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A Simple Method of Genomic DNA Extraction from Human Samples for PCR-RFLP Analysis

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Isolation of DNA from blood and buccal swabs in adequate quantities is an integral part of forensic research and analysis. The present study was performed to determine the quality and the quantity of DNA extracted from four commonly available samples and to estimate the time duration of the ensuing PCR amplification. Here, we demonstrate that hair and urine samples can also become an alternate source for reliably obtaining a small quantity of PCR-ready DNA. We developed a rapid, cost-effective, and noninvasive method of sample collection and simple DNA extraction from buccal swabs, urine, and hair using the phenol-chloroform method. Buccal samples were subjected to DNA extraction, immediately or after refrigeration (4 – 6°C) for 3 days. The purity and the concentration of the extracted DNA were determined spectrophotometerically, and the adequacy of DNA extracts for the PCR-based assay was assessed by amplifying a 1030-bp region of the mitochondrial D-loop. Although DNA from all the samples was suitable for PCR, the blood and hair samples provided a good quality DNA for restriction analysis of the PCR product compared with the buccal swab and urine samples. In the present study, hair samples proved to be a good source of genomic DNA for PCR-based methods. Hence, DNA of hair samples can also be used for the genomic disorder analysis in addition to the forensic analysis as a result of the ease of sample collection in a noninvasive manner, lower sample volume requirements, and good storage capability.

KEY WORDS: mitochondrial, quick-prep, restriction enzyme

INTRODUCTION

Recent research advances in genomic disorders have necessitated the collection of large amounts of good quality DNA that needs to be obtained from different sample sources. DNA typing is currently the most validated method for the personal identification of human bodily fluid stains found at crime scenes. In a wide variety of genetic studies, the commonly used method is to obtain genomic DNA from nucleated cells of peripheral blood; as a result of the invasiveness of this approach, it may be difficult to obtain samples from the study subjects.[1,2] The isolation schemes have been tedious, and total analysis times have also been rather long. Other alternative sources of DNA isolation include buccal cell, hair with follicle, and urine, which are easier to obtain in a noninvasive manner than by an invasive blood collection.[2] Buccal cell collection can be performed easily by a buccal swab with a cotton swab or using a mouth-wash procedure.[3] DNA isolation using buccal swabs provides many advantages, such as cost-effective processing, lower sample volume requirement, long-term archiving, and suitability of self-collection. It is more comfortable for the patient, and the buccal swabs provide sufficient DNA for the PCRs, as they demands only a few nanograms of DNA.[3]

Human hair is one of the most common biological materials associated with legal investigations and has been used for the statistics-based population work and DNA-based analysis in criminology.[4] The most valuable method of DNA testing is short tandem repeat analysis of nuclear DNA.[5] This is possible when the root portion of the hair and/or adhering tissue is present. However, telogen hairs (shed hair), often associated with a crime scene, may not contain any nuclear material.[5] Cellular mitochondria and mitochondrial DNA (mtDNA) still remains intact,[5,6] while the nucleus degrades as the hair shaft hardens during keratinization, and mtDNA analysis is feasible from the keratinized hair. Unfortunately, the protein-rich nature of hair samples requires additional steps to break down the shaft and release the DNA (such as fragmentation using a microscopic glass grinder, followed by an organic solvent extraction)[7–9], thus exposing the specimen to increased risk of contamination. Forensic investigation of human urine stains is of great importance when identifying the exact location of a crime and the type of death.[10] Human urine is

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a suitable sample for toxicological analysis in doping and drug-screening tests.[11]

However, owing to the practical difficulties and methodological reasons, it is essential to optimize the conditions to maximize the yield and purity of DNA obtained from different kinds of samples using various methods. A simplified method, demonstrated in the present study, for the extraction of DNA from hair shafts that reduces unnecessary steps virtually eliminates the contamination of DNA and also substantially conserves the time duration of the analysis that would be useful to the forensic community as well as to the population-based research community. In addition, the requirement of lower sample volume coupled with sample collection in a noninvasive manner allows pediatric sampling that readily manifests in broader study recruitment in population-based case studies. Furthermore, it can be envisaged to develop this simplified method as it may be applied as a medical diagnostic tool with DNA analysis that can be done on a reasonably short time scale (~8 h) to detect disease states, which the current diagnostic medical field eagerly desires.

