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Evaluation of Generating DNA Profile from Keratinous Tissues

Mohammed M. Al-Zubaidi*, Miriam Jasim Shehab , Thoalnoon Younis Salih, Rasha Sadeq Ameen

Department of Forensic Biology, Higher Institute of Forensic Sciences, Al-Nahrain University, Jadriya, Baghdad, Iraq.

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Abstract

Fragmented DNA samples became serious issue in crime scene and all most result in incomplete DNA profile. Keratinous tissues such as hair and nails contain highly fragmented DNA and could be seen as biological evidence at crime scene. This study aimed to evaluation of generating DNA profile from keratinous tissues. Ten samples (five from hair and five from nails) collected from volunteers. Extraction of DNA was carried out by using PrepFiler™ forensic DNA extraction kit. The quantities of DNA were measured using real-time PCR, while the amplification of eight loci (D13S31, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO, FGA) and amelogenin was performed using the AmpFLSTR™ MiniFiler™ PCR Amplification Kit. The genetic Analyzer 3130 XL system and GeneMapper ID software v.3.2 were used to perform capillary electrophoresis and data analysis. The results indicated that the mean quantities of DNA isolated from hair (3.34 ± 1.7) and the DNA isolated from nails (4.36 ± 3.02). Analysis of MiniFiler results revealed that most of hair samples generated partial DNA profile while all samples of nail produced full profile and the percentage of allele's appearance for hair samples range between (30%-90%). Whereas, nail samples alleles appearance was 100%. Conclusion, although both nail and hair tissues are subject to DNA fragmentation due to their keratinized structure and cellular composition, nail samples produces more consistent and complete DNA profiles compared to hair samples when analyzed using MiniFiler kit. This is likely attributed to the relatively lower degradation and greater stability of DNA in nails. In contrast, hair samples especially those lacking follicles exhibited a high degree of allele drop-out, particularly in loci with large amplicon sizes.

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*Corresponding author: mohammed.mahdi@nahrainuniv.edu.i

1. Introduction

Short tandem repeats (STRs) are repetitive DNA sequences found in non-coding regions of the genome that vary in length and are highly polymorphic. This makes them ideal markers for DNA profiling. Amplification of these repetitive by using polymerase chain reaction (PCR) is a highly effective method used in forensic science for identifying individuals and establishing relationships between them [1]. One of the major challenges in forensic DNA analysis is the presence of degraded DNA or templates with low copy numbers (LCN) [2,3]. DNA degradation refers to the breakdown of the DNA molecule, wherein the sugar-phosphate backbone responsible for maintaining the structural integrity of the DNA strand weakens and fragments. As a result, the nitrogenous bases attached to the backbone also become fragmented, producing small, disjointed pieces of DNA [4,5]. DNA degradation can occur naturally, as seen in keratinous tissues such as hair and nails, or due to various external factors. These include exposure to ultraviolet (UV) light, microbial activity (e.g., bacteria and mold), excessive moisture, fluctuations in pH levels, and temperature extremes. Keratinous tissues have been utilized as a source of genetic

material for over 20 years. Keratinous tissues are often found at crime scenes. Although DNA extracted from keratinous tissues is often highly fragmented and contaminated due to the unique composition and structure of these tissues, where DNA is embedded within keratinized cells that limit the yield and quality of extraction they still offer notable advantages. These include ease of sample collection and the relative stability of DNA over time [6-10]. In degraded DNA samples, high molecular weight STR markers often fail to amplify, resulting in incomplete DNA profiles [4]. The use of mini primer sets, an approach that reduces the size of STR amplicons enhances the efficiency of DNA analysis, particularly in degraded samples [11]. The present study aimed to evaluation of generating DNA profile from keratinous tissues by using AmpFLSTR™ MiniFiler™ PCR Amplification Kit.

2. Materials and Methods

2.1. Ethical Considerations

The study was conducted in compliance with the Declaration of Helsinki and received approval from the Forensic DNA Research and Training Center, Al-Nahrain University. All participants provided written informed consent.

2.2. Samples collection and DNA extraction

Samples were collected from unrelated adult volunteers, A total of ten samples were analyzed, comprising five nail samples (5 mg each) and five hair samples (5 cm in length). Among the hair samples 3 were taken from the shaft only, while 2 included both the shaft and the follicle. All samples were stored at room temperature until DNA extraction. PrepFiler™ forensic DNA extraction kit (Applied Biosystems, Foster City, CA) was used to extracted DNA from all samples according to manufacture instruction [12].

