BRIEF COMMUNICATION

Kathryn Sinclair,[1] *Ph.D. and Victoria M. McKechnie,*[1] *Ph.D.*

DNA Extraction from Stamps and Envelope Flaps Using QIAamp and QIAshredder

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ABSTRACT: The use of Qiagen QIAamp and QIAshredder for extracting DNA from envelope flaps and stamps is reported. The stamps or flaps can be added directly to extraction solutions and the DNA is bound to a spin column containing a silicon membrane for washing prior to elution. DNA extracted from four stamps and four envelope flaps was amplified and analyzed using a multiplex PCR system. Complete DNA profiles were obtained from five samples.

KEYWORDS: forensic science, DNA typing, DNA extraction, saliva, stamps, envelope flaps

The analysis of saliva on evidential material such as stamps and envelope flaps can provide important information to the forensic scientist. DNA can be extracted from the epithelial cells present in saliva and, using the current PCR technology, a profile determined. A number of different approaches have been used in the extraction of DNA from saliva on stamps, including phenol-chloroform (1) and Chelex (2).

Here we present the use of Qiagen's QIAamp and QIAshredder as a method for extracting DNA from envelope flaps and stamps. The DNA extracted using this method is suitable for analysis by the PCR-based typing system currently used in the authors' laboratory.

Materials and Methods

DNA was extracted from four stamps and four envelope flaps using QIAamp and QIAshredder kits supplied by Qiagen U.K. (3). The age of the samples was approximately 7 to 10 days, including postage and storage at room temperature in the laboratory. A 25 mm $\times$ 12.5 mm area of stamp or envelope flap was removed and cut into small pieces. No attempt was made to separate the two paper layers. The paper fragments were incubated in 180 μL QIAamp buffer ATL and 20 μL proteinase K (20 mg/mL) overnight at 56°C. The lysate and filter pieces were transferred to a QIA shredder column and centrifuged 13 500 g/5 min. The column was removed and stored at −20°C for sample preservation and 200 μL QIAamp buffer AL added to the flow through. Following incubation 70°C/10 min, 210 μL absolute alcohol (99.86%) was added and the mixture applied to a QIAamp spin column and centrifuged 6000 g for 1 min. The column was washed twice with 500 μL ethanol containing high salt buffer (QIAamp buffer AW) followed by 1 min centrifugation after each wash. The DNA was eluted in 50 μL QIAamp buffer AE (preheated to 70°C) by centrifugation of the column for 1 min.

DNA yields were estimated using the Aces 2.0 Human DNA System (Gibco BRL, Paisley, Scotland) according to the manufacturer's instructions.

DNA was amplified using a multiplex PCR system developed by the Forensic Science Service (U.K.) of six variable regions and one sex-specific region (4). This system is currently in use in this laboratory for routine casework. Briefly, 1 ng DNA was added to a 50 μL reaction mixture containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 1% Triton X-100, 1.25 U AmpliTaq gold, 200 μM each dNTP, 0.044 μM Amelogenin 1 and 2, 0.22 μM HUMvWFA31A 1 and 2, 0.0864 μM HUMTHO1 1 and 2, 0.642 μM D8S1179 1 and 2, 0.08 μM HUMFIBRA 1 and 2, 0.236 μM D21S11 1 and 2 and 0.04 μM D18S51 1 and 2. One primer of each pair was fluorescently labeled. For those samples with DNA concentration $<$0.03 ng/μL (flaps 1 and 4), the maximum volume of 30 μL and therefore the maximum concentration of DNA was amplified. The amplification conditions, on a PE 480 thermal cycler, were 95°C for 15 min followed by 30 cycles of 93°C for 80 s, 58°C for 2 min, 72°C for 1 min and a final step of 72°C for 30 min.

Amplification reaction mixtures (1.5 μL) were combined with an internal lane standard, GS350, and resolved on 6% polyacrylamide denaturing gel using an ABD automated DNA sequencer model 373A (5). Fragment sizes were determined using Genescan 672 software. Allele designations were made by reference to allelic ladders employing Genotyper version 2.0 software.

Results and Discussion

DNA was extracted from the stamps and envelope flaps of four standard white envelopes (Table 1). An advantage of this extraction technique is that the paper fragments are added directly to the buffers without the need to separate the paper layers or swab either surface of the paper. DNA profiles have been obtained using this technique from swabbings of envelope flaps submitted as routine casework (data not shown). The paper layers had been separated and the exposed surfaces swabbed with sterile water-soaked cotton. This represents an alternative preparation method if sample preservation for fingerprint examination is required. The cell lysate is passed through the QIAshredder while the paper fragments are re-

[1] Forensic scientist and forensic technician, respectively, Lothian and Borders Police Forensic Science Laboratory, Edinburgh, Scotland.

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TABLE 1—*Total DNA yield from stamps and envelope flaps.*

	Sample	Yield, ng*
Stamps	A	5
	B	50
	C	20
	D	5
Flaps	1	1
	2	30
	3	10
	4	1

* Yield determined following comparison with known standards.

TABLE 2—*DNA profiles obtained from stamps and envelope flaps.*

Sample		Amelogenin	D18851	D21S11	D8S1179	HUMFIBRA	HUMTHO1	HUMvWFA31A
Stamps	A	X,X	12,13	67,70	10,13	21,23	7,8	17,18
	B	X,X	11,21	65,68	10,13	22,23	6,7	17,17
	C	X,X	12,15	61,65	12,14	19,25	7,9.3	19,19
	D	no result						
Flaps	1	no result						
	2	X,X	11,21	65,68	10,13	22,23	6,7	17,17
	3	X,X	12,15	61,65	12,14	19,25	7,9.3	19,19
	4	no result						

tained in the column removing them from future steps. In addition, further purification of the DNA is obtained using the QIAamp column, eliminating the need for toxic chemicals, e.g., phenol. Up to 12 samples (depending on the size of the centrifuge rotor) can be extracted simultaneously, and DNA is obtained in less than 30 min following an overnight incubation.

Six samples (A–D, 2,3) were amplified using 1 ng DNA and two samples, 1 and 4, using 0.6 ng. This represented the maximum volume (30 μL) of sample in the 50 μL amplification reaction.

The quality of the DNA obtained from stamps A to C and flaps 2 and 3 was sufficient to obtain full DNA profiles (Table 2). Although DNA was extracted from stamp D, no DNA profile was obtained perhaps due to the presence of inhibitors, insufficient purification of DNA or degradation of DNA. Further studies in this laboratory aim to address these issues. Preliminary observations (data not shown) suggest that washing and concentration of DNA through a micron 30 column (Millipore U.K. Ltd.) may increase the DNA profiling success rate for envelope flaps and other sample types, e.g., dyed bloodstain fabric, semen stains. The total amount of DNA in the reactions for flaps 1 and 4 was 0.6 ng. Since this DNA profiling system is optimized for 1 ng DNA, the absence of profiles for these samples is not unexpected.

The use of QIAamp and QIAshredder represents an alternative method for the extraction of DNA from stamps and envelope flaps and subsequent STR analysis in forensic casework.

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Additional information and reprint requests:
Kathryn Sinclair, Ph.D.
Lothian and Borders Police Forensic Science Laboratory
11 Howden Hall Road
Edinburgh EH16 6TF
Scotland