DNA recovery from forensic clothing samples by tape-lift

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Introduction

Two collection methods, double swab and soaking, have been commonly used to recover trace DNA from forensic biological evidences. However, double swab collection method may lead to irreproducible amount of epithelial cells collected due to the non-sticky cotton was used, whereas excision method alters the original forensic evidence, increases contamination, and tends to include increasing amount of PCR inhibitors [1-4].

The advent tape-lift method with adhesive water-soluble tape has been recently demonstrated as an alternative for collection of skin epithelial cells and DNA profile analysis [4-5]. Tape-lifting has been suggested to be more advantageous than earlier methods because it is not only help to effective collect the epithelial cells, easy to use, quick, and cost effective but also this would not alter the original forensic evidence. However, there is no report whether it is possible to apply tape-lift method for DNA recovery from other types of sample.

This study was designed to explore possibilities of DNA recovery from clothing samples by tape-lift method followed by DNA profile analysis with the intention to develop a practical method to be used in forensic science.

Materials and Methods

1. Clothing samples preparation

. The pure cotton white T-shirts were successively washed by hands coupled with a commercial detergent and completely air-dried without laundry before storing in a sealed paper bag. The shirts were worn only once for 12 hours by volunteers with outdoor activity before collection of skin epithelial cells as clothing DNA samples.

2. Epithelial cell collection

The tape-lift method with adhesive soluble tape was used to collect the skin cells. Epithelial cells were collected from both the volunteer's skin as reference DNA profile and corresponding clothing DNA sample after wearing for 12 hours. Piece of water- soluble tape (Scothch® 3M No.5414, 2.5 cm x 6 cm) was used in collecting skin epithelial cells the upper right back position from each volunteer. Another piece of the tape (2.5 cm x 4 cm) was applied in collecting epithelial cells from the inner collar of clothing sample. The tape was pressed firmly to the skin (or cloth collar) followed by lifting it free. The pressing-lifting cycle was repeated 40 times for each sample. The

tape was fold over onto itself and stored separately in a sealed plastic bag at room temperature until use for DNA extraction.

3. DNA extraction

Three different extraction methods were performed, Chelex® 100 (BIO-RAD, CA, USA), ChargeSwitch® Forensic DNA Purification Kit (Invitrogen, CA, USA), and QIAamp® DNA Mini Kit (QIAGEN, CA, USA). Each piece of the tape-lift was cut into 2 x 2.5 cm and placed in each extraction tube. Otherwise 20 uL of blood sample was used instead.

The extraction procedure *by Chelex® 100* was performed as previously described [6-7]. Briefly, 1 mL of sterile double distilled water was added into a sterile 1.5 mL microcentrifuge tube containing a tape-lift sample. The tube was incubated at room temperature for 30 min followed by centrifugation at 13,000 rpm 5 min. The supernatant was removed and discarded. The remaining (about 100 µL) was added with 200 µL of 5% Chelex stock followed by 20 µL of Proteinaes K (20 mg/mL Promega, CA, USA). The tube was further incubated at 56^{o}C 30 min with additional 8 min at 100^{o}C. Finally the tube was centrifuged at 13,000 rpm for 5 min and about 250 µL was transferred into a new 1.5 mL sterile microcentrifuge tube and stored at 4^{o}C for subsequent DNA analysis.

DNA extraction by ChargeSwitch® Forensic DNA Purification Kit was performed following the manufacturer's instructions [8] except the placing time of tube in MagnaRack™ was increased up to 3 min and three wash steps were repeated. DNA extracted from tape-lift was eluted in 30 µL of buffer and stored at 4^{o}C for subsequent DNA analysis.

DNA extraction by QIAamp® DNA Mini Kit was also performed following the manufacturer's instructions [9]. However the viscosity of mixture of the soluble tape-buffer was very high and not suitable for extraction DNA from the tape-lift.

4. DNA quantitation by multiplex polymerase chain reaction

The amount of DNA in all samples was quantified using Real-Time PCR technique (ABI 7000 Sequence Detection System, Applied Biosystems) [10]. After quantitation, DNA extracted from the samples by ChargeSwitch® Forensic DNA Purification Kit were processed for DNA profile analysis. Between 1.0-4.6 ng of the template DNA were amplified in total reaction volume of 25 and 50 µL using AmpF*l*STR® Identifiler™ PCR Amplification Kit in a thermocycler (GeneAmp® PCR System 9700, Applied Biosystems for 28 PCR cycles[11].

5. Human DNA typing and match probabilty

DNA profiles of the amplified PCR products were analyzed by using *capillary electrophoresis* on ABI 3100 Genetic Analyzer (Applied Biosystems) with GeneMapper™ *ID* Software version 3.1 [12] with peak amplitude threshold (PAT) setting at 100 RFU. Match probabilities of DNA profiles between skin epithelial cells of each volunteer (positive control) and those from corresponding clothing sample were calculated by using reference allele frequencies among Thai population [13]. The locus

genotype proportions and match probability was calculated according to the Hardy-Weinberg formulae [14-17].