2.3. Quantification of DNA

DNA quantification was performed using the Quantifiler Duo DNA Quantification Kit (Applied Biosystems, Foster City, CA, USA) in conjunction with the 7500 Fast Real-Time PCR System and 7500 SDS software to determine the concentration of the extracted DNA, following the manufacturer's instructions. All samples were analyzed in duplicate to ensure the reproducibility and reliability of the quantification results. Each amplification run included a positive control provided with the kit to verify proper amplification performance, as well as a no-template negative control to monitor potential contamination. The baseline and threshold settings were automatically determined by the real-time PCR instrument software according to the manufacturer's recommended parameters. DNA quantification was performed using a standard curve generated from a series of standards with an initial concentration of 50 ng/µL (Std1), which were serially diluted to obtain concentrations down to 0.023 ng/µL (23 pg/µL) (Std8). DNA concentrations of the samples were calculated from the corresponding cycle threshold (Ct) values obtained during amplification. The real-time PCR reaction mixture consisted of 10.5 µL of Quantifiler Duo primer mix, 12.5 µL of Quantifiler Duo reaction mix, and 2.0 µL of DNA template. Amplification was carried out under the following thermal cycling conditions: an initial incubation at 60 °C for 2 minutes, followed by denaturation at 95 °C for 10 minutes, and 28 amplification cycles consisting of 15 seconds at 95 °C and 1 minute at 60 °C.

2.4. Polymerase chain reaction amplification

Eight loci, namely D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO, FGA, along with the sex-typing locus amelogenin ,were amplified using the PCR reaction.Amount of DNA templet that amplified in each sample was 0.5ng according to manufacture instruction of AmpFLSTR™ MiniFiler™ PCR Amplification Kit [13] and reaction carried out with Applied Biosystems Veriti® PCR System.

2.5. Capillary electrophoresis and detection of alleles:

Genetic Analyzer 3130 XL 16- capillary array system (Applied Biosystems, Foster City, CA, USA) was used for separation of amplicons and allelic detection .To each well of the reaction plate 7.8 µl of Hi-Di formamaide, 0.3 µl of GeneScan™ 50 LIZ size standard, and 1 µl of PCR product or AmpFLSTR™ MiniFiler™ Allelic Ladder were added. The reaction plate underwent denaturation at 95°C for 3 minutes, cooling for 3 minutes on a freezer block, and then subjected to capillary electrophoresis. To ensure the reliability of the genotyping results, positive and negative PCR controls were included in every amplification batch. The positive control consisted of control DNA supplied with the amplification kit to verify successful amplification and instrument performance, while a no-template negative control was used to detect any potential contamination during PCR setup. The Data collection v.2.0 software (Applied Biosystems, Foster City, CA, USA) was employed for data collection and

the GeneMapper IDv.3.2 software, was used for analyzing the samples.

2.6. Allele Appearance and Locus-Specific Dropout

The interpretation of allele appearance and locus-specific dropout was performed based on the electropherogram profiles generated from the amplified DNA samples. Allele detection was carried out using the GeneMapper ID software, which enabled accurate identification of allelic peaks according to the corresponding allelic ladder and internal size standard. An allele was considered successfully detected when a clear peak exceeding the analytical threshold was observed at the expected allele position within the locus. In this study, the allele appearance rate was calculated individually for each allele rather than per locus in order to obtain a more precise estimation of amplification success across the analyzed loci. The percentage of allele appearance was determined by dividing the number of observed alleles by the total number of expected alleles for each marker, and the results were expressed as a percentage. This approach allowed the evaluation of locus-specific amplification efficiency and the identification of loci with higher susceptibility to allelic dropout, particularly in low-template or degraded DNA samples.

3. Results and Discussion

The present study employed the AmpFlSTR MiniFiler PCR Amplification Kit to generate DNA profiles from keratinous tissues. The DNA yield obtained from the samples is presented in Table 1 and was quantified using real-time PCR. The results indicated that DNA concentrations ranged from 1.4 to 5.6 ng/µL in hair samples and 1.2 to 8.2 ng/µL in nail samples.

Table -1: The DNA concentration (ng/ µl) by real time-PCR according to sample type hair and nails).

Sample type	Sample code	DNA concentration	Sample type	Sample code	DNA concentration
Hair	H1	4.0	Nail	N1	1.9
	H2	5.6		N2	3.8
	H3	4.1		N3	8.2
	H4	1.6		N4	6.7
	H5	1.4		N5	1.2
Mean ±SD		3.34 ± 1.7	Mean ±SD		4.36 ± 3.02

Analysis of MiniFiler results revealed that most of hair samples produce partial DNA profile while all samples of nail except one generated full profile (table 2).

