

Reliable extraction of DNA from Whatman™ FTA™ cards

This study examined the yield and quality of DNA from samples applied to Whatman FTA cards, using five common methods of DNA extraction. The data demonstrate that all five methods yield purified DNA of sufficient quantity and quality for both quantitative PCR and short tandem repeat analyses. In addition, EasiCollect™ device for buccal sample collection was shown to be compatible with all five DNA extraction methods, and provided final DNA yields equal to or higher than those from separate foam swabs.

Introduction

Whatman FTA family of products (Fig 1) facilitates collection, transport, purification, and long-term, room-temperature storage of nucleic acids, all in a single device. FTA technology has the ability to lyse cells on contact, denature proteins, remove contaminants, and protect DNA from degradation. In the classic scenario for FTA cards, purified DNA is analyzed while still bound to the matrix (i.e., using the “punch-in” method). This study examined an alternative option for FTA cards—DNA extraction into solution.

Materials and methods

Sample collection

Whole blood and buccal cells were collected onto FTA cards.

Blood

Blood from ten unrelated individuals was collected by venipuncture into vacuum collection tubes containing dipotassium EDTA. Blood (40 µl) was applied to each FTA Micro Card. Cards were dried for at least 3 h at room temperature.



Fig 1. Whatman FTA family of products.

Buccal samples

Buccal samples were collected from ten unrelated individuals, using either EasiCollect devices from GE Healthcare (Fig 2) or foam swabs. Samples were applied to FTA cards according to the manufacturer's instructions. Cards were dried for at least 2 h at room temperature.



Fig 2. EasiCollect is an all-in-one device that collects buccal samples and transfers them to an integrated FTA card.



Methods for DNA extraction

DNA was extracted or isolated from FTA cards using five different methods, exactly as described in the following protocols. These methods include:

- Organic extraction
- Chelex 100 resin (142-1253, Bio-Rad Laboratories, Hercules, CA, USA)
- QIAamp™ DNA Investigator Kit (56504, Qiagen, Valencia, CA, USA)
- illustra™ tissue and cells genomicPrep Mini Spin Kit (GE Healthcare)
- DNA IQ™ Kit (TB297, Promega Corp, Madison, WI, USA)

Organic extraction of DNA

Prepare extraction buffer and Proteinase K stock solution.

Extraction buffer: 10 mM Tris-HCl, pH 8.0; 10 mM EDTA, disodium salt, pH 8.0; 100 mM sodium chloride; and 2% v/v SDS.

Proteinase K stock solution: 20 mg/ml in sterile distilled water.

1. Use a Harris Uni-Core disposable punch (1.2, 2.0, 3.0, or 6.0 mm) to remove a disc from the center of a dried sample spot. Place the disc in a clean RNase/DNase-free 1.5 ml microcentrifuge tube.
2. Add 500 µl of extraction buffer and 20 µl of Proteinase K stock solution.
3. Vortex and incubate at 56°C overnight with agitation.
4. Add an equal volume of buffered phenol (pH 8.0), vortex briefly, and centrifuge for 10 min at maximum speed in a microcentrifuge.
5. Transfer the upper, aqueous phase to a new microcentrifuge tube that contains 500 µl of chloroform.
6. Vortex thoroughly and centrifuge for 10 min at maximum speed in a microcentrifuge.
7. Transfer the upper, aqueous phase to a new tube containing 50 µl of 3 M sodium acetate, pH 5.2.
8. Add 800 µl of 100% ethanol, vortex, and precipitate at -20°C for at least 1.5 h.
9. Recover DNA by centrifuging for 30 min at maximum speed in a microcentrifuge and decanting the supernatant.
10. Add 1 ml of 70% ethanol to the pellet and centrifuge for 20 min at maximum speed in a microcentrifuge.
11. Dry the pellet in a vacuum centrifuge for 30 min.
12. Dissolve the pellet in 50 µl of TE buffer or water.

DNA isolation using Chelex 100 resin

Prepare a 5% w/v suspension of Chelex 100 resin in sterile water prior to use; stir to maintain a suspension.

