Chapter

# Genetic DNA Identification from Bone Remains in Kinship Analysis Using Automate Extraction System

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## Abstract

The first ever human identification through DNA analysis was done in the year 1987. Since then, this test has been used, not only in the ruling of civil and juridical cases, but also for human identification of missing persons and mass disaster victims. In this chapter we will present the usefulness of genetic DNA testing of skeletonized remains for human identification, by using automate DNA extraction from three different human bone types: tooth, femur and petrous pyramid. For each case, we obtained saliva samples on buccal swabs from relatives. After the bones were washed and cleaned, Bead Balls Mill Mix 20 (Tehtnica Domel, Slovenia), was used to obtain the bone powder. The DNA extraction from bone samples was performed on the automate Maxwell RSC 48 Instrument (Promega, USA), using the Maxwell FSC DNA IQ Casework Kit (Promega, USA). Power Quant System (Promega, USA) was used for DNA quantification of the samples. The DNA samples were amplified on a Pro Flex PCR System (Thermo Fischer, USA), using the Global Filer PCR Amplification Kit (Applied Biosystems, USA). PCR products were run on a 3500 Genetic Analyzer (Thermo Fischer, USA). Data analysis was performed by Gene Mapper 1.4. Considering that these cases involved DNA extraction from teeth, bones and old human remains, automate system was felt to be the best option to reduce handling errors and increase the possibilities of obtaining good quality DNA.

**Keywords:** deoxyribonucleic acid (DNA), human identification, postmortem, bone remains, International Society of Forensic Genetics (ISFG)

## 1. Introduction

During the last three decades, the field of forensic genetics that applies genetic science for human identification has experienced new changes and advances in molecular sciences, owing to recent discoveries in the field of molecular biology. With respect to human DNA identification, establishing kinship between individuals is based on Mendel's laws, which stipulate that individual inherit genetic traits

from their biological parents in equal contribution. Thus, an individual's genetic DNA profile contains all the ancestor's DNA and is unique, excepting the case of monozygotic twins. Genetic identification using autosomal short tandem repeat (STR) markers is based on this principle. Genetic human identification can also be done on DNA haploid markers, which are the markers found on X-chromosomes and Y-chromosomes. These markers are inherent in maternal or paternal lines and are used in cases where one of the biological parents is dead [1].

In forensic genetics, short tandem repeats (STR) are the genetic markers used for human identification in cases of kinship determination, paternity or maternity rulings, crimes, sexual assaults, and burglaries. DNA samples obtained from different items can be tested to prove the presence or absence of a person in a crime scene, thus serving an important purpose in the criminal justice system [2]. Obtaining DNA profiles from bone remains and teeth is an important procedure that helps in human identification in cases of mass disasters or unidentified human remains. Human remains are the only biological samples that remain after destructive events, after exposure to harmful environmental conditions or in cases where a long time has passed since the death of the person [3]. DNA molecules degrade gradually in hard tissues, such as bones and teeth, which makes them available for DNA extraction for longer periods of time [4]. Many external factors such as humidity, temperature, pH, geochemical properties of the soil, presence or absence of micro-organisms, storage time and conditions, and various other factors can affect the preservation of DNA molecules in skeletal remains [5, 6].

It is known that after death of the organism, DNA damage and bone fragmentation can occur, thus it is especially important to understand the composition of bone and teeth and their decomposing process. The root of the tooth is covered with cementum, a biochemical structure containing hydroxyapatite, collagen, and other non-collagenous proteins. On the other side, dental pulp represents an important source for DNA extraction. According to international recommend It is known that after death of the organism, DNA damage and bone fragmentation can occur, thus it is especially important to understand the composition of bone and teeth and their decomposing process. The root of the tooth is covered with cementum, a biochemical structure containing hydroxyapatite, collagen, and other non-collagenous proteins [7]. On the other side, dental pulp represents an important source for DNA extraction. According to international recommendations the most suitable teeth are molars, premolars, and canines.

Regarding the bones, compact bone tissue makes the outer part of the ends of long bones (epiphysis) and flat bones and the middle part of the long bones (diaphysis). The spongy part of the bones is found inside the flat bones and at the ends of the long bones. Because over time the porosity of the bones will increase, bacteria and fungi who are present on soils, and cyanobacteria present in water will penetrate more easily inside the bone tissue [8].

