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Title: DNA Profiles Generated from a Range of Touched Sample Types

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Highlights

- Comparison of direct PCR and extraction-based PCR on a range of substrate types within this study supports previous findings in the application of direct PCR for touch DNA.
- Touch DNA was successfully amplified using both GlobalFiler® and Identifiler® Plus from a range of substrate types.
- The type of STR kit used for DNA amplification impacts the profiles obtained.
- Identifiler® Plus produced profiles of increased allele numbers when compared with GlobalFiler®.
- Mixed DNA profiles are often obtained from touch DNA samples and are able to be deconvoluted.

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 seconds 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.

Keywords
Direct PCR; STR; DNA Profiling; Touch DNA; Human Identification

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 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 ^{o}C for 10 to 15 minutes, 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 minutes before touching the items, as per regular use, for a maximum of 15 seconds. During the 15 minutes 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 minutes [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 profiles with single peak allele drop-in at low RFU.

3.1 Direct PCR vs Extraction

SUGGESTED FIGURE 1

To compare data obtained from the direct PCR approach to that obtained using a commonly used extraction process, four volunteers touched four items (unfired aluminium bullet, insulated wire, circuit board, ziplock bag) in triplicate. These 48 samples were analysed using GlobalFiler® or Identifiler® Plus STR kits employing direct PCR and Identifiler® Plus using the extraction method. Identifiler® Plus was used for all extracted samples as it was found to out-perform GlobalFiler® in the direct PCR trials. The difference in the average percentage of informative profiles between substrate types, considering all data for each of the four volunteers, can be seen in Figure 1. Each of the 48 samples submitted for extraction were amplified using Identifiler® Plus. Quantification was performed to inform approximate quantities of DNA within the touch DNA samples taken. Of the 48 samples submitted for extraction 17 were quantified as 20 pg/µL or more, which is the quantification threshold of Qubit. Only 13 of the 48 samples produced any alleles; three of which were informative with 14, 15 and 21 alleles, including amelogenin, and a maximum of 9 alleles were observed in the remaining profiles. These alleles matched those of the volunteers. Insufficient DNA was obtained after the DNA extraction process for successful amplification for the remaining 12 samples. These results were not unexpected as it has been reported that up to 76 % of the DNA collected by a swab is lost during an extraction protocol [13] (Supp. Table S1).

The poor success of allele amplification after an extraction process contrasts with the direct PCR approach. An overall average of 77 % of the 48 samples processed using GlobalFiler® produced informative profiles, compared with 100 % of those processed with Identifiler® Plus, and only 6 % of those processed with an extraction prior to amplification. As previously noted, samples processed using Identifiler® Plus produced more informative profiles than those processed with GlobalFiler®.

These data suggest that exhibits suspected to contain limited DNA, such as those touched for a short period of time, can be processed by direct PCR with either the GlobalFiler® or Identifiler® Plus STR kits and achieve significantly greater success rates than if the sample underwent extraction prior to amplification. While these data are supported by previous studies [6, 7, 14], our additional data supports the use of Identifiler® Plus for the amplification of touch DNA.

3.2 GlobalFiler vs Identifiler

STR data generated using direct PCR applying either the GlobalFiler® or Identifiler® Plus amplification kits, after four volunteers touched four items for a maximum of 15 seconds, are shown in Figure 2. All tests were performed in triplicate. Full Identifiler® Plus DNA profiles were obtained from all four items touched by volunteers 2 and 4. Data from volunteer 1 shows that full Identifiler® Plus profiles were obtained from 2 of the items (circuit board, ziplock bag) with an average of 98 % of alleles being present from the aluminium cartridge case and 99 % present from the insulated wire. The average percentage of alleles present, using Identifiler® Plus, for volunteer 5 was 88 % for the

aluminium cartridge case, 75 % from the insulating wire, 91% from the circuit board and 94% from the ziplock bag. The data for Identifiler® Plus contrasts with that of GlobalFiler® where none of the samples touched by the four volunteers generated full DNA profiles. The highest percentage of alleles was an average of 92% for the circuit board (volunteer 4) with the least being an average of 20% for the aluminium cartridge case (volunteer 5).

