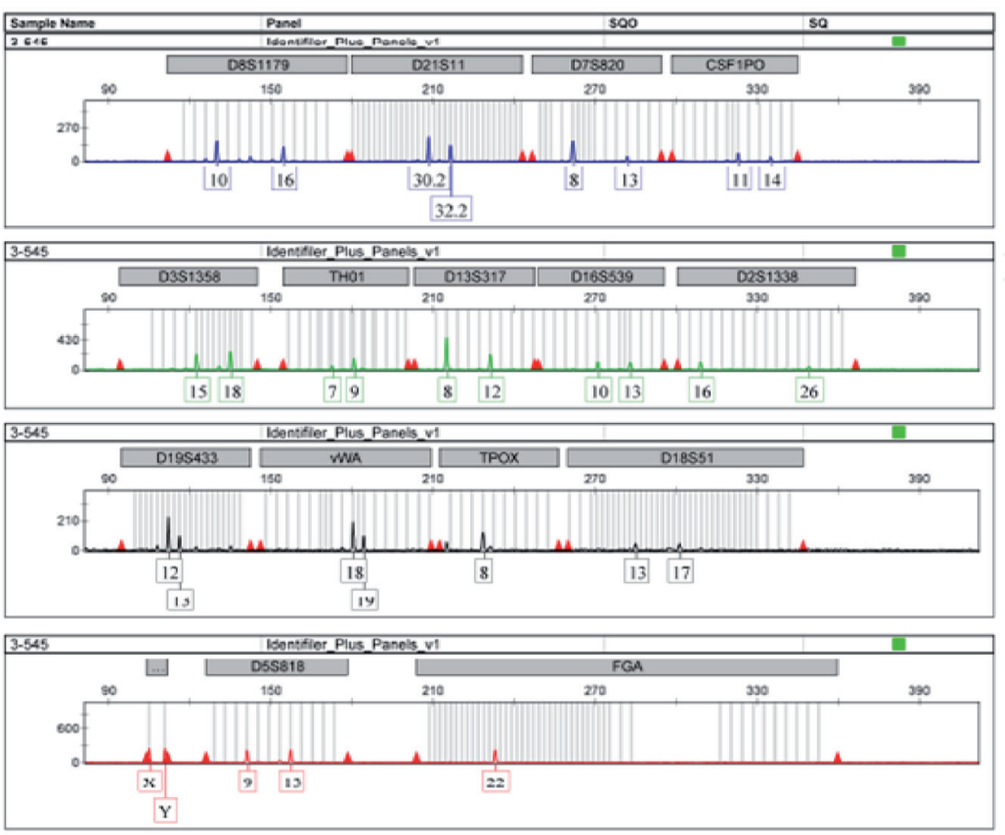

Figure 17.
Autosomal STR profiling from the burnt phalange.

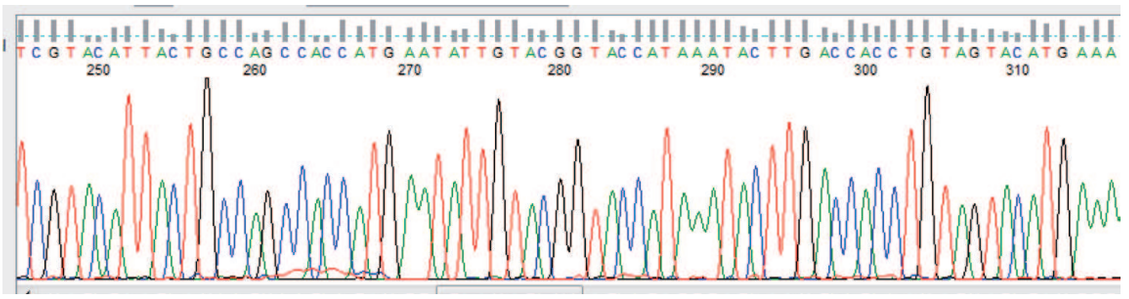

Figure 18.
Partial HVS1 sequence for DNA sample extracted from the burnt liver.

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CLUSTAL format alignment by MAFFT (v7.452)


CRS             tcacgggagctctccatgcatttggtattttcgtctgggggggtatgcacgcga--tagca
1-1             ---------------------------------------------------------
1-5             --------------------------------cgtctgggggggtgtgcacgcga--tagca
rel             ---------------------------------ctgggggggtgtgcacgcgaataagca


CRS             ttgcgagacgctggagccggagcaccctatgtcgcagtatctgtctttgattcctgcctc
1-1             ----------------------cccctatgtcgcagtatctgtctttgattcctgcctc
1-5             ttgcgagacgctggagccggagcaccctatgtcgcagtatctgtctttgattcctgcctc
rel             ttgcgagacgctggagccggagcaccctatgtcgcagtatctgtctttgattcctgcctc
                                 ***********************************


CRS             atcctattatttatcgcacctacgttcaatattacaggcgaacatacttactaaagtgtg
1-1             ctcctattatttatcgcacctacgttcaatattacaggcgaacatacctactaaagtgtg
1-5             atcctattatttatcgcacctacgttcaatattacaggcgaacatacctactaaagtgtg
rel             atcctattatttatcgcacctacgttcaatattacaggcgaacatacttactaaagtgtg
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CRS             ttaattaattaatgcttgtaggacataataataacaattgaatgtctgcacagccacttt
1-1             ttaattaattaatgcttgtaggacataataataacaattgaatgtctgcacagccgcttt
1-5             ttaattaattaatgcttgtaggacataataataacaattgaatgtctgcacagccgcttt
rel             ttaattaattaatgcttgtaggacataataataacaattgaatgtctgcacagccgcttt
                ************************************************.****