Based on the current recommendations for sampling forensic bone remains, long leg bones (femur and tibia) and teeth are the most suitable for DNA extraction, while spongy and flat bones such as skull, ribs and vertebrae are less suitable.

In such cases, the possibility of isolating DNA from hard tissue serves to benefit forensic science [9]. After bone cleaning and DNA isolation from biological samples, laboratory protocols require the following steps to be taken to perform human identification on DNA samples: DNA quantification, amplification of DNA products using polymerase chain reaction (PCR) and visualization of the PCR products using capillary electrophoresis.

In this chapter, we will show the advantages of DNA human identification from bone remains, the concentration of which will differ depending on the type of the

bone being tested. We will present three cases of human DNA identification, performed on three different types of human bones.

## 2. Materials and methods

Between 1st of January 2019 and 1st of January 2021, the police brought six different cases of human bones remains to the Institute of Forensic Medicine in Timisoara, Romania, for the purpose of DNA identification.

**Case 1.** The corpse of a male was found in an advanced putrefaction stage. A bone fragment was obtained from the left femur during the autopsy procedure at the Institute of Forensic Medicine in Timisoara, Romania, for further genetic identification. In this case, police provided the saliva reference sample of the suspected presumptive son of the deceased victim, to confirm the identity of the victim, based on the DNA profile (**Figure 1**).

**Case 2.** A woman had been reported missing a few months ago. Police got an information that a shopping cart had been spotted in the woods, with clothes and human remains. Police obtained the hair and skull of the victim from the crime