MATERIALS AND METHODS

Sample Collection and Processing

In the current study, five healthy adult volunteers were recruited (age range, 22–35 years), and demographic information, including age, health condition, sex, population ancestry, hair color, and hair treatments, was collected. The volunteers recruited were asked to rinse their mouth with tap water, 30 s before sampling of buccal swabs, to avoid the contamination as a result of food particles. For each individual, both sides of buccal mucosa were wiped with a cotton swab for 15 s, and a total of five samples was collected in 500 μl 10 M Tris-HCl, 10 mM EDTA, 2% SDS, containing 1.5-ml microcentrifuge tubes. Isolation of DNA from cotton swabs was performed (vide infra).

Hair samples (three hairs each) from the five subjects were washed by immersing them in fresh water to remove the surface dirt and other contaminants. The hair samples were picked with clean forceps, washed with 500 μl 70% ethanol in a 1.5-ml microcentrifuge tube, and then kept in a tube containing sterile, deionized water. The hair samples were examined further under a magnifying glass for removing any body fluids if present. The hairs were cut off 5–10 mm of the proximal (root) end for digestion.

All recruited volunteers were fully informed about the study and instructed accordingly for urine collection. Urine specimens were collected in sterile sample bottles and were mixed by gentle inversions for at least 30 min before processing. To avoid contamination as a result of repeated sampling and to study the impact of storage effects on the sample integrity, samples of each urine specimen were aliquoted further (5 ml) in appropriate containers. PBS (500 μl) was added in 1 ml urine sample containing a 2-ml microcentrifuge tube with 0.5 M EDTA (pH 8.0) to a final concentration of 10 mM EDTA to inhibit any possible nuclease activity in urine sample. Tubes were then vortexed thoroughly for 1 min. Urine solutions were used immediately or frozen (−20°C).

Blood specimens were also obtained from the same donors by finger-pricking using sterile lancets by aseptic techniques and were kept in an EDTA-rinsed microcentrifuge tube. Blood samples (50 μl) were processed fresh and served as the subjects' DNA isolation reference.

DNA Extraction from Buccal Swabs

The buccal swab samples were suspended in 500 μl lysis buffer [10 mM Tris (pH 8.0), 10 mM EDTA, and 2.0% SDS], and 50 μl 10% SDS, followed by 5–10 μl 20 mg/ml proteinase K (Himedia, Mumbai, India), was added. The samples were incubated 1–3 h at 56°C until the tissue was totally dissolved. The DNA was then extracted from each sample with an equal volume of phenol:chloroform: isoamyl alcohol solution (25:24:1) and mixed gently by inverting the tubes for 3 min. The samples were then centrifuged (Eppendorf 5415R; Hamburg, Germany) for 10 min with 10,000 *g* (4°C), and the upper aqueous layer was transferred to a fresh, sterilized microcentrifuge tube. RNase A (10 μl of 10 mg/ml; Fermentas, Thermo Scientific, Germany) was added, and the solution was incubated at 37°C for 30 min. Equal volumes of chloroform:isoamyl alcohol solution were added and centrifuged (Eppendorf 5415R), again with 10,000 *g* (4°C) for 10 min. The upper aqueous layer was transferred to a sterilized microcentrifuge tube, and double the volume of chilled isopropanol (Merck, Whitehouse Station, NJ, USA) was added, along with one-tenth volume of 3 M sodium acetate, and chilled at −20°C for 1 h for precipitation. After 1 h, the sample was centrifuged (Eppendorf 5415R) at 10,000 *g* (4°C) for 10 min. After decanting the supernatant, 250 μl 70% ethanol (Merck) was added, and the pellet was dissolved; the mixture was centrifuged at 10,000 rpm for 10 min, and the supernatant was decanted gently. The pellet was air-dried under laminar air flow, and the dried pellet was resuspended in 50 μl nuclease-free water or 1× 10 mM Tris-HCl, 1 mM EDTA, pH 7.6 (TE), buffer and frozen at −20°C or at −80°C for storage.

