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Challenges Facing Us in DNA Analysis from Human Hair Samples: A Review

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Abstract

Human hair is a valuable tool in criminology, aiding in population studies through statistics and DNA analysis. It's easily identifiable at crime scenes, often clinging to clothing, carpets, and various surfaces, including animal fur, through a process known as secondary transfer. Forensic analysis of hair evidence serves several purposes; it can help establish the possibility of a connection between a suspect and a crime scene or between a suspect and a victim. It can demonstrate that there is no evidence linking the perpetrator to the crime scene or the suspect to the victim. While microscopic hair analysis cannot definitively identify a specific individual as the source of the hair, it does provide a solid basis for association. The wealth of macroscopic and microscopic details available in hair examination can also provide strong evidence for the defense. To understand the challenges of conducting DNA tests on hair, it's essential to study hair structure and composition. DNA in hair is not evenly distributed throughout every part of the hair; it exists in both nuclear and mitochondrial forms.

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1. Introduction

One of the most commonly used biological products in legal science is human hair, which has been employed in criminology for population study using statistics and DNA analysis [1, 2]. Rudolf Virchow used human hair forensic comparison for the first time in 1861[3]. Actually, at a crime scene, hair can be identified easily. It can stick to clothing, carpets, and many other surfaces and adhere to other areas, which is called secondary transfer and is particularly prevalent with animal fur [4]. Forensic analysis for hair evidence can be highly helpful in evaluating physical evidence by (1) demonstrating the possibility that a suspect and a crime scene, or a suspect and a victim, may have had a relationship. (2) Demonstrating that there is no evidence of an association between the perpetrator and the crime scene or the suspect and the victim [5]. Despite the fact that the science of microscopic hair analysis can never lead to identification, that is, concluding that a hair came from one person and not another, hair examination may provide a reasonable foundation for an association and definitely offers strong exculpatory evidence due to the large amount of both macroscopic and microscopic details available. [6]. It is mandatory to study hair structure and composition in order to understand the difficulties of carrying out a hair DNA test. The restricted distribution of DNA in every part of the hair also needs to be recognized (nuclear and mitochondrial DNA). In this study, the main anatomical and physiological aspects of the various types of human hair were summarized and the clinical importance of the different structures and basis of DNA hair analysis was considered.

2. Hair Structure and Composition

Hair consists of three concentric layers. The medulla referred to as the innermost layer; the marrow, is a soft structure made up of circular cells that are often known as the marrow. The pigments are located in the middle region that gives the hair color and elasticity, referred to as the cortex. The hair cuticle is the outer layer. This rigid, translucent outer layer surrounds and protects the hair's inner layers and offers that distinctive bright shine to hair as well. Below the scalp, the hair root is embedded and contained inside a hair follicle. This is linked through the dermal papilla to the blood stream (figure 1).

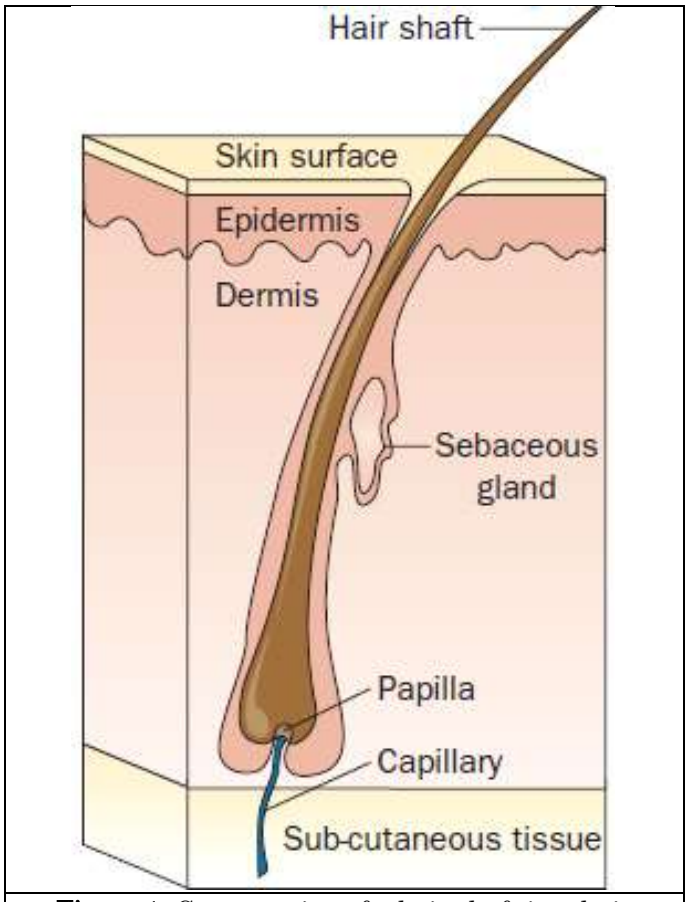


Figure 1. Cross-section of a hair shaft in a hair follicle in the skin.[1]

