



Short communication

Evaluation of three methods for effective extraction of DNA from human hair

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Abstract

In this paper we evaluate three different methods for extracting DNA from human hair i.e. the Chelex method, the QIAamp[®] DNA Mini Kit method and the ISOHAIR[®] method. Analysis of DNA prepared from dyed hairs with the ISOHAIR[®] method suggested that the DNA extracts contained PCR inhibitors. On the other hand, few inhibition was observed when DNA from dyed hairs were extracted using the Chelex method and the QIAamp[®] DNA Mini Kit method. In conclusion, the Chelex method is recommended for PCR experiments in view of its simplicity and cost-effectiveness. To assess the reliability of the Chelex method for the extraction of genomic DNA from both natural and dyed hair samples, minisatellite variant repeat (MVR)-polymerase chain reaction (PCR) patterns of Chelex-extracted DNA were compared using hairs (three natural black hairs and three dyed hairs) with buccal swabs from six individuals. Complete agreement was observed between hair and swab samples in each individual, proving the utility of the Chelex method.

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1. Introduction

Recently, the polymerase chain reaction (PCR) has enabled the analysis of trace amount of DNA in hair, an important evidence in forensic science [1,2]. However, hairs contain extremely small quantities of DNA [1]. Many studies have employed relatively abundant mtDNA because the content of nuclear DNAs are too small for amplification, particularly those from naturally shed hairs or hair shafts rather than hair root [3–5]. In addition, even if sufficient amounts of DNA were extracted from hair, the DNA are not always successfully amplified by PCR, suggesting the presence of PCR inhibitors in the extracted samples. Previous works have revealed that the hair pigment melanin was a strong inhibitor of the PCR process [6–8]. Specifically, hair-dyeing has a strong influence on PCR [6]. In this paper we evaluate three different methods for extracting DNA from human hair, namely

the Chelex method [9], the QIAamp[®] DNA Mini Kit method and the ISOHAIR[®] method, in order to obtain DNA without PCR inhibiting compounds, in the amplification of the D1S8 (MS32) locus using minisatellite variant repeat (MVR)-PCR [10].

Finally to ascertain whether DNA extracted by the Chelex method from hairs, especially dyed hairs, represents genomic DNA, the MVR-PCR patterns of DNA extracts from hair roots were compared to those of DNA extracts from buccal swabs.

2. Experimental

2.1. Materials

Natural black hairs were plucked from 26 Japanese individuals and dyed hairs were plucked from 15 Japanese individuals. Chelex[®] 100 was purchased from BIORAD (Rich-

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mond, CA, USA). QIAamp[®] DNA Mini Kit was purchased from QIAGEN (Hilden, Germany). ISOHAIR[®], Proteinase K, Gene Taq for PCR and Marker 5 (ϕ X174/Hinc[®] digest), Agarose 21 for gel electrophoresis were obtained from NIPPON GENE (Toyama, Japan). ISOHAIR[®] consisted of extraction buffer, enzyme solution, lysis solution and Ethachinmate. The primers [10] were prepared by Sawady Technology (Tokyo, Japan). PicoGreen[®] dsDNA Quantitation Kit and SYBR[®] Gold Nucleic Acid Gel Stain as a staining reagent for DNA was purchased from Molecular Probes (Eugene, OR, USA). Water was purified using a Milli-Q system (Millipore; Bedford, MA, USA). The other reagents and buffers used were of guaranteed grade.

2.2. DNA extraction from human hair

Individual two hair root segments (1 cm in length) were treated by three different methods: (1) the Chelex method, (2) the QIAamp[®] DNA Mini Kit method and (3) the ISOHAIR[®] method.

Two pieces of hair were washed with 500 μ L of 100% ethanol in a small polypropylene test tube. After air-drying, the hair was placed in a 1.5-mL microcentrifuge tube.

2.2.1. The Chelex method

Two hundred micro liters of 5% Chelex[®] 100 and 10 μ L of 10 mg/mL Proteinase K were added to two pieces of hair placed in a 1.5-mL microcentrifuge tube and mixed well. The solution was incubated at 55 °C for at least 6–8 h, or alternatively overnight. The mixture was vortexed and incubated in a boiling-water bath for 8 min. After centrifugation at 10,000–15,000 \times g for 2–3 min, the supernatant was transferred to another 1.5-mL microcentrifuge tube and used for PCR amplification.

In addition to Chelex[®] 100, the following two commercial DNA extraction kits were used; the manufacturers gave no information on the composition of the ingredients of their commercial kits.

