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Repeated extraction of DNA from FTA cards

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ABSTRACT

Extraction of DNA using magnetic bead based techniques on automated DNA extraction instruments provides a fast, reliable and reproducible method for DNA extraction from various matrices. However, the yield of extracted DNA from FTA-cards is typically low. Here, we demonstrate that it is possible to repeatedly extract DNA from the processed FTA-disk. The method increases the yield from the nanogram range to the microgram range.

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1. Introduction

Biological material (e.g. blood or buccal cells) may be collected on FTA-cards. STR typing may be performed directly on a small disk of the FTA-card [1] or after extraction of DNA from the disk. Here, we demonstrate that it is possible to repeatedly extract DNA from the processed FTA-disk and obtain micrograms of DNA.

2. Materials and methods

2.1. Samples

Buccal cells were isolated with a sterile foam-tipped applicator (GE Healthcare) and applied to indicating FTA-cards (GE Healthcare). Fingertip blood was isolated with a Tenderlett® finger incision device (1.75 mm, ITC) and applying two drops of blood to an FTA-card. For EDTA blood samples, 200 μL of blood was manually transferred to FTA-cards. The FTA-cards were dried over night at room temperature. A 1 cm² piece of the card containing biological material was manually isolated with a sterile scalpel and placed in a 2 mL sample tube (Eppendorf).

2.2. Pre-treatment and DNA extraction on the QIAsymphony SP

The pre-treatment protocol recommended by Qiagen was compared to an in-house optimized protocol. Samples pre-treated using the Qiagen recommended pre-treatment were added 180 μL of buffer ATL and 20 μL proteinase K. Both part of the QIAsymphony DNA Investigator kit (Qiagen). Following vortexing on an IKA Vibrax® VXR shaker (IKA® Works), samples were incubated for 15 min at 56 °C in shaking incubators (VorTemp 56, Labnet International, Inc.). Using a manual plunger operated pipette, the lysate was transferred to new sample tubes, loaded onto the QIAsymphony SP (Qiagen). Samples pre-treated using an in-house optimized pre-treatment protocol were added 190 μL 0.5× ATL buffer and 10 μL proteinase K. Samples were incubated for 15 min at 56 °C in a heating block (Techne Dri-Block, DB-3D) interrupted by vortexing every 5 min followed by 5 min at 95 °C. After vortexing, sample tubes were loaded onto the QIAsymphony SP leaving the pieces of FTA-cards in the tubes.

Samples were extracted using the ACS_REF_200 protocol. DNA was eluted in 100 μL water, which was included in the kit in semi-skirted 96-well twin.tec plates (Eppendorf). Sample tubes containing the pieces of FTA-cards were subjected to a new round of pretreatment and extraction procedure or stored in the sample tube for 1 year at 4 °C.

2.3. DNA extraction using manual Chelex®

Sample tubes containing pieces of FTA-cards were added 1 mL autoclaved MilliQ water, vortexed and incubated at room temperature for 15 min interrupted by vortexing every 5 min. Following centrifugation in a bench-top centrifuge (5417C, Eppendorf) at 11,000 RCF for 2 min, the supernatant was removed leaving 30 μL in the tubes. To each tube 20% Chelex® solution pH > 10.5 (Chelex® 100 Resin, 100–200 mesh Na-form, BIO-RAD

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Laboratories) was added to a final volume of 200 μL. The Chelex® particles were prevented from sedimenting by continuous stirring on a magnetic stirrer (Variomag, Compact HP1). Subsequently, 2 μL of a 10 mg/mL solution of proteinase K (Qiagen) was added to the samples. Following vortexing on an IKA Vibrax® VXR shaker, the samples were incubated for 1 h at 56 °C in a heating block (Techne Dri-Block, DB-3D). The samples were vigorously mixed for 20 s and incubated for 20 min at 100 °C, vigorously mixed for 20 s and centrifuged at 11,000 RCF for 1 min. The supernatant and the pieces of FTA-card were manually transferred to a new sample tubes leaving the Chelex beads in the tubes. Sample tubes containing the pieces of FTA-card were subjected to another round of extraction. Supernatant were stored at –20 °C. The process was repeated a total of 6 times for each piece of FTA-card.

2.4. DNA quantification, PCR and capillary electrophoresis

Extracted DNA was quantified using the Quantifiler® Human DNA Quantification Kit (Applied Biosystems (AB)) and an AB 7900HT. All samples were quantified in triplicate.

AmpFISTR® Identifier® PCR Amplification Kit PCR master mix (AB) in a total reaction volume of 10 μL. The PCR was prepared by combining 2 μL of the eluted DNA with 8 μL of the PCR master mix. PCR setup was accomplished with a Biomek® 3000 (Beckman Coulter (BC)) laboratory-automated workstation, using an in-house method. PCR with 27 cycles was performed in 96-well microtiter plates (Eppendorf) on Gold-plated 96-well GeneAmp® PCR systems 9700 (AB).

A total of 1.5 μL PCR product was combined with 15 μL formamide (AB) and size standards (AB) in a 96-well electrophoresis plate (Axxygen Scientific Inc.) on a Biomek® 3000 using an in-house method. Capillary electrophoresis was performed on ABI 3130xl. Genetic Analyzers (AB). Results were analyzed using Genescan Analysis version 3.7 and Genotyper version 3.7 (AB).

3. Results and discussion

Three different protocols were compared. The QIAsymphony with the in-house optimized pre-treatment, a manual Chelex protocol and the QIAsymphony with the Qiagen recommended pre-treatment. The protocols were evaluated using three, one and two sample types, respectively. Each sample type was evaluated using 8 different samples. Repeated extractions of pieces of FTA-cards indicated that the DNA bound to the FTA membrane was not eluted in the first extraction. The results also indicated significant differences between sample types and extraction methods (Fig. 1A). Combining the DNA extracts (Fig. 1B) obtained from the six rounds of extractions may increase the yield from the nanogram range to the microgram range.

A total of 8 FTA-cards were subjected to one round of DNA extraction followed by storage in the sample tube for 1 year at 4 °C. The results also demonstrated that FTA-card pieces containing buccal samples may be archived for a prolonged period and used for another round of extraction if the initial extraction procedure fails. Re-extraction of FTA-pieces containing buccal samples may be archived for a prolonged period and used for another round of extraction if the initial extraction procedure fails. Re-extraction of FTA-card pieces following long term storage did not affect the quality of the DNA profile (data not shown).

4. Conclusion

Extraction of DNA from FTA-cards with buccal cells or blood was possible using a QIAsymphony. Blood resulted in higher yields than buccal samples. For blood samples, the in-house pre-treatment resulted in higher yields than the Qiagen recommended protocol. None of the methods isolated the entire amount of bound DNA in the first extraction round.

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None.

Conflict of interest

None.

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Reference