



Investigation of DNA extraction from hair shafts

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Abstract

Human hair shafts can be important forensic evidence for identification, but DNA typing, even of mitochondrial DNA (mtDNA), presented certain difficulties. We describe three DNA extraction methods from hair shafts, such as the phenol/chloroform method, NaI treatment method, and silica-beads method. In order to make an investigation of mtDNA amplification rate and efficiency, the amplifications of the mtDNA control region (D-loop) HV1A (15997–16262) used FAM-labeled forward primer. As a result of the extractions from different lengths of fresh hair shafts and the variations of the template volume, fluorescent peak heights as DNA recovery by three methods were sufficiently high. In the degraded sample, a high fluorescent peak height enough to sequence mtDNA could be obtained from our NaI method and silica-beads method.

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1. Introduction

In forensic investigations, shed human hair is one of the most common types of biological evidence [1]. Shed hairs are usually telogen phase hairs containing little nuclear DNA. But large numbers of mtDNA are found in each cell [2,3], allowing the analysis of single hair shafts [4]. MtDNA displays considerable sequence variation between individuals, with much of this variation concentrated in two hypervariable regions that enclose the

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non-coding D-loop region. It has been confirmed that water-soluble eumelanins often extracted together with DNA from Japanese natural black hairs act as an inhibitor of Taq polymerase in the PCR. We describe two new protocols that enable mtDNA extraction and amplification from human hair shafts, then compare these new methods to the standard phenol/chloroform method.

2. Materials and methods

2.1. DNA extraction

2.1.1. Phenol/chloroform method

The hair shafts samples used were 1–10-cm long (separated from the root end by 1 cm), dissolved according to the ISOHAIR (NipponGene) protocol. Hair shafts cut into lengths of 5 mm were placed in a tube containing the extraction buffer. Following the addition of the enzyme solution and the lysis solution to the tube, the resulting mixture was gently agitated. After the mixture was incubated, the digestion product was extracted with the phenol/chloroform and centrifuged. The aqueous phase was transferred to a new tube, followed by the addition of 3 M sodium acetate and ethachimate. After gently mixing, the ethanol was added and the tube was centrifuged. This washing process was repeated. The precipitate was dried and 30 μ l D.W. was added. The final DNA extraction volume was 30 μ l.

2.1.2. NaI method

Following treatment using ISOHAIR digestion, the NaI method was employed using the DNA Extractor Kit—Genome (Wako). To the digestion solution, the NaI was added and vortex-mixed. The mixture was incubated, and centrifuged. Without sucking the pellet off, the solution was transferred to a new tube and isopropyl alcohol added and vortex-mixed. After being allowed to stand for 15 min at room temperature, the tube was centrifuged. The washing solution B was then added and vortex-mixed. The centrifugation was repeated and the precipitation was allowed to dry out and digested 30 μ l D.W.

2.1.3. Silica-beads method

Following treatment using ISOHAIR digestion, the silica-beads method based on the MagExtractor Genome (TOYOBO) protocol was employed. To the digestion solution, the digestive and adhered solution and magnetic beads were added and mixed using the tube mixer. The supernatant was removed by setting the tube set on the magnetic stand and the washing buffer was added and vortex-mixed. The tube set the magnetic stand for 30 s, and then the supernatant was removed. This washing process was then repeated. Following the addition of 70% ethanol, the tube was vortex-mixed, and ethanol-washing process repeated. A volume of 30 μ l D.W. was added to the magnetic beads, and the tube mixed for 10 min using the tube mixer. The tube set the magnetic stand according to collecting the magnetic beads. The supernatant was transferred to the new tube.

2.2. DNA amplification

The segment HV1A of the mtDNA control region HV1 was amplified using the primers L15997 and H16236.

L15997 (5'-FAM-CACCATTAGCACCCAAAGCT-3')

H16236 (5'-CTTTGGAGTTGCAGTTGATG-3')

The primer L15997 was labeled with FAM at the 5' end. The reaction was performed with several volumes of the hair extract in a 25 μ l volume containing PCR buffer, Taq polymerase (Takara) and the each primer. The thermal cycling conditions were 1 min at 95 $^{\circ}$ C, followed by 36 cycles of 95 $^{\circ}$ C for 10 s, 60 $^{\circ}$ C for 30 s, and 72 $^{\circ}$ C for 30 s. Following thermal cycling, the samples were maintained at 15 $^{\circ}$ C for 10 min. All PCR reactions were performed in a Thermal Cycler 9700 (Perkin Elmer).

