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A modified direct PCR amplification method using the GlobalFiler™ PCR Amplification Kit on bloodstains collected using microFLOQ™ direct swabs

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ABSTRACT

The standard forensic DNA analysis workflow typically encompasses DNA extraction, quantification, STR-PCR amplification, and CE detection. A direct PCR amplification method eliminates the extraction and quantification steps, shortening the turnaround time for DNA profiling. However, a limitation to the direct PCR amplification method lies in its inability to allow for additional PCR amplification on the same sample. We found that replicate PCR amplifications can be afforded with the following modification to the direct PCR amplification method: after sample collection, the microFLOQ™ Direct swabs were incubated in low TE buffer prior to PCR amplification of the lysate. With replicate amplifications, the impact of stochastic effects during STR-PCR amplification on DNA profile interpretation would be reduced. Additionally, adjustments can be made to template volumes in subsequent amplifications, preventing oversaturated PCR reactions. Our results showed that this modified direct amplification method gave comparable median peak heights, allele recovery and intra-locus peak-height-ratio to those of the standard workflow, while maintaining the advantage of minimal evidence consumption.

1. Introduction

We previously demonstrated that direct PCR amplification of DNA from blood samples using microFLOQ™ Direct swabs allowed the rapid generation of DNA profiles within three hours, providing early leads to law enforcement agencies. The established method involved swabbing the stain-of-interest and snapping the swab tip directly into a PCR tube for a single STR-PCR amplification [1].

While the direct PCR amplification method using microFLOQ™ Direct swabs is rapid, improved collection efficiency, and required only a thermal cycler and genetic analyser (which are standard in forensic DNA laboratories), the method suffered from a drawback – absence of liquid DNA [1]. This can be a limitation to laboratories that require replicate amplifications towards a consensus-based interpretation of DNA mixture profiles [2,3]. Additionally, shoulder peaks and pull-up artefacts caused by excessive DNA template can occur due to the inability to control the template quantity used in the PCR reaction [1]. Sherier et al. had demonstrated that their modified direct PCR method, which involved eluting DNA from microFLOQ™ Direct swabs using a commercial kit, also allowed for better control over the template quantity used for PCR amplification [4]. With these considerations, we sought to modify our direct PCR amplification method to generate a liquid lysate, allowing for the ability to perform replicate PCR amplifications with adjustable lysate volumes.

The modified direct PCR amplification method (hereinafter referred to as the modified method) described in this study incorporates a 30 min pre-amplification process, where samples collected on the microFLOQ™ Direct swab tip is incubated with heating in 35 µL of low TE buffer. The resultant cell lysate is sufficient for at least two PCR amplifications. Low TE is a readily available buffer, and can serve as an alternative to commercial direct amplification kits. The results demonstrate the effectiveness of our modified method as compared to the standard forensic DNA analysis workflow.

2. Materials and methods

2.1. Preparation of blood-stained substrates

Ten different substrate types were used in this study: denim, leather, metal objects, rusty objects, tissue paper, fabric, soil-stained fabric,