[1] Figure taken from Neutron Activation Analysis of Hair, chapter three; The Study of Hair, http://ngl.cengage.com/assets/downloads/forsci.pdf.

3. Microscopic Examinations of Hair

In the follicles, there are also hormones and receptors that help control hair growth. Hairs are basically made up of a protein named keratin that is fibrous. Keratin is also the main component of skin, hoofs of animals and nails, despite the fact that cells make up a portion of the hair, they do not contain appropriate content for DNA analysis [8, 9, 10, and 11]. A microscopic comparison is carried out as the first step of the inspection process. This includes determining whether the questioned hair can be excluded or included from the source of a known sample by assessing the microscopic characteristics of the hair samples and reviewing the points for comparison. Some primary physical characteristics that are correlated with hair from various broad racial groups have been reported by hair examiners. These features are only generalities and may not be available to individuals of certain races.

Furthermore, since the characteristics of a particular hair are poorly described or difficult to calculate, it may be impossible to assign it to a specific race. [13,14]. The physical characteristics of hair can provide clues to an individual's large racial history. In forensic investigations, three ethnic groups are used: European, Asian, and African [5]. In figure 2, the broad characteristics of hair from different breeds are compared. Hair comes in a variety of shapes, sizes, diameters, textures, and colors. Hair curl is affected by the hair's cross-section, which can be circular, triangular, irregular, and flattened Contents in table 1. Hair texture ranges from coarse (such as in whiskers) to fine (such as in younger children) [13].

Table 1. Racial characteristics of hair [4]

Race	Includes	Diameter	Cross Section	Pigmentation	Cuticle	Undulation
Negroid	Blacks	60-90 μm	Flat	Dense and clumped	—	Prevalent
Caucasoid	American, European, Mexican, and Middle Eastern	70-100 μm	Oval	Evenly distributed	Medium	Uncommon
Mongoloid	Orientals and American Indian	90-120 μm	Round	Dense auburn	Thick	Never

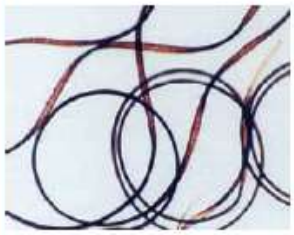
Race	Appearance	Pigment Granules	Cross Section	Other
European	Generally straight or wavy 	Small and evenly distributed	Oval or round of moderate diameter with minimal variation 	Color may be blond, red, brown, or black
Asian	Straight 	Densely distributed	Round with large diameter 	Shaft tends to be coarse and straight Thick cuticle Continuous medulla
African	Kinky, curly, or coiled 	Densely distributed, clumped, may differ in size and shape	Flattened with moderate to small diameter and considerable variation 	

Figure 2. A study of some common physical features of hair from various races.

Even though animal-hair comparisons are less significant than human-hair comparisons, this does not negate their possible use in forensic investigations. The appearance of dog hair on a victim's object that can be microscopically compared to a confirmed dog hair sample from the suspect's dog may be critical (see figure 3) [6].