1. Use a Harris Uni-Core disposable punch (1.2, 2.0, 3.0, or 6.0 mm) to remove a sample disc from the center of a dried sample spot. Place the disc in a clean RNase/DNase-free 1.5 ml microcentrifuge tube.
2. Macerate the FTA disc with a 20-gauge needle.
3. Wash the disc by adding 1 ml of sterile water to each sample tube and incubating at room temperature for 10 min with occasional vortexing. Remove the water.
4. Repeat the wash by adding 1 ml of sterile water to each sample tube and incubating at room temperature for 10 min with occasional vortexing.
5. Centrifuge samples for 3 min at 20 000 × g.
6. Remove and discard the supernatant.
7. Add 200 µl of Chelex 100 suspension using a large-bore pipette tip.
8. Incubate the sample at 56°C for 20 min.
9. Vortex for approximately 15 sec.
10. Incubate at 100°C for 8 min.
13. Vortex for approximately 15 sec.
14. Centrifuge the sample for 3 min at 20 000 × g.
15. Transfer the supernatant to a new sterile pre-labeled microcentrifuge tube, being careful to avoid transfer of the Chelex 100 resin pellet.
16. Store DNA at 2°C–8°C for short periods or at -20°C for extended periods.

DNA isolation using QIAamp DNA Investigator Kit

Reagents and columns are provided with the kit.

1. Use a Harris Uni-Core disposable punch (1.2, 2.0, 3.0, or 6.0 mm) to remove a sample disc from the center of a dried sample spot. Place the disc in a clean RNase/DNase-free 1.5 ml microcentrifuge tube.
2. Add 280 µl of ATL buffer to the tube.
3. Add 20 µl of Proteinase K and vortex for 30 sec.
4. Heat for 60 min at 56°C, vortexing for 30 sec every 10 min.
5. Centrifuge at maximum speed in a microcentrifuge for 30 sec.
6. Add 300 µl of AL buffer and vortex for 10 sec.
7. Incubate at 70°C for 10 min, vortexing for 10 sec every 3 min.
8. Centrifuge at maximum speed for 30 sec and transfer the lysate to a minElute column.
9. Centrifuge at 6000 × g for 1 min and discard the flowthrough.

10. Add 700 μ l of AW2 buffer and centrifuge for 1 min at 6000 \times g. Discard the flowthrough.
11. Add 700 μ l of 100% ethanol and centrifuge for 1 minute at 6000 \times g. Discard the flowthrough.
12. Centrifuge at 20 000 \times g for 3 min, then open the column lid for 10 min at room temperature.
13. Add 50 μ l of distilled water and incubate for 5 min at room temperature.
14. Centrifuge at 20 000 \times g for 1 min. Store the flowthrough, which contains the extracted DNA, at 4°C until needed.

DNA isolation using illustra tissue and cells genomicPrep Mini Spin Kit

Reagents and columns are provided with the kit. Phosphate-buffered saline (PBS) is also required.

1. Use a Harris Uni-Core disposable punch (1.2, 2.0, 3.0, or 6.0 mm) to remove a sample disc from the center of a dried sample spot. Place the disc in a clean RNase/DNase-free 1.5 ml microcentrifuge tube.
2. Add 1 ml of PBS and centrifuge at 16 000 \times g.
3. Macerate the disc using a 20-gauge syringe needle.
4. Centrifuge for 10 sec at 2000 \times g.
5. Add 50 μ l of buffer 1 to each sample.
6. Add 10 μ l of Proteinase K to each sample and vortex for 15 sec.
7. Incubate for 1 h at 56°C.
8. Centrifuge for 10 sec at 2000 \times g. Add 5 μ l of RNase A (20 mg/ml). Incubate for 15 min at room temperature.
9. Add 500 μ l of buffer 4 and vortex for 15 sec.
10. Incubate for 10 min at room temperature.
11. Pipette the sample into a mini column (placed in a collection tube) and centrifuge for 1 min at 11 000 \times g. Discard the flowthrough.
12. Add 500 μ l of buffer 4 to each column. Centrifuge for 1 min at 11 000 \times g and discard the flowthrough.
13. Place the column into a collection tube and add 500 μ l of buffer 6 to the column.
14. Centrifuge for 3 min at 11 000 \times g. Transfer the column to a new 1.5 ml tube.
15. Add 50 μ l of pre-warmed elution buffer (buffer 6) to the column.
16. Incubate for 1 min at room temperature.
17. Centrifuge for 1 min at 11 000 \times g. Discard the column and store the DNA at 4°C until needed.