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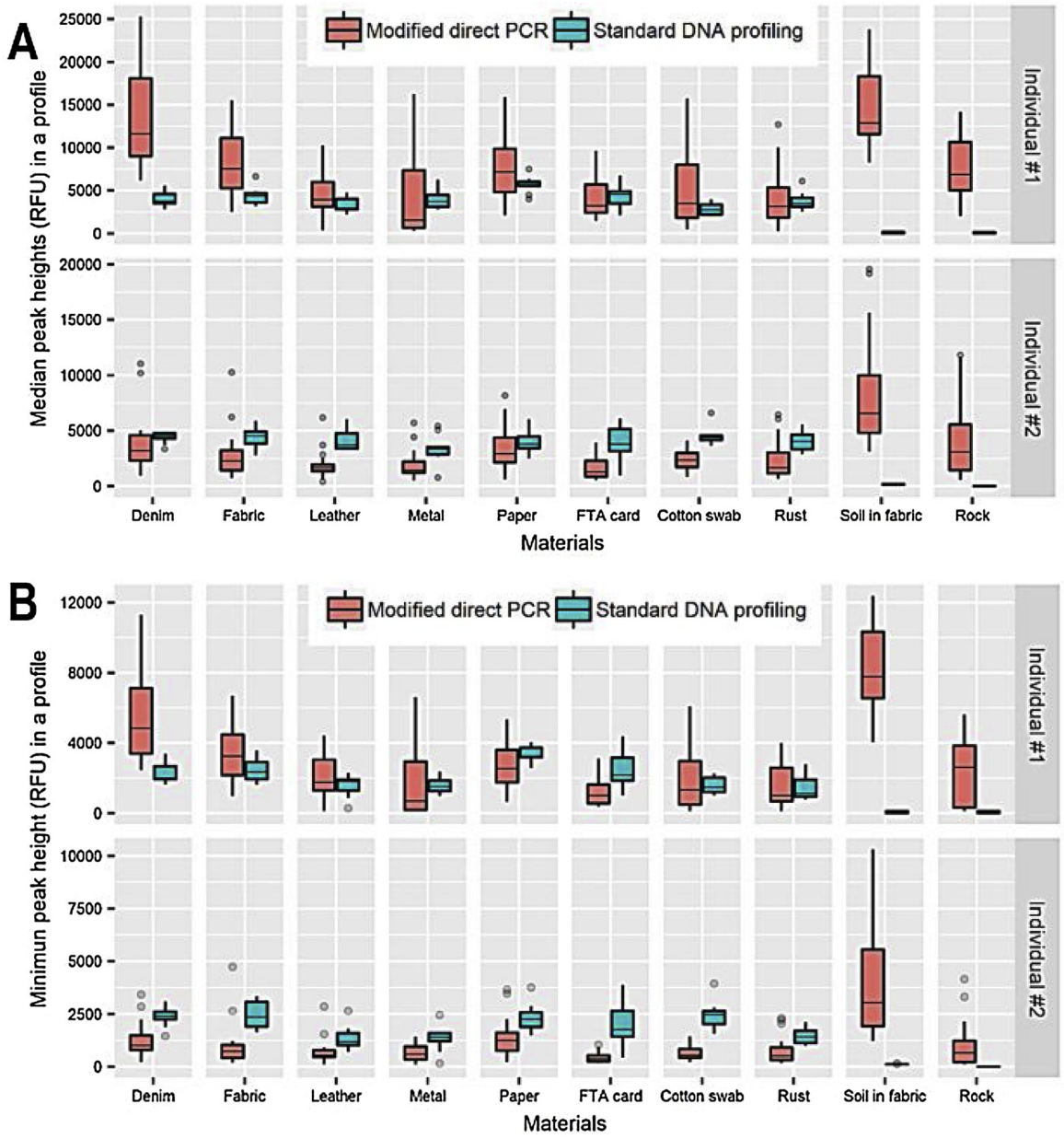

Fig. 1. Peak heights observed from the modified and standard methods. (A) Median and (B) minimum peak heights observed in a DNA profile. The overall median peak heights for both methods were comparable. The overall minimum peak heights for both methods were above the laboratory's analytical and stochastic thresholds.

rocks, FTA cards and cotton swabs. To prepare the soil-stained fabric, the fabric was soaked in soil solution (3 tablespoons of soil in 25 mL of water) for 5 min and dried overnight. Subsequently, ten spots of 5 µL neat blood were deposited onto each substrate type and dried overnight at room temperature before the samples were collected using the microFLOQ™ Direct swab.