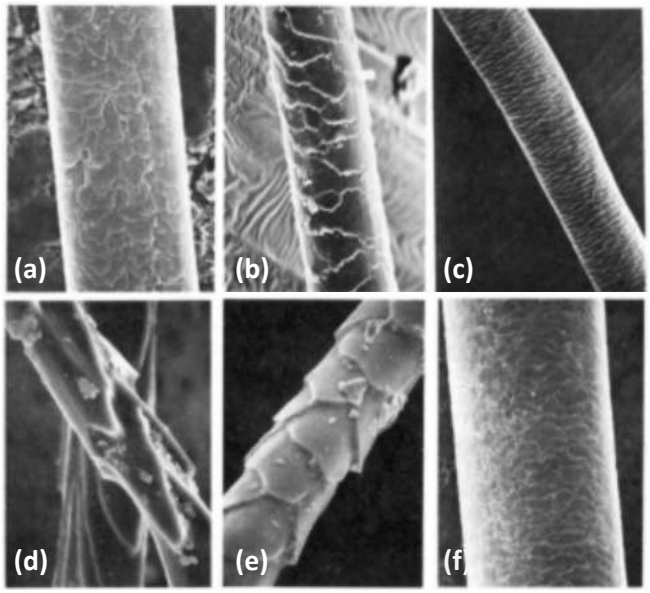


Figure 3. Several different styles of hair have different scales or cuticle patterns. a. head Human hair (600x), b. dog fur (1250x), c. Deer hair (120x), d. Rabbit fur (300x), e. Cat fur (2000x), f. Horse hair (450x) [6].

4. Hair Evidence Value

a. Hairs are common source of forensic evidence, and they can be analyzed at crime scenes or secondary locations [16].
b. Hair does not readily decompose, even when exposed to moisture and subsequent tissue decomposition [4].
c. A chemical examination will expose a person's history of using medications and other chemicals, detect heavy metals, and determine nutritional deficiency [17].
d. Hairs with roots are optimal for nuclear DNA testing; when the follicle of a hair is present, DNA evidence can be produced. The results of DNA tests are not considered class evidence. It is preferable because it will aid in individual recognition, making it personal proof [18].
e. Hair shafts, present in the higher copy number, can be examined with mtDNA. [19].
f. First Hair DNA demonstration: Higuchi, 1988[20].
g. Due to its tough outer covering, hair does not decompose easily [21].

5. Nuclear and Mitochondrial DNA Availability in Hair Samples

Molecular biology tools have allowed forensic scientists to classify both nuclear and mtDNA biological evidence at the DNA level (see table 2). Any material containing nucleated cells, including hair, Nuclear DNA typing can theoretically be used to manipulate this. For successful nuclear DNA typing, these hairs typically have to contain sheath material (figure 4).

Table 2. Comparison between mitochondrial DNA and nuclear DNA characteristics

Mitochondrial DNA	Nuclear DNA
Circular molecule with a closed shape	linear molecular structure
Size of 16, 569 bp	Size nearly 3 billion bp
Noncoding region nearly 1100 bp	non-coding region comprises a large portion
maternal inheritance	biparental inheritance
recombination absent	Recombination present
50 to several thousand copies per cell	two copies per cell

Figure 4. A female's DNA profile demonstrating good PCR amplification for all 16 loci with amelogenin

When sheath material is found, the safest, most discriminating technique for comparing a questioned hair sample to a known sample is nuclear DNA testing. In case the unknown hair is microscopically related to a known sample and there is suitable root material, nuclear DNA analysis of this hair should be performed [5, 22]. PCR technique made it possible to examine the small amount of hair DNA, which is in forensic science considered a valuable piece of evidence [23, 24]. Hairs, however, contain extremely small amounts of DNA [23]. Even if hairs contain roots, the possibility of a complete DNA profile being successfully extracted using PCR technology is somewhere between 60-70%. In rare cases, when a hair's root is absent, analysis may produce a nuclear DNA profile [13, 25]. This is possible in cases where, at the tip of the hair shaft, live hair cells are still present or where a partial breakdown of nuclear DNA has occurred during the hair keratinous process [21], as indicated above. Degraded DNA data at the larger STR loci seen as a "decay curve" is recognized by a decrease or complete loss of genotype information. Data will exhibit a "wedge" shape with a sharp decrease in the height of allelic peak as the length of the PCR amplicon increases [26], which leads to

improvement: New Higher Sensitivity Nuclear DNA Assays (mini STRs) characterization of new Mini STR loci [28], Degraded sample analysis aid (see figure 5), often DNA degradation occurred during tissue biogenesis such as hair [29] or loss by dye, hematin, and microbial DNA effects, all of these factors are widely recognized inhibitors[30].

Figure 5. Successful PCR amplification for 9 mini STRs Assays with amylogenin in a male DNA profile.

6. Mitochondrial DNA Hair Analysis

Since the content of nuclear DNAs is too small to amplify Instead of hair root hair, especially from naturally shed hair or shafts of hair, many studies have focused on relatively abundant mtDNA., [31], although DNA is not always completely amplified by PCR even if adequate quantities of DNA from hair have been extracted, indicating existence of PCR inhibitors in the extracted hair samples [32]. Hair pigment melanin is primarily a strong inhibitor of the PCR process, according to previous studies; hair dyeing has a strong effect on PCR [7, 13, and 33]. In forensic casework or human identification cases when observing damaged skeletal remains or hair without roots, mitochondrial DNA analysis is used. [34], two key advantages over nuclear DNA analysis are provided by mitochondrial DNA [35]:

1. There are thousands of copies of mtDNA in a cell compared to two copies of nuclear DNA, resulting in increased sensitivity.
2. mtDNA is inherited maternally.

