DNA elution from buccal cells stored on Whatman FTA Classic Cards using a modified methanol fixation method

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We describe here a method for DNA elution from buccal cells and whole blood both collected onto Whatman FTA technology, using methanol fixation followed by an elution PCR program. Extracted DNA is comparable in quality to published Whatman FTA protocols, as judged by PCR-based genotyping. Elution of DNA from the dried sample is a known rate-limiting step in the published Whatman FTA protocol; this method enables the use of each 3-mm punch of sample for several PCR reactions instead of the standard, one PCR reaction per sample punch. This optimized protocol therefore extends the usefulness and cost effectiveness of each buccal swab sample collected, when used for nucleic acid PCR and genotyping.

Whatman FTA Classic Cards (Cat. no. WB120205; Whatman International Ltd, Piscataway, NJ, USA) combined with sterile foam tipped applicators (Cat. no. WB100032) are used to painlessly collect loose inner-cheek (buccal) cells, including those that are floating in saliva. FTA technology prevents the degradation of genomic DNA at room temperature, and PCR amplification of viable DNA after long-term archiving is possible (1). For analysis, a small disc is punched from the dried sample area.

Whatman standard protocols require the disc to be washed in FTA purification reagent (Cat. no. WB120204), dried, and the entire sample disc then incorporated directly into the PCR amplification reaction. The protocols do not adjust reaction volume or PCR conditions to the presence of the disc (www.whatman.com/References/51613rerevised.pdf).

It has been found that the use of saliva samples is a good alternative to blood samples (2) to obtain genomic DNA of high quality, and that their use increases the response rate considerably in epidemiologic studies (3). Theoretically, buccal cells have the potential to generate more DNA product per volume than blood. With improved stability and lack of discomfort during collection, they are becoming the sample of choice allowing easy collection in any field situation. However, the time-consuming sample punch process is a known rate-limiting step, which is exacerbated if a particular analysis requires multiple sample preparations (4) (http://microscopy.tamu.edu/lab-protocols/protocols-FTA_paper_processing.pdf). Another issue with this is the relatively fast consumption of biological sample. Methanol fixation during DNA extraction has been used previously for blood samples stored on Guthrie cards (5). Notably, the effects of different fixation techniques on chromatin ultrastructure and DNA extraction have shown cell morphology to be compromised with an increased yield of genomic DNA using a methanol-based fixative (6,7).

We describe here for the first time DNA elution from buccal cells collected on Whatman FTA Classic Cards using a modified methanol fixation method (5,8). This enables a decrease in sample processing time and an increase in the number of PCR reactions per sample punch.

This revised method was tested in comparison with the standard Whatman FTA protocol for both buccal cells on Whatman FTA Classic Cards (stored for 0–1 years) and blood spots (stored for 2–5 years) on 1-mm Whatman BFC 180 filter paper blood sample collection cards (Figure 1). Participants were sourced from a study approved by the University of Queensland Medical Research Ethics Committee in accordance with the National Health and Medical Research Council's guidelines.

Sample collection. A DNA self-collection kit was designed consisting of a Whatman FTA Classic Card individually sealed in a 9 × 15-cm transparent plastic zipper storage bag (Cat. no. 7233; Glad Products Australia, Padstow, NSW, Australia) and then placed into a sealed envelope with a single Whatman sterile foam-tipped applicator. Participants collected the sample as directed [http://www.whatman.com/References/BUCCAL(1).pdf] and all samples were dried, resealed in the plastic bag, then stored at room temperature.

Sample preparation. A single 3-mm diameter circle punch was obtained from each sample using a manual plier one-hole punch (Cat. no. 23517097; Fiskars Brands Inc., Madison, WI, USA) from which the confetti trap was removed (http://microscopy.tamu.edu/lab-protocols/protocols-FTA_paper_processing.pdf). The paper punch was cleaned between samples by rigorous manual wiping with 70% ethanol. It was then used to punch 3 holes from a separate clean, unused blood sample collection card, prior to performing a single sample punch. Sample spots were then processed according to either the Whatman FTA protocol, or the alternative method.

