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Implementation of Prep-n-Go™ Buffer for DNA extraction from buccal swabs

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ABSTRACT

The efficacy of two extraction methods; room temperature and heat protocols was assessed for buccal swabs using the Prep-n-Go™ Buffer. DNA was extracted from buccal swabs using both extraction methods and their effectiveness to produce good quality DNA profiles was evaluated. Heat protocol was found to yield more DNA, however room temperature protocol produced better quality DNA profiles with fewer artefacts when the samples from both extraction methods were amplified directly without any normalisation with the VeriFiler™ Express PCR Amplification Kit.

1. Introduction

DNA extraction from buccal swabs has been widely practiced due to the non-invasive nature of sample collection and most importantly, high DNA yield [1]. At Anglia DNA Services, buccal swabs for paternity and relationship testing were processed using the QIAamp® DNA Mini Kit [2]. DNA extraction using this kit took approximately 2 h and involved several tube transfers. To reduce the turnaround time and avoid any cross contamination or sample mix-up, a simpler and quicker DNA extraction method; Prep-n-Go™ Buffer was tested and implemented.

2. Materials and methods

2.1. Sample collection and DNA extraction

Two buccal swabs from each individual were collected from 45 anonymous volunteers with informed consent. One swab was extracted using the room temperature protocol and the other with the heat protocol following manufacturer's recommendation [3]. A volume of 400 µl Prep-n-Go™ Buffer (Applied Biosystems™) was used for both protocols. After adding the buffer to the swabs in the 1.5 ml Eppendorf® tubes, room temperature protocol samples where let to stand for 20 min at room temperature (20 °C to 25 °C) to lyse the sample. The heat protocol samples where incubated for 20 min in the preheated heat block (90 °C) to lyse the sample and thereafter let to stand at room temperature for at least 15 min to cool the lysates.

2.2. DNA quantification

Extracted DNA samples were quantified on the QuantStudio™ 5 Real-Time PCR System (Applied Biosystems™) using the Quantifiler™ HP DNA Quantification Kit (Applied Biosystems™) following manufacturer's recommendation. This step was carried out to identify the amount of DNA obtained from both extraction methods and the results were not used for normalisation.

2.3. PCR amplification

Samples were amplified directly (without normalisation) using the VeriFiler™ Express PCR Amplification Kit (Applied Biosystems™). Each PCR reaction comprised 10 µl Master Mix, 10 µl Primer Sets and 5 µl template DNA; and amplified on the GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems™) following manufacturer's recommendation.

2.4. Electrophoresis and analysis

Capillary electrophoresis was carried out on the ABI Prism® 3500xL Genetic Analyzer (Applied Biosystems™) and alleles were determined using the GeneMapper® ID-X v1.3 software (Applied Biosystems™). Statistical analysis (t-test) was carried out using the R Studio software with $\alpha = 0.05$.

3. Results

Based on the quantification results, the heat protocol produced higher DNA yield from the buccal swabs compared to the room temperature protocol (Fig. 1). The average DNA concentration for the heat protocol was 8.48 ng/µl (standard deviation: 5.87 ng/µl) compared to the room temperature protocol with 2.84 ng/µl (standard deviation:

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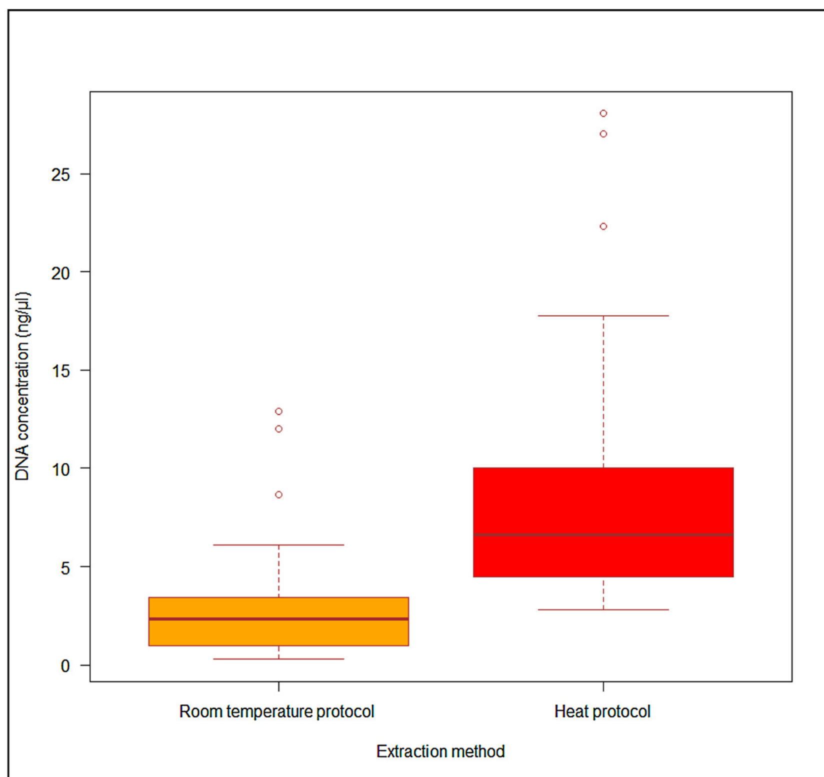

Fig. 1. Boxplots generated from the concentrations of DNA extracted using room temperature and heat protocols. Each boxplot represents 45 samples.