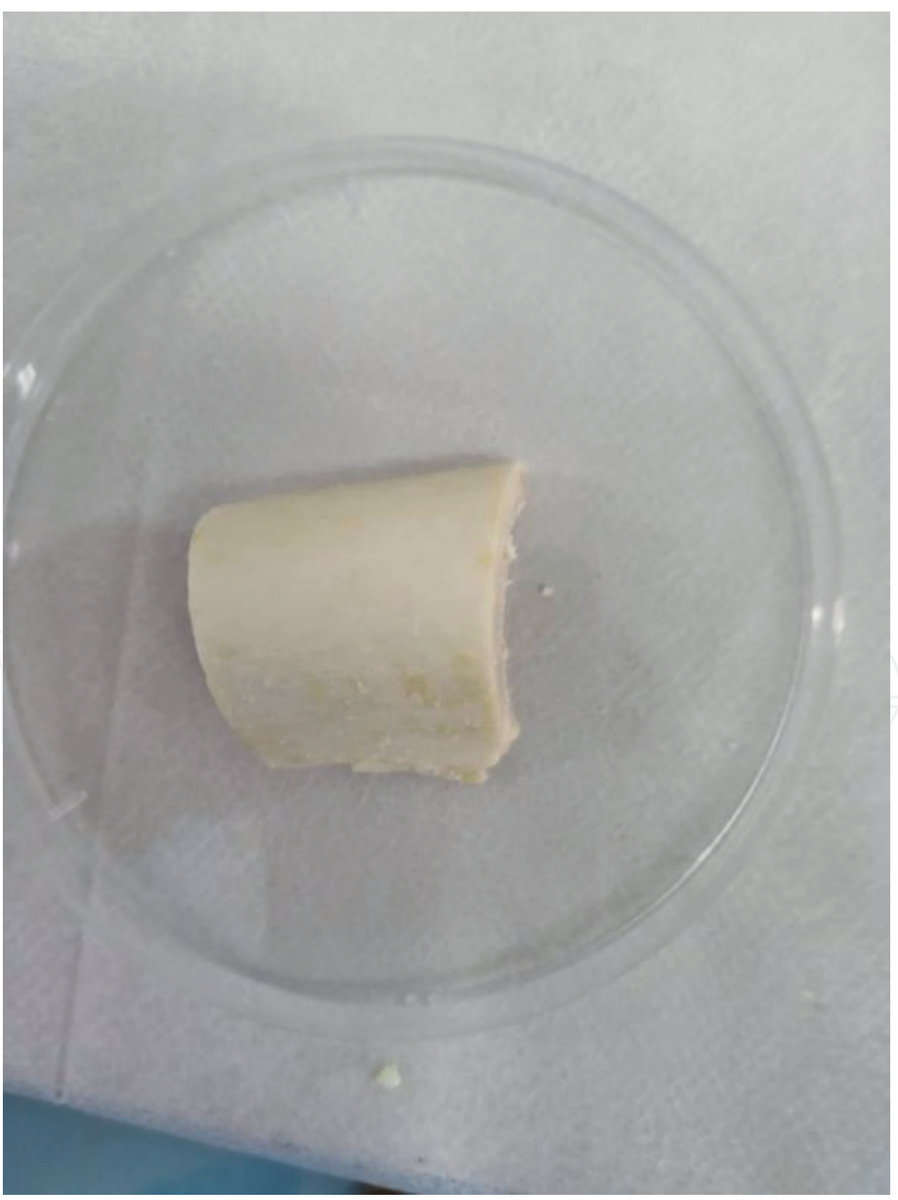

**Figure 1.**
*Left femur bone fragment after cleaning.*

scene and sent them to our laboratory for DNA identification. They also found one of the daughters of the probable victim and sent her saliva reference samples to facilitate victim identification. In this case, we used a fragment of the petrous pyramid from the victim's skull to perform DNA identification (**Figure 2**).

**Case 3.** A man committed suicide by self-immolation. As the body was in a highly destructed state, the police decided to confirm the identity of the victim through DNA identification, by comparing his DNA with that of his presumptive daughter. To this case, we obtained a canine tooth from the deceased, during the autopsy procedure (**Figure 3**).

### 2.1 Bone pre-processing

The collected bone remains were washed using a rough part of a sterilized and UV irradiated dish sponge, and then washed three times in sterile bi-distilled water (Merck Millipore, USA), using a mild detergent (few ml of detergent, measuring up to 5% of the water). The washed bone fragments were left overnight to dry. After this, the bone powder was obtained by grinded the bones in small pieces at 30 Hz for 1–2 minutes, using the Bead Beater Mill Mix 20 (Tehtnica Domel, Slovenia).

**Figure 2.**
*Skull recovered from the crime scene.*

**Figure 3.**
*Canine tooth after cleaning.*

After pre-processing, the Bone DNA Extraction Kit, Custom (Promega, USA) was used to perform the demineralization buffer, by following the FSC DNA IQ Maxwell protocol. 100 mg of pulverized bone powder was weighed on an electronic balance and transferred into 1.5 mL Eppendorf tubes (Eppendorf, Germany). The kit was used for the demineralization of the tooth and the rest of the bone remains, by preparing two cocktails lysis, A and B (**Figure 4**).

First, we prepared the bone lysis cocktail A in the following manner: 400 μL of demineralization buffer, 40 μL of proteinase K and 10 μL of 1-thyoglicerol were mixed by vortex for 10 seconds. After this, 400 μL of bone lysis cocktail A was transferred into a new, sterile 1.5 mL Eppendorf tube (Eppendorf, Germany). As per protocol, the tube was left to incubate in the Eppendorf thermomixer C (Eppendorf, Germany) for 2.5 hours, with intermittent shaking at 1000 rpm. Following this, the sample was centrifugated at 13.300 rpm for 5 minutes, after which the supernatant was transferred into another clean, sterile 1.5 mL Eppendorf tube.

Next, we prepared bone lysis cocktail B in the following manner: In a clean, sterile 1.5 mL Eppendorf tube, 990 μL lysis buffer was added to 10 μL 1-thyoglicerol. From the bone lysis cocktail B, 800 μL was transferred into another clean, sterile 1.5 mL Eppendorf tube and vortexed for 10 seconds to ensure proper mixing.

The entire volume of the previously obtained eluate was added to well 1 of the Maxwell 48 RSC Instrument (Promega, USA). In wells 8, the plungers and 50 μL of the elution buffer were added in 0.5 mL elution tubes supplied by the producer. The

**Figure 4.**
*Pulverized bone powder.*

FSC DNA IQ Casework protocol was executed from the instrument, to obtain the DNA from the bone fragments and the tooth. After the run was complete, the obtained DNA samples were stored at T = 4°C, till the time of analysis. The extraction of reference DNA samples from relatives of the deceased was performed on the automate Maxwell® 48 RSC instrument (Promega, USA), using the Maxwell® FSC DNA IQ™ Casework (Promega, USA).

After DNA extraction, biological samples were kept at −86°C for 24–72 hours till the genetic analysis.

