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An Effective Method for DNA Extraction From Human Hair Samples

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Abstract

Introduction: In historical cases, mass disasters, missing person's identification, and Archaeogenetic investigations, the bone, teeth, and hair samples are often the best and the only biological materials available for DNA typing. In the present study, an extremely effective protocol for DNA recovery of hair has been demonstrated.

Materials and Methods: Obtaining genetic information from hair instance for DNA Typing includes the following steps: sterilization, DNA extraction, quality control, polymerase chain reaction (PCR) and profiling. After performing a new extracting ancient DNA (aDNA) from the hair, autosomal short tandem repeat (STR) analyses were repeated for each sample using the AmpFℓSTR® MiniFiler™ and Identifiler™ kits.

Results: The extracted DNA was evaluated quantitatively and qualitatively. The qualitative analysis of the DNA which had been obtained by electrophoresis showed that the quality of the extracted samples was very appropriate and that electrophoresis bands were observed.

Conclusions: The results of our experiment demonstrated that the Fast DNA Extraction of Hair (FDEH) method is both cheaper and faster than commercial kits. Moreover, this new method enhances DNA recovery from hair. Because of the simple protocol and high quality, this method can be utilized in medicine.

Keywords: Hair, DNA Extraction, PCR, DNA Profiling, Identification

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Introduction

In natural disasters such as flood, earthquake, and also accidents which occur because of human beings such as war, driving accident, air crash, etc. Many people may be killed and their identity may not be recognizable. This is while it can be possible to identify them by using genetic methods called DNA typing or DNA profiling.[1] We can clearly define unidentified bodies utilizing their genetic information and compare this with the genetic database or genetic information of their birth parents and their relatives. Genetic information is based on inter-individual differences which results from the difference in the genetic information held in their DNA.[2,3] A total of 99% of human's DNA have similarity in different individuals,[4] and in fact, a relatively small amounts of DNA are different from each other, which is equal to one nucleotide per 1000 nucleotides. Considering this difference, genetic tests can thus be performed to distinguish individuals.[5]

Variable genomic regions that appear frequently are introduced as genetic markers in various forms.[6] There are several types of these genetic markers at the DNA surface that are used for identification purposes.[7,8] Most usable markers are variable number tandem repeats (VNTRs), short tandem repeats (STRs), SNPs, mtDNA, and Y chromosomes.[9,10] Considering limitations and capabilities as well as the type of database application, one can use each of these markers. All of these methods have the capability to identify corpses, who are unidentifiable, by using the genetic information of corpses and comparing with the genetic database, or parents', descendants', and siblings.[6]

The DNA extraction from ancient samples is a new method that helps archaeologists to have a more exact hypothesis about nationality and identity of ancient people.[11,12] Ancient DNA (aDNA) analysis can inspire both the public and scientific community.[4,13,14] Knowing about the ancient human genome and comparing it with the modern human being genome can give us a new perspective about the evolution and migration of human during the history.[15,16] Contamination is the biggest technical problem while studying aDNA.[17-19] Contamination by modern human DNA may be encountered during exploration, occurring in post-exploration phases or in the laboratory.[2,3,5] Most of contamination is modern human DNA, that in contrast to aDNA and forensic DNA, which relatively

healthy and found in far larger amounts. Any contact by skin cells, transpiration, or modern human saliva poses a serious threat of contamination to the forensic specimens and aDNA, which means that contamination by modern DNA can make wrong results. Consequently, instead of aDNA and forensic DNA, the modern DNA will generally can be identified by polymerase chain reaction (PCR).[2,3,18] These are some of the reasons for working on the human remains such as hair, teeth, and bones.[20] Most of the methods which use commercial kits to extract DNA from these samples are expensive.[21,22]

The aim of this study is to obtain a cheap and efficient method for extracting the DNA of the human hair sample for DNA typing studies.

Materials and Methods

Before anything, in order to inhibit the potential contaminations, laboratory instruments were decontaminated through bleaching with 5% active chlorine. They were then immersed in sterile distilled water, and finally, all surfaces were irradiated by UV for 60 minutes. All buffers and materials were autoclaved and sterilized. All steps were taken in separately under sterile conditions. Negative and positive controls were used for all stages of extraction, PCR and Profiling. Obtaining genetic information from hair instance for DNA typing includes the following steps: Sterilization, DNA extraction, quality control, PCR and profiling.