Table-2: Complete and partial DNA profiles with alleles percentage appearance obtained from hair and nails samples that amplified by MiniFiler Kit.

Sample	STR marker (Allele1/ Allele2)							
	D13S317	D7S820	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
H 1	8/11	10/13	20/23	29/30	8/10	13/15	9/12	21/21
H 2	11/0	0/0	20/25	28/28	8/8	14/16	9/10	20/22
H 3	0/0	9/0	25/25	0/0	8/0	12/0	11/0	0/0
H 4	0/0	0/0	20/0	0/0	0/0	0/0	0/0	0/0
H 5	0/0	0/0	20/25	0/0	0/0	14/16	9/10	0/0
Allele appearance %	30%	30%	90%	40%	50%	70%	70%	40%
N 1	8/11	10/13	20/23	27/30	10/10	13/15	9/12	21/21
N 2	11/11	7/8	17/24	29/32	8/8	11/17	11/12	23/23
N 3	11/12	8/10	21/23	29/32	11/12	14/15	10/12	22/22
N 4	8/12	9/9	23/25	32/33	12/12	12/15	11/12	20/20
N 5	11/13	10/12	17/17	32/32	12/13	12/12	10/12	0/0
Allele appearance %	100%	100%	100%	100%	100%	100%	100%	80%

The percentage of allele detection in hair samples ranged from 30% to 90%. In contrast, most nail samples showed 100% allele detection. The electropherogram for D13S317 and D7S820 loci illustrated allele drop-out in most of hair samples (Figure 1).