SUGGESTED FIGURE 2

These data are shown using the percentage of alleles obtained considering a full profile. It should be noted that GlobalFiler® amplifies 24 loci, however this includes two Y-chromosome makers that will only amplify DNA from the male volunteers. The total number of alleles expected is therefore dependent on gender; 44 for female and 46 for male. This compares to the 32 alleles, from 16 loci, generated from a full Identifiler® Plus profile.

The profiles analysed using the Identifiler® Plus kit showed consistently higher quality, regarding profile completeness, when compared to those processed using GlobalFiler®. The variation between replicates in allele presence was also greater when examining the GlobalFiler® samples compared with the Identifiler® Plus samples, shown by the standard deviations in Figure 2. It should be noted that an average of 36 (+/- 15) alleles were present in GlobalFiler® profiles while amplification with Identifiler® Plus generated an average of 31 (+/- 1) alleles. Although the average number of alleles per profile is higher in GlobalFiler®, compared with Identifiler® Plus, the standard deviation is also much higher therefore for increased consistency in the discriminatory power of the profiles generated it is advised that Identifiler® Plus be used. A comparison between STR profiles generated from one sample type using GlobalFiler® and Identifiler® Plus is shown in Figure S1.

As GlobalFiler® consists of a greater number of loci than present in Identifiler® Plus, a smaller percent of the profile is required to make the profile informative (considered up-loadable to the Australian DNA database). A profile is considered informative when there are 12 or more alleles, disregarding amelogenin, are present.

SUGGESTED FIGURE 3

When considering the informative power of the profiles, Figure 3 shows a closer correlation between the kits used. Although the number of GlobalFiler® samples producing informative profiles is similar to the Identifiler® Plus samples in eight of sixteen comparative sets, between sample types per volunteer (Figure 3), the same trend is observed as previous; the GlobalFiler® kit is not as efficient at amplifying direct PCR samples as the Identifiler® Plus kit, even when considering the requirement of only 12 alleles. Between 30-100 % of profiles produced, depending on the individual, were informative using the GlobalFiler® kit while 100 % of profiles produced were informative using the Identifiler® Plus kit from all four volunteers.

These data suggest that there is a very high chance of generating informative STR DNA data from items touched for only a few seconds using direct PCR techniques. It is noted that the STR kit chosen has a significant effect on the potential for informative profile generation. The data indicates that Identifiler® Plus should be used to further increase the profiling success potential of touch DNA samples. The difference observed between the kits may be due to the superior buffer found in the Identifiler® Plus kit.

3.3 Identifiler substrate trials

Due to the superior data from Identifiler® Plus in the previous study, this kit was used to amplify an extended sample set including eight items touched by seven volunteers (additional items were a mobile phone, sim card, fuse and fingermark on glass slide). Cumulative data from these sample types, performed in triplicate, are shown in Figure 4.

SUGGESTED FIGURE 4

Touching these items for a maximum of 15 seconds resulted in full, or near full, DNA profiles for three of the four males (PRI 01, PRI 02, PRI 04). The exception was volunteer PRI 03, who was a poor shedder, where an average of 49 % of alleles per profile were present and an average of 38 % of profiles were informative. This is still far more successful that a total average of 4 % when extractions were performed. Predominantly male donors presented higher profile coverage, or increased allele numbers, than those from female donors; standard deviations between replicates were also lower (Figure 4). This is not unexpected as previous research has shown that males, on average, shed more DNA than females [18]. The cause of the significant drop in the amplification success of PRI03 is unknown but it may be due to their propensity to deposit DNA, activities performed before item handling, or difference in touching location to that which was swabbed.

PRI05 and PRI06 had been previously determined to be low shedders, therefore it is of note to observe informative profiles generated from items touched by these individuals; this further highlights the potential application of direct PCR to touched items.