DNA Extraction from Hair Sample

DNA was isolated from hair shafts using modified versions of the microscopic glass-grinding and organic solvent extraction protocol.[12–14] As these protocols expose the specimen to increased risks of contamination, the present study has replaced the tedious physical digestion method with a

smooth chemical digestion method using dithiothreitol (DTT) (Hi-media) as it is a strong reducing agent with relatively high salt content and also an anionic detergent. Digestion buffer (500 µl; 10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 20% SDS, pH 7.5) was added to a 1.5-ml microcentrifuge tube, along with 40 µl of 1 M DTT (to a final concentration of ~80 mM, 240 mM of sodium acetate, pH 5.2) and 15 µl of 10 mg/ml proteinase K (to a final concentration of ~0.3 mg/ml; Himedia). Hair sample was added to this solution before vortexing and incubating for 2 h at 56°C. After 2 h of incubation, the sample tube was vortexed again, and an additional 40 µl of 1 M DTT and 15 µl of 10 mg/ml proteinase K were added, followed by gentle mixing and incubation at 60°C for 2 more h or until hair was dissolved completely.

The DNA was then extracted from each sample with an equal volume of phenol:chloroform: isoamyl alcohol solution (25:24:1) and mixed gently by inverting the tube for a few minutes. The samples were centrifuged (Eppendorf 5415R) for 10 min with 10,000 *g* (4°C), followed by transferring the upper aqueous layer into a fresh, sterilized microcentrifuge tube. RNaseA (10 µl of 10 mg/ml; Fermentas, Thermo Scientific) was added and kept for incubation at 37°C for 30 min. An equal volume of chloroform: isoamyl alcohol was added, and the tube was centrifuged (Eppendorf 5415R) again at10,000 *g* (4°C) for 10 min. The upper aqueous layer was transferred into a fresh, sterilized microcentrifuge tube before double the volume of chilled isopropanol and one-tenth volume of 3 M sodium acetate were added. The sample was chilled at −20°C for 1 h for the DNA precipitation to occur. The sample was centrifuged (Eppendorf 5415R) at 10,000 *g* (4°C) for 10 min. The supernatant was discarded, 250 µl 70% ethanol was added, and the pellet was tapped gently before further centrifugation (Eppendorf 5415R) at 10,000 rpm for 10 min. The supernatant was discarded, and the pellet was air-dried in a laminar air flow, resuspended in 50 µl nuclease-free water or 1× TE buffer, and frozen at −20°C or −80°C for storage.

DNA Extraction from Urine Sample

Frozen urine samples were thawed at room temperature and then placed immediately in ice before DNA isolation. The urine specimen was inverted or swirled in a specimen cup to create a homogenous suspension of cells. One milliliter of the specimen was transferred into an Eppendorf tube and centrifuged (Eppendorf 5415R) for 10 min at 10,000 *g* (4°C). The supernatant was removed, and a dry pellet containing cells was chilled at −20°C for 15 min. Lysis buffer (500 µl; 10 mM Tris, 1.2 mM EDTA, 10% SDS, pH 9.0) was added to the dry pellet, and the sample was vortexed to resuspend the pellet. Proteinase K (20 µl of 20 mg/ml; Himedia) was added, and the tube was incubated in a water bath (CW-30G; Jeio Tech, Seoul, Korea) at 56°C for 2 h. Sodium acetate (60 µl of 3 M) and 0.5 ml cold isopropanol were added, mixed, and chilled at −20°C for 1 h, followed by centrifugation at 10,000 *g* (at 4°C) for 20 min. The supernatant was discarded, 250 µl 70% ethanol was added, and the pellet was tapped gently, followed by centrifugation at 10,000 rpm for 10 min before the supernatant was discarded gently. The pellet was air-dried in a laminar air flow, and the dried pellet was resuspended in 50 µl nuclease-free water or 1× TE buffer and frozen at −20°C or −80°C for storage.

