Successful Generation of DNA profiles using Direct PCR

Yuvaneswari C. Swaran[a,b] and Lindsey Welch[a]

[a] *Centre for Forensic Science, University of Strathclyde, UK*
[b] *Forensic Division, Department of Chemistry Malaysia, Jalan Sultan, 46661Petaling Jaya*

ABSTRACT: It has been reported that it is possible to obtain DNA profiles from trace amounts of DNA from touched surfaces using various enhancement techniques. Some of the enhancement techniques used are known to cause peak imbalance, allelic drop-in and allelic drop-out, which complicate interpretation. Here we used Direct PCR where DNA profiles are generated without performing an extraction process or using any enhancement techniques. By removing the extraction process, Direct PCR is observed to be more sensitive as there is no associated loss of DNA. The Direct PCR technique was compared with the QIAamp® DNA Micro Kit extraction using low amounts of DNA recovered from glass, a commonly found substrate at any crime scene. Five different concentrations of a commercially available DNA solution were used to compare the DNA profiles generated from these two techniques.

Keywords: DNA, PCR, profile

Introduction

It is common crime scene practice to swab items that may have come into contact with either the victim or the perpetrator in the hope that it may yield sufficient DNA to produce a DNA profile using various enhancement techniques [1]. Low Copy Number (LCN) DNA profiling is a popular enhancement technique used when low amounts of DNA are obtained from these items, however, it has been documented that this technique has its disadvantages. Peak imbalance, allelic drop-in and allelic drop-out are common artefacts encountered in a DNA profile that has been subjected to the LCN technique, which can complicate data interpretation [2]. Here we describe a technique called Direct PCR where low amounts of DNA (up to 0.1 ng) are successfully amplified from glass without any enhancement techniques. This technique has been tried on fabric and it has proved to be a success [3]. This technique is capable of saving time and cost of purchasing extraction and quantitation kits. Moreover, the chances of cross contamination and technical errors can also be reduced as this technique reduces sample transfers and handling time. In this experiment, we compare the ability of the Direct PCR and QiaAmp DNA Micro kit (Qiagen Ltd.) to produce DNA profiles from glass surfaces using the PowerPlex 16 HS system (Promega Corp.).

Materials and Methods

Human Placental DNA (Cambio Ltd.) solution was diluted to five different concentrations: 0.1 ng/µL, 0.075 ng/µL, 0.05 ng/µL, 0.025 ng/µL and 0.01 ng/µL. Two sets of samples were prepared by smearing 10 µL of diluted sample onto sterile, DNA-free glass microscope slides and left to dry overnight at room temperature. Two sets were prepared; one for Direct PCR and the other for QiaAmp DNA Micro kit extraction.

A cotton swab was moistened with 40 µL of sterile deionised water. The surface smeared with DNA was then swabbed. An extract negative was taken by swabbing an area of the microscope slide not smeared with DNA. One set of swabs was subjected to Direct PCR and the other set subjected to extraction. For Direct PCR, only a $2mm^2$ area of the cotton swab was cut and placed directly into an amplification tube. For extraction, the whole swab was cut and placed in a 1.5 mL tube and extracted following the QiaAmp DNA Micro kit Swab protocol [4]. A positive control (Buccal swab) was extracted together with the samples.

Amplification was carried out using the PowerPlex 16 HS kit following the manufacturer's recommended protocol [5] on the Applied Biosystem's 2720 thermal cycler together with positive and negative controls. Fragment analysis was carried out on a 310 Genetic Analyzer (Applied Biosystems).

The whole experiment was repeated a total of three times to observe reproducibility.

Results

The results indicated that Direct PCR was able to generate a full DNA profile with total DNA concentrations of 1.0 ng (**Fig. 1**) and a partial DNA profile with total DNA concentrations of 0.1ng (**Fig. 3**). No alleles were observed with the QiaAmp DNA Micro kit extraction with total DNA concentrations of 1.0 ng and 0.1 ng (**Fig. 2** and **Fig. 4**).