DNA isolation using DNA IQ Kit

Reagents and columns are provided with the kit. A magnetic separation stand is also required.

1. Use a Harris Uni-Core disposable punch (1.2, 2.0, 3.0, or 6.0 mm) to remove a sample disc from the center of a dried sample spot. Place the disc in a clean RNase/DNase-free 1.5 ml microcentrifuge tube.
2. Add 100 μ l of lysis buffer and incubate at 70°C for 30 min.
3. Transfer the contents to a spin column seated in a 1.5 ml tube and centrifuge at maximum speed for 2 min. Discard the spin column.
4. Vortex the stock resin until thoroughly mixed, and add 7 μ l of resin for the 1.2 mm disc, 7 μ l for the 2.0 mm disc, 16 μ l for the 3.0 mm disc, or 28 μ l for the 6.0 mm disc.
5. Vortex tubes for 3 sec and incubate at room temperature for 5 min, vortexing for 3 sec once every min.
6. Vortex the tube for 2 sec and place on a magnetic stand.
7. Decant the supernatant without disturbing the pellet.
8. Add 100 μ l of lysis buffer. Remove the tube from the magnetic stand and vortex for 2 sec.
9. Return the tube to the magnetic stand and decant the lysis buffer.
10. Add 100 μ l of 1 \times wash buffer. Remove the tube from the stand and vortex for 2 sec.
11. Return the tube to the magnetic stand and decant the wash buffer.
12. Repeat wash steps 10 and 11 two more times for a total of three washes. Decant the wash buffer following the final wash.
13. With the tube in the magnetic stand, open the lid and air-dry for 10 min.
14. Add 100 μ l of elution buffer. Close the lid and vortex for 2 min. Incubate for 5 min at 65°C.
15. Remove the tube from the heat and vortex for 2 sec, then immediately place the tube in a magnetic stand.
16. Transfer the eluate to a fresh tube and store the eluted DNA at 4°C until needed.

DNA analyses

DNA extracted from FTA cards was measured using quantitative PCR (qPCR). DNA was subjected to short tandem repeat (STR) analysis to assess its quality.

DNA quantitation by qPCR

Extracted DNA was measured using quantitative PCR (qPCR) and an ABI™ 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Reactions were prepared using the Quantifiler™ Human DNA Quantification Kit (4343895, Applied Biosystems). The DNA quantitation assay combines two 5' nuclease assays. The first is a target-specific human DNA assay consisting of two primers for amplifying human DNA and one TaqMan™ MGB probe labeled with FAM™ dye for detecting the amplified sequence. The second assay is an internal PCR control (IPC) assay consisting of two primers for amplifying the IPC template DNA and one TaqMan MGB probe labeled with VIC™ dye for detecting the amplified IPC DNA. Standard curves for DNA quantitation were prepared using control DNA supplied with the kit.

Short tandem repeat analysis

STR analysis of extracted DNA was conducted using the Powerplex™ 16 System (Promega) according to the manufacturer's instructions. Amplification was performed in a 9700 thermal cycler (Applied Biosystems), and amplification products were analyzed on an ABI PRISM™ 3130XL Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions.

Results and discussion

DNA quantitation by qPCR

DNA was extracted from FTA discs containing blood or buccal cells from ten unrelated individuals. Four sizes of disc were used for each extraction method, and the total quantity of extracted DNA per disc was determined as described in *Materials and Methods*.

For each of the five extraction methods, a minimum of 10 ng of genomic DNA was recovered from blood samples on FTA cards (see Fig 3). The amount of DNA extracted using each method increased as the disc size increased.

