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DNA profiles generated from a range of touched sample types

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ABSTRACT

Direct PCR from touch DNA has a range of potential applications in the field of forensic investigation for exhibit examination that, under standard extraction methods, rarely produce informative DNA profiles. Previous studies from 'touch DNA' have focussed on fingermarks created under laboratory conditions. Here we report on successful STR DNA profiling from a range of touched items. Direct PCR, with no increase in cycle number, was performed after eight different sample types, typical of those submitted for forensic investigation, were handled by volunteers for a maximum of 15 s to deposit trace amounts of their DNA. Amplifications were performed using either GlobalFiler® or Identifiler® Plus following manufacturer's instructions. These two kits were chosen deliberately as many laboratories worldwide have adopted and validated them in their workflow, thus allowing for direct PCR to be incorporated within their practises easily. It was found that informative STR profiles were obtained from all eight substrates using both STR kits. Identifiler® Plus out-performed GlobalFiler® in terms of the percentage of alleles amplified using the direct PCR approach. Both generated informative profiles from all items and all individuals, at different rates, with Identifiler® Plus being informative in a larger percentage of samples. GlobalFiler® produced profiles with an average of 60% ± 24% (36 ± 15 alleles) alleles present while Identifiler® Plus produced profiles with an average of 96% ± 4% (31 ± 1 alleles) alleles present. A comparison was made between the direct PCR approach and subjecting touched samples to a standard DNA extraction process, both using Identifiler®. An average of 4% of profiles were informative for samples that underwent extraction with 100% being informative from the same subset of samples amplified by direct PCR. Our findings further demonstrate the success of direct PCR to enhance the STR DNA profiles from touch DNA. Further, Identifiler® Plus was found to generate informative profiles more often than GlobalFiler®. Direct PCR is fast, simple, and non-destructive of evidence with the ability to generate informative genetic data where standard methods are likely to fail.

1. Introduction

As cellular material sheds from our skin, DNA will be deposited on a surface as touch DNA in circumstances such as: inserting a bullet cartridge into a firearm; using a mobile phone; inserting a sim card into a mobile phone; opening and closing ziplock bags; and holding wires and circuit boards, which may be involved in a terrorist act. The ability to obtain informative STR profiles from items such as these, if handled for a short period of time, is very limited. However, the importance of obtaining DNA profiles from trace or touch DNA evidence is increasing, as DNA is considered to be the gold standard in forensic evidence, and touch DNA evidence may be all that is collected from a crime.

There is a growing interest in the use of direct PCR to maximise the amount of DNA profile information obtained from forensic evidence, particularly from trace or touch DNA samples. Since its first application in forensic science in 2010 [1], direct PCR has been applied to single hairs [2], nails [3], fibres [4], bullet cartridges [5], different surface types [6,7], and more recently fingermarks [7–9]. Direct PCR has also been the subject of a recent review article where its informative power and benefits in niche application were outlined [10]. The aim of direct PCR is to maximise the amount of DNA collected from the substrate and made available for PCR template, thereby increasing the sensitivity of DNA profiling from trace biological material, by omitting the DNA extraction process. Extraction protocols fulfil the function of removing inhibitors from a sample for the downstream PCR process, which may be essential, for instance in the case of blood where haem is a known inhibitor [11], but less essential for touch DNA, where fewer inhibitors are present and DNA can be found cell-free [12]. It has been reported that extraction methods can result in the loss of 76% of the DNA within a sample [13]. By omitting the DNA extraction step, the process from sample receipt to capillary electrophoresis is faster, and cheaper (as no extraction costs, and has fewer tube changes). A consequence of this is

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that a quantification step is also omitted and there is no sample pool to re-test if the PCR fails or if required by another agency. This can be detrimental only if there is a requirement for the quantity of DNA to be recorded or if the provision for re-testing is mandatory.

Previous studies on direct PCR investigated a single commercial STR kit that was available at the time of their studies. The first use of direct PCR employed SGM Plus® [1] with further studies using NGM SElect™ [2,4,8]. As the commercially available kits have increased the number of loci available to amplify, so has the ability of the buffers used to overcome inhibitors [7] as well as the activity of the enzyme. GlobalFiler® is one of the latest commercial STR kits launched by Thermo Fisher Scientific and amplifies 24 loci, comprising 21 autosomal STRs, 1 Y-STRs and 1 Indel. Recently, GlobalFiler® was used to compare the success rate of amplifying DNA from blood, saliva, and semen using direct PCR methods compared to standard DNA extraction processes [14,15].

The use of a hot start enzyme, where the sample is heated to 95°C for 10 to 15 min, is to the benefit of direct PCR as this heating will break open any cellular material, releasing the DNA into the PCR matrix. By comparison to GlobalFiler®, the AmpFLSTR® Identifiler® Plus amplification kit has a hot start enzyme, and a buffer with different constituents potentially more adept to overcome inhibitors.