2.2.2. The QIAamp[®] DNA Mini Kit method

Two hundred micro liters Buffer X1 (10 mM Tris–HCl buffer, pH 8.0, 10 mM EDTA, 100 mM NaCl, 40 mM dithiothreitol, 2% SDS, 250 μ g/mL Proteinase K) was added to two pieces of hair placed in a 1.5-mL microcentrifuge tube and the tube was incubated at 55 °C for at least 1 h until the sample was dissolved. Two hundred micro liters of Buffer AL (Lysis Buffer) and 200 μ L of ethanol were added. After vortexing, the solution was transferred to a QIAamp spin column, 500 μ L of Buffer AW1 (Washing Buffer) was added, and then centrifuged at 6000 \times g for 1 min. After placing the QIAamp spin column in a clean 2-mL collection tube, 500 μ L of Buffer AW2 (Washing Buffer) containing sodium azide was added to the QIAamp spin column and then centrifuged at 20,000 \times g for 3 min. After placing the QIAamp spin column in a clean 1.5-mL microcentrifuge tube, 50–100 μ L of

Buffer AE (Elution Buffer) was added to the spin column. After allowing to stand at room temperature for 1 min, the tube was centrifuged at 6000 \times g for 1 min. The eluate containing DNA was collected in a 1.5-mL microcentrifuge tube.

2.2.3. The ISOHAIR[®] method

Two hundred micro liters of extraction buffer, 5 μ L of enzyme solution and 8 μ L of lysis solution were added to two pieces of hair placed in a 1.5-mL microcentrifuge tube before incubating at 55 °C for 20 min. Five micro liters of enzyme solution was added and the solution was incubated at 55 °C for 5–10 min. Two hundred micro liters of a phenol–chloroform–isoamylalcohol (25:24:1, v/v/v) mixture was added and gently mixed for a few times by inverting the tube. The solution was centrifuged at 11,000 \times g for 5 min. The upper layer was transferred to another tube and mixed with 20 μ L of 3 M sodium acetate (pH 5.2) and 2 μ L of Ethachinmate. The latter was added to facilitate the precipitation of DNA when treated with ethanol in the next step. After mixing by inverting the tube, 400 μ L ethanol was added and then centrifuged at 11,000 \times g for 15 min. After removing the supernatant, the precipitate was washed with 70% ethanol and dried in vacuo. The DNA was dissolved in 50 μ L of TE buffer (10 mM Tris–HCl buffer containing 1 mM EDTA, final pH 8.0) before it was used in PCR.

2.3. DNA extraction from buccal swab using the Chelex method

To collect a sample, swab was scraped with a cotton-stick against the inside of each cheek six times. After drying at room temperature for 2 h, the swab coated on cotton was cut from the stick with scissors. Five hundred micro liters of 5% Chelex[®] 100 and 10 μ L of 10 mg/mL Proteinase K were added to cotton swab placed in a 1.5-mL microcentrifuge tube and mixed well. The solution was incubated at 55 °C for 30 min and vortexed. The mixture was incubated in a boiling-water bath for 8 min and applied to tube with a hole in its bottom placed in a clean 1.5-mL microcentrifuge tube. After centrifugation at 6000 \times g for 5–10 min, the supernatant of the collection tube was transferred to another 1.5 mL microcentrifuge tube and used for PCR amplification.

2.4. MVR-PCR

About 10 ng (quantified by PicoGreen[®] dsDNA Quantitation Kit) of each DNA sample was employed for PCR amplification.

Reaction conditions reported by Jeffreys et al. [10] was slightly modified as follows. Samples of genomic DNA were amplified in 50 μ L of PCR solution containing 1 μ M primer 32D (5'-CGACGCGCAGATGG-AGCAATGGCC-3'), 1 μ M primer TAG (5'-TCATGCG-TCCATGGTCCGGA-3'), 2.5 unit Gene Taq DNA polymerase and either 0.2 μ M 32-TAG-A (5'-TCATGCGT-CCATGGTCCGGACATTCTGAGTCACCCCTGGC-3') or

0.4 μ M 32-TAG-T (5'-TCATCGTCCATGGTCCGGAC-ATTCTGAGTCACCCCTGGT-3') [10]. DNA was denatured at 94 °C for 5 min, then Taq polymerase was added and reactions were performed at 94 °C for 1.3 min, at 68 °C for 1 min and at 70 °C for 5 min in the first 30 cycles, followed by incubation at 68 °C for 1 min and at 70 °C for 10 min in two further cycles using a DNA thermal cycler (TAKARA BIO INC., Shiga, Japan).

2.5. Electrophoresis and detection of PCR products

For analysis of PCR products, a submerged gel electrophoresis system (ATTO, Tokyo, Japan) and an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA), a microchip electrophoresis apparatus with a laser induced fluorescence detector were used.