Mixed profiles were prevalent in this study with 60 % of the samples tested containing two or more contributors. This is in line with previous studies into direct PCR from fingermarks on glass, one of the sample types chosen in this study [8]. This was most likely due to the volunteer's actions having no restrictions during the 15 minutes post-hand washing, it is expected that a volunteer will touch items during this time that may have been previously handled by others. It has been shown that non-self DNA is present on an individual which would explain the prevalence of mixtures within this study, especially considering the sensitivity of the technique [16, 18-21]. Mixed profiles often included only a few low-level non-donor alleles and where a larger number of non-donor alleles were

present they exhibited a clear major contributor, with the exception of PRI03 whose profiles presented mixed profiles closer to a 1:1 ratio. The alleles generated within the mixed DNA profiles could be deconvoluted when compared against a database containing volunteers, office staff, and laboratory staff. The exception for this was that unaccounted for alleles were present in PRI03's profiles as profiles of individuals with which PRI03 shares common space with were not able to be obtained.

SUGGESTED FIGURE 5

When all data from each volunteer are averaged dependant on sample type, the expected resultant profiles from each object can be assessed (Figure 5). Most of the variation seen was due to PRI03. Figure 5 contains data from this volunteer to account for the occasion of an equally poor DNA donor in the general population. Even with the lowered mean and increased standard deviations from the average profile coverage percentage, due to PRI 03, a sample processed via direct PCR using the Identifiler® Plus kit produced a profile with an average profile coverage of 82 %- 96 %, dependant on sample type. Glass substrates, such as the fingermark on glass and the fuse, generated fewer loci when amplified than the plastic and metal substrates. The sample produced from the ziplock bag produced the most profile coverage on average. It should be noted that the swabbing method conducted in this study targets the inner surface of the bag opening and the seam along which the bag is closed. This may be in contrast to sampling a large surface area of the exterior of the bag, leading to the potential to collect DNA from a large number of donors if multiple people have had cause to handle the bag.

4. Conclusion

Touch DNA data can be crucial to a criminal case or for intelligence purposes to determine who may have made contact with an exhibit. In this study a range of substrates were chosen that are typical of such items: ziplock bags may be used to store controlled substances, wires and fuses are found in timing devices, communication uses mobile phones and sim cards, and generating data from cartridge cases has obvious implications. Direct PCR has niche application for exhibits that may be unlikely to produce informative DNA profiles if swabs taken from them are subjected to extraction-based STR typing methods; results post-extraction are particularly unlikely if an individual had made brief contact with an item. Although only 4 % of samples processed after extraction, using Identifiler® Plus, produced informative profiles when direct PCR methods were employed 77 % of those processed using GlobalFiler® and an overall average of 90 % of those processed using Identifiler® Plus produced informative profiles.

A previous study DNA typing touch DNA used only marks after thumbs or fingers were placed on glass [8]. Successful STR DNA typing was performed using NGM SElect™ and highlighted the potential of direct PCR use to obtain STR DNA data from these trace sources. A fingermark on a glass slide was included in this study and data from these samples, amplified using

Identifiler® Plus resulted in 86 % informative profiles compared with 71 % in previous research amplified using NGM SElect™ [8]. This is comparable when considering the differences in the kits used, with respect to total alleles amplified within the kit and the buffer systems used. Although unfired cartridge cases were tested in this study, testing fired cartridges would represent a more commonly found evidence type.

Mixed DNA profiles were obtained from a number of samples, this was expected as volunteer activities between washing and DNA deposition were not controlled. This would also be expected in case-work. These were typically able to be deconvoluted easily without the use of software as there were only a few low-level alleles or a clear major or minor was present. There is potential that the few DNA profiles with ratios closer to 1:1 could be analysed using software such as STRmix™.

Both Identifiler® Plus and GlobalFiler® generated informative STR profiles from touch DNA samples when no, or very few, alleles were obtained if the same touched items were subjected to an extraction process. The extraction process used was the DNA IQ™ System, which uses magnetic beads. Other extraction processes are available, such as Chelex® or solid phase silica based methods, although previous studies have also shown that these methods result in a high percentage loss of DNA [7, 13, 15].

The evidence presented in this study indicates that the STR kit employed for amplification impacts the quality of the DNA profile obtained. There are three variables within these kits that may be responsible for the advantage Identifiler® Plus displays over GlobalFiler®; this advantage may be due to the inherent benefit that hot start enzymes give, the length of the 95°C pre-PCR hold or the buffer supplied with Identifiler® Plus having an increased ability to overcome inhibitors and facilitate amplification. Considering the data presented in this study, with the stated restriction in ascertaining the variable of advantage, the recommendation remains that Identifiler® Plus be used in preference of GlobalFiler® for the amplification of touch DNA samples.