DNA Extraction from Blood Sample

Lymphocytes from whole blood were separated by lysing the red blood cells (RBCs) using a hypotonic buffer (ammonium bicarbonate and ammonium chloride; Himedia) with minimal lysing effect on lymphocytes. Three volumes of RBC lysis buffer was added to blood sample and mixed by vortexing and inverting thoroughly for 5 min and centrifuged (Eppendorf 5415R) at 20,00 *g* for 10 min. The supernatant was mostly discarded, leaving behind ~1 ml to prevent loss of cells. To the pellet, 3 vol RBC lysis buffer was added, and vortexing, inverting, and centrifuging steps were repeated two to three times until a clear supernatant and a clean white pellet were obtained. After the final wash, the supernatant was discarded completely, and the pellet was resuspended in 500 µl PBS, followed by addition of 400 µl cell lysis buffer (10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 10% SDS, pH 7.5) and 10 µl proteinase K (10 mg/ml stock; Himedia). The sample was vortexed to dissolve the pellet completely and incubated for 2 h at 56°C in a water bath (CW-30G; Jeio Tech) for lysis. An equal volume of phenol (equilibrated with Tris, pH 8) was subsequently added to the tube and mixed well by inverting for 1 min. The tube was centrifuged at 10,000 *g* (at 4°C) for 10 min, and the aqueous upper layer was transferred to a fresh tube containing equal volumes (1:1) of phenol and chloroform:isoamyl alcohol (24:1). The tube was mixed by inverting for 1 min and centrifuged for 10 min at 10,000 *g* (at 4°C). The supernatant was then transferred to a fresh tube, and 10 µl of 10 mg/ml RNase A (Fermentas, Thermo Scientific) was added.

The sample was incubated at 37°C for 30 min before an equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed by inverting the tube for 1 min and centrifuging at 10,000 *g* (at 4°C) for 10 min. The supernatant was transferred to a fresh tube, and twice the volume of absolute alcohol (Merck) was added and inverted gently a few times and chilled at −20°C, followed by centrifugation at 10,000 *g* at (4°C) for 20 min. The supernatant was discarded, 250 µl 70% ethanol was added, and the pellet

was tapped gently, followed by centrifugation at 10,000 rpm for 10 min and decanting the supernatant gently. The pellet was air-dried in a laminar air flow, and the dried pellet was resuspended in 50 μl nuclease-free water or 1× TE buffer and frozen at −20°C or −80°C for storage.

Concentration and Purity Determination

A quantitative spectrophotometric assay of DNA was performed using a Cary 60 UV-visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Absorbance was measured at wavelengths of 260 and 280 (A_{260} and A_{280}, respectively) nm. The absorbance quotient (OD_{260}/OD_{280}) provides an estimate of DNA purity. An absorbance quotient value of $1.8 < \text{ratio (R)} < 2.0$ was considered to be good, purified DNA. A ratio of <1.8 is indicative of protein contamination, where as a ratio of >2.0 indicates RNA contamination.

DNA Integrity

The integrity of genomic DNA was tested by resolving DNA extracts on a 0.8% agarose gel by electrophoresis (Bio-Rad, Hercules, CA, USA), followed by visualization with ethidium bromide staining. Each DNA sample was graded, according to the electrophoretic migration of sample DNA compared with a known molecular weight marker (Fermentas, Thermo Scientific).

PCR Amplification of mtDNA D-Loop Region for PCR-Based Assays

The adequacy of buccal, hair, urine, and blood DNA extracts for the PCR-based assays was assessed by amplifying the mtDNA D-loop region, which was amplified by PCR using primers human mitochondrial (HMt)-F (5′-CACCATTAGCACCCAAAGCT-3′) and HMt-R (5′-CTGTTAAAAGTGCATACCGCCA-3′), as described by Salas et al.[12] for the HVI region. PCR (vapo.protect; Eppendorf) was carried out in 25 μl total reaction volumes, each containing 100 ng template DNA, 0.2 pM of each primer, 2.5 μl 10× PCR buffer (final 1× PCR buffer), 1.5 mM $MgCl_2$, 200 mM dNTPs, and 1 unit Taq DNA polymerase. The reaction mixture was heated to 94°C for 5 min, followed by 40 cycles, each consisting of 1 min denaturation at 94°C, 1 min annealing at 63°C, 1.5 min extension at 72°C, and a final 10-min extension at 72°C. The PCR amplification products (10 μl) were subjected to electrophoresis (Bio-Rad) on 1.2% agarose gel in 1× Tris-acetate-EDTA buffer at 80 V for 30 min and stained with ethidium bromide (Himedia), and images were obtained in gel documentation (G-Box; Syngene, Cambridge, UK) systems.