## 2.2 Quantification of the extracted DNA samples

The Power Quant™ System Kit (Promega, USA) was used for the quantification of the DNA samples. In this case, following the manufacturer's recommendations, for each sample, a mix solution with a final volume of 18 μL, consisting of 10 μL of Power Quant 2 x Master Mix, 7 μL of Amplification grade water and 1 μL of Power Quant 20 x Primer Mix was prepared. The quantification was performed on a 7500 real time PCR system (Thermo Fischer Scientific, USA), using the HID Real-Time PCR Analysis Software v 2.0.6. The DNA concentrations in the saliva, bone fragments and tooth are presented in **Tables 1–3**.

| Case 1 | |
|---|---|
| **Person's Identity** | **DNA concentrations (ng /μL) in saliva and femur bone fragment** |
| Unknown victim | 1.183 |
| Presumptive son | 34.05 |

**Table 1.**
*DNA concentrations from saliva and femur bone fragment.*

| Case 2 | |
|---|---|
| **Person's Identity** | **DNA concentrations (ng/μL) in saliva and petrous pyramid** |
| Unknown victim | 1.635 |
| Presumptive daughter | 10.41 |

**Table 2.**
*DNA concentrations from saliva and petrous pyramid.*

| Case 3 | |
|---|---|
| **Person's Identity** | **DNA concentrations (ng/μL) saliva and canine tooth** |
| Unknown victim | 6.18 |
| Presumptive son | 21.35 |

**Table 3.**
*DNA concentrations from saliva and canine tooth.*

## 2.3 Amplification of the DNA samples

The DNA samples were amplified for the STR markers using the Global Filer™ PCR Amplification Kit (Thermo Fischer Scientific, USA). The analysis was done as per the recommendations of the manufacturers. In this step, the amplification of the DNA samples was performed on a Pro Flex PCR System (Thermo Fischer Scientific, USA). The PCR reactions of the DNA samples were carried out in a total volume of 25 μL, which contained: Master Mix: 7.5 μL, Primer Set: 2.5 μL, nuclease-free water: 12.5 μL and template DNA: 2.5 μL. The Global Filer PCR Amplification Kit (Thermo Fischer Scientific, USA) contains 21 autosomal STR markers, as follows: D1S1656; D2S441; D2S1338; D3S1358; D5S818; D7S820; D8S1179; D10S1248; D12S391; D13S317; D16S539; D18S51; D19S433; D21S11; D22S1045; CSF1PO; FGA; TH01; TPOX; vWA; SE33, plus DYS391 and the gender-specific marker, amelogenin. In all amplification reactions, positive and a negative PCR controls were used.

## 2.4 Capillary electrophoresis of the amplified DNA samples

During the capillary electrophoresis, the samples were analyzed on a 3500 Genetic Analyzer (Applied Biosystems, USA), as per the manufacturer's recommendations. For the autosomal STR markers, 1 μL of the amplified PCR product (for each DNA sample) and 1 μL of the allelic ladder (AL) were used. These were added into a mix containing 9.6 μL of Hi-Di™ Formamide (Applied Biosystems, USA), 0.4 μL Gene Scan™ and 600 LIZ™ Size Standard v.2.0 (Applied Biosystems, USA). Gene Mapper ID-X Software version 1.4 (Applied Biosystems, USA) was used to analyze the obtained data.

## 2.5 Statistical analyses

The genetic profiles obtained from bones and the reference sample obtained from salivas, were compared with an estimation of the familiar relationships. In these cases, the calculation of likelihood ratio (LR) and posterior probability (PP) were performed using the DNA VIEW software and the allele frequencies from the Romanian population [10]. The genetic profiles obtained from the bones were also

compared with the genetic profiles belonging to the persons included in the elimination database to monitor the possible contamination.

## 3. Results

The bone powder obtained during bone pre-processing was used for DNA extraction. In this case, bone demineralization was performed. The DNA concentrations of the saliva, petrous pyramid and tooth after quantification using the Power Quant® System (Promega, USA) are presented in **Tables 1–3** for STR markers.