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Conflicts of Interest

The authors declare no conflict of interest

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Figure 1 Average percentage of informative profiles, containing 12 or more alleles, excluding amelogenin, of all profiles obtained from each sample type. Each of the four participants handled each sample type in triplicate for each processing method. Comparison is between direct PCR using GlobalFiler® or Identifiler® Plus STR kits and the extraction-based profiling method using Identifiler® Plus. No informative profiles were obtained from the circuit board and ziplock bag sample types when an extraction was performed.

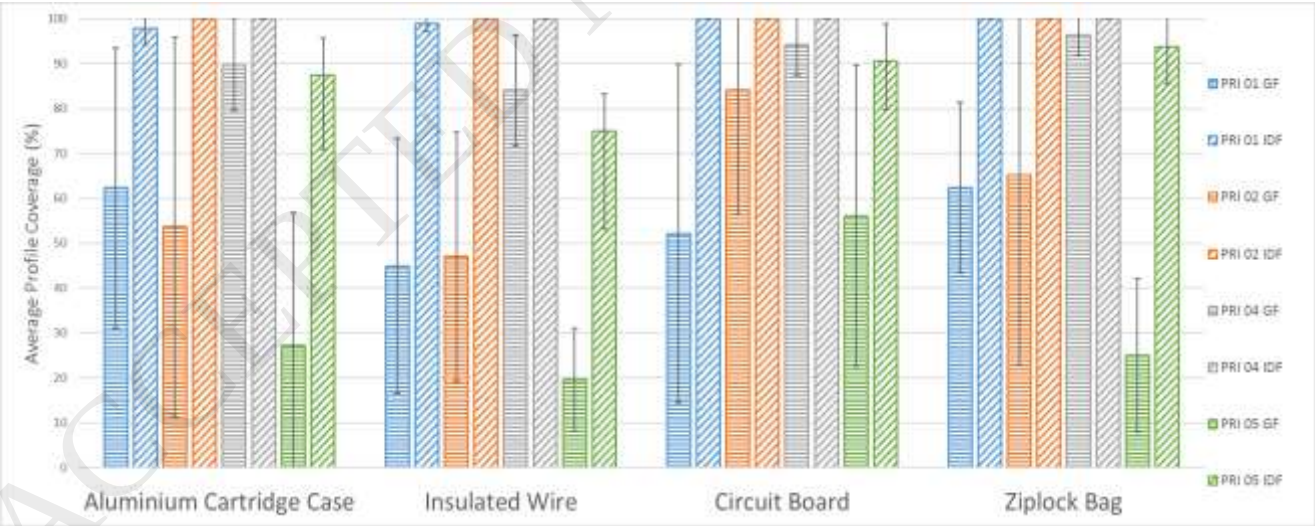

Figure 2 Difference in the average percentage of profile coverage (total number of alleles expected are 44 or 46 with GlobalFiler® or 32 with Identifiler® Plus) on each substrate, for each volunteer, using direct PCR between the GlobalFiler® and Identifiler® Plus STR kits. Error bars are the standard deviation in the percentage of profile coverage.

