Applications

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Automated PCR of buccal-swab FTA cards in a 96-well format using *forensicGEM*™ and the Liquid-Handling Workstation epMotion® 5075 MC

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Abstract

A rapid, simple and fully automated, DNA extraction and PCR based DNA forensic profiling has been demonstrated on the epMotion 5075 MC. The 35 min procedure, extracts DNA from FTA card punches using a proprietary heat inactivated protease *(forensicGEM)*. The PCR set up, plate loading, sealing and PCR protocol was also fully automated on the epMotion 5075 MC (liquid handler with a fully integrated thermal cycler). The resultant Identifiler Profiles of DNA were run on an ABI 3730 sequencer. Importantly we were able to demonstrate that there was no detectable contamination of samples from well to well. The results demonstrate that the epMotion 5075 MC is ideally suited to automating DNA extraction from FTA cards using the *forensicGEM* enzyme.

Introduction

An expanding area in forensic DNA profiling involves databases of reference DNA profiles from known individuals. These profiles can be compared against others from crimes with no known suspect [1]. Correspondence between a sample and the database, allows linkage of an individual to a crime-scene and this may lead to a conviction. Reference DNA databases are usually compiled from past offenders or suspects (depending on jurisdictional legislation) and most often use buccal swabs to obtain reference profiles. Buccal swabs are usually then transferred to FTA® cards (Whatman) for long-term storage.

FTA® products contain protein denaturants, chelating agents and a free radical trap designed to 'protect and entrap nucleic acids' [2]. The DNA in the sample binds to the substrate whilst other material in the sample (inhibitors such as heme) are removed by a series of washing steps.

Commonly, the DNA extraction procedures used for FTA cards are time-consuming and involve repeated sample transfers. Typical methods use Chelex®-based protocols and require boiling and centrifugation steps, which are difficult to automate. Furthermore, such steps expose the sample to potential contamination – a feature that makes them unattractive for forensic biology. Alternative strategies have been devised, but generally, these are expensive when used in high-throughput (for example spin columns or magnetic beads).

The relative uniformity and predictability of FTA® samples lend them to processing with automated procedures. With automation, throughput can be increased, contamination can be minimised and laboratory staff can be freed for other purposes.

In this article, we describe a new method that uses forensicGEM™ (ZyGEM Corporation Ltd). This is a thermophilic proteinase with characteristics making it ideal for forensic DNA extraction [3].

The method is rapid, reliable and because of its simplicity, is suited for robotic automation using an epMotion® Liquid-Handling Workstation.

Methods

CHELEX® Extraction

1. 5 x 1.2 mm punches were taken from buccal-swab FTA cards and placed into an Eppendorf Safe-Lock tube.
2. 1 ml of water was added and the samples left on bench-top for 30 minutes.
3. Samples were centrifuged at 13,000 x g for 3 minutes.
4. The supernatant was removed and 170 µL of 20% Chelex® added.
5. 20 µl of 20 mg / ml proteinase K added.
6. Samples were incubated at 55°C for 20 minutes.
7. Samples were mixed gently and then boiled for 8 minutes.
8. Samples were vortexed for 30 seconds.
9. Samples centrifuged at 13,000 x g for 3 minutes.

Time: Approximately 70 minutes.
Technician Time: 10 minutes throughout.

ForensicGEM™ Extraction

1. 1 x 1.2 mm punches were taken from buccal-swab FTA cards and placed into Eppendorf Safe-Lock tube.
2. 100 µL of 20 mM HEPES (pH 7.7 at room temp = pH 7 at 75°C) and 1 U of *forensicGEM* were added.
3. Samples were incubated at 75°C for 15 minutes.
4. Samples were incubated at 95°C for 15 minutes.

Time: Approximately 35 min.
Technician Time: 4 min initially.

epMotion Extraction and PCR

The worktable of the epMotion 5075 MC was equipped as on Fig. 1.