2.3. Analysis

The PCR products were analyzed using 310 Genetic Analyzer and Gene Scan Analysis software. A fluorescent peak appeared at 274 bp, and the peak heights of each sample were compared. Extraction, amplification and electrophoresis were performed three times for all samples.

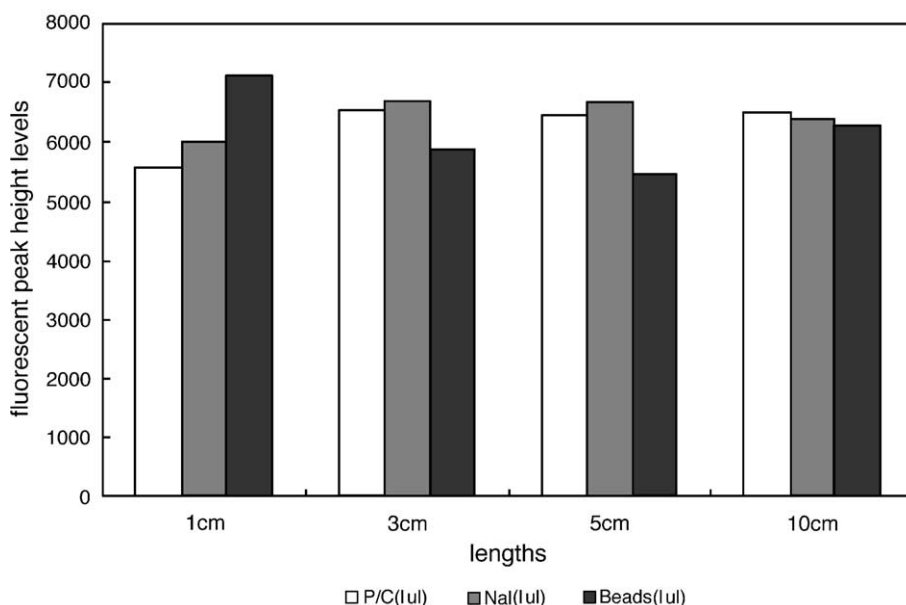


Fig. 1. Comparison of fluorescent peak height levels amplified from different sample lengths.

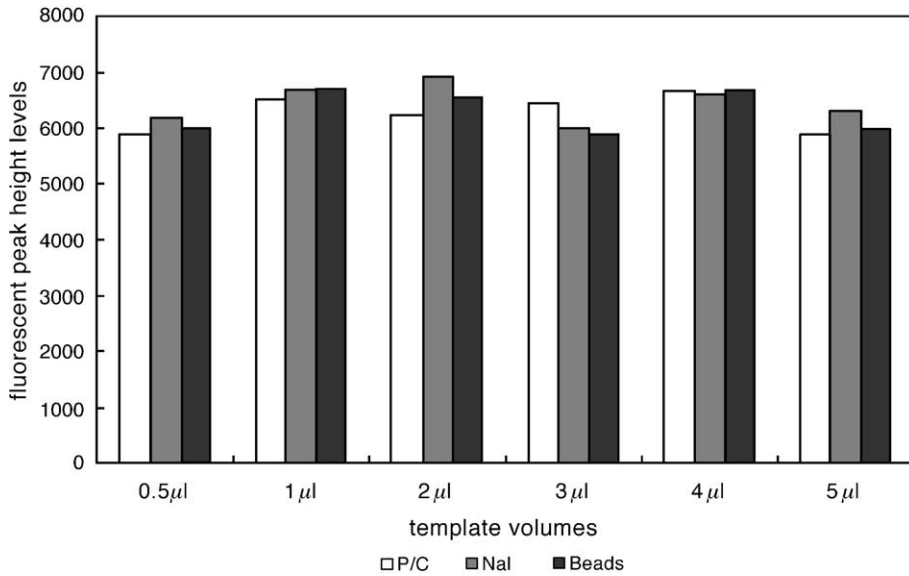


Fig. 2. Comparison of fluorescent peak height levels amplified from different template volumes.