Restriction Digestion of the mtDNA D-Loop Region PCR Product

Restriction fragment-length polymorphism (RFLP) of the mtDNA D-loop region was performed to check the contamination in the isolated DNA.[13] PCR products were digested with *Hae*III and *Alu*I (Fermentas, Thermo Scientific) in a total volume of 20 μl (10 μl reaction solutions, 2 μl enzyme buffers, 0.2 μl enzymes, and 7.8 μl distilled water) and placed in the incubator at 37°C for 4 h. The restriction products were analyzed by electrophoresis (Bio-Rad) on a 2% agarose gel, and the molecular weight of restricted fragments was analyzed by gel documentation systems (G-Box; Syngene) after ethidium bromide (Himedia) staining.

RESULTS

In the present study, we demonstrated a rapid, reliable, and robust method for obtaining PCR-ready genomic DNA from human buccal swabs, hair, and urine samples, demanding very low sample volume with an isolation/amplification time that is, at least, a factor of two shorter when compared with the conventional methods. Blood was used as a reference sample for DNA isolation. By modifying the conventional phenol-chloroform method, we also successfully developed and demonstrated a reliable protocol that is rapid, cost-effective, and readily implemented for the isolation of DNA with optimal concentration and purity.

Yield and Purity

The yield of the extracted DNA from the four different sample sources was evaluated using a double-beam UV-visible spectrophotometer and the gel electrophoresis (Fig. 1). Small-scale DNA extraction from buccal swabs (from 1 ml) resulted in 60–85 ng/μl genomic DNA/isolation, 49–72 ng/μl in hair (from four pieces), 25–42 ng/μl in urine (from 5 ml), and 57–94 ng/μl in blood (from 50 μl) samples (Table 1). Similarly, the purity of the extracted DNA from the urine (1.42–1.58) and buccal swab samples (1.54–1.67) was lower than the blood (1.76–1.86) and the hair (1.72–1.97) specimens (Table 1). The storage of extracted DNA from urine, hair, blood, and buccal swabs, for over 1 month, frozen at −20°C, did not affect the PCR performance.

Effect of Sample Processing on DNA Integrity

Different band intensities were observed for the extracted DNA from freshly collected and/or stored samples. There was no degradation in the DNA that was isolated immediately after the urine and buccal sample collection. However, isolation of DNA from the stored buccal swabs and the urine samples exhibited bands with some degree of DNA degradation (low-intensity bands) and the concomitant smearing in the lane. However, for the hair and the

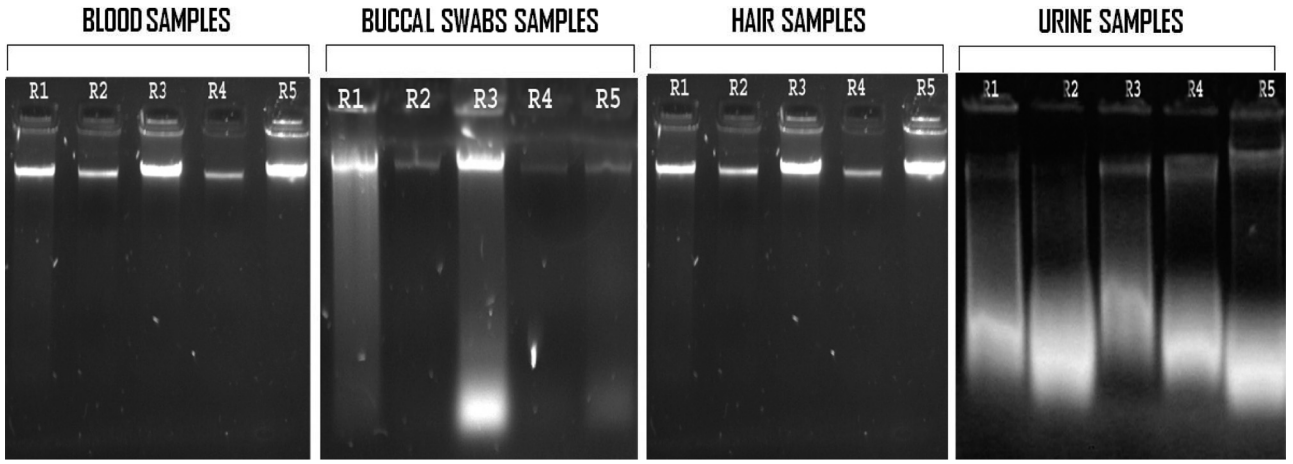

FIGURE 1

Isolated genomic mtDNA from blood, buccal swab, hair and urine samples (R1 – R5: five individual replicates).

blood samples, storage by freezing (−20°C or −80°C) had no effect on the integrity of the extracted DNA (Table 1).