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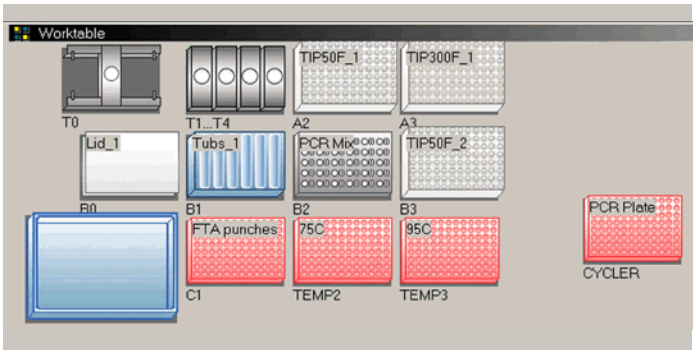


Figure 1: worktable set-up (Screenshot from the epMotion® Editor)
Pos T0: Gripper tool
Pos A2 and B3: epT.I.P.S. 1 – 50 µl
Pos A3: epT.I.P.S. 20 – 300 µl
Pos B0: CycleLock
Pos B1: 30 ml Reservoir
Pos B2: Thermorack for 24 Safe-Lock Eppendorf tubes®
Pos C1 and CYCLER: twin.tec PCR plate 96, skirted.
Pos TEMP2 and TEMP3: Peltier blocks.

The twin.tec plate 96-well plated at position C1 contained within each well a single 1.2 mm punch from a FTA® card. These were arranged in a checkerboard pattern to test for cross-contamination between wells (Fig. 2).
The thermoracks were pre-heated to the required temperatures. Using the multichannel pipettor, 100 µl of the extraction solution was added to each well of the PCR plate at position C1.

The plate was then transported sequentially to the 75°C and then to the 95°C Thermoracks with incubations on each for 15 minutes. On completion, the plate was transferred back to position C1.

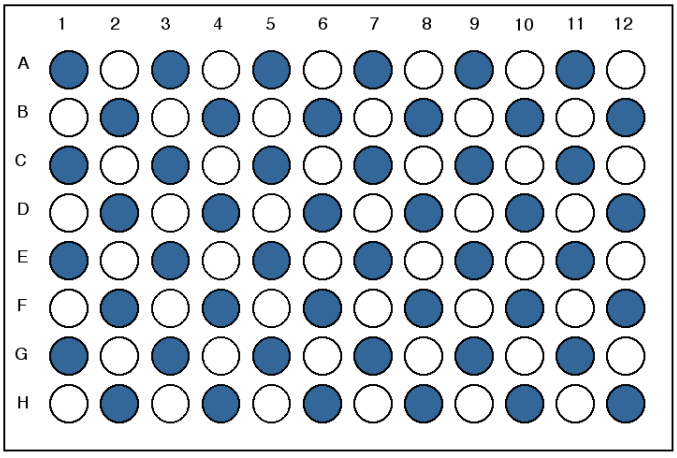


Figure 2: Distribution of FTA card punches in the 96 well PCR plate at position C1.

23 µl of PCR mastermix was pipetted into a 96-well plate on the thermal cycler and 2 µl of the extracted DNA pipetted into corresponding wells.
For this experiment, PCR primers were used to amplify a human nuclear gene. The primer sequences were as follows:
Forward: TTCACCACCATGGAGAAGGCTGGG
Reverse: GTCCACCACCCTGTTGCTGTAGCC
The primers are designed to amplify a 650 base pair fragment of the human gene encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH).
A CycleLock lid was transported to the thermal cycler and a PCR was performed with cycling as follows:
94°C - 4 min; 94°C-30 s, 59°C–30 s, 72°C–60 s x 30;
72°C 5 min.

Results

5 µl of the PCR product was resolved by gel electrophoresis (Fig. 3).