2.2. Standard forensic DNA analysis workflow

Samples were collected on cotton swabs. DNA was extracted with the Promega Casework Extraction Kit and DNA IQ™ Casework Pro Kit on the Maxwell® FSC Instrument. DNA was eluted in a final volume of 50 µL and quantified with the Quantifiler® Duo DNA Quantification Kit. STR-PCR amplification was performed with the GlobalFiler™ PCR Amplification Kit. CE was performed on the 3500xL Genetic Analyzer and analysed with the laboratory's analytical and stochastic thresholds. This method is hereinafter referred to as the standard method [1].

2.3. Modified direct PCR method

Two blood-saturated microFLOQ™ Direct swab tips were incubated in 35 µL of low TE (10 mM Tris, 0.1 mM EDTA, pH 8.0) buffer at 70°C for 30 min, shaking at 1000 rpm in a thermomixer. 15 µL of lysate was used for PCR amplification. Thermal cycling and CE parameters were identical to those used in the standard method [1].

3. Results and discussion

3.1. Comparison of DNA yield obtained from the modified and standard methods

DNA concentrations were determined from the 50 µL liquid DNA and 35 µL cell lysate obtained from the standard and modified methods, respectively. DNA was detected from bloodstained denim, leather, metal objects, rusty objects, tissue paper, fabric, FTA cards and cotton

swabs processed with the standard method. Similarly, DNA was detected from these materials processed with the modified method, albeit at a lower DNA yield. This was to be expected as only approximately 1–1.5 mm diameter area was swabbed and collected using the two microFLOQ™ Direct swabs.

Interestingly, DNA was detected for soil in fabric and rock samples processed with the modified method, but not the standard method. There was no shift in the C_T value of the internal positive controls in the quantification assays of samples from both modified and standard methods. This suggested the lack of DNA detection in soil in fabric and rock samples processed with the standard method was not due to inhibitory substances. Rather, it was due to little or no DNA being extracted by the standard method as humic acid in the soil may competitively sequester the released DNA during DNA extraction [5].

3.2. Comparison of DNA profile peak heights between modified and standard methods

To evaluate the performance of the modified method, peak heights obtained from the modified and standard methods were compared (Fig. 1). The overall median peak heights across all the substrate types for both methods were generally comparable, at 3516 Relative Fluorescence Unit (RFU) and 3566 RFU for the modified and standard methods, respectively. The inter-quantile ranges (IQR) were 5536 RFU and 2103 RFU, respectively. The wider IQR in the modified method is likely a consequence of both the biological variations between the individuals' blood and the non-normalised DNA input used for amplification in the modified method. The overall minimum peak heights across all the substrate types (and IQR) were 1179 (2421) and 1664 (1407) RFU, for the modified and standard methods, respectively. While the minimum peak heights from the modified method were lower than those from the standard method, they were well above the laboratory's analytical and stochastic thresholds. Hence, the genotypes generated via the modified method can be reported reliably.

3.3. Comparison of allele recovery and intra-locus peak-height-ratio between modified and standard methods

Although lower minimum peak heights were obtained from the modified method, full DNA profiles were obtained from all substrates. In contrast, the overall median (and IQR) percentage of alleles recovered for soil in fabric and rock samples using the standard method were 15% (31%) and 0% (2.5%), respectively. Intra-locus peak-height-ratios (PHRs) were also evaluated. Median (and IQR) of 0.88 (0.14) and 0.89 (0.13) were obtained for the modified and standard methods, respectively, suggesting comparable DNA quality from the two methods.

4. Conclusion

This study demonstrated that modified direct PCR amplification method had comparable median peak heights, allele recovery, and intra-locus PHR to the standard laboratory method. For substrates involving soil, the modified method out-performed the standard method in terms of allele recovery and peak heights. Additionally, there were less shoulder peaks and pull-up artefacts observed for the modified method as compared to the direct method [1]. Although the additional pre-amplification process added 30 min to the processing time, the modified method conferred the significant advantage of allowing for replicate amplifications which would facilitate (i) consensus-based interpretation of DNA mixture profiles, and (ii) control of lysate volume in additional PCR amplifications.

Declaration of Competing Interest

None.

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