2.74 ng/µl). Statistical analysis showed a significant difference between both extraction methods (p-value < 0.05).

All extracted samples were amplified directly without any normalisation using the VeriFiler™ Express PCR Amplification Kit. Electropherograms showed that good quality DNA profiles were obtained from the room temperature protocol while the heat protocol produced electropherograms with artefacts such as split peaks and pull-ups. The average heterozygote peak heights for room temperature protocol were between 3379–9734 relative fluorescence units (RFU) while the heat protocol produced average heterozygote peak heights between 12934–35011 RFU (Fig. 2). Statistical analysis showed a significant difference between both extraction methods for each locus (p-value < 0.05). The heterozygote peak height ratio was better in the heat protocol with the average being 0.92 (standard deviation: 0.05) while it was 0.84 (standard deviation: 0.12) in the room temperature protocol.

4. Discussion

The aim of this study was to evaluate and implement a reliable buccal swab extraction method for the Prep-n-Go™ Buffer. Based on the DNA yield, the heat protocol was more robust than the room temperature protocol; however room temperature protocol produced better quality electropherograms when the samples from both extraction methods were amplified directly without any normalisation with the VeriFiler™ Express PCR Amplification Kit. Previous studies have also

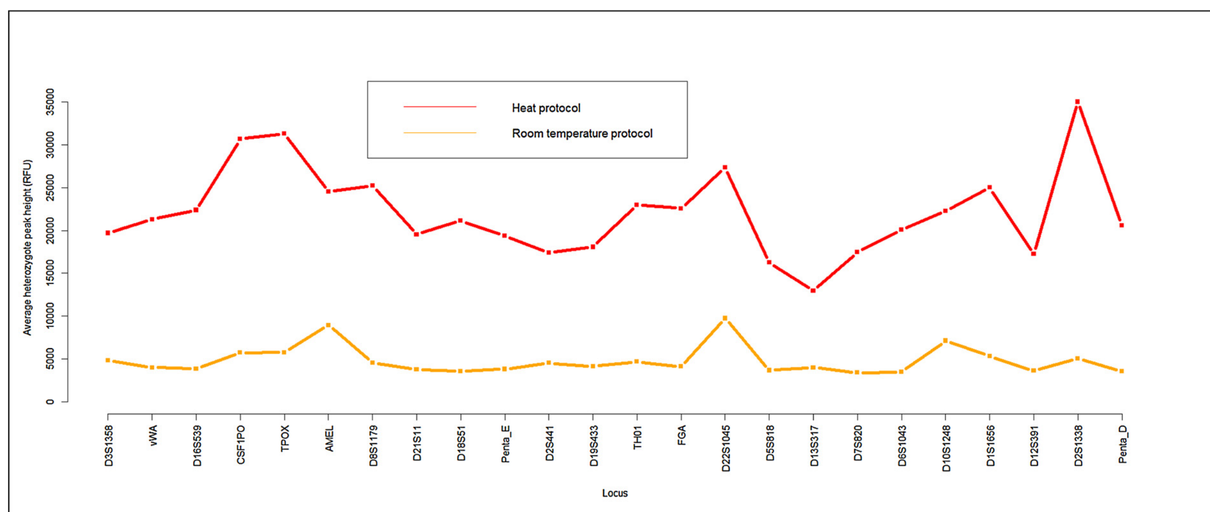

Fig. 2. Graph generated from the average heterozygote peak height for each locus from room temperature and heat protocols. A volume of 5 µl of each extract was used in the PCR amplification.

shown that the direct amplification can be carried out with other biological samples such as hair [4] and blood [5] extracted using the Prep-n-Go™ Buffer when processed with the compatible amplification kits such as the GlobalFiler™ Express Kit and the Yfiler™ Plus Kit.

According to the VeriFiler™ Express PCR Amplification Kit manufacturer, the optimum PCR cycle number should generate profiles with no instances of allelic dropout and minimal occurrence of off-scale allele peaks with heterozygote peak heights between 3000–12000 RFU [3]. Based on this, the room temperature protocol was the better option for the buccal swab samples, where the extracted samples can be amplified directly without the DNA quantification step.

5. Conclusion

Following the results found in this study, room temperature protocol was implemented to extract DNA from the buccal swabs using the Prep-n-Go™ Buffer at Anglia DNA Services. Direct amplification on extracted sample from this protocol eliminated the DNA quantification cost and also reduced the turnaround time. Heat protocol was also implemented to obtain full DNA profile from the second swab if the first swab extracted using the room temperature protocol produced a weak/partial DNA profile.

Declaration of Competing Interest

None.

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