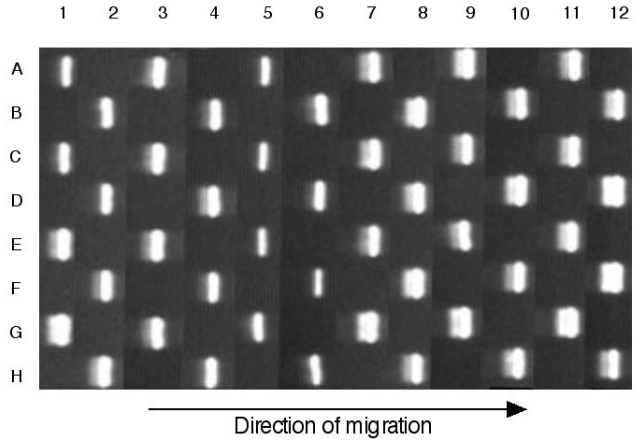
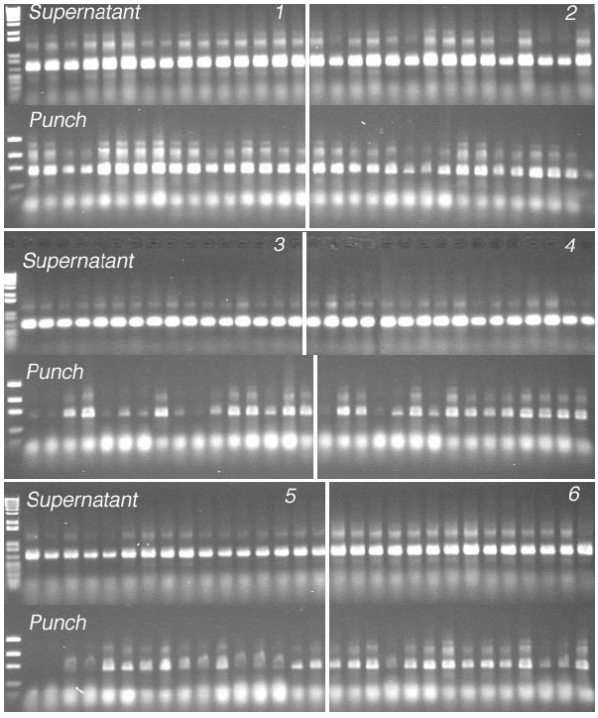


Figure 3: Composite picture of the PCR products as visualised by agarose gel electrophoresis. The gel strips are arranged to correspond to the wells of the PCR plate shown in Figure 2.

The acid-test for any forensic DNA extraction is the ability of the DNA to produce reliable STR profiles using industry-standard STR kits.
The yield of DNA extraction from FTA cards is dependent on a number of factors: (i) the individual from whom the sample is taken (ii) the technique used in the sampling and (iii) the uneven distribution of epithelial cells on the FTA card. The prohibitive cost of DNA profiling means that few forensic laboratories can afford a high failure rate. Consequently, quantification is necessary prior to the profiling reaction.
DNA extracts were produced in a second experiment using 92 of the wells in a 96-well microtitre dish (with four negative controls). Again, the reactions were performed using an epMotion 5075 MC. DNA yields were quantified using the QuantiBlot system (Applied Biosystems), and GAPDH PCR's were carried out to indicate whether the DNA was amplifiable (Fig. 4).



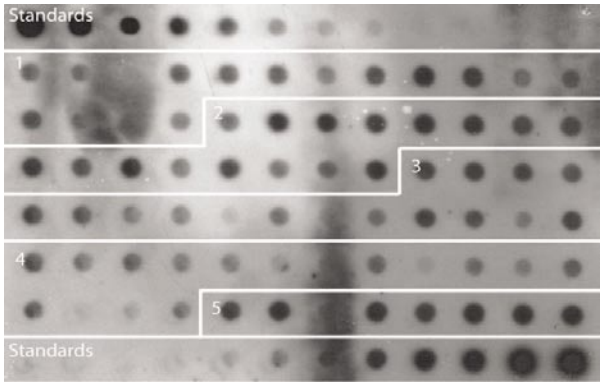


Figure 4: Left: GAPDH PCR products from 92 extractions performed on the epMotion 5075 MC workstation. Top: Quantification using QuantiBlot of 72 of the samples. Top and bottom rows are the standards.

A random selection of the extracts were subjected to a full forensic profile using the AmpFISTR® Identifiler™ PCR Amplification Kit (Applied Biosystems). The profile from one FTA card is shown in Fig. 5 alongside a profile of the same DNA using the standard CHELEX® method.