Disadvantages of checking with mtDNA:

1. Low discrimination capacity.
2. Intensive Labor.
3. Expensive.

There are several areas of the control region in mitochondrial DNA that are extremely variable from person to person. The variation of the sequence inside two hypervariable regions, (HV1) and (HV2), is usually examined during forensic examination. At least HV1 has a range of 16024 to 16365, and HV2 has a range of 73 to 340 [16, 36]. The first mtDNA analysis helped with a criminal investigation was in Tennessee in 1996 on a suspect convicted of a teenage girl rape and murder. Since then, hundreds of cases have been investigated using mitochondrial DNA analysis, and hundreds of them have been successfully prosecuted in court. [37]. Because of the high copy number of mtDNA, data on mitochondrial DNA can be certainly produced for aged specimens with highly fragmented and degraded DNA when trials to form nuclear DNA markers fail to generate a profile [38-40]. Hair shafts and aged fingernails

are two examples of samples that have a lot of mtDNA but little or no intact nuclear DNA [41].

7. Forensic Mitochondrial DNA Research Protocols

To determine the mitochondrial DNA form, editing the sequence data and compiling a consensus sequence was performed to be compared to the revised Cambridge Reference Sequence (rCRS), sequence data produced by the ABI 3130xl is compiled into a project for analysis. It will be one of the following Match requirements for sequencing data [41, 42]:

1. Concordance: When, in the overlapping regions, two sequences of mtDNA of different samples (e.g. from two pieces of evidence or from evidence and a reference source for the maternal family) are compatible with each other, The probability of the two samples coming from the same source or having a maternal connection, respectively cannot be excluded.
2. Inconclusive: In case of two mtDNA sequences from distinct samples have one variation; the resulting comparison will be called inconclusive. Other reference sources and further analysis for obtaining further sequence data could be useful in these situations.
3. Exclusion: Exclusion would be considered to be the resulting comparison if two mtDNA sequences from different samples have two or more variations.

8. Conclusions

A combination of microscopic hair testing then nuclear DNA or mtDNA analysis will provide the most accurate data of questioned hair. First, microscopic analysis, followed by DNA inspection, must be done since the part of the hair used in DNA analysis would be damaged, rendering it unable to be microscopically examined.

In case of presence of hair root of, to produce a DNA profile, DNA can be collected, amplified, and compared to established samples for identification. If the samples are extremely degraded, then mini STRs assays will be carried out. If root is lacked, mitochondrial DNA analysis will match the hair. Matching conditions for mitochondrial DNA sequencing: concordance, Inconclusive and Exclusion. Hairs are not a form of positive identification in the forensic science community, but because of the variation in hair between individuals, they may provide important information.

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Conflict of Interest:

The authors declare no conflict of interest.

References

[1] Suenaga, E.; Nakamura, H.; "Evaluation of three methods for effective extraction of DNA from human hair". J. Chromatogr. A., 820(1): 137-141; 2005.

[2] Ghatak, S.; Muthukumaran, R.B.; Nachimuthu, S.K.; "A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis". J. Biomol. Tech., 24(4): 224, 2013.

[3] Bisbing, R.E.; Wolner, M.F.; "Microscopical discrimination of twins' head hair". J. Forensic Sci., 29(3): 780-786, 1984.

[4] Cortes-Figueiredo, F.; Carvalho, F.S.; Fonseca, A.C.; Paul, F.; Ferro, J.M.; Schönherr, S.; Morais, V.A.; "From Forensics to Clinical Research: Expanding the Variant Calling Pipeline for the Precision ID mtDNA Whole Genome Panel". Int. J. Mol. Sci., 22(21):12031, 2021.

[5] Cortellini, V.; Carobbio A.; Brescia G.; Cerri N.; Verzeletti A.; "A Comparative Study of Human and Animal Hairs: Microscopic Hair Comparison and Cytochrome: c: Oxidase I Species Identification". J. Forensic Sci. Med., 5(1): 20-3, 2019.