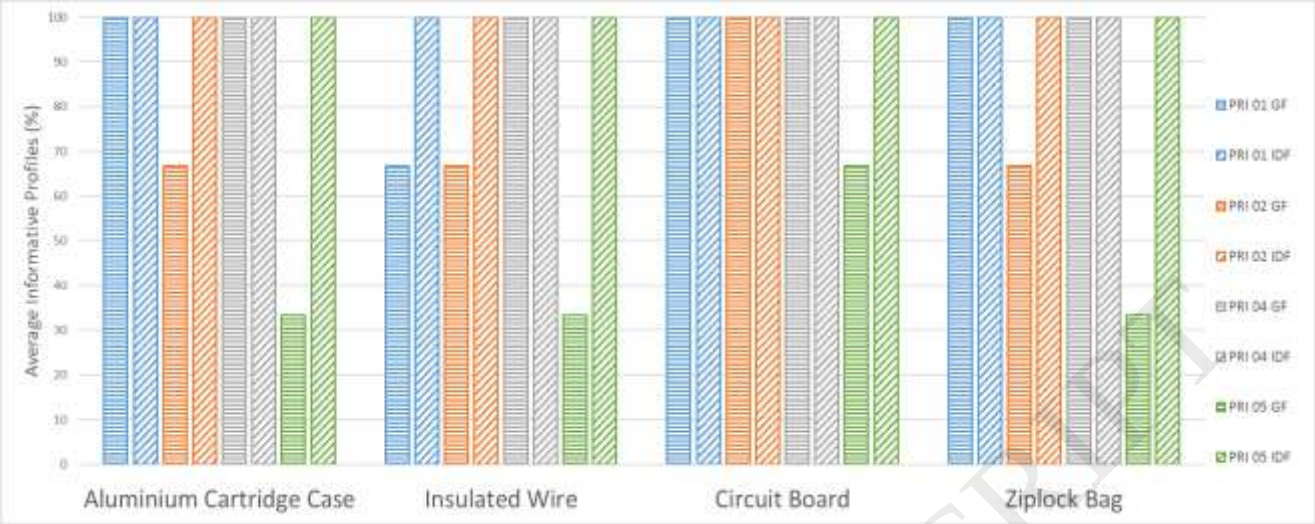

Figure 3 Difference in the average percentage of informative profiles on each substrate, for each volunteer, using direct PCR between the GlobalFiler® (left) and Identifiler Plus® (right) STR kits. Error bars are the standard deviation in the percentage of informative profiles. A profile is considered informative when 12 or more alleles, discounting amelogenin, are present.

