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Automating PrepFiler® forensic DNA extraction kit: Optimization and validation on Freedom EVO® 150

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ABSTRACT

To handle the growing number of forensic casework samples we decided to automate our trace sample workflow using PrepFiler® a magnetic bead based DNA extraction kit, on a Tecan liquid handling workstation Freedom EVO® 150. Even though there was a manufacturer-supplied validated protocol for the automated PrepFiler® DNA extraction, substantial changes were needed in order to obtain the optimal DNA yield and elution volume to meet the needs of our laboratory for further downstream applications. In this study, optimal lysis condition, elution volume and elution temperature were determined for the automated PrepFiler® extraction using blood samples on cotton swabs. Additionally, a comparison with the manual in-house method Chelex-100® was made using a variety of forensic mock samples on swabs. We tested blood, saliva and contact traces from a variety of substrates, with 10 replicates of each type. With the automated PrepFiler® system, the DNA yield obtained was around 30–70% lower than with Chelex-100®. However, the purity of the extracts was higher. Allele recovery of the STR profiles obtained with AmpFlSTR® NGM SElect™ kit was comparable. Furthermore we found that, besides the kit-specific optimization factors, swab characteristics are of major importance for the success of PrepFiler® DNA extraction. We incorporated the optimized PrepFiler® protocol on Freedom EVO® 150 into our routine workflow and included quality controls to maintain contamination-free and efficient DNA extraction with no evidence for loss of magnetic beads.

1. Introduction

The majority of the casework samples received in our laboratory are contact stains collected on swabs. To enhance the throughput of these samples, we decided to automate our extraction method for stains using PrepFiler®, a magnetic bead based DNA extraction kit, on a Tecan liquid handling workstation Freedom EVO® 150. Using EVOware, the Freedom EVO® 150 can be programmed, allowing customization of each step of the extraction. The PrepFiler® kit was designed for extracting and purifying DNA from a variety of common forensic sample types [1]. The polymer-embedded magnetic particles are relatively small, resulting in a large surface area with high DNA binding capacity [2]. In this study, we examine the optimal lysis and elution conditions for the automated PrepFiler® DNA extraction on Freedom EVO® 150 and compare it to our manual in-house method Chelex-100®.

2. Materials and Methods

The PrepFiler® DNA extraction was installed on a Freedom EVO® 150 liquid handling workstation equipped with a robotic manipulator arm. To optimize the automated PrepFiler® extraction, 1 μl of blood on cotton swabs ($n = 19$) was extracted for each condition tested. To assess the performance of our optimized automated PrepFiler® DNA extraction protocol (70 °C o/n lysis, 11 min elution time, 71 °C elution temperature and 100 μl of elution volume), a comparison to our manual Chelex-100® protocol (1000 μl of 5% Chelex-100®, overnight incubation at 56 °C, 100 °C for 8 min, transfer into Amicon Ultra Centrifugal Tube following centrifugation at 3500 × *g* till volume reduction to approximately 100 μl, 2 ml TE buffer addition and volume reduction to 100 μl by centrifugation at 3500 × *g*) was done. Therefor 6 different kinds of forensic mock samples on swabs in 10 replicates of each type were used. The extracted DNA samples were quantified using Quantifiler® Human DNA Quantification kit (Applied Biosystems) on ABI Prism 7000 according to manufacturer's instructions. Quantified DNA from each sample was normalized and an input amount of either 200 pg or, in case of low concentration 10 μl, was amplified using AmpFlSTR® NGM

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Table 1
Comparison of average DNA yield for optimizing PrepFiler® Automated DNA extraction kit: different lysis conditions, elution volumes, elution times and elution temperatures were tested using blood samples on cotton swabs $n = 19$, respectively. *Personal communication from another lab.

	Parameters tested	Avg DNA yield (ng)	STD	Lysis conditions	Elution volumes (μl)	Elution times (min)	Elution temperatures (°C)
Elution volume	85 μl	12	±5	56 °C o/n	85	11	71
	100 μl	15	±3	56 °C o/n	100	11	71
Elution time	11 min	24.8	±8	70 °C 40 min	100	11	71
	12 min	20.2	±8	70 °C 40 min	100	12	71
Elution temperature	71 °C 11 min	14	±6.5	70 °C 40 min	100	11	71
	77 °C 20 min*	13	±4	70 °C 40 min	100	20	77
Lysis condition	70 °C o/n	14.5	±4	70 °C o/n	100	11	71
	56 °C o/n	12.5	±4	56 °C o/n	100	11	71
	70 °C 40 min	7.6	±3	70 °C 40 min	100	11	71

SElect™ PCR Amplification kit (Applied Biosystems). Capillary electrophoresis was then performed with AB 3500. The results were analyzed using GeneMapper ID-X. Subsequently, the occurrence of cross-contamination was investigated carrying out four runs of 88 samples each, containing positive and negative controls arranged in 4 different patterns.