[6] Lanning, K.; Michaud, A.; Bisbing, R.; Springer, F.; Tridico, D.S.; "Scientific Working Group on Materials Analysis Position oFn Hair Evidence". J. Forensic Sci., 54:1198–1202, 2018.

[7] Houck, M.M.; Budowle, B.; "Correlation of microscopic and mitochondrial DNA hair comparisons". J. Forensic Sci., 47(5): 1-4, 2002.

[8] Suenaga, E.; Nakamura, H.; "Evaluation of three methods for effective extraction of DNA from human hair". J. Chromatogr. A; 820(1); 137-141; 2005.

[9] Linch, C.A.; "The ultrastructure of tissue attached to telogen hair roots". J. Forensic Sci.; 53(6), 1363-1366; 2008.

[10] Wang, X.; Shi, Y.; Zhou, Q.; Liu, X.; Xu, S.; Lei, T.; "Detailed histological structure of human hair follicle bulge region at different ages: a visible niche for nesting adult stem cells". J. Huazhong Univ. Sci. Technolog Med. Sci., 32(5): 648-656, 2012.

[11] Buffoli, B.; Rinaldi, F.; Labanca, M.; Sorbellini, E; Trink, A.; Guanziroli, E.; Rodella, L.F.; "The human hair: from anatomy to physiology". Int. J. Dermatol.,53(3): 331-341, 2014.