We report on data obtained using direct PCR after items, typical of those submitted as part of a forensic investigation, were touched for a short period of time. Eight sample types were chosen, which included a fingermark as a comparative control. The fingermark sample type allowed comparison with previously published results to ensure the data presented here was congruent with previous research [8,9]. A comparisons is made between GlobalFiler® and Identifiler® Plus, using direct PCR methods. Through analysis of the same sample types, and amplification with Identifiler® Plus, we further compare the process of direct PCR with using standard extraction processes.

2. Methods

2.1. Exhibits and volunteers

Four items were chosen to emulate potential real-life exhibits which comprised of: unfired aluminium cartridge case, insulated wire, circuit board, and a ziplock bag. Four volunteers (designated PRI 01, PRI 02, PRI 04, and PRI 05) were used for these tests, and each item was prepared in nine replicates giving a total of 144 samples. The nine replicates were separated, at random, into three groups for a total of three replicates per volunteer per group, these groups being: GlobalFiler® using direct PCR, Identifiler® Plus using direct PCR, and Identifiler® Plus using standard extraction methods.

A set of eight items, including the four previously tested were chosen to extend the number of exhibits. The four additional items were as follows: mobile phone, sim card, fuse, and glass slide. Seven volunteers (designated PRI 01, PRI 02, PRI 03, PRI 04, PRI 05, PRI 06, and PRI 07) were used for these tests, and each item was prepared in triplicate for a total of 168 samples. PRI 01-04 were male, and PRI 05-07 were female; PRI 01, PRI 02, PRI 04 and PRI 05 are the same volunteers as above.

The shedder status of all volunteers had been previously determined to ensure there was a wide range of DNA deposition rates within the volunteers used. Shedder status was determined following the method of Kanokwongnuwut et al., under review. PRI 01 was found to be a high shedder, PRI 02, PRI 04, and PRI 07 were intermediate shedders and PRI 03, PRI 05, and PRI 06 were poor shedders.

2.2. Deposition of DNA

All items were cleaned with 3% bleach, wiped, and allowed to air dry in an isolated clean room, to ensure no DNA was present on the items prior to the deposition of DNA by the volunteers. Negative control samples were collected from a set of cleaned items. Negative controls were performed in triplicate from each item. Participants were asked to wash their hands, without soap, to remove excess cellular and cell-free DNA. They then waited 15 min before touching the items, as per regular use, for a maximum of 15 s. During the 15 min intervals the volunteers conducted normal activities with the exception of wearing gloves or washing their hands again. These times were chosen as previous studies have shown that DNA is present on an individual's hands and profiles were obtainable after 15 min [8,16,17].

2.3. Collection of DNA from exhibits

Each sample was double-swabbed using a nylon ultra-fine micro-applicator (City Dental, Adelaide). Each swab head was moistened with 2 µL of 0.1% Triton™ X-100 (Sigma, Victoria, Australia), with the exception of the unfired aluminium cartridge where 5 µL of 0.1% Triton™ X-100 was added to the exhibit prior to each swabbing action. Sampling area was dependant on sample type; the same areas were targeted with both swabs on small items, while on larger items each swab was used in a different location suspected of being touched.

2.4. DNA extraction

DNA extractions were performed using the DNA IQ™ System (Promega, Sydney, Australia) using the 'cotton swab' method, following the manufacturer's protocol, with a final elution volume of 30 µL.

2.5. DNA quantification

The DNA in all samples was quantified after the DNA extraction process using Qubit® dsDNA HS assay (Thermo Fisher Scientific, Melbourne, Australia). Quantification followed the manufacturer's protocol for High Sensitivity.

2.6. DNA amplification

Direct PCR was performed on each sample using either the GlobalFiler® kit (Thermo Fisher Scientific, Melbourne, Australia) or the AmpFLSTR® Identifiler® Plus kit (Thermo Fisher Scientific) by removing the two swab heads, with a sterile scalpel blade, directly into a 0.2 mL thin-walled PCR tube.

Amplifications were performed in 25 µL following the manufacturer's protocol, 30 cycles using GlobalFiler® or 29 using Identifiler® Plus, with exception of 2 µL of Prep-n-Go™ (Thermo Fisher Scientific) and Low TE Buffer (Thermo Fisher Scientific) replacing water. All amplifications were performed on a ProFlex™ thermal-cycler (Thermo Fisher Scientific). PCR product (1 µL) was added to 8.7 µL Hi-Di formamide and 0.3 µL 600 LIZ® (Thermo Fisher Scientific) and separated on a 3500 Genetic Analyser (Thermo Fisher Scientific).

All extracted samples were processed for STR typing. Amplification of extracted DNA samples were performed in 25 µL using Identifiler® Plus, following manufacturer's protocols, with 10 uL of the DNA extract added to the PCR.

2.7. Data analysis

Data were analysed using GeneMapper® ID-X (version 1.4).The quality of the profiles, with respect to peak morphology, peak balance and artifact incidence were observed and the number of alleles present from the donor were recorded. Peaks were recorded if they were of 50 RFU or above. Peaks were considered for homozygosity if they were of 150 RFU or above.

3. Results and discussion

All negative controls taken from each item retuned blank profiles or