DNA extracted from blood on FTA cards

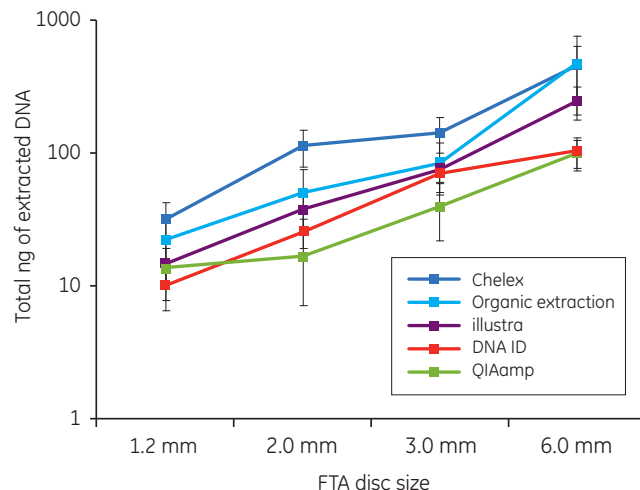


Fig 3. Total DNA (ng) isolated from discs taken from the center of FTA cards spotted with 40 μ l of whole human blood. Each point on the graph represents the mean recovery of DNA from a single disc from ten individuals. Error bars represent standard deviations. Note that the y axis represents a logarithmic scale.

Buccal samples have historically produced greater variability in DNA yield than blood samples. The variability in buccal DNA yield is most likely due to clumping of cells on the matrix. As for blood samples, the amount of DNA recovered with each extraction method tended to increase as the disc size increased. Note that higher DNA yields were often obtained from buccal samples collected with EasiCollect devices than from samples collected with foam swabs (Fig 4).

The data shown in Figures 3 and 4 illustrate that commonly-used DNA extraction methods and kits do provide DNA of sufficient quantity and quality for successful qPCR.

DNA extracted from collected buccal samples

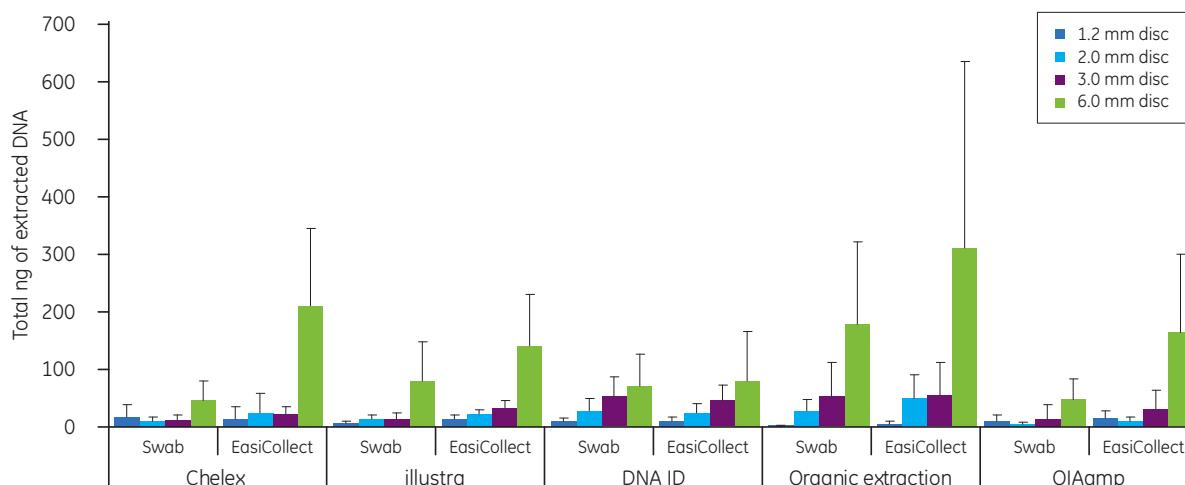


Fig 4. Total DNA (ng) isolated from discs taken from the center of an FTA card containing buccal cells, applied with either a foam applicator or an EasiCollect device as indicated.

Short tandem repeat analysis

Short tandem repeat (STR) profiles are often used for the purpose of forensic human identification. Figure 5 displays STR profiles of DNA extracted from FTA cards containing blood or buccal samples from Test Subject 1. Buccal samples were collected with EasiCollect devices or foam swabs as indicated and applied to FTA cards. DNA was extracted from individual discs (3.0 mm) removed from the FTA cards, using the five methods described

in *Materials and Methods*. STR was then performed as described. As shown in Figure 5, all of the DNA extraction methods tested yielded sufficient DNA for effective amplification and STR analysis. Profiles of samples collected with EasiCollect devices were nearly identical to profiles from whole blood or samples collected with foam swabs, as were those from buccal samples versus whole blood.