DNA elution – methanol fixation method. The sample spot was fixed by overlaying with methanol (4 drops delivered via Pasteur pipette; AJAX, Taren Pt, NSW, Australia) three times and allowed to air-dry in between (the first time at room temperature for 20 min and the next two times incubated at 37°C for 40 min each). Genomic DNA was then eluted from the paper in a heat incubation step run on a MyCycler thermal cycler (Bio-Rad, Hercules, CA, USA). Using flat nosed forceps, dry sample spots were transferred to a thin-walled 0.2-mL PCR tube containing 5 µL 10× PCR buffer (Invitrogen, Mt Waverley, VIC, Australia) and 45 µL water. The tube was subjected to one cycle of 60°C for 30 min, 99.9°C

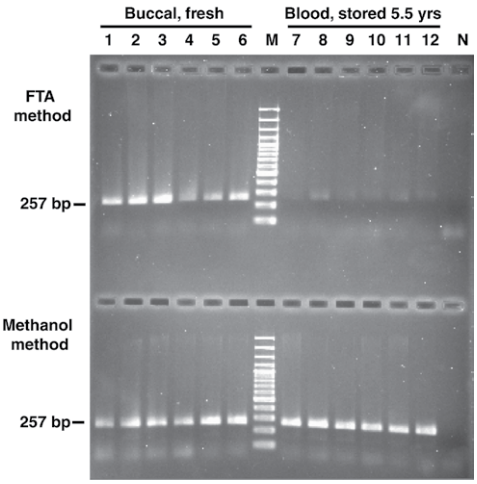

Figure 1. Comparative PCR amplification of *TYR* exon 4 using DNA extracted from dried buccal and blood spots via the Whatman FTA protocol and the methanol fixation method. PCR conditions: Total reaction volume 25 µL containing 2 µL human genomic DNA template (2 µL eluant or 3-mm treated sample disc), 0.25 µM both Tyr4f and Tyr4r primers (9), 200 µM each dNTP (Bioline, Alexandria, NSW, Australia), 1× PCR buffer (Invitrogen), 2.0 mM $MgCl_2$ (Invitrogen) and 1 U *Taq* polymerase (Invitrogen). PCR products visualized on 1.5% agarose gel (Quantum Scientific Probiogen Biochemicals, Murarrie, QLD, Australia) in 1× Tris-acetate-EDTA (TAE) and stained with ethidium bromide (Sigma-Aldrich, Sydney, NSW, Australia). Lanes 1–12, samples from multiple subjects; Lane N, no DNA control; Lane M, molecular size marker (GeneRuler 100-bp DNA Ladder Plus; Quantum Scientific Fermentas, Murarrie, QLD, Australia). Buccal spots were <1 month old and fresh-collected onto Whatman FTA Classic Cards. Blood spots were stored for 5.5 yrs on 1-mm Whatman BFC 180 filter paper.

for 10 min, then cooled to 4°C. The FTA paper was left in the tube with the eluant and stored at 4°C. PCR reactions were performed using 2 µL extracted product.

The quality of genomic DNA eluted after methanol fixation was determined through PCR amplification and sequencing of human *TYR* gene exons 1–5 (fragment range overall was 106–257 bp in length). These fragments were amplified using primer pairs Tyr1.1, Tyr1.2, Tyr2, Tyr3, Tyr4, Tyr5 and the protocol as published (9) (Figure 1 and data not shown). We did not attempt to sequence the weak PCR fragments from blood samples purified via the FTA protocol: in our experience, yields of this level are routinely unable to be sequenced.

We have used the methanol method to extract DNA from blood spots on neonatal cards since 2003 and from buccal spots on FTA Classic Cards since 2007. Inclusion of samples from multiple family groups has consistently shown expected Mendelian inheritance patterns. Best results were achieved when the sample spot was completely dried before the thermal elution step.

This comparative study demonstrates that amplimers of expected size were able to be detected via both DNA extraction methods for buccal and blood samples collected on each of the two types of Whatman paper media (Figure 1). However, the methanol method was consistent for each biological sample and the FTA method showed a marked decrease in yield for the blood spots on BFC 180 filter paper (hemolysed reaction noted). Both methods tested for buccal cells yielded equal quantities of DNA per PCR reaction. Readable sequence for the region of interest was able to be generated from DNA extracted from both buccal and blood samples via the methanol elution method (Figure 2).