Results

1. DNA extraction from human blood

No suitable DNA extraction method from tape-lift has been documentary established. Therefore, 3 commonly used DNA extraction methods were selected to determine the ost appropriate one in this study. To standardize the methods, extraction of human blood DNA was performed in comparison. Blood DNA at dilutions between 1:16 to 1:1024 folds could be extracted by all 3 extraction methods used except no extractable DNA found at 1:4 diution by ChargeSwitch® (Figure 1). QIAamp® and Chelex® 100 showed better yields of DNA extraction at all blood dilutions than those obtained by ChargeSwitch®.

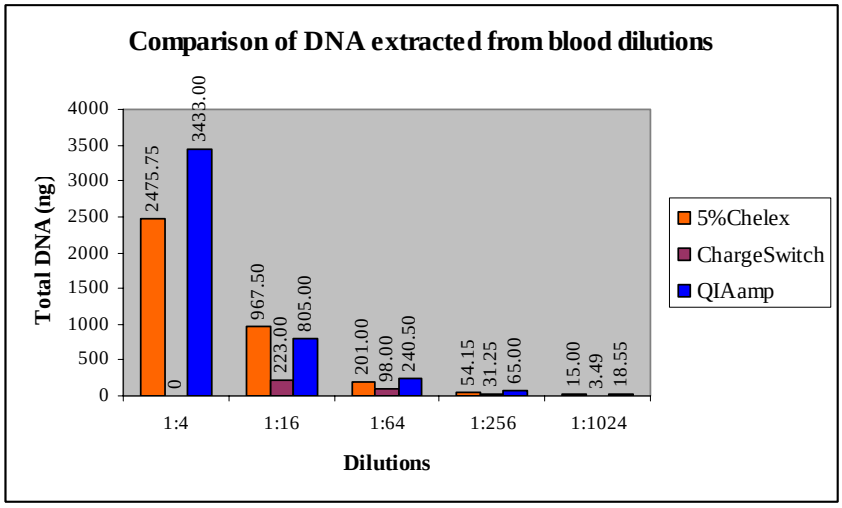

Figure 1 Amounts of of human blood DNA extracted by Chelex® 100, ChargeSwitch® Forensic DNA Purification Kit and QIAamp® DNA Mini Kit. 200 uL each of blood dilutions (1:4 to 1:1024) was extracted as described in the Methods.

2. DNA extraction from tape-lift

2.1 DNA from skin epithelial cells

All 3 different extraction methods were applied recovering DNA from tape-lift samples. Measurable amounts of DNA collected from skin epithelial cells of all volunteers were extracted either by Chelex® 100 or ChargeSwitch® (Figure 2). Larger range of DNA quantities among the samples was obtained from Chelex® 100 extraction than from the ChargeSwitch®. Particularly, the amount of DNA from volunteer no.8 (1.13 ng) extracted by the Chelex® 100 method was almost undetected. No DNA was extracted from all tape-lift samples by the QIAamp® DNA method. Therefore, the method was not continued further.

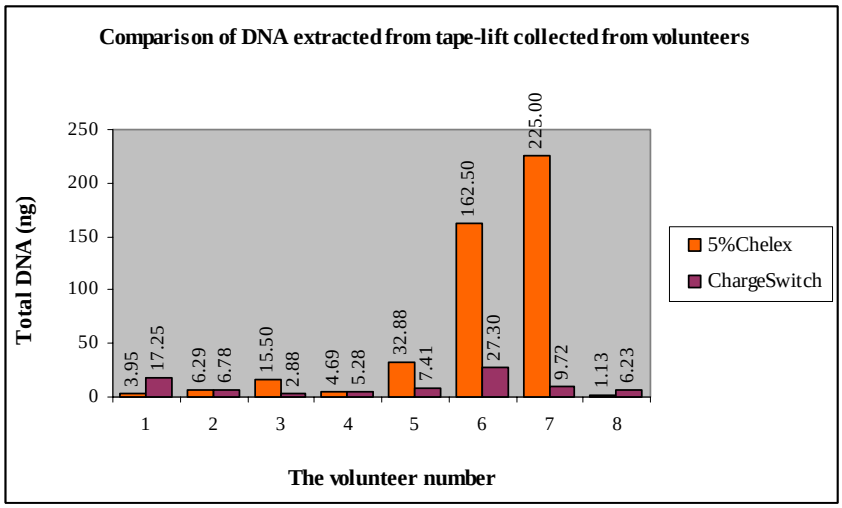

Figure 2 Amounts of DNA on tape-lift collected from 8 volunteers'epithelial cells, after extraction by Chelex® 100 and ChargeSwitch® methods. DNA quantitation was performed by Real-Time PCR as described in the Methods.