Using capillary electrophoresis, we obtained the genotypes on STR markers as follows: in **Case 1 –** the paternity relationship between the presumptive son and his deceased father was confirmed (**Table 4**); **Case 2 –** the maternity relationship between the presumptive daughter and the victim found in the woods was proven (**Table 5**); **Case 3 –** the kinship between the unrecognizable burn victim and his presumptive daughter was established (**Table 6**).

| DNA markers | Genetic DNA profile of the presumptive son | Genetic DNA profile obtained from the femur bone |
|---|---|---|
| CSF1PO | 12;**12** | 12;**12** |
| D10S1248 | 14;**15** | **15**;16 |
| D12S391 | **18**;19 | **18**;21 |
| D13S317 | 9;**11** | **11**;14 |
| D16S539 | **11**;12 | **11**;11 |
| D18S51 | 14;**15** | 12;**15** |
| D19S433 | **14**;16 | **14**;14 |
| D1S1656 | **12**;15 | **12**;15 |
| D21S11 | 28;**28** | **28**;28 |
| D22S1045 | **16**;18 | **16**;16 |
| D2S1338 | 17;**25** | 20;**25** |
| D2S441 | **11**;11.3 | **11**;14 |
| D3S1358 | **14**;15 | **14**;17 |
| D5S818 | **10**;12 | **10**;11 |
| D7S820 | 8;**10** | 7;**10** |
| D8S1179 | **13**;14 | 12;**13** |
| FGA | **22**;24 | 20;**22** |
| SE33 | 21;**30.2** | 13.2;**30.2** |
| TH01 | **6**;8 | **6**;6 |
| TPOX | 8;**11** | 11;**11** |
| vWA | 17;**19** | 14;**19** |
| AMELOGENINA | XY | XY |
| CUMULATIVE PATERNITY PROBABILITY (CPP) | | (CPP) = 99.99989% |
| PATERNITY INDEX (PI) | | (PI) = $9.147 \times 10^3$ |

**Table 4.**
*Genetic DNA profiles in Case 1. The paternal alleles are shown colored.*

| DNA markers | Genetic DNA profile obtained from the petrous pyramid | Genetic DNA profile of the presumptive daughter |
|---|---|---|
| CSF1PO | **10;** 12 | **10;**11 |
| D10S1248 | 15;**16** | 14;**16** |
| D12S391 | 18;**21** | **21;**21 |
| D13S317 | **8;** 9 | **8;**11 |
| D16S539 | 12;**13** | 12;**13** |
| D18S51 | 16;**16** | 12;**16** |
| D19S433 | **12;**15 | **12;**13 |
| D1S1656 | **12;**17 | **12;**14 |
| D21S11 | **28;**30 | **28;**31 |
| D22S1045 | **14;**15 | **14;**18 |
| D2S1338 | **16;**19 | **16;**22 |
| D2S441 | 10;**10** | **10;**11 |
| D3S1358 | 18;**18** | 14;**18** |
| D5S818 | **12;**13 | **12;**12 |
| D7S820 | **10;**12 | **10;**12 |
| D8S1179 | 13;**13** | **13;**14 |
| FGA | 23;**26** | 21;**26** |
| SE33 | 19;27.2 | 19;19 |
| TH01 | **7;**9 | **7;**9.3 |
| TPOX | 8;**10** | 8;**10** |
| vWA | **16;**17 | **16;**19 |
| AMELOGENINA | XX | XX |
| CUMULATIVE MATERNITY PROBABILITY (CMP) | | (CPP) = 99.99956% |
| MATERNITY INDEX (MI) | | (PI) = $7.391 \times 10^3$ |

**Table 5.**
*Genetic DNA profiles in Case 2. The maternal alleles are shown colored.*

| DNA markers | Genetic DNA profile of the presumptive son | Genetic DNA profile obtained from the canine tooth |
|---|---|---|
| CSF1PO | 10;**10** | 10;**10** |
| D10S1248 | **13**;14 | 13;**13** |
| D12S391 | 19;**22** | 22;**22** |
| D13S317 | 8;**11** | 8;**11** |
| D16S539 | **11**;14 | **11**;12 |
| D18S51 | 13;**17** | **17**;19 |
| D19S433 | 14;**14** | **14**;15 |
| D1S1656 | **15**;17.3 | **15**;16 |
| D21S11 | **26**;32.2 | **26**;30.2 |
| D22S1045 | 15;**15** | **15**;16 |