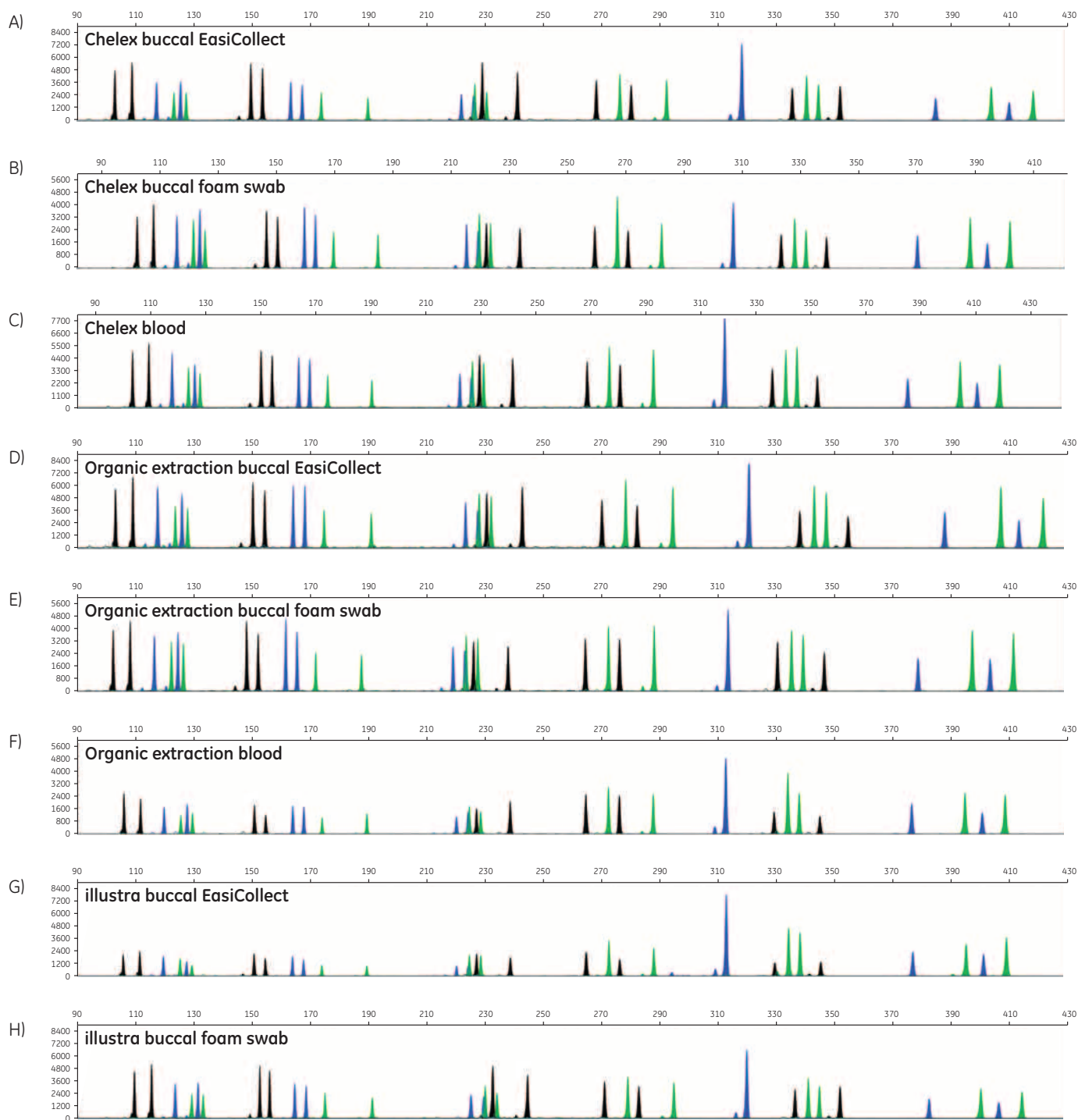


Fig 5 A-H. Figure continues on next page.