Effect of Sample Processing on PCR-RFLP

To check the quality of the isolated DNA on a small scale from buccal swabs, hair, urine, and blood samples, we amplified the mtDNA D-loop region using a PCR assay. The mtDNA D-loop region was amplified successfully from all samples, irrespective of the status of the sample, whether processed immediately after collection or stored (Fig. 2). Therefore, urine, buccal swab, and hair are a good, alternative source, in addition to the blood sample, when PCR-ready genomic DNA is required. However, a significant variation in the yield, as well as the quality or the purity among the sample types, is observed. Hence, a reliable PCR reaction can be performed using even a much smaller quantity of the isolated DNA. The PCR product intensity was higher for blood and hair template DNA than with urine and buccal swab specimens. The PCR- RFLP band patterns were good in the case of hair and blood DNA, but urine and buccal swab samples did not exhibit satisfactory band intensity. However, there was no detectable contamination among the isolated DNA samples, as all digested samples were the same length (Figs. 3 and 4).

DISCUSSION

Yields of DNA obtained from buccal swabs and urine are highly altered depending on the swab or the urine type, the individual being swabbed, the swabbing technique, and the number of cells captured on the swab and in the urine.[6,11] The expected yield using this protocol is 60–85 ng/μl/swab and for urine, 25–45 ng/μl/15 ml collection (Table 1), which is, at least, a factor of two higher when compared with the conventional methods. The yield and purity of isolated DNA are also dependent on the researchers' handling procedures. A decrease in DNA quality and quantity was observed when the material was not placed immediately in cell lysis buffer for further processing. The degradation of DNA bands was observed in buccal swabs and urine specimen processed with the time delay, whereas degradation in blood and hair sample is not observed, probably as a result of the nature of the sample and the extent of nuclease enzyme concentration in the sample before digestion. Although there was a certain degree of DNA degradation in samples stored under cold temperature condition for 3 days, the present study did not exhibit any significant difference between the extracted DNA PCR amplification products from the buccal cells immediately after the sample collection or from the buccal cells frozen 3 days at −20°C. Moreover, 1 week of storage, as refrigerated at 4°C or frozen at −20°C, also did not affect the yield of the extracted DNA or PCR amplification of DNA. In the case of hair and blood samples, a high quality of DNA can be obtained for later use, even after storage of the hair sample in ethanol for more than 2 months and the blood sample in the EDTA-coated vial for more than 4 months at −20°C.

In general, for DNA-typing studies, fresh whole blood or blood-stained material is the primary source of an individual's DNA "fingerprint" and is used as a standard for comparison. The findings presented here allowed us to demonstrate use of the buccal swabs and urine as alternative sources for extraction of DNA. However, there is a marked difference between male and female urine samples regarding the quantity of DNA available; as in most cases, there is no information regarding the gender when the urine is collected from a crime scene,[10] it should always be collected from the largest urine stains that are available. Large vol-

TABLE 1

Total DNA Yield and Quality by Immediate or Storage Processing of the Biological Samples

	Spectrophotometry																	
	Immediate processing									Storage processing								
	Total DNA yield (ng/μl)					A_{260}:A_{280}				Total DNA yield (ng/μl)					A_{260}:A_{280}			
Biological sample	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5
Blood sample	86	74	92	57	94	1.82	1.86	1.77	1.82	1.76	79	91	83	82	71	1.89	1.65	1.81	1.77	1.81
Buccal swab sample	83	62	85	60	65	1.57	1.62	1.54	1.67	1.62	55	51	58	42	47	1.62	1.67	1.62	1.83	1.75
Hair sample	49	62	68	57	72	1.72	1.82	1.87	1.74	1.97	58	52	58	61	57	1.82	1.82	1.83	1.84	1.82
Urine sample	31	25	37	42	39	1.48	1.42	1.58	1.52	1.58	23	36	36	20	23	1.48	1.46	1.48	1.55	1.53

R1–R5, Five individual replicates; Immediate processing, DNA extraction was performed immediately after sample collection; Storage processing, DNA extraction was performed 3 days after sample collection.