2.5.1. Analysis using agarose gel electrophoresis

Ten micro liters of PCR product was loaded on the gel. The ladder of PCR products was separated on 3% Agarose 21 (NIPPON GENE) in TBE buffer [89 mM Tris–89 mM boric acid–2 mM EDTA·2Na (pH 8.3)] at a constant voltage (60 V). As a staining reagent, SYBR® Gold Nucleic Acid Gel Stain was used and the amplified DNA bands were detected with a fluoroimage analyzer FLA-2000 (FUJIFILM, Tokyo, Japan).

2.5.2. Analysis using a microchip electrophoresis apparatus

MVR-PCR products obtained from hair and buccal swab were assessed using the Agilent 2100 bioanalyzer in conjunction with the DNA 1000 Labchip kits. One μ L of PCR products was used for each analysis. Size separation of DNA was achieved during migration through interconnected microchannels filled with a sieving polymer.

3. Results and discussion

3.1. Evaluation of extraction methods for hair root DNA

The D1S8 locus is located on chromosome 1 at 1q42 → q43 [11,12]. The minisatellite consists of two major classes of repeat units (29 bp) [12], designated a-type and t-type, which differ by a single base substitution within a repeat unit, and show highly diverse dispersion patterns within alleles [11]. MVR specific primers (32-TAG-A and 32-TAG-T) [10] incorporate only a single base difference at the 3' end to distinguish precisely two major classes of repeat unit. The size of the first repeat unit is expected 293 bp. The ladder of PCR products show variant pattern depending on individual.

Fig. 1 shows the agarose gel electropherograms of the PCR products (t-type) obtained from dyed hair extracts and natural black hair extracts using the three different extraction methods. When the Chelex method (lane 1, lane 4) or the QIAamp® DNA Mini Kit method (lane 2, lane 5) was used, PCR products were consistently observed from all samples.

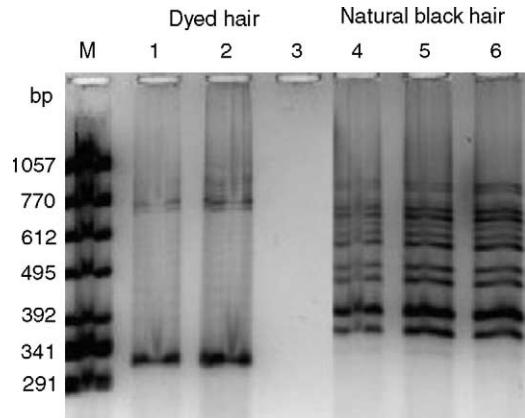


Fig. 1. Gel electrophoresis of PCR products for DNA extracted from human hair using three different extraction methods. Lane M: Marker 5 (ϕ X174/Hinc II digest); lane 1: the Chelex method (dyed hair); lane 2: the QIAamp® DNA Mini Kit method (dyed hair); lane 3: the ISOHAIR® method (dyed hair); lane 4: the Chelex method (natural black hair); lane 5: the QIAamp® DNA Mini Kit method (natural black hair); lane 6: the ISOHAIR® method (natural black hair). Ten microliters of PCR products were electrophoresed through a 3% Agarose 21 and visualized by SYBR® Gold staining.

The Chelex method and the QIAamp® DNA Mini Kit method resulted in success in 15 out of 15 dyed hair analyses and in 26 out of 26 natural black hair analyses. On the other hand, DNA extracted using the ISOHAIR® method (lane 3, lane 6) was not always successfully amplified. Specifically, the DNA extracts from dyed hair failed to produce satisfactory amount of PCR products. The ISOHAIR® method failed in 6 out of 15 dyed hair analyses and in 1 out of 26 natural black hair analyses. The PCR analysis of DNA extracts from dyed hair using the ISOHAIR® method indicated that the extracts contained PCR inhibitors.

The yield of extracted DNA was quantified using PicoGreen® dsDNA Quantitation Kit. This method was used because compounds such as RNA, melanin and aromatic amines present in hair dyes [13] were expected to be present in the extract and these compound shows significant UV absorbance at 260 nm. The ISOHAIR® method gave higher extraction yields compared to the two other methods. When using the ISOHAIR® method, more than 150 ng of DNA was extracted. On the other hand, 120–140 ng of DNA was extracted by the Chelex method, while the QIAamp® DNA Mini Kit method extracted less than 120 ng of DNA. Thus, the yield of DNA extracted with the QIAamp® DNA Mini Kit was quite low. In addition, the QIAamp® DNA Mini Kit method was relatively expensive. On the other hand, the cost of the Chelex method was reasonably low.