Sterilization

Sterilization involves eliminating contamination from the surface of samples and destroying external DNA molecules. At this stage, the samples were washed once with sodium hypochlorite solution 5% and then they were floated in sterile water for three times. After that, samples were exposed by UV irradiation (10 minutes) to remove external contamination.

DNA Extraction

The first step after sterilization is digestion of the tissue.[3] For digestion, 300 µL cell lysis buffer and 50 µL proteinase K were added to samples and were then incubated at 56°C. The appropriate time for hair samples is 2 hours. After complete digestion, the solution was spun for 5 minutes at 4000 RPM. The next stage is extraction of DNA from the other contents of the cell. In this stage 100 µL of Nacl (5M) and an equal volume of chloroform were added. Then, they were briefly vortexed and centrifuged at 14 000 RPM. After on the supernatant was removed and cold ethanol (100%) 2 volumes were added and placed at -20°C for 1 hour. The solution was centrifuged at 14 000 RPM for 10 minutes. At this stage, the supernatant was discarded and the remained sediment would keep working on it in the next phase.

To purify DNA, the following steps were done: The Chelex solution (5%) 200 µL, and Tris solution (1M) 0.1 volume were added to the remaining sediment. The samples were incubated at 56°C for 1 hour. Then they were centrifuged at 3000 RPM for 1 minute. The supernatant was transferred to 1.5 mL microtube and added 1000 µL cold ethanol (100%) and centrifuged at 14 000 RPM for 5 minutes. At this phase, the supernatant was removed carefully, and the tube was left at room temperature to completely evaporate its alcohol. Finally 50 µL distilled water was added to sediment. When this stage completed, samples were ready for PCR.

Quality Control

Between two stages of extraction and PCR, it is necessary to control samples quantitatively and qualitatively. Hence, the NanoDrop (IMPLAN) instrument was used. This device can detect very low levels of DNA, RNA, and protein by ultraviolet light spectroscopy. This device calculates the absorption of DNA, RNA, and protein at different wavelengths. The wavelength of 260 nm wavelength shows the absorption rate of DNA. The wavelength of 280 nm indicates the absorption of aromatic amino acids.

Finally, the wavelength of 230 nm shows the absorption rate of peptide bonds and also the contaminations caused by buffers such as EDTA and buffer salts. The closer ratio of 260/280 to 1.8, the better the quality of extracted DNA. Furthermore, the electrophoresis method is used to control the quality of samples which uses a 1% agarose gel in a tank containing 0.5 TBE and a voltage of 110 V for 15 minutes.

Polymerase Chain Reaction

After performing the quality control steps, the number of desired sequences (STRs) was increased by the PCR method. The AmpF/STR MiniFiler kit of the ABI Company was used to carry out the PCR procedure. This kit duplicates nine standard loci of Codis.

Profiling

The samples were run in ABI 3130XL genetic analyzer (Applied Biosystems). The system of this instrument is based on the capillary electrophoresis and Argon laser. After performing the profiling, data were analyzed in the GeneMapper ID software.

Results

The extracted DNA was evaluated quantitatively and qualitatively. The qualitative analysis of the DNA which had been obtained by electrophoresis showed that the quality of the extracted samples was very appropriate and electrophoresis bands had been observed (Figure 1).

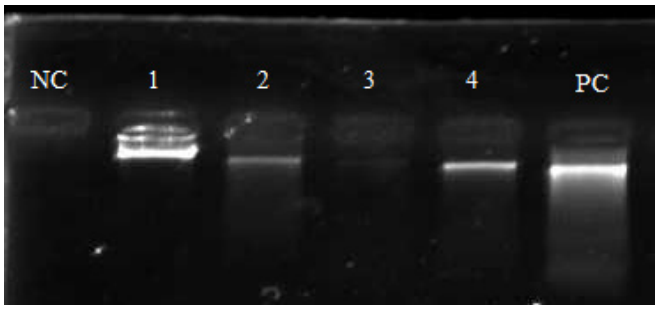

Figure 1. The Qualitative Analysis Of DNA Which Had Been Extracted by FDEH Method Showed That the Quality of The Extracted Samples Was Excellent and We Had Obvious Electrophoresis Bands (Lane 1-4); NC: Negative control; PC: Positive control.

