Robotic DNA extraction system as a new way to process sweat traces rapidly and efficiently

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Abstract. A modified DNA IQ ™ System (Promega Corporation, USA) was used on a variety of exhibits collected at different crime scenes, potentially interested by sweat traces and analysed by means of a fully automated extraction (MultiProbe II Plus EX by Perkin Elmer LAS, USA). As is well known, sweat can be soaked into large portions of fabrics and clothes; as a result, preliminary treatment steps are needed to isolate the few cells that remain in each sample. The main goal of this application was to establish a dedicated robotic extraction to manage large volumes of lysis buffer, to facilitate processing of large amounts of evidence. Using an initial volume of 10–15 ml, between 500 and 2000 pg of purified DNA was recovered, thus allowing us to obtain full STR profiles, saving time, improving the rate of success on LCN samples and reducing the risk of possible contamination. © 2005 Published by Elsevier B.V.

Keywords: Automation; DNA extraction; Magnetic bead

1. Introduction

DNA extraction efficiency with silica chemistry on magnetic beads is well documented [1,2]. The protocol procedures are completely independent of starting volume; hence, one can consider an automated procedure able to manage several millilitres of lysis buffer. With sweat on absorbent fabrics, the number of residual cells potentially present on such material is extremely low and usually found in the lower layers of the fabric too, unless one processes a larger surface area. The main problem is the casting of a magnetic support for several samples and the relative tubes used for samples (the appropriate deep of well

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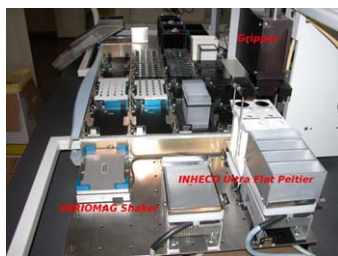
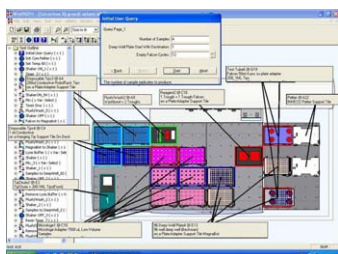Fig. 1. View of MultiProbeII arranged for large volume extraction.

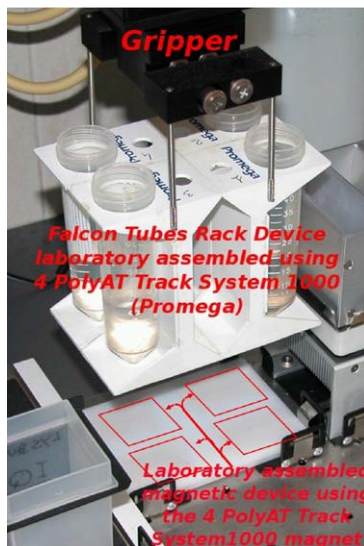Fig. 3. Software overview window of assembled labware.

Fig. 2. Large volume extraction device.

plates). The main parameters that need to be established are incubation times, shaking speed, number of wash steps and final recovery extraction volume.

2. Materials and methods

Some manual steps are needed to transfer the residual cells present on the fabric into the solution, for example understanding the area of interest by latent biological fluids and subsequently cutting in small pieces the relative tissues. A lysis step was performed in a 50-ml falcon tube using a starting volume of lysis buffer between 10 and 25 ml, depending upon the fabric thickness and width (for example: jacket collar – case A; and cuff – case B) or expected area of material which came in contact with the forehead (for the wool hats – case C). Lysis incubation was performed overnight adding dithiothritol and proteinase K as recommended in the IQ protocol. After removal of the cut fabrics, the tubes were processed modifying the format of the PolyATract® System 1000 Magnetic Separation

Fig. 4. Evidence A.

Fig. 5. Evidence B.

Fig. 6. Evidence C.

Table 1
DNA quantification and quality of obtained profile

Case	QuantiBlot® (pg/μl)	Quantifiler® (pg/μl)	Well-typed loci	RFU range
A	118	–	15	100–800
B	–	155	15	400–1600
C	320	–	15	1200–3000

Stand (Promega) for a rack suitable for four 50-ml falcon tubes and for the MultiProbe II Gripper Arm (Figs. 1 and 2 – MultiProbe II Plus EX by Perkin Elmer LAS, USA). The automated workstation offers the user a predefined procedure, which only requires the user to input the width and volume to process, variable from one case to another (Fig. 3 – WinPrep® v. 1.20.0238 Perkin Elmer LAS). A 3-min incubation step on the magnetic support followed by a shaking step (at 800 rpm) using a shaker specifically designed for automation labware (Variomag® Teleshake) and three additional wash steps were needed. Final elution was performed in 50 μl of penta-deionised water.

Quantification was performed by 'Slot-Blot' procedure using QuantiBlot® kit (ABD), or alternatively Real-time PCR was performed using Quantifiler™ Human DNA Quantification kit (ABD). Genotyping was performed by PCR using Identifiler kit (ABD) according to the original protocols. PCR fragments were separated with capillary electrophoresis using ABI Prism 3100 Automatic Sequencer (ABD) and the size call allele was done by GeneMapper v. 3.2 (ABD).

3. Results and discussion

The evidence and sampled fabric areas are shown in Figs. 4–6 and the quantification of the IQ DNA extraction as a yield of successful 'full profile typing' is shown in Table 1.

In conclusion, the results demonstrate that an almost fully automated DNA extraction from difficult sweat traces is possible.

As more and more forensic applications become well known, especially by those committing crimes, finding biological evidence at crime scenes becomes more difficult for the investigators. Sweat or perspiration is a colourless and odourless form of evidence, that even the most intelligent criminal cannot avoid leaving at the crime scene. Recent experience shows that the demands for sweat DNA analysis has grown significantly in the last 2 years and, despite the time demands, such analysis is being requested more and more to help resolve serious crimes. Automation of sweat DNA extraction is the only way to allow larger sampling of this kind of evidence and further optimization of chemistries and liquid handling automation are in progress in order to better achieve this strategic objective.

References

[1] S.A. Greenspoon, et al., J. Forensic Sci. 49 (1) (2004) 3179–3491.
[2] D.I. Komonski, et al., Can. Soc. Forensic Sci. J. 37 (2) (2004) 103–109.