2.2 DNA from clothing samples

Extraction of DNA from tape-lift of clothing samples was performed using the Charge Switch® method. The Chelex® 100 method showed irreproducible results and no extactable DNA of some samples. DNA from 8 clothing samples were satisfactorily extracted at different quantities ranging from 5.06 ng to 15.60 ng (clothing no.8 and no.7 respectively) (Figure 3).

Total amounts of DNA extracted from skin epithelial cells and clothing samples of all 8 volunteer were summarized in Table 1. Most of the samples contained satisfactory amounts of DNA, however, few samples particularly, VT-3, VT-4 and CT-8 showed relatively lower amount of extracted DNA than others. This might interefere subsequent DNA profile analysis.

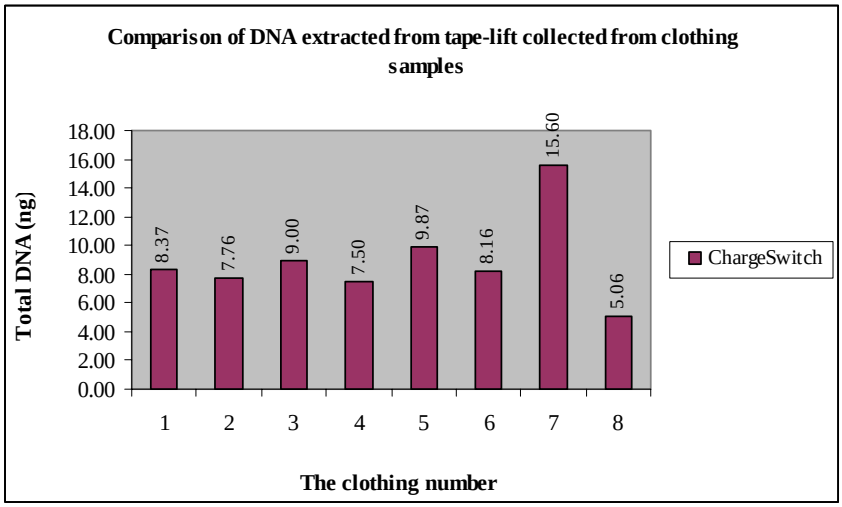

Figure 3 Amounts of DNA extracted from tape-lift of 8 clothing samples after extraction by ChargeSwitch® and quantified by Real-Time PCR. The clothing numbers correspond to the volunteer numbers indicated in Figure 2

Table 1 Total amounts of DNA extracted from skin epithelial cells (VT) and clothing samples (CT) of all 8 volunteers

Samples no.	Total amount of extracted DNA (ng)*	
	Volunteers(VT)	Clothings (CT)
1	17.25	8.37
2	6.78	7.76
3	2.88	9.00
4	5.28	7.50
5	7.41	9.87
6	27.30	8.16
7	9.72	15.60
8	6.23	5.06

* The amount was calculated from the reaction tube (see Methods) for total DNA on each piece of tape-lift.

3. DNA profile analyses

DNA profiles were investigated to match identities of DNA obtained from volunteers' skin epithelial cells (positive control reference) and those from the clothing samples. A representative electropherogram of DNA profile from epithelial cells of a volunteer was demonstrated (Figure 4). DNA profile matching were analyed between volunteers' epithelial cells and those collected from corresponding clothing samples (Table 2).