[12] Wolfram, L.J.; "Human hair: a unique physicochemical composite". J. Am. Acad. Dermatol, 48(6): S106-S114, 2003.
[13] Alvarez-Cubero, M.J.; Saiz, M.; Martinez-Gonzalez, L.J.; Alvarez, J.C.; Eisenberg, A.J.; Budowle, B.; Lorente, J.A.; "Genetic identification of missing persons: DNA analysis of human remains and compromised samples". Pathobiology, 79,(5): 2012.
[14] Lanning, K.; Michaud, A.; Bisbing, R.; Springer, F.; Tridico, D.S.; "Scientific Working Group on Materials Analysis Position on Hair Evidence". J. Forensic Sci., 54(5): 1198–1202, 2009.
[15] Melton, T.; Holland, C.; Holland, M.; "Forensic mitochondria DNA analysis: Current practice and future potential". Forensic Sci. Rev., 24(2): 101, 2012.
[16] Machado, H.; Granja, R.; "DNA Technologies in Criminal Investigation and Courts. In: Forensic Genetics in the Governance of Crime. Palgrave Pivot,Singapore,(2020).
[17] Finaughty, C.; Heathfield, L.J.; Kemp, V.; Márquez-Grant, N.; "Forensic DNA extraction methods for human hard tissue: A systematic literature review and meta-analysis of technologies and sample type". Forensic Sci. Int. Genet., 63: 102818, 2023.
[18] Just, R.S.; Irwin, J.A.; Parson, W.; "Mitochondrial DNA heteroplasmy in the emerging field of massively parallel sequencing". Forensic Sci. Int. Genet. 18: 131-139, 2015.
[19] Higuchi, R.; von-Beroldingen, C.H.; Sensabaugh, G.F.; Erlich, H.A.; "DNA typing from single hairs". Nature, 332(6164): 543-546, 1988.
[20] Campos, P.F.; Gilbert, M.T.P.; "DNA Extraction from Keratin and Chitin". Methods Mol. Biol.,1963:57-63, 2019.
[21] Wilson, M.R.; Polanskey, D.; Butler, J.; Di-Zinno, J.A.; Replogle, J.; Budowle, B.; "Extraction, PCR amplification and sequencing of mitochondrial DNA from human hair shafts". Biotechniques, 18, 662-669, 1995.
[22] Higuchi, R.; von-Beroldingen, C.H.; Sensabaugh, G.F.; Erlich, H.A.; "DNA typing from single hairs". Nature, 332(6164): 543-546, 1988.
[23] Sullivan, K.M.; Hopgood, R.; Gill, P.; "Identification of human remains by amplification and automated sequencing of mitochondrial DNA". Int. J. Legal Med.,105(2): 83-86, 1992.
[24] Grisedale, K.S.; Murphy, G.M.; Brown, H.; Wilson, M.R.; Sinha, S.K.; "Successful nuclear DNA profiling of rootless hair shafts: a novel approach". Int. J. Legal Med., 132(1): 107–115, 2018.
[25] Chung, D.T.; Drábek, J.; Opel, K.L.; Butler, J.M.; McCord, B.R.; "A study on the effects of degradation and template concentration on the amplification efficiency of the STR Miniplex primer sets". J. Forensic Sci., 49(4): 1-8, 2004.
[26] Coble, M.D.; Butler, J.M.; "Characterization of new miniSTR loci to aid analysis of degraded DNA". J. Forensic Sci., 50(1): 43–53, 2005.
[27] Alberts, C.C.; Ribeiro-Paes, J.T.; Aranda-Selverio, G.; Cursino-Santos, J.R.; Moreno-Cotulio, V.R.; Oliveira, A.L.D.; Souza, E.B.D.; "DNA extraction from hair shafts of wild Brazilian felids and canids". Genet. Mol. Res., 9: 2429-2435, 2010.
[28] Schneider, P.M.; Bender, K.; Mayr, W.R.; Parson, W.; Hoste, B.; Decorte, R.; Greenhalgh, M.; "STR analysis of artificially degraded DNA— results of a collaborative European exercise". Forensic Sci. Int., 139(2-3): 123-134, 2004.
[29] Suenaga, E.; Nakamura, H.; "Evaluation of three methods for effective extraction of DNA from human hair". J. Chromatogr. A., 820(1): 137-141, 2005.
[30] Romeika, J.M.; Yan, F.; " Recent advances in forensic DNA analysis". J. Forensic Sci., S13: 001, 2013.
[31] Suenaga, E.; Nakamura, H.; "Evaluation of three methods for effective extraction of DNA from human hair". J. Chromatogr. B., 820(1):137-141, 2005.
[32] Allen, M.; Engström, A.S.; Meyers, S.; Handt, O.; Saldeen, T.; von-Haeseler, A.; Gyllensten, U.; "Mitochondrial DNA sequencing of shed hairs and saliva on robbery caps: sensitivity and matching probabilities". J. Forensic Sci., 43(3): 453-464, 1998.
[33] Sujirachato, K.; Tirawatnapong, S.; Mimae, N.; Inon, S.; Kaewdouengdee, A.; Worasuwannarak, W.; "The Study of the Yield of DNA Extracted From Hairs of Postmortem Cases". Ramathibodi Med. J., 43: 9–18, 2020
[34] Anderson, S.; Bankier, A.T.; Barrell, B.G.; de-Bruijn, M.H.; Coulson, A.R.; Drouin, J.; Young, I.G.; " Sequence and organization of the humanmitochondrial.genome". Nature, 290(5806): 457-465, 1981.
[35] Nilsson, M.; " Sequencing of mtDNA in Shed Hairs: A Retrospective Analysis of Material from Forensic Cases and a Pre-Screening Method". Open Forensic Sci. J., 5(1): 13–22, 2012.
[36] Lindahl, T.; Andersson, A.; "Rate of chain breakage at apurinic sites in double-stranded deoxyribonucleicacid". J. Biochem., 11(19): 3618 - 3623; 1972.
[37] Just, R.S.; Irwin, J.A.; Parson, W.; "Mitochondrial DNA heteroplasmy in the emerging field of massively parallel sequencing". Forensic Sci. Int. Genet., 18: 131-139, 2015.
[38] Higuchi, R.; von-Beroldingen, C.H.; Sensa-baugh, G.F.; Erlich, H.A.; "DNA typing from single hairs". Nature, 332(6164): 543-546, 1988.

[39] Anderson, T.D.; Ross, J.P.; Roby, R.K.; Lee, D.A.; Holland, M.M.; "A validation study for the extraction and analysis of DNA from human nail material and its application to forensic casework". J. Forensic Sci., 44(5): 1053-1056, 1999.

[40] Templeton, J.E.; Brotherton, P.M.; Llamas, B.; Soubrier, J.; Haak, W.; Cooper, A.; Austin, J.J.; "DNA capture and next-generation sequencing can recover whole mitochondrial genomes from highly degraded samples for human identification". Investig. Genet. 4(1): 1-13, 2013.

[41] Kuznetsov, A.V.; Ausserlechner, M.J.; "Research of Mitochondrial Function, Structure, Dynamics and Intracellular Organization". Int. J. Mol. Sci., 24(1): 886, 2023.

[42] Ambers, A.; Wiley, R.; Novroski, N.; Budowle, B.; "Direct PCR amplification of DNA from human bloodstains, saliva, and touch samples collected with microFLOQ® swabs". Forensic Sci. Int. Genet., 32: 80–87, 2018.