Table 1. DNA Quantity Review by Using NanoDrop Instrument

Sample ID	A260 (ng/µL)	A280 (ng/µL)	260/280 (ng/µL)	260/230 (ng/µL)	Concentration (ng/µL)	Result of Profiling
1	4.013	2.026	1.98	2.13	159	OK
2	0.135	0.087	1.56	0.5	5.5	Fail
3	3.054	1.528	2	1.92	121	OK
4	0.363	0.195	1.86	0.72	16.9	OK
6	1.931	1.009	1.91	1.24	75.2	OK
7	1.753	0.913	1.92	1.92	68.9	OK
8	0.389	0.226	1.72	0.59	16.8	Fail
9	2.38	1.188	2	1.21	96.5	OK
10	1.448	0.729	1.99	0.41	62.8	OK
11	0.402	0.213	1.89	0.08	22.5	OK
12	7.402	3.697	2.01	1.98	221	OK
13	0.409	0.227	1.8	0.29	17.6	OK
14	0.544	0.312	1.75	1.4	19.2	OK
15	0.367	0.203	1.81	0.44	14.5	OK
16	0.308	0.155	1.99	0.04	12.5	Fail
17	3.928	1.929	2.04	0.95	155	OK
18	0.362	0.194	1.86	0.72	14.7	OK
19	0.352	0.183	1.92	0.65	17.7	OK
20	0.589	0.328	1.79	1.85	25.3	OK

Also, the quantity of DNA was evaluated by using a NanoDrop instrument which expressed relatively good values of DNA (Table 1). Besides this, the evaluation of nine locations of STRs, which was profiled by the 3130x1 genetic analyzer instrument and its analysis with the GeneMapper ID software, showed no contamination in samples (Figure 2).

Discussion

DNA fingerprinting method was introduced by Alec Jeffries in 1984 for personal identification. He observed that DNA has many repetitive sequences, such as STRs that have a sequence length of 2 to 6 bp. Based on this repeated region, the primary STR multiplex kit was developed as quadruplex which targeted four STR loci. Today, current multi-locus kits are available and has been used by experts to solve the crimes in order to bring justice to societies. Actually it can help to identify the criminal and can also provide the required details about the actual occurrence. Hair is frequently found in crime scenes and can be a valuable source of DNA. The efficient amount of template DNA necessary for PCR-based STR profiling is approximately 1 ng and the root sheath will contain sufficient quantities.

In the Fast DNA Extraction of Hair (FDEH) method, we are able to extract DNA from the human hair samples by using a series of primary raw materials instead of the commercial kits. Then, their STR sequences are matched with the related families. Meanwhile, DNA extraction was performed to compare the FDEH method with the Qiagen Kit method. Hence, 9 positions of STR were identified in both methods. Achieved results of both methods were perfectly matched with each other which indicated the fact that the FDEH method is standard. Also, the analysis of the results with the GeneMapper ID software showed the desired quality of the extracted DNA with appropriate peak heights, and without any contamination in each of the STR location.

Conclusions

The results of our experiment demonstrated that the FDEH method is both cheaper and faster than commercial kits; moreover, this new method enhances DNA recovery from hair. Because of the simple protocol and high quality, this method can be utilized in the medical and forensic genetics laboratories as a viable alternative. Significantly, because of its simplicity, this new method is faster than other protocols and decreases contamination. This protocol can produce more DNA concentration so it is more proper for such a forensic human identification kit like STR MiniFiler kit.