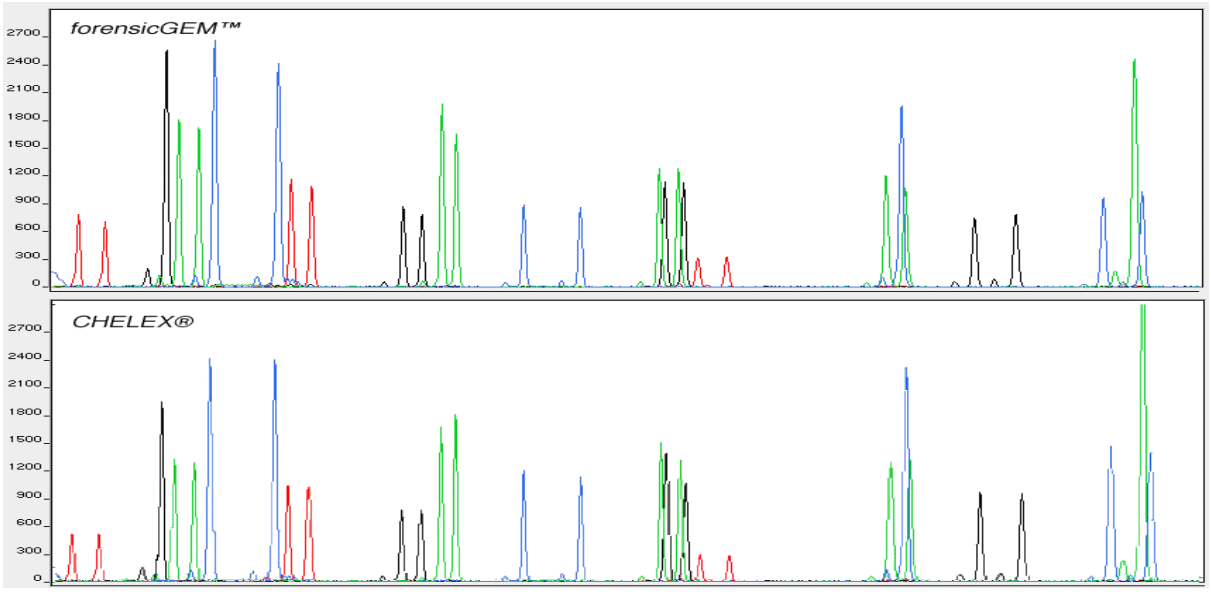


Fig. 5: Identifiler Profiles of DNA extracted buccal swab FTA punches by two different methods.
Top panel: DNA extracted manually using the current best-practise CHELEX method.
Bottom Panel: DNA extracted using an epMotion 5075 Liquid-Handling Workstation and the new *forensicGEM™* procedure.

Discussion

The results demonstrate that the method using *forensicGEM™* is ideally suited for the automation of DNA extraction from FTA cards. Furthermore, the non-contact dispensing of the epMotion Workstation makes it ideal for forensic applications; the epMotion is capable of pipetting in an 96-well format with no detectable cross contamination between wells.
The time taken for the new extraction process is approximately 35 minutes with only 4-5 minutes of technician time required initially to prepare the reactions. This time is highly favourable when compared with the manual method which typically takes 70 minutes and the technician is required throughout the process. The brief and concentrated technician time with the new method means that the new procedure when exectuted on an epMotion workstation, allows a technician to parallel-process many samples simultaneously.
The epMotion with its accuracy down to 1 µl, has allowed us to reliably set-up PCR's using only 2 µl of template in a 25 µl polymerase chain reaction. All wells containing a FTA punch produced sufficient DNA for the amplification of a low-copy human gene.

Quantification is not possible with a simple PCR (the geometric amplification process tends towards the normalisation of product yields) but the Quantiblot results highlight the level of variation typical for FTA punches from buccal swabs. The results emphasize the need for accurate quantification of template prior to the more expensive steps of DNA profiling. This necessity can be accomodated on the epMotion with a new import function on PC editor software which modifies reagent volumes on the basis of individual need. This research used an epMotion 5075 MC with Peltier plates installed in two locations and a thermal cycler. The chemistry of *forensicGEM™* is ideally suited for this configuration. However, the method can be easily adapted to simpler models of Eppendorf robotic workstations (e.g. epMotion 5075 LH); the two-temperature incubation can be programmed into any thermal cycler.

The key significance of the results is that a bottleneck in high-throughput processing of forensic samples can be automated by using the new method and an Eppendorf epMotion workstation.

References

[1] Ruitberg, C M, Reeder, D J and Butler, J M, STRBase: A Short Tandem Repeat DNA Database for the Human Identity Testing Community. Nucleic Acids Research, 2001. 29(1): 320-322.

[2] Whatman, B, FTA Technology: Collect, Transport, Archive and Purify Nucleic Acids (User's Manual). 2001.

[3] Moss D, Harbison S-A and Saul D. J (2003) An easily-automated, closed-tube forensic DNA extraction using a thermostable proteinase. Int. J. Legal. Med 117:340-349

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