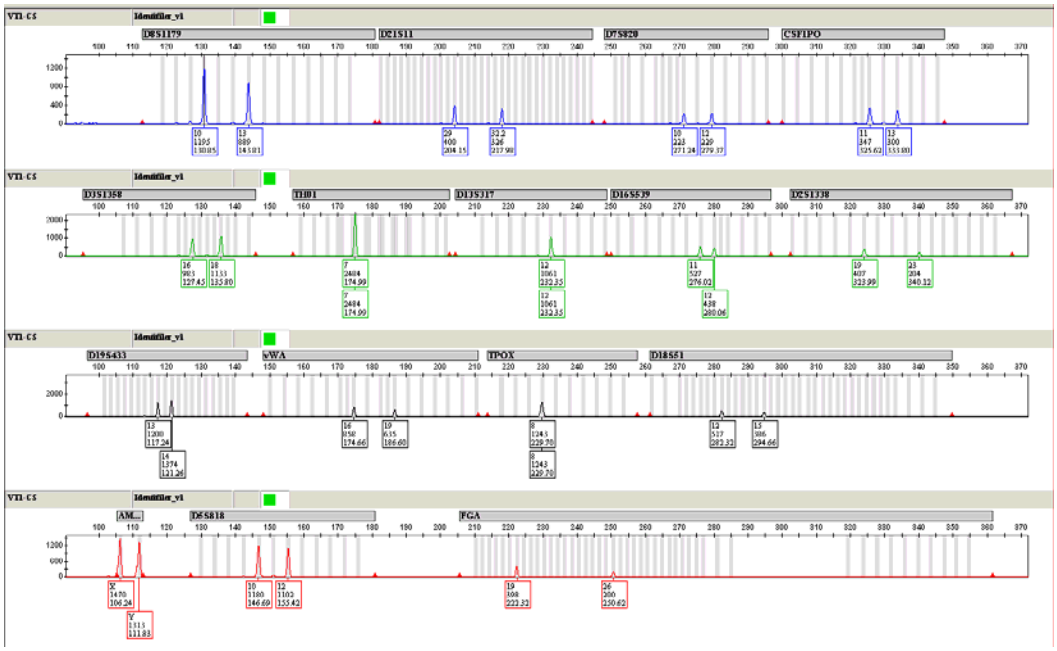

Figure 4 A representative electropherogram of DNA profile from skin epithelial cells of a volunteer (VT-1).

From total 8 volunteers, completely matching of STR 15 loci were observed in 5 samples, 14 loci in 2 samples and 13 loci in 1 sample with highest match probability at 1.02×10^{-18} (Table 2)

Table 2 Match probabilities of DNA profiles between skin epithelial cells of volunteers (VT) and clothing samples (CT)

Compared DNA profile*	Number of matching loci	Match probability
VT1 and CT1	Completed (15 loci)	8.88×10^{-19}
VT2 and CT2	14 loci	4.21×10^{-18}
VT3 and CT3	14 loci	2.07×10^{-19}
VT4 and CT4	13 loci + 2 half loci	1.02×10^{-18}
VT5 and CT5	Completed (15 loci)	7.26×10^{-20}
VT6 and CT6	Completed (15 loci)	8.00×10^{-21}
VT7 and CT7	Completed (15 loci)	5.26×10^{-19}
VT8 and CT8	Completed (15 loci)	1.48×10^{-18}

* Epithelial cells (VT1-VT8) and clothing samples (CT1-CT8) of volunteer numbers1-8, respectively. The data were summarized from 16 electropherograms.

Discussions

Recovery of human DNA from clothing samples by tape-lifting was investigated. DNA extraction from the tape-lift by appropriate extraction method was also demonstrated providing sufficient quantity for DNA profile analysis. Using 15 loci STR analysis, identical DNA profiles were observed between the samples and volunteers wearing the clothings with match probability less than 1.0×10^{-10}.

Human blood DNA could be extracted by all the methods investigated. High yield of DNA recovery by extraction method agreed with previous reprt probably due to optimal binding of DNA to the QIAamp silica-membrane aswell as removal of PCR inhibitors [9]. Unfortunately, the QIAamp® method could not extract DNA out from the tape-lift and therefore not suitable in the present study. On the other hand, ChargeSwitch® method is not suitable for extraction of blood DNA in large amount due to possible contamination of the purified DNA with heme [8]. This was supported by the absence of extracted DNA in our experiment (Figure1). However, the method provided reproducible recovery yields of extracted DNA on tape-lift from both clothing samples and skin epithelial cells. In comparison, Chelex® 100 method extraction showed minute amount of DNA from epithelial cells number 8 (Figure2) and no DNA from some of clothing samples (data not shown). Therefore, ChargeSwitch® method appeared to be most suitable for DNA extraction from tape-lift as earlier suggested [4-5].

Different amounts of DNA extracted from the tape-lift (Table 1) were not due to extraction recovery as repeat extraction produced similar results (data not

shown). This appeared to be related to the amount of epithelial cells shedding from the skin. The tape contains a water-soluble adhesive layer, cell collection property is critically affected by moisture [2, 17]. Narrower range of DNA amounts observed from clothing than those from volunteer skin [Table1] suggested better DNA recovery of tape-lift on dry samples. Preparation of reference DNA profiles from blood or buccal cells volunteer's might be other alternatives.

Interestingly, complete DNA profiles of all 15 loci STR observed in 5 from 8 clothing samples, despites 2 and 1 from 5 samples showed 14 loci and 13 loci matching to the owner reference DNA profiles (Table 2). Incomplete matching of 3 from 5 samples might be due partial degradation of the extracted DNA templates and other related factors [18-22]. Interference occurred from unexpected cross contact by other individuals on the clothing samples might also be possible to produce the incomplete matching. However, very low match probability indicated reliability of identical DNA profiles from clothing samples and skin epithelial cells of the corresponding volunteers.

Conclusions

DNA on clothing samples was successfully recovered by tape-lifting and ChargeSwitch® extraction method. The investigation also demonstrated for the first time of DNA profile matching between cells collected by tape-lifting from clothing samples and skin cells from individuals wearing the clothes. The results demonstrated possible development of the study for potential application in forensic samples.

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