Authors' Contributions

AM conceived the original idea; supervised the project, methodology and carried out the experiments. MK carried out the experiments and wrote the manuscript. MS developed the theoretical formalism. PZ edited the manuscript and FS prepared the samples.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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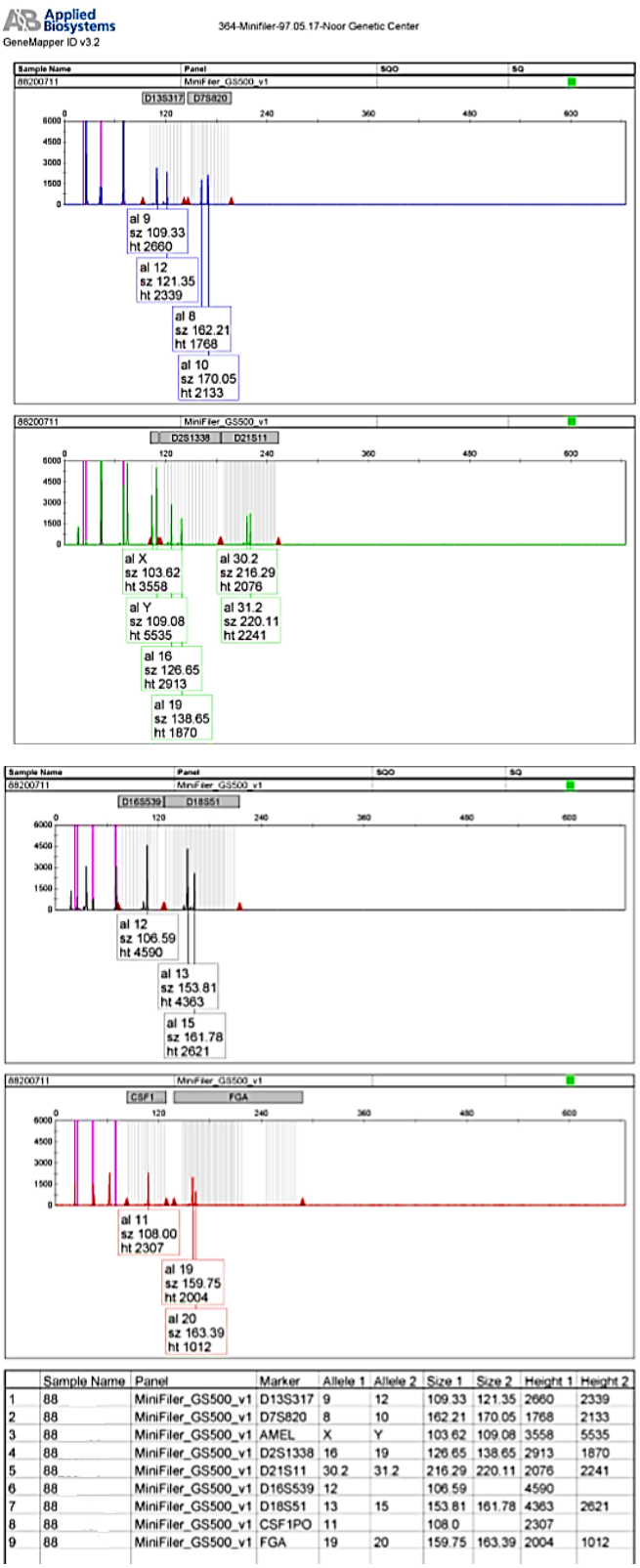

	Sample Name	Panel	Marker	Allele 1	Allele 2	Size 1	Size 2	Height 1	Height 2
1	88	MiniFiler_GS500_v1	D13S317	9	12	109.33	121.35	2660	2339
2	88	MiniFiler_GS500_v1	D7S820	8	10	162.21	170.05	1768	2133
3	88	MiniFiler_GS500_v1	AMEL	X	Y	103.62	109.08	3558	5535
4	88	MiniFiler_GS500_v1	D2S1338	16	19	126.65	138.65	2913	1870
5	88	MiniFiler_GS500_v1	D21S11	30.2	31.2	216.29	220.11	2076	2241
6	88	MiniFiler_GS500_v1	D16S539	12		106.59		4590	
7	88	MiniFiler_GS500_v1	D18S51	13	15	153.81	161.78	4363	2621
8	88	MiniFiler_GS500_v1	CSF1PO	11		108.0		2307	
9	88	MiniFiler_GS500_v1	FGA	19	20	159.75	163.39	2004	1012

Figure 2. The Assessment of the 9 Locations of the STRs, Which Were Determined by the 3130x1 Genetic Analyzer System and Its Analysis With the Gene Mapper ID Software.

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