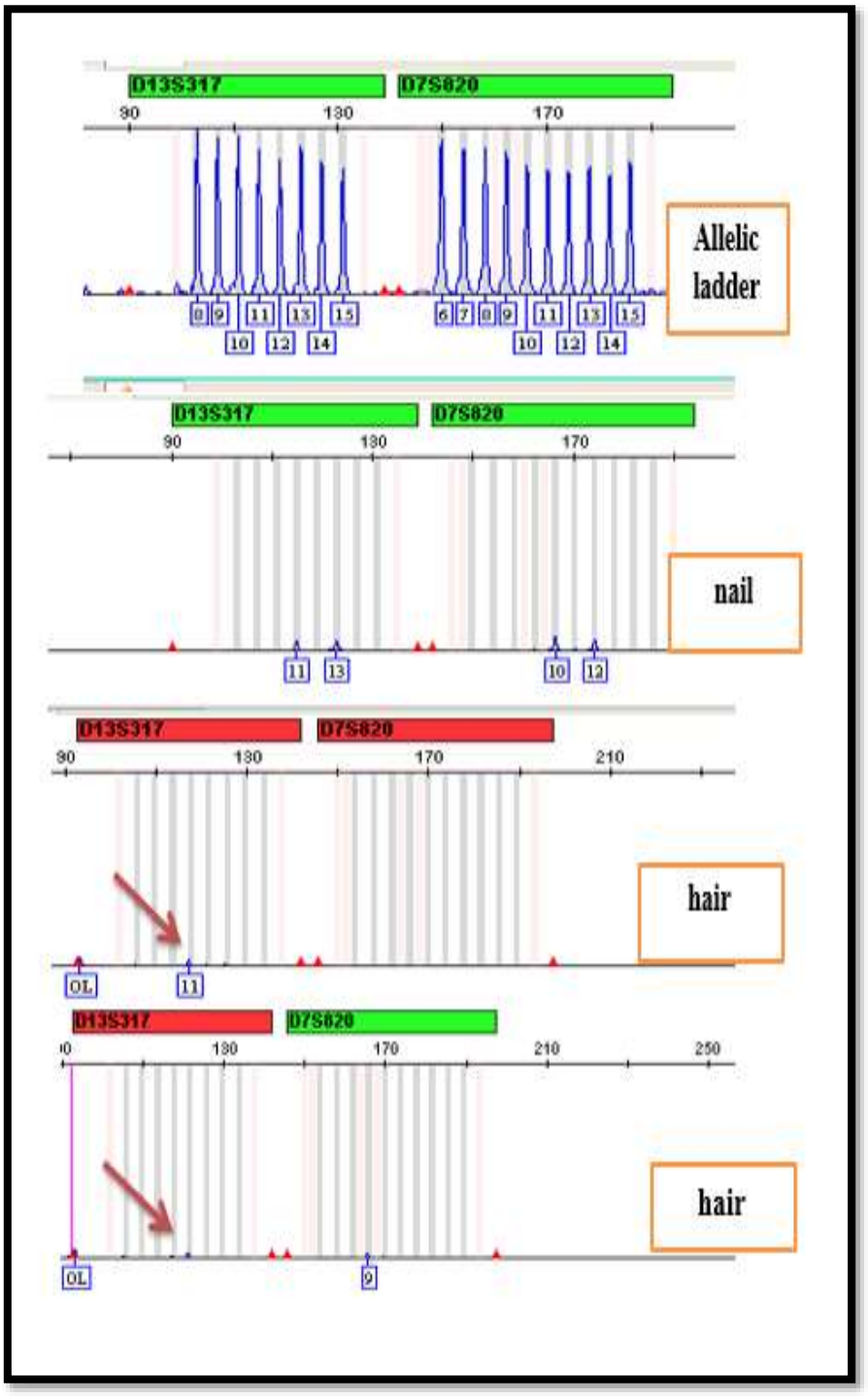

Figure 1: Electropherogram of D13S317 and D7S820 loci obtained from nail and hair samples along with allelic ladder that amplified by MiniFiler Kit. Photographic taken directly from 3130 XL Genetic Analyzer instrument.

In the present study, the variability in DNA quantities may be related to the type of sample. The quality and quantity of DNA obtained from hair and nail samples can differ substantially between individuals, even within the same species. This is due to the fact that hair and nails consist of dead cells that lack a nucleus, which is where most of DNA in living cells is found. The DNA present in the hair and nails comes from the hair bulb and nail matrix, which contain small amount of nuclear DNA and mitochondrial DNA, which inherited only from the mother. Furthermore, the amount and quality of DNA that can be extracted from hair and nails depend on various factor, such as the type of hair and nail, the age of sample and the methods used for collection, preservation, extraction and amplification [14-17]. The AmpFlSTR MiniFiler PCR Amplification Kit generates relatively short amplicons ranging from approximately 70 to 283 base pairs, which are considerably smaller than those produced by many conventional multiplex STR kits that typically amplify fragments between 100 and 450 base pairs. This design improves the amplification success of degraded DNA samples [13, 18]. In the present study, several hair samples exhibited allelic dropout at multiple loci amplified by the MiniFiler kit, resulting in a reduced allele recovery rate. A lower percentage of allele appearance was particularly observed at the loci D13S317 and D7S820, where the allele detection rate was approximately 30%. Allelic dropout refers to the failure to detect one or both alleles at a specific locus despite the presence of amplifiable DNA.

The observed reduction in allele detection is most likely associated with low-template DNA quantities and stochastic amplification effects, which are common in degraded keratinous samples such as hair. These stochastic effects may lead to preferential amplification or loss of alleles during PCR, especially when the DNA template is limited or partially degraded rather than being primarily related to the size of the amplified loci [19–21]. Hair is common sample that could found in crime scenes. DNA extraction from hair can be performed on two parts: the hair follicle and the hair shaft. Isolation of DNA from the hair follicle can yield both cellular and mitochondrial DNA (mtDNA). However, the hair shaft typically only contains mtDNA and a limited amount of nuclear material, due to the hardening and degradation of the nucleus caused by keratinization. Shed hairs, which are commonly found at crime scenes, are typically composed of the hair shaft and thus have limited nuclear DNA. This may result in a partial DNA profile, as there may not be enough intact DNA to generate a complete profile with the MiniFiler™ kit [18, 20, 21]. Although the nails are type of keratinous tissue and undergo programmed cell death which leads to considerable DNA fragmentation, results of this study showed that nails samples consistently generated full DNA profiles when analyzed using MiniFiler™ kit. Compared to hair samples, nail derived DNA appeared less degraded and less susceptible to environmental factors. This likely contributed to the higher DNA yield and the successful generation of complete profiles using the MiniFiler™ kit [8, 22-24]. Result in this study corresponded with previous study which stated that nails specimens generated partial DNA profiles due to drop-out one or more STR markers when used AmpFlSTR® Identifiler™ kit to amplified 15 STR Loci, and was attributed this result to highly fragmented DNA that present in nails samples, and the researchers suggested that use MiniFiler™ kit may be benefit to generate complete profile [25].

4. Conclusions

The keratinous tissues such as hair and nails can be valuable tools in forensic investigations, particularly when using amplification strategies optimized for degraded or low template samples. While both tissue types are subject to DNA fragmentation due to their keratinized structure and cellular composition, nail samples produces more consistent and complete DNA profiles compared to hair samples when analyzed with MiniFiler kit. This is likely due to the relatively lower degradation and greater stability of DNA in nails. In contrast, hair samples especially those lacking follicles showed high degree of allele drop-out, particularly in loci with large amplicon sizes.

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