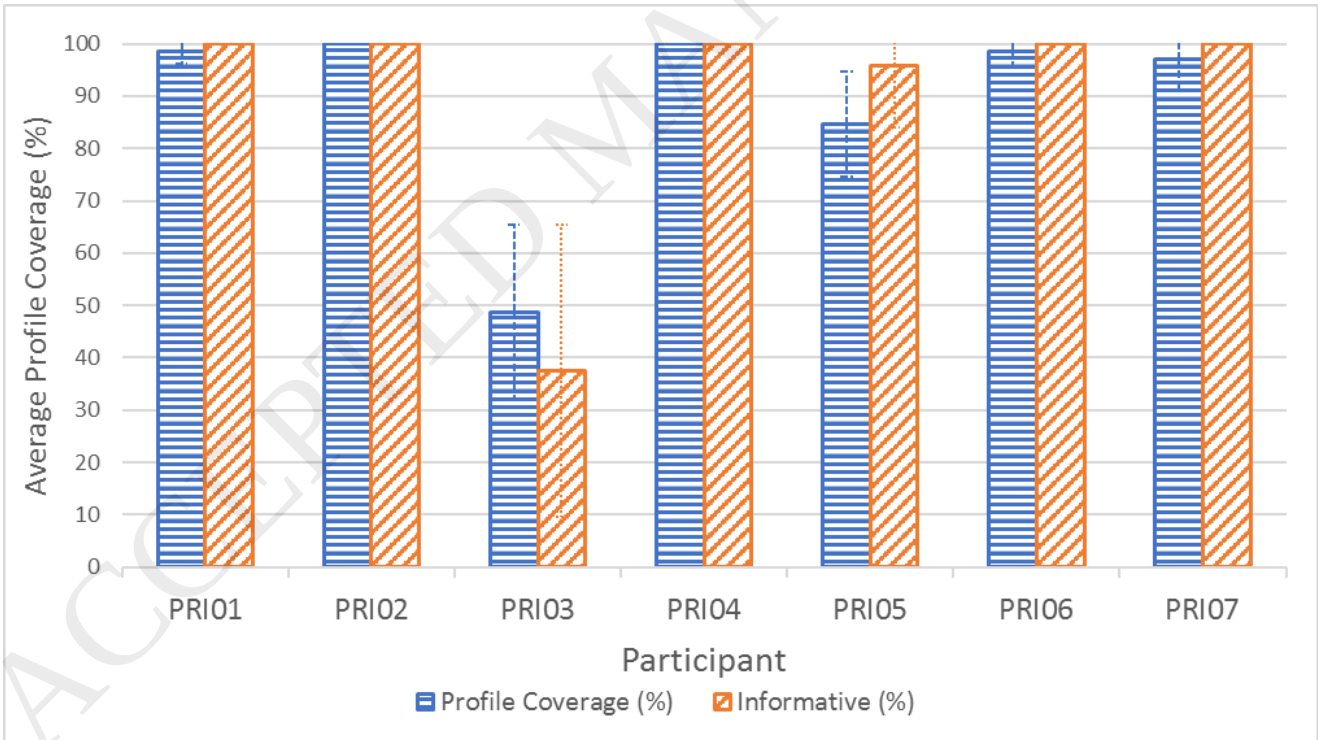

Figure 4 Difference in the average percentage of profile coverage (out of a total number of 32 alleles) of all profiles from each exhibit between the volunteers. PRI 01-04 were male while PRI 05-07 were female. Each exhibit was performed in triplicate. Amplification was performed using Identifiler® Plus.

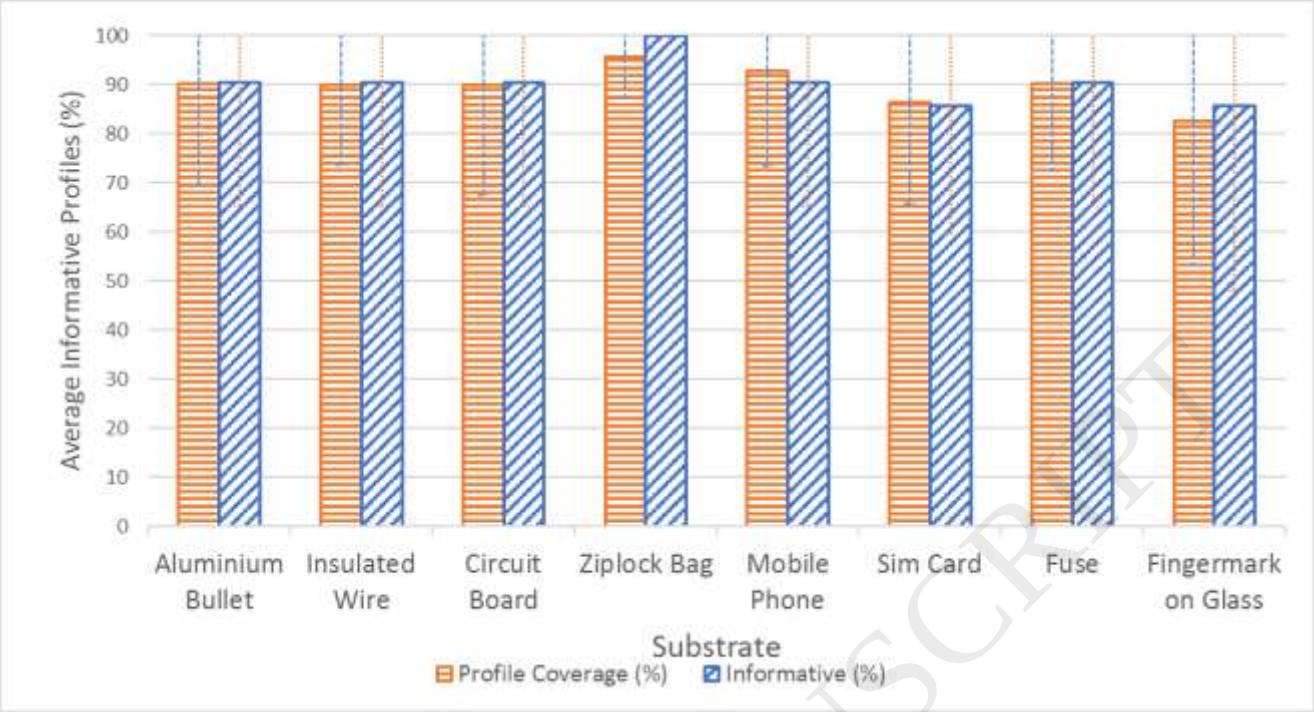

Figure 5 Difference in the average percentage of profile coverage (out of a total number of 32 alleles) of all profiles from each volunteer between the exhibits. Each volunteer touched each exhibit in triplicate. Amplification was performed using Identifiler® Plus.