FIGURE 2

PCR amplification of mtDNA D-loop region. M – Marker, BL – Blood DNA, H – Hair DNA, BS – Buccal Swab DNA, U – Urine DNA.

umes of saliva and urine can be collected in a noninvasive manner without any pain. In fact, a buccal swabbing and the urine were easily obtained with minimum fuss for a detailed analysis, even from an infant.[14] We can also amplify viral and bacterial genes from the DNA of urine and buccal swabs for the study of a presence or absence of any pathogens using the reported PCR assay.

The isolated DNA from all samples has generated PCR products of a similar base-pair size of the target mitochondrial gene. However, in the case of restriction digestion, hair and blood PCR products produced excellent digested products. This may be a result of the presence of good concentration of PCR amplification products and the absence of any impurity in the PCR sample, rather than urine and buccal swab samples that were not digested properly by the restriction enzyme. Hence, we can use hair samples instead of blood samples for the PCR-RFLP-based molecular analysis.

There is a possibility for low levels of contaminants that may be present in the DNA isolated from the serum or plasma that are much less abundant rather than those that exist in urine and buccal swab specimens.[15] In these techniques, there are fewer amounts of contaminants, so the

FIGURE 3

Restriction digestion of the PCR product of mtDNA D-loop region by AluI. M – Marker, BS – Buccal Swab DNA, U – Urine DNA , H – Hair DNA, BL – Blood DNA.

FIGURE 4

Restriction digestion of the PCR product of mtDNA D-loop region by HaeIII. M – Marker, BS – Buccal Swab DNA, U – Urine DNA , H – Hair DNA, BL – Blood DNA.

probability of interference of the contaminants during the PCR process is very low.

Harty et al.[16] have reported that PCR amplification was successful after a long storage of samples, even though the storage has reduced the yield of DNA. In the present study, the isolated DNA from all samples stored as frozen at −20°C was suitable for later use. The DNA extracted from the urine sample that was stored for over 1 month frozen at −20°C performed as good as a fresh urine sample. Although the DNA in the urine specimen seems to degrade over a period of time, the urine sample, stored up to 3 months frozen at −20°C, may still be used for PCR amplification. Isolated DNA from the hair and the blood is very good on the basis of the stability of DNA for storage as frozen at −20°C for further analysis.

Conclusion

The successful sample collection and the extraction of genomic DNA from buccal swabs, urine, and hair are noninvasive and reliable alternatives to the prickly invasive blood sampling, both for subjects and sample collectors.[17–20] We have demonstrated here a simple and novel method of the sample collection and DNA extraction, which is cost-effective, easy, and rapid, providing a sufficient quantity and quality of DNA for PCR-RFLP-based analysis. Comparison of the extraction procedures shows that the simple phenol-chloroform method is the most suitable for DNA extraction from buccal swab, urine, hair, and blood samples. Under appropriate storage conditions, DNA isolated from buccal cells, urine, and hair can be successfully used to perform PCR-based assays. The DTT, high-salt, anionic detergent solution mtDNA extraction method developed in this study represents a rapid and simple protocol that excels the mtDNA amplification success rate of the standard glass-grinding/organic solvent extraction techniques currently used by many forensic laboratories. The relatively lesser number of steps used in this method facilitates shorter time duration and in addition, results in a significantly reduced probability of contamination with a minimal sample loss. The DTT chemical digestion method uses reagents, supplies, and equipment readily available in any basic laboratory. Its ease will help in mtDNA analyses in those laboratories that have yet to undertake forensic mtDNA testing, as well as a population-based study using hair samples. However, important questions still remain to be explored regarding the yield and quality of human DNA that can be obtained from different DNA extraction methods.