3. Results

Optimization results of the automated PrepFiler® DNA extractions are presented in Table 1. Optimal DNA yield was obtained with an overnight lysis at 70 °C. A hundred microliter elution volume and 11 min elution time at 71 °C gave not only the best DNA yield, but also a final elution volume of around 57 μl, sufficiently high for further downstream analysis. Extracted DNA quantities and obtained peak heights resulting from each of the two methods are presented in Fig. 1A and 1B. In comparison to the manual DNA extraction procedure with Chelex-100®, the automated PrepFiler® extraction obtained 30–70% lower DNA yield, depending on the stain. However, the average peak height was higher. The allele recovery of the STR profiles were comparable (data not shown). No cross-contamination could be detected for the specific cross-contamination tests.

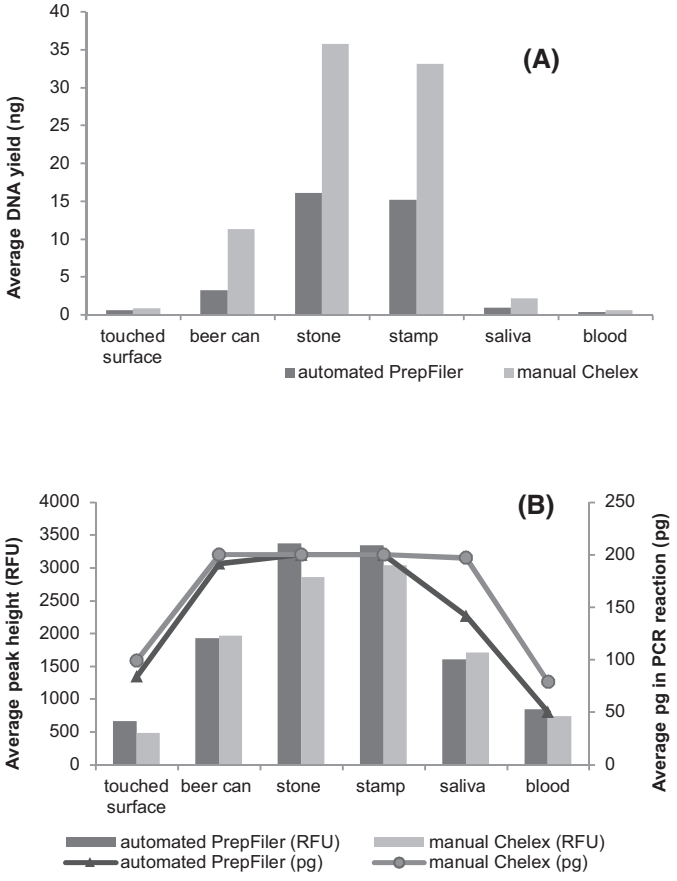

Fig. 1. Comparison of average DNA yield (A), average peak height and average DNA quantity added in PCR reaction (B) using optimized PrepFiler® Automated DNA extraction and manual Chelex-100® method. 10 Replicates of each set were extracted except for blood, for which 6 samples were analysed.

4. Discussion

Although the automated PrepFiler® extraction, compared to manual extraction, resulted in lower DNA yield, and less DNA was utilized for the PCR, the average peak height of the samples was comparable, indicating a better purity of extracts.

To maintain the elution temperature applied in our optimization studies, the heating ability of Te-Shake during elution is monitored every 3 months. An elution temperature higher than 65 °C at the end of the elution time is essential for an optimal release of DNA from magnetic beads [1]. Heating causes half of the elution to evaporate, resulting in recovery of 57 μl from the initial 100 μl. To avoid high salt concentration in our DNA extracts, the PrepFiler® elution buffer was diluted 1:2 with water.

To ensure optimal extraction with no loss of magnetic beads for different sample types, a second 96 deep-well plate was additionally implemented to collect the lysate isopropanol solution of each sample from the processing plate, which is normally discarded during the run. After the run, this waste plate is put on the magnetic ring stand and visually examined. In case of any magnetic beads presence in the waste plate, these samples are processed again.

It is also important to note that besides kit-specific factors, choosing the right swab is essential for the success of PrepFiler® DNA extraction. Good results were obtained with Puritan® cotton swabs. While using another swab type, during the PrepFiler® lysis a partial dissolution was observed, and almost no lysis buffer could be separated from the swab.

5. Conclusion

With the optimized PrepFiler® protocol implemented on Freedom EVO® 150 the results were comparable to our routine manual Chelex-100® extraction. In contrast to other preprogrammed automated extraction systems, using Freedom EVO® 150 for automation offers the flexibility to program and customize every step of DNA extraction and also to install different extraction methods. The drawbacks, however, are a time-consuming

installation and in-house validation, necessary to integrate such a system into the routine workflow. Such a validation was done in our lab and as our data indicate, optimizing PrepFiler® extraction conditions such as for elution results in better DNA yield and is of major consequence.

Role of funding

None.

Conflict of interest

None.

References

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