As described above, analysis of DNA prepared from dyed hairs with the ISOHAIR® method suggested that the DNA extracts contained PCR inhibitors. Yoshii et al. [6] reported that DNA isolated from dyed hairs contained melanin species, probable PCR inhibitors. Hydrogen peroxide (a component of hair-dyeing agent) may transform water-insoluble melanins to water-soluble melanins [6]. Because the water-

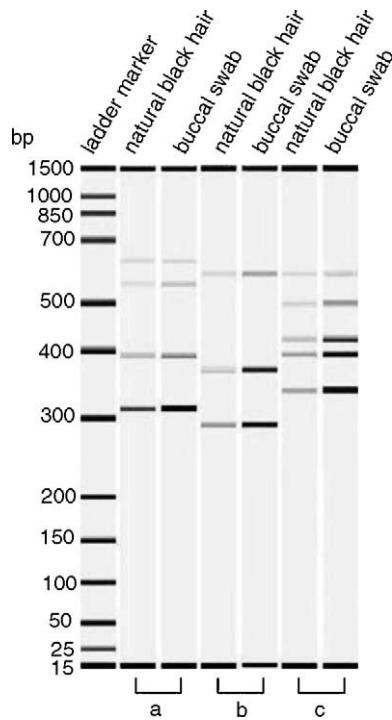


Fig. 2. Microchip gel electrophoresis of PCR (t-type) products for DNA extracted using the Chelex method from natural black hair and buccal swab originating from three individuals a–c.

soluble melanins behave in the same manner as DNA in the purification process of DNA, they probably remain in the DNA sample solution to inhibit PCR, especially in ISOHAIR® method, by which DNA was separated from other components based upon the difference in their solubility into a phenol–chloroform–isoamylalcohol mixture and ethanol. Actually, the DNA preparations prepared by the ISOHAIR® method showed a dark-brown color. On the other hand, inhibition which affected to PCR analysis was not observed when DNA from dyed hairs were extracted by the Chelex method and the QIAamp® DNA Mini Kit method. The DNA extracts prepared by the Chelex method and the QIAamp® DNA Mini Kit method showed no color. These results suggest that the latter two methods provide DNA preparations free from water-soluble melanins, which are probable PCR inhibitors. Of the two methods, the Chelex method is recommended for DNA extraction from human hair (roots) because of its simplicity and cost-effectiveness.

3.2. Comparison of MVR-PCR patterns for DNA extracts from hair and buccal swab samples

To ascertain whether DNA extracted by the Chelex method from hairs represents genomic DNA and also whether hair dyeing cause damage to native DNA and affect to MVR-PCR analysis, D1S8 locus MVR-PCR patterns for DNA were compared using hairs with buccal swabs, which were selected as samples free from the affect of hair dyeing, from six individuals. When PCR products (t-type) obtained from natural

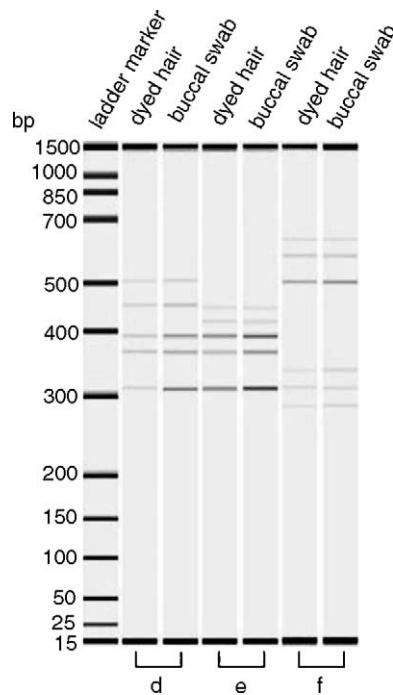


Fig. 3. Microchip gel electrophoresis of PCR (t-type) products for DNA extracted using the Chelex method from dyed hair and buccal swab originating from three individuals d–f.

black hair and buccal swab extracts using the Chelex method were electrophoresed on a microchip electrophoresis apparatus, amplified band positions and their relative intensities of D1S8 locus variation observed in natural black hair samples matched those of DNA extracted from buccal swab samples from each individual (Fig. 2). The fact indicates that this method gives genomic DNA and is highly reproducible. In the case of dyed hair, D1S8 locus variation observed in dyed hair also matched those of DNA extracted from buccal swab samples (Fig. 3). These results additionally proved that hair dyes does not impact minisatellite repeat analysis.

Consequently, the Chelex method seems likely to become an effective tool for DNA extraction from human hair regardless of dyeing.

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