CRS             ccacacagacatcataacaaaaaatttccaccaaaccccccct-cccccgcttctggcca
1-1             ccacacagacatcataacaaaaaatttccaccaaaccccccctccccccgcttctggcca
1-5             ccacacagacatcataacaaaaaatttccaccaaaccccccctcccccgcttctggcca
rel             ccacacagacaccataacaaaaaatttccaccaaaccccccctcccccgcttctggcca
                **********.****************************** ***************


CRS             cagcacttaaacacatctctgccaaaccccaaaaacaaagaaccctaacaccagcctaac
1-1             cagcacttaaacacatctctgccaaaccccaaaaacaaagaaccctaacaccagcctaac
1-5             cagcacttaaacacatctctgccaaaccccaaaaacaaagaaccctaacaccagcctaac
rel             cagcacttaaacacatctctgccaaaccccaaaaacaaagaaccctaacaccagcctaac
                ************************************************************


CRS             cagatttcaaattttatctttt----------------------
1-1             cagatttcaaattttatcttttggcggtatgcacttttaacaga
1-5             cagatttcaaattttatcttttggcggtatgcacttttaacaga
rel             cagatttcaaattttatcttttggcggtatgcacttttaacaga
                **********************
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Figure 19.
mtDNA HVS1 sequences alignment in DNA samples extracted from burnt liver fragment (1–1), long bone (1–5) and victim's sister buccal swab (rel) (by MAFFT program (https://mafft.cbrc.jp/) compared to the Cambridge reference sequence (CRS)).

Both autosomal and Y-STR loci profiles were determined using the AmpFLSTR® Identifiler® Plus PCR Amplification Kit and AmpFlSTR® Yfiler ™ PCR Amplification Kit (Applied Biosystems, USA). At the same time, the profile of autosomal DNA and Y-chromosome DNA were able to be successfully established in the objects removed from the fire pit, which completely coincided with the profiles

obtained in the material of the lower extremities, burnt liver fragments (**Figure 16**), burnt finger phalange (**Figure 17**) and the victim's clothes.

According to the preliminary investigation, the victim had a sister so in order to complete human identification, mtDNA analysis was carried out. Hypervariable regions, HVS-1 and HVS-2, of mtDNA D-loop sequencing was performed by the MitoPlex system (Gordiz, Russia). Individual sequences were also determined in the DNA samples extracted from lower limbs, burnt liver fragments and clothing items of the missing man, as well as from the buccal swabs of the woman believed to be the sister of the deceased man (**Figures 18** and **19**).

Thus, the testing that was conducted made it possible not only to identify the deceased man but also to prove the circumstances of the crime committed. This assisted the process of gauging the circumstances of the offending and the seriousness of the crime committed.

4. Conclusion

Based on our data, when analyzing highly degraded DNA, it is preferable to use information from all types of DNA markers such as autosomal STRs, X- and Y-STRs and mitochondrial hypervariable region sequence to get the best possibleindividual DNA profile, especially in difficult cases.

In the near future, in order to obtain the fullest possible individual DNA profile, it will be useful to include information about phenotyping features (such as skin, hair or eye color, skeletal particularities etc.) and biological age based on microarray analysis, next-generation sequencing SNP data, state of methylation and specific gene expression analysis.

But the highest percentage of reliable and reproducible DNA profiles can be obtained from the odontoid process of the 2nd cervical vertebra, long tubular bones of the lower (femoral) and upper (shoulder) limbs, as well as from teeth. In addition, even single hairs and burnt tissues can constitute a source for successful DNA identification.

The results of our analysis made it possible to formulate a number of recommendations regarding the collection of human remains from the crime scene in order to enable successful DNA analysis.

If a complete skeleton or unidentified corpse is found:

- in the absence of deeply penetrated putrefactive changes, it is advisable to provide bones with remnants of soft tissues, not only their single fragments;
- it makes sense to provide, first of all, ribs and long tubular bones of the lower and upper limbs as well as 2nd cervical vertebrae;
- it is advisable to provide several bones from one body, such as a rib, long bone, or a tooth;
- if an incomplete skeleton or individual bones are found, it is advisable to provide all the discovered bone remains, so that the forensic geneticist can independently decide which of the bones to examine first;
- if a body is found after a fire, it is good practice to provide tissue fragments (liver, blood vessels, etc.) for DNA identification.

When working at the crime scene special attention should be paid to searching and removal of small bones and fragments to make it possible to obtain complete genetic profiles. However, it is necessary to understand that not all objects allow the preservation of DNA for a long time [13] and bone remains are one of the most controversial objects. On the one hand, the physiological structure of the bone allows DNA preservation for a long time, but on the other hand, it is very difficult to extract DNA from some types of bones.

Besides, in the process of DNA extraction, a sample can be severely damaged or even destroyed, and as a result a false-positive result can be obtained (such as by contamination by other DNA samples) or a false-negative result (such as by the presence of inhibitors). That is why it is very important to eliminate the errors due to the reproducibility in DNA analysis.

Moreover, probability estimation in identification analysis also plays an important role in DNA analysis interpretation. Based on our experience, the Bayesian probability gives the most reliable conclusion about the non-random coincidence of determining genetic profile in case of positive individual identification [14]. In addition, it should be taken into account that for working with degraded DNA samples qualifications and experience of a geneticist should be confirmed with special competence documents.

Finally, it is important that the results of DNA identification should not be the only prosecution evidence. Where there are discrepancies with the materials of the investigation, it is necessary for further expert studies to be undertaken which take into account all the circumstances of the crime that has been committed [15, 16].

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Conflict of interest

The authors declare no conflict of interest.

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