| DNA markers | Genetic DNA profile of the presumptive son | Genetic DNA profile obtained from the canine tooth |
|---|---|---|
| D2S1338 | 23;**23** | 21;**23** |
| D2S441 | **11**;13 | **11**;14 |
| D3S1358 | 15;**17** | 16;**17** |
| D5S818 | 12;**12** | 11;**12** |
| D7S820 | 8;**10** | 10;**10** |
| D8S1179 | 13;**14** | 14;**14** |
| FGA | 23;**23** | 18;**23** |
| SE33 | 9.2;**22.2** | **22.2**;28.2 |
| TH01 | 6;**9.3** | 6;**9.3** |
| TPOX | 8;**11** | 11;**11** |
| vWA | **16**;18 | 16;**16** |
| AMELOGENINA | XY | XY |
| CUMULATIVE PATERNITY PROBABILITY (CPP) | | CPP=99.99926% |
| PATERNITY INDEX (PI) | | PI=6.84 X $10^3$ |

**Table 6.**
*Genetic DNA profiles in Case 3. The paternal alleles are shown colored.*

The Gene Mapper ID-X software version 1.4 (Thermo Fischer Scientific, USA) was used to analyze the data.

## 4. Discussion

Genetic analysis is a fundamental tool that is used for the identification of skeletonized remains. In forensic genetics, the most important steps for genetic identification are DNA extraction from biological samples and their quantification.

In our cases, from the biological samples that were analyzed: the saliva provided by the first-degree relatives of the deceased as reference sample in all cases, and a canine tooth, a femur bone fragment, and a petrous pyramid extracted from the victims, all had good concentrations on autosomal STR markers.

The DNA concentrations of the biological samples are presented in **Tables 1–3**. An important aspect to look out for while performing genetic identification of skeletonized remains relates to the relationship between the types of bones and the DNA concentration obtained during the analysis. Many studies have demonstrated that compact bones yield a greater amount of DNA when compared to spongy bones [11–13]. In **Tables 4–6**, the genetic profiles obtained from bones remains are presented together with the genetic profiles of the first degree relatives, thus being demonstrated the kinship relationship between them. In these studies, it was demonstrated that compact bones from the lower limbs are more effective as DNA concentrations when compared to compact bones from the upper limbs [14].

Many studies classified the types of human bones that are recommended for forensic genetic identification depending on the amount of DNA that can be obtained, such as: tooth, talus, tarsal bones, petrous temporal bone, vertebra, femur and tibial metatarsal [15]. In our cases, our results conformed to the results obtained by other forensic genetics laboratories, in that the DNA concentrations differed

based on the bone type. The tooth was observed to have the greatest DNA concentration when compared to the petrous temporal bone or the femur bone.

During bone demineralization, some laboratories had included liquid nitrogen in their grinding procedure and mill freeze and bone powder incubation was observed for 72 hours [16–18]. Following the advances made in molecular techniques for DNA extraction from skeletonized remains, in 2019, Promega Company introduced a new kit for DNA extraction from highly degraded bones. This kit optimized the process of DNA extraction and PCR amplification, thereby enabling forensic laboratories to obtain good results while causing minimal destruction to bone samples and using a minimal time of only three hours for DNA extraction [19–21].

Also, in some cases where human remains are found and no relatives have been identified for genetic identification, forensic anthropology tries to provide information regarding the gender and height of the deceased and helps in estimating the time since death and the cause of death (if the remains provide relevant evidence). Approximate height can be determined by looking at the measurements of the bones; the best way to find approximate height is to measure the femur bone.

In forensic anthropology, the preferred method for establishing the identity of skeletal remains is dental identification. In some cases, when the teeth or skull may be missing, other alternatives to dental identification may be used.

Advancements in the field of forensic genetics through automate systems have aided in reducing the time that is spent on obtaining genetic profiles. Also, the introduction of next-generation system (NGS) and massive parallel sequencing (MPS) systems has enabled the concomitant analysis of multiple STR, and SNPs of human remains to identify the person [22–26].

To our knowledge, this is the first forensic laboratory in Romania regarding forensic human DNA identification from bones and tooth, where liquid nitrogen was not used with an automate protocol and DNA extraction from skeletonized remains was performed within three hours.

## 5. Conclusion

The biological material subjected to DNA identification analyzes in forensic medicine is in different taphonomy conditions and the selection of appropriate working samples is crucial.

In genetic human identification, automate system for DNA extraction from different biological samples is better than manual extraction, which is time consuming and presents the risk of errors.

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