Direct qPCR Quantification of Unprocessed Forensic Casework Samples

ABSTRACT
The current STR typing workflow involves sample collection, sample screening, DNA extraction, DNA qPCR quantification, PCR amplification and CE analysis. Since not every forensic sample contains probative DNA, subjecting non-probative samples to STR analysis wastes time and resources. Although the information obtained from qPCR quantification is useful for identifying samples with non-probative values, samples have to be extracted and purified before they can be quantified. By the time samples are ready for quantification, significant amounts of time and resources have been spent on extracting samples of non-probative value. To meet customers’ demand, we developed the direct qPCR quantification technology which allows forensic scientists to directly quantify forensic samples without the need for sample preparation. The direct qPCR quantification is possible with the invention of the PE-Swab, which also enables direct PCR amplification. The direct quantification and direct amplification technologies represent major advancements in forensic STR typing workflow.

INTRODUCTION
The increasing number of case work samples collected and submitted, especially those from property crimes, have made the timely processing of all the samples especially challenging. As a result, DNA backlog has been growing. Some laboratories also limit the number and the type of samples that can be submitted in each case. Law enforcement officers are making difficult decisions on which DNA samples to submit, and this may leave more probative samples left un-submitted. Therefore, a simple, fast, and inexpensive screening tool capable of measuring DNA quantity and the sample gender is highly desired.
In this presentation we will describe the proof of concept studies of a new forensic DNA sample screening technique called direct qPCR quantification. This technique allows the DNA quantity and the sample gender information to be obtained before the sample is processed for DNA extraction and purification.

MATERIALS AND METHODS
Touch DNA samples were collected on transparent films. 10 µL of ethanol was applied to each touch sample before being swabbed using a 5 mm PE-Swab. Dry blood stain samples were prepared by pipetting 20 µL blood from a female donor onto five different types of substrates. The liquid blood was spread out with a pipette tip and was set out to dry for five days. Before swabbing, 40 µL de-ionized water was applied to each dry blood stain. Because the amount of dry blood on the substrate is much higher than on the touch DNA samples, a larger 20 mm PE-Swab was used to swab each dry blood stain.

RESULTS

Figure 1. The PE-Swab
A PE-Swab consists of three components: a filter paper strip, a holder and a clip. A PE-Swab was assembled by wrapping a filter paper strip around the holder and then securing the paper strip on the holder using the clip at the end of the PE-Swab handle. An example of a functional PE-Swab is shown in Figure 1A. PE-Swabs were used to swab objects of interest that contain touch DNA samples or blood samples. When a swabbing liquid was used, it was applied to the object of interest before swabbing. After swabbing, the filter paper strip was detached from the swab holder and was air dried before punching (See Figure 1B). Using a Harris Uni-Core™ punch, punches of the desired size were generated from the active sampling area of the filter paper for a direct qPCR quantification assay or a direct STR amplification assay.

Figure 2. Direct quantification of unprocessed touch DNA samples

The feasibility of direct quantification was demonstrated using Quantifiler® Duo DNA Quantification kit on 19 unprocessed touch samples (See Figure 2). No human or Y DNA was detected in any of the four female samples. Among the 15 male samples, both human and Y DNA were detected in 13 samples. The two exceptions were sample Male_B_S1 (only Human DNA detected, 41pg) and Male_D_S3 (only Y DNA detected, 32pg). The failed detection of both targets is likely due to the stochastic effect which is common for low copy number samples.

Other than the samples from donor Male_E, the PIC Ct values for all samples were within 0.5 Ct of the punch NTC (29.1, average: 0.19, standard deviation) indicating minimal inhibition even with unprocessed samples. Interestingly, the PIC Ct values from all three samples of donor Male_E (27.7, average: 0.09, standard deviation) are more than one Ct lower than the rest of the samples (29.1, average: 0.14, standard deviation). As expected, after DNA extraction and purification using AutoMate Express™, the PIC Ct discrepancy between donor Male_E and rest of the samples disappeared (The average PIC Ct for all purified samples was 28.6 with standard deviation of 0.11). The lowered PIC Ct values in unprocessed Male_E samples suggest that some composition in these samples actually enhances qPCR efficiency. The exact reasons are not clear at this point. Nevertheless, this result clearly demonstrated the feasibility of direct qPCR quantification of unprocessed touch DNA samples.

Figure 3. The correlation between direct qPCR quantification, post extraction quantification and post extraction STR typing

After taking a 0.5 mm punch for direct qPCR, the remaining sample from all 19 touch samples shown in Figure 2 were further extracted and purified using an AutoMate Express™ Forensic DNA Extraction System. 2 µL purified DNA was quantified. Based on the post extraction quantification result, 15 µL purified DNA from each of the 19 samples was amplified using GlobalFlex™ PCR Kit. The quantification results from the direct qPCR, the post extraction qPCR and the percentage alleles recovered from STR typing were plotted in Figure 3. The samples were ranked from the highest to the lowest based on the direct qPCR human quantity (the solid blue line). The trend of the human quantity from the post extraction qPCR (the dotted red line) follows that of the direct qPCR. The fluctuation of the post extraction qPCR line is likely caused by the non-uniformity of the sample on a PE-Swab and the stochastic effect from low copy number samples.

The direct quantification results also correlated with the percentage alleles recovered (the solid green line) from the STR analysis. The top five ranked samples on average have 98% of the alleles recovered, while the bottom five ranked samples on average only have 8% of the alleles recovered. This result clearly demonstrated that the direct qPCR with the Quantifiler® Duo kit is effective at prioritizing a large number of samples.

Figure 4. Direct quantification of unprocessed blood samples

The feasibility of direct quantification of unprocessed blood samples was also demonstrated (See Figure 4). No male DNA was detected in any of the five samples, which agrees with the fact that the blood is from a female donor. Although the blood samples were unprocessed, only the IPC Ct for black leather and brown leather is higher than the average IPC Ct value for NTC. The IPC Ct for cement, denim 1 and denim 2 are less than 0.13 Ct higher than the average IPC Ct value of two NTC reactions.

Figure 5. Direct qPCR/direct STR amplification of dry blood stains on various substrates

Based on the direct qPCR quantitation results shown in Figure 4, optimal punch size and number of punches were used in the direct STR reactions. Figure 5 shows the correlation between the DNA input amount and the average peak height of the STR profiles. Full STR profiles with high profile quality were obtained from all five dry blood stain samples.

CONCLUSIONS
A workflow for direct qPCR quantification of unprocessed forensic casework samples has been developed and demonstrated. By collecting the forensic casework sample on a PE-Swab, a paper punch containing unprocessed DNA sample can be generated and placed directly in a Quantifiler® Duo assay to assess the gender and the DNA quantity of the sample. Since the direct qPCR quantitation is correlated to the post extraction qPCR quantitation as well as the post extraction STR typing results, the direct qPCR can be used to prioritize both touch DNA samples and blood stains so that crime laboratories have the choice to process the samples with better chance of obtaining probative STR profiles first. The direct qPCR results can also be used to estimate the optimal DNA input for the direct STR amplification of the sample from the same PE-Swab. The direct qPCR and the direct STR amplification of unprocessed forensic casework samples collected on the same PE-Swab could become an attractive alternative STR typing workflow.

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