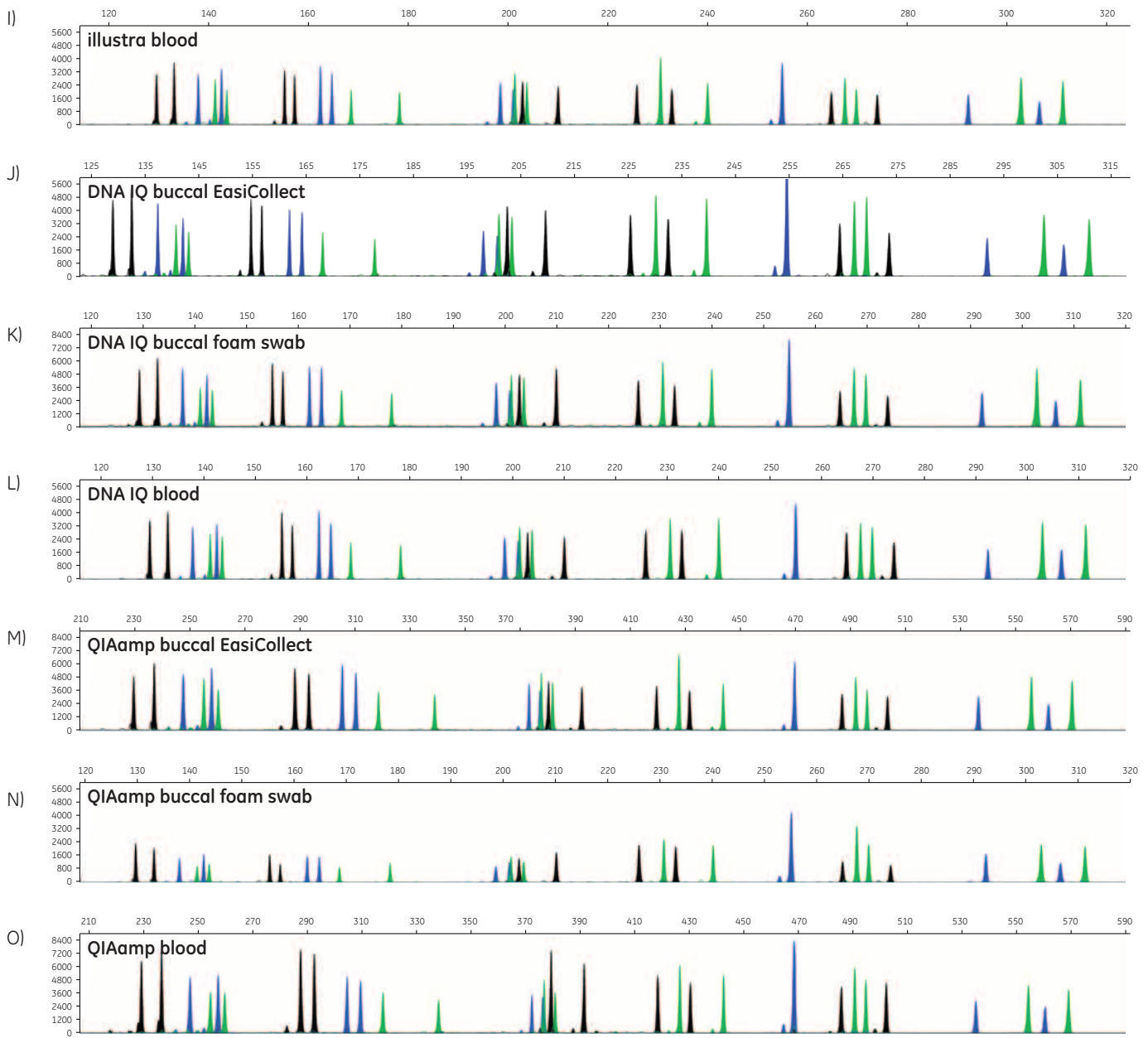


Fig 5 I-O. STR profiles of DNA extracted from FTA cards containing blood or buccal samples from Test Subject 1.