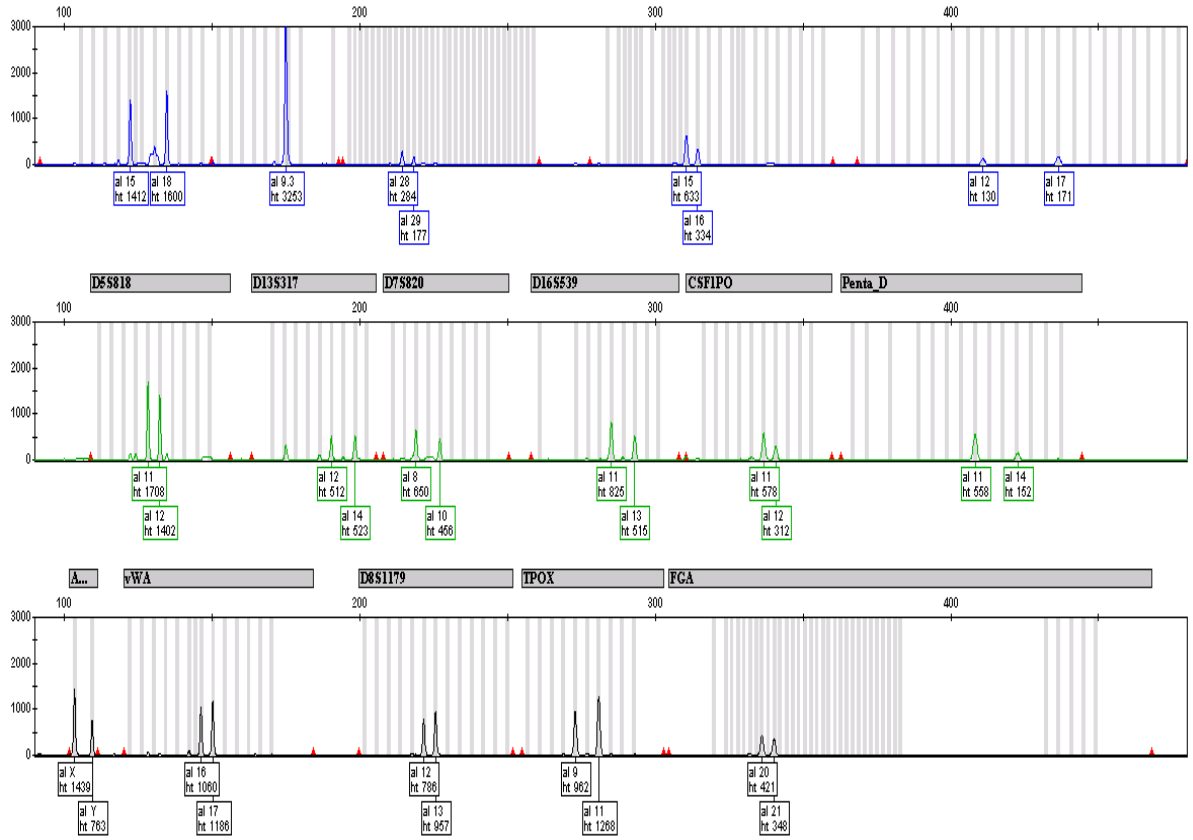

Fig. 1: DNA profile of 1.0 ng total DNA from glass using Direct PCR

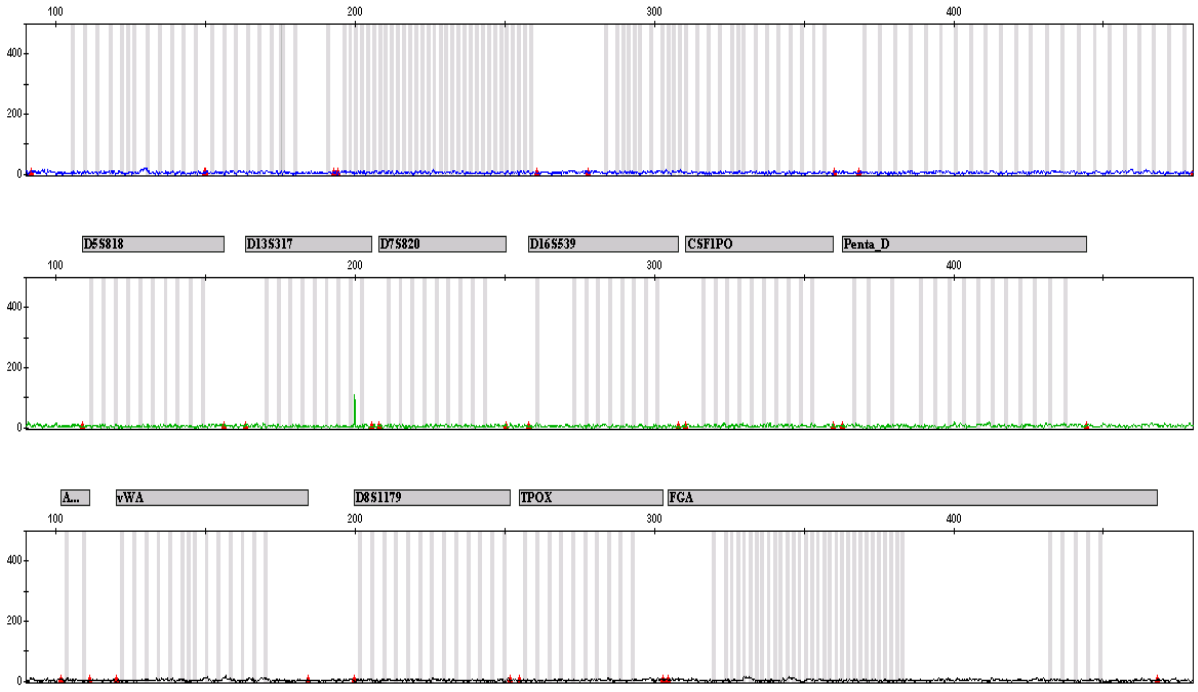

Fig. 2: DNA profile of 1.0 ng total DNA from glass using QiaAmp DNA Micro kit

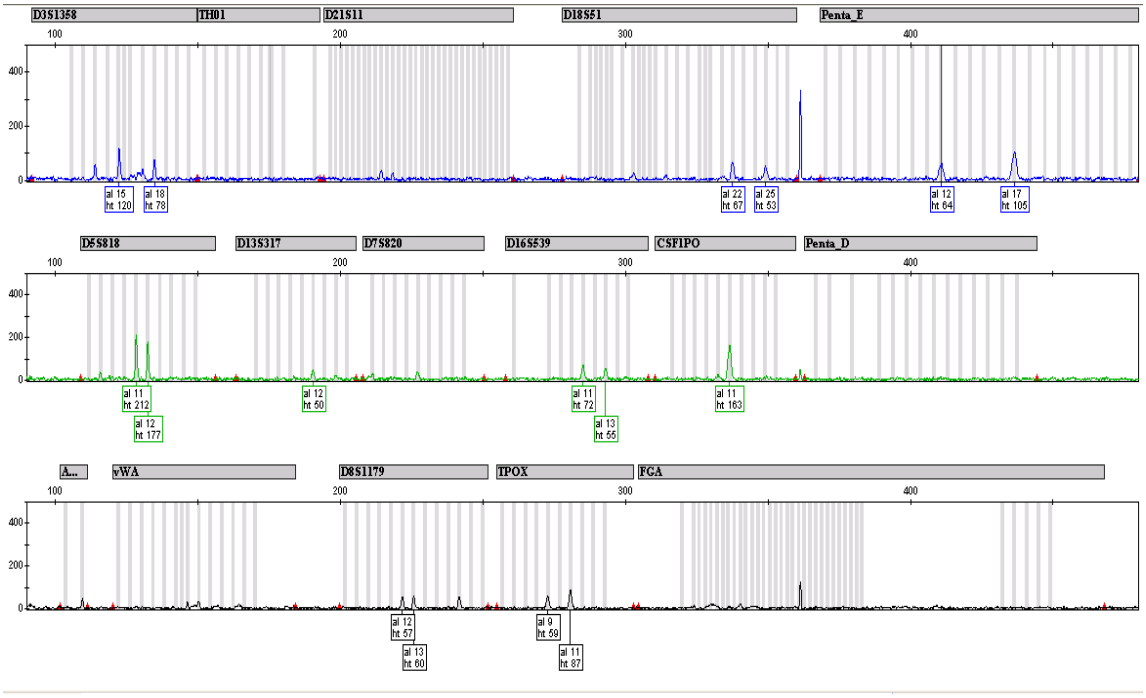

Fig. 3: DNA profile of 0.1 ng total DNA from glass using Direct PCR

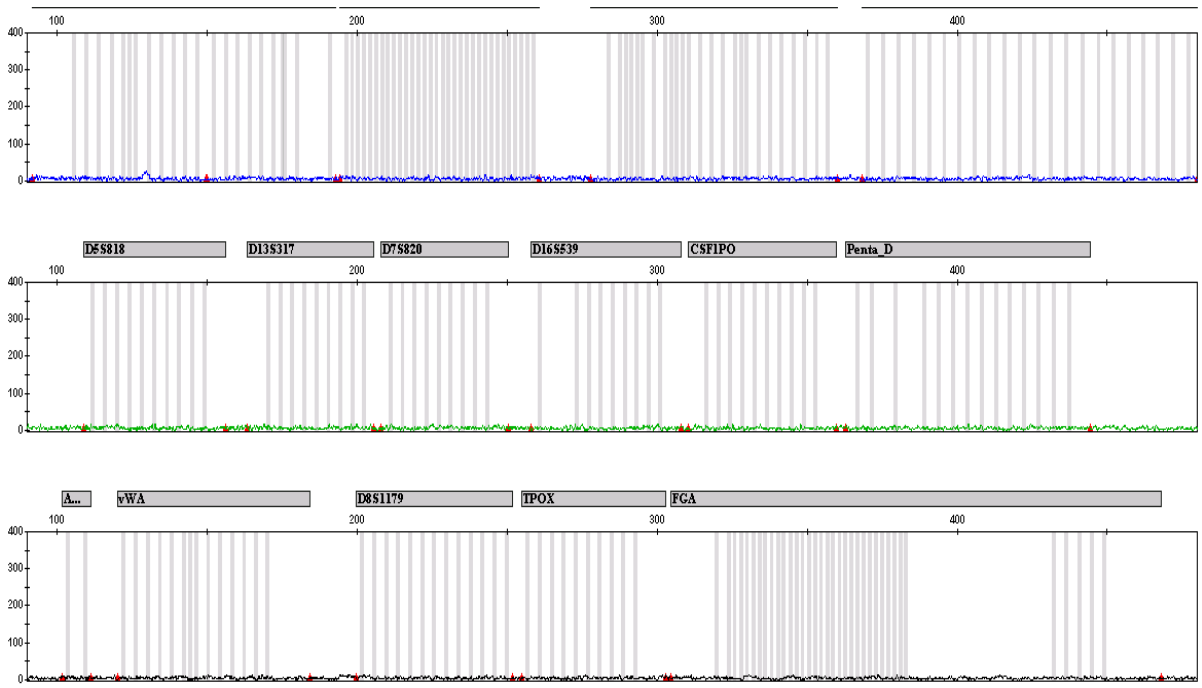

Fig. 4: DNA profile of 0.1 ng total DNA from glass using QiaAmp DNA Micro kit

Discussion and Conclusion

Direct PCR exhibits greater sensitivity on glass when compared to the QiaAmp DNA Micro kit extraction. This may be due to the fact that there is no associated loss of DNA with Direct PCR as there is with DNA extraction. It has been proven that some extraction techniques lose DNA significantly due to the number of transfer steps involved [6]. This is not the case with Direct PCR as there are no additional transfer steps after the initial transfer of sample to the PCR tube and almost all the DNA recovered on the swab is subjected to STR amplification.

Though Direct PCR has shown great success in obtaining DNA profiles with sub-optimal amounts of DNA, a second analysis of the sample is currently not possible as there is no extract available for re-analysis. More research needs to be carried out in order to rectify this problem.

References

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Additional information and reprint requests:
Yuvaneswari C. Swaran
Department of Forensic, Kimia Malaysia,
Jalan Sultan, 46661 Petaling Jaya, Selangor