2.4. Sequencing and sequence analysis

The amplification products were purified by Microcon 100, and 1 μl of solution was used for the sequence reaction performed using the Big Dye Terminator Cycle Sequencing

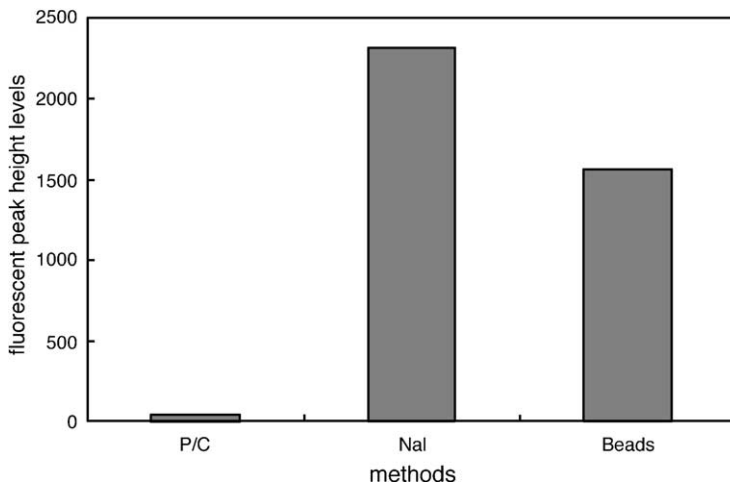


Fig. 3. DNA extraction and amplification from 11-year-old hair shafts.

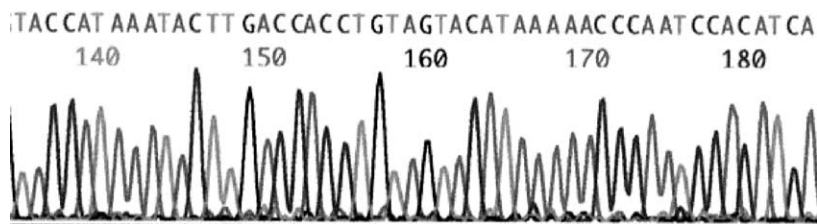


Fig. 4. Sequencing data for mtDNA extracted from 11-year-old hair shaft using the NaI method.

FS Kit (Perkin Elmer) in accordance with the ABI protocol. Electrophoresis and sequence analysis were performed on a 310 Genetic Analyzer.

3. Results

3.1. Analysis of length variation

Hair shaft samples collected from a single individual were cut into lengths of 1, 3, 5, and 10 cm. DNA was then extracted from these samples by three different methods. A 1- μ l volume of the DNA solution was used as a template for amplification. Thus, average fluorescent peak heights were at near maximum levels for all three methods (Fig. 1).

3.2. Analysis of template volumes

To compare amplification rates between different template volumes, hair shafts from a single individual were cut into lengths of 3 cm. Following DNA extracted by the three different methods, amplification was performed using 1–5 μ l volume templates. With these three methods, the average fluorescent peak height levels were almost same maximum for all volumes (Fig. 2). That is, there was no difference stemming from template volume.

3.3. Sequence analysis of 11-year-old hair shafts

Using 3-cm-long hair shafts stored for 11 years at room temperature, DNA extraction was performed by the three different methods. Following amplification using 1 μ l template, no significant fluorescent peaks were found with the phenol/chloroform method. However, sufficient peak heights were found by both the NaI method and silica-beads method (Fig. 3). A sequence reaction was performed for a 1- μ l PCR product of NaI method to enable sequence analysis of mtDNA (Fig. 4).

4. Discussion

Our results demonstrated that each of the phenol/chloroform, NaI and Silica-beads methods are effective in extracting DNA from fresh hair shafts of 1–10-cm lengths.

However, when the samples are old or degraded, DNA extraction by the NaI or silica-beads methods becomes valid for mtDNA sequencing. The phenol/chloroform method requires a degree of technical proficiency, while the NaI method offers a stable extraction procedure, and the silica-beads method offers the advantage of short extraction time and simple procedure.

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