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REFERENCES

1. Le Marchand L, Lum-Jones A, Saltzman B, Visaya V, Nomura AM, Kolonel LN. Feasibility of collecting buccal cell DNA by mail in a cohort study. *Cancer Epidemiol Biomarkers Prev* 2001; 10:701–703.
2. Hansen TV, Simonsen MK, Nielsen FC, Hundrup YA. Collection of blood, saliva, and buccal cell samples in a pilot study on the Danish nurse cohort: comparison of the response rate and quality of genomic DNA. *Cancer Epidemiol Biomarkers Prev* 2007;16:2072–2076.
3. Van-Wieren-de-Wijer DB, Maitland-van-der-Zee AH, de Boer A, et al. Determinants of DNA yield and purity collected with buccal cell samples. *Eur J Epidemiol* 2009;24:677–682.
4. Emi S, Hiroshi N. Evaluation of three methods for effective extraction of DNA from human hair. *J Chromatog B* 2005;820: 137–141.
5. Graffy EA, Foran DR. A simplified method for mitochondrial DNA extraction from head hair shafts. *J Forensic Sci* 2005;50: 119–1122.
6. Mulot C, Stücker I, Clavel J, Beaune P, Loriot MA. Collection of human genomic DNA from buccal cells for genetics studies: comparison between cytobrush, mouthwash, and treated card. *J Biomed Biotechnol* 2005;2005:291–296.
7. Wilson MR, Polanskey D, Butler J, DiZinno JA, Repogle J, Budowle B. Extraction, PCR amplification and sequencing of mitochondrial DNA from human hair shafts. *BioTechniques* 1995;18:662–669.
8. Wilson MR, DiZinno JA, Polanskey D, Repogle J, Budowle B. Validation of mitochondrial DNA sequencing for forensic casework analysis. *Int J Legal Med* 1995;108:68–74.
9. Bisbing RE. The forensic identification and association of human hair. In Saferstein R (ed): *Forensic Science Handbook*, vol 1. EnglewoodCliffs, NJ, USA: Prentice Hall Regents, 1982;184–221.
10. Yasuda T, Iida R, Takeshita H, et al. A simple method of DNA extraction and STR typing from urine samples using a commercially available DNA/RNA extraction kit. *J Forensic Sci* 2003;48:108–110.
11. Deelman LE, Esther A, Wouden VD, Duin M, Henning RH. A method for the ultra rapid isolation of PCR-ready DNA from urine and buccal swabs. *Mol Biol Today* 2002;3:51–54.
12. Salas A, Lareu MV, Carracedo A. Heteroplasmy in mtDNA and the weight of evidence in forensic mtDNA analysis: a case report. *Int J Legal Med* 2001;114:186–190.

13. Doosti A, Ghasemi-Dehkordi P. Genetic polymorphisms of mitochondrial genome D-loop region in Bakhtiarian population by PCR-RFLP. *Int J Biol* 2011;3:41–46.
14. Tsung-Hsing C, Sheng-Pyng C, Tzu-Chuan L, Wen-Chi C, Jenn-Shing S, Yi-Shing S. Optimal DNA extraction from buccal swab samples. *J Med Sci* 2010;30:149–154.
15. Chan AK, Chiu RW, Lo YM, Clinical Sciences Reviews Committee of the Association of Clinical Biochemists. Cell-free nucleic acids in plasma, serum and urine: a new tool in molecular diagnosis. *Ann Clin Biochem* 2003;40:122–130.
16. Harty LC, Garcia-Closas M, Rothman N, Reid YA, Tucker MA, Hartge P. Collection of buccal cell DNA using treated cards. *Cancer Epidemiol Biomarkers Prev* 2000;9:501–506.
17. Freeman B, Powell J, Ball D, Hill L, Craig I, Plomin R. DNA by mail: an inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. *Behav Genet May* 1997;27:251–257.
18. King IB, Satia-Abouta J, Thornquist MD, et al. Buccal cell DNA yield, quality, and collection costs: comparison of methods for large scale studies. *Cancer Epidemiol Biomarkers Prev* 2002;11:1130–1133.
19. Russell H, Von-Beroldingen CH, Sensabaugh GF, Erlich HA. DNA typing from single hairs. *Nature* 1988;332:543–546.
20. Linch CA, Whiting DA, Holland MM. Human hair histogenesis for the mitochondrial DNA forensic scientist. *J Forensic Sci* 2001;46:844–853.