We suggest the following alternative applications that add to the benefit of the methanol method for blood spots and hair bulbs respectively, which have been successfully used by V.H. (data not shown).

Immediate methanol fixation. Fix the entire collection card with methanol immediately following collection in the field and air-dry. Upon returning to the laboratory, fix the entire card once more by soaking in methanol. This could potentially increase the shelf life of the sample and will also enable the fixation step to be skipped when preparing sample spots, thus decreasing processing time even further.

Proteinase K step. To potentially increase the DNA yield even further, add Proteinase K (50 µg/mL; Roche, Castle Hill, NSW, Australia) to the thermal incubation elution step. The Proteinase K would be destroyed in the 99.9°C incubation step.

The methanol DNA elution method provides many advantages. The buccal cell DNA entrapped on the Whatman FTA card can be eluted from each sample spot, enabling as little as 2 µL of product to be used per PCR reaction instead of

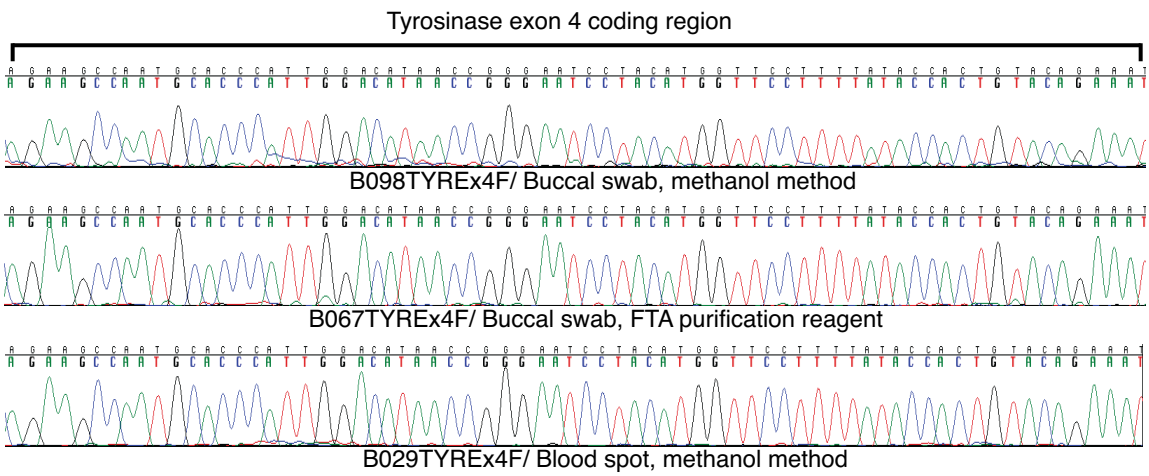

Figure 2. *TYR* exon 4 coding region sequence of 3 separate subjects. Displayed are the comparative results for DNA from dried buccal sample stored on Whatman FTA Classic Cards extracted via the methanol DNA elution method (top) and using FTA purification reagent (middle). Also shown is dried blood stored on 1-mm Whatman BFC 180 filter paper sample collection cards, extracted via the methanol DNA elution method (bottom). PCR product was separated by agarose gel electrophoresis and purified using the QIAEXII Gel Extraction Kit (QIAGEN, Doncaster, VIC, Australia). Sequence was generated using AB BigDye version 3.1 automated cycle sequencing (AGRF, Brisbane, QLD, Australia). Sequences were edited using Sequencher 4.2 software (Gene Codes Corporation, Ann Arbor, MI, USA).

the entire sample disc (equal to a 25-fold reaction increase). The method consistently yields DNA of adequate quantity and quality to be successfully used in PCR amplification and automated sequencing. Results are comparable with those found in the Whatman FTA protocol. The methanol method increases the amount of PCR reactions able to be processed per sample punch without compromising result output.

The simplicity of this method combined with overall time and cost saving extends the life of the biological sample and increases the beneficial value of the Whatman FTA card system.

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The authors declare no competing interests.

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