STR data were generated to determine the accuracy of allele calls for DNA extracted from FTA cards. For each DNA extraction method, duplicates of DNA from FTA cards were run for three blood samples, three buccal samples collected using EasiCollect devices, and three buccal samples collected using separate foam swabs, resulting in a total of 18 samples per DNA extraction method. Note that the three samples were from three test subjects. Of the 90 STR profiles collected, 87 samples provided quality data with accurate allele calls on the first attempt; three samples required a reinjection for 100% accurate allele calls. These data illustrate that commonly-used DNA extraction methods and kits provide DNA of sufficient quantity and quality to support allele call accuracy as high as 100% in STR analysis.

Table 1. STR data generated from 90 samples

	Chelex	Organic extraction	illustra	DNA IQ	QIAamp
Total samples	18	18	18	18	18
Total possible allele calls	288	288	288	288	288
Total correct allele calls	288	288*	288	288†	288
% accuracy	100%	100%*	100%	100% †	100%

* After two sample reinjections due to failed capillaries

† After one sample reinjection due to failed capillary

Conclusions

This study shows that five common DNA extraction methods yielded enough DNA from FTA cards for downstream quantitative analysis. This DNA was of sufficient quality for determining human identity via STR profiling.

In addition, collection of buccal cells using the EasiCollect device often resulted in a higher yield of DNA than collection using a foam swab. These data demonstrate that the EasiCollect device is compatible with current validated laboratory protocols for DNA extraction.

References

1. Baechtel, F. S. The extraction, purification and quantification of DNA, in *Proceedings of the International Symposium on the Forensic Aspects of DNA Analysis*, U.S. Government Printing Office, Washington, D.C., pp. 25–28 (1989).
2. Bustin S. A. *et al.* The MIQE Guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* **55**, 611–622 (2009).

Ordering Information

FTA cards and Indicating FTA cards

Product	Quantity (cards/pack)	Sample areas/card	Maximum volume/sample area (µl)	Maximum total volume/card (µl)	Code number
FTA Classic Card	100	4	125	500	WB120205
Indicating FTA Classic Card ¹	100	4	125	500	WB120206
FTA Mini Card	100	2	125	250	WB120055
Indicating FTA Mini Card ¹	100	2	125	250	WB120056
FTA Micro Card	100	1	125	125	WB120210
Indicating FTA Micro Card ¹	100	1	125	125	WB120211
FTA Gene Card ²	100	3	75	225	WB120208
PlantSaver™ Card ³	100	4	25	100	WB120065
CloneSaver™ Card	5	96	5	480	WB120028
EasiCollect	50	1	125	125	WB120462
FTA Elute Micro Card	100	4	30	120	WB120410
FTA Elute Micro Card	25	4	30	120	WB120401

¹ Indicating FTA Cards change color from pink to white when sample is applied. They are recommended for use with clear samples

² FTA Gene Cards are compatible for use with automated liquid sampling systems when used with FTA Gene Card Trays (see below)

³ Plant homogenate; 10 mg/50 µl PBS

Reagents, Kits, and Accessories

Product	Quantity	Code number
illustra tissue and cells genomicPrep Mini Spin Kit	50 preps	28-9042-75
	250 preps	28-9042-76
FTA Purification Reagent	500 ml	WB120204
FTA Kit	1	WB120067
Sterile Foam Tipped Applicator Swabs	100	WB100032
Desiccant (1 g)	1000	WB100003
Multi-Barrier Pouch, Small (for Micro Cards)	100	WB100036
Multi-Barrier Pouch, Large (for Classic Cards)	100	WB100037
Harris Micro Punch 1.2 mm (with Mat)	1	WB100005
Harris Uni-Core Disposable 1.25 mm Punches (with 2 Mats)	4	WB100028
Harris Micro Punch 2.0 mm (with Mat)	1	WB100007
Harris Uni-Core Disposable 2.0 mm Punches (with 2 Mats)	4	WB100029
Harris Micro Punch 3.0 mm (with Mat)	1	WB100038
Harris Uni-Core Disposable 3.0 mm Punches (with 2 Mats)	4	WB100039
Harris Uni-Core Disposable 6.0 mm Punches (with 2 Mats)	4	WB100040
Replacement Cutting Mat	1	WB100020
Replacement Tip 1.2 mm	1	WB100006
Replacement Tip 2.0 mm	1	WB100008
Replacement Tip 3.0 mm	1	WB100042

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