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Preliminary evaluation of microfloq® capabilities to capture dna from buccal and bloodstained swabs with globalfiler express kit

Research article

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Abstract

Direct amplification of crime scene samples has become a trending method in recent years. Many laboratories worldwide longed for this type of method in order to reduce case backlogs, save cost for extraction, save time and human labour. MicroFLOQ® swabs offered direct amplification method with promising evaluation results. Not only in saving time and cost for eliminating extraction procedure, this study has found that with a minimum of 8µl of GlobalFiler™ Express Kit (GFE), a full DNA profile could be generated from both buccal swab samples and blood samples. At 25 cycles, buccal swab samples could generate full profiles with 8µl of GFE while blood swabs require 27 cycles with the same volume of GFE. These results were a stepping stone to achieve a no- extraction procedure for screening purposes of crime scene samples. Thus, selective sampling could be carry out to fit the purpose of reducing cases turnaround time and budgeting for DNA laboratories.

Keywords:

direct PCR, buccal sample, bloodstained swab, microFLOQ®, GlobalFiler™ Express

Introduction

DNA profiling from crime scene samples like swabs, stained-cloth and other evidences were time consuming and the method is often conventional. Most forensic laboratories preferred to stick with the methods in order to save cost. However, due to high number of national backlogs for casework samples [1], these laboratories' capabilities were questioned and the government has proposed the idea of having more high technology and robust analysis which require shorter time of getting the profiles.

Inventors and R&D around the world have designed many tools to fit the purpose which includes the user friendly criteria, easy application and compact as well as simple tools for extraction purposes in order to meet the turnaround time for analysis of DNA profiling. To begin with, COPAN has produced nylon flocked swabs, 4N6FLOQ® swabs which intended for buccal sampling usage and crime scene recovery [2].

This study was carried out to evaluate the capability of microFLOQ® swab to capture DNA and to determine the optimum cycle numbers of PCRas well as volume of GlobalFiler™ Express Kit (GFE) used,which will benefit the DNA laboratories in the future.

Based on previous study, microFloq®has been proved to be more effective in producing high recoveries of DNA compared to manual extraction and this device was also tested with various human amplification kits worldwide

[3,4]. This promising results were used as our references to elaborate more on finding the best cycle number to amplify the microFLOQ® swabs but at the same time enabled us in saving costs of extraction and amplification kits.

Materials and method

Blood and buccal samples were collected from two different individuals and in accordance with DNA Act 2009 (Act 699) in Malaysia.

Buccal samples

4N6FLOQ®Swabs reference collection was used to collect the buccal samples from individuals. They were dried in the fume hood at room temperature.

Blood samples

Blood samples were collected and stained on 4N6FLOQ®Swabs reference collection. They were dried in the fume hood at room temperature.

Sample collection with microFLOQ® Direct swabs

Sample collection was carried out following manufacturer's protocol. The tip of the swab was touched on the sample and labelled accordingly to cycle number and volume of master mix. For example, B25A which the letter B represented the Buccal sample while D represented the Blood sample. Number 25 indicated the cycle number used for amplification process and A represented the volume of 14.5 µl of GlobalFiler Express used while B is 10 µl and C is 8 µl.

PCR amplification of microFLOQ® Direct Swabs

After sample collection, the tips of microFLOQ® Direct swabs were splitted into 96-well plate and amplified using the GlobalFiler™ Express (GFE) PCR Amplification Kit (Thermo Fisher Scientific). There were three different final volumes of GFE used for amplification process which were 14.5µl, 10µl and 8µl. Each volume was represented as A, B and C respectively. Amplification was performed on an ABI GeneAmp 9700 PCR System for 25, 26 and 27 cycles. Positive control of DNA 007 with the presence of microFLOQ® as well as negative control that containing microFLOQ® only were included in the assay.

DNA detection, separation and analysis

The amplified products (amplicons) were analysed using ABI 3500xl Genetic Analyser (Life Technologies). 1µl of PCR products, 9.5µl Hi-Di™formamide and 0.5µl Genescan™ Liz Size Standard v2.0 (Thermo Fisher Scientific) were used on this size-separation analysis. Allelic ladder was also included in every injection on the 96 well-plate with 1µl of volume. Samples were denatured for 3 minutes at 95 °c and cooled on ice afterwards for 3 minutes. Electrophoresis were performed with 24-cm capillary array with validated protocols using POP-4™ polymer with standard injection parameters. Data obtained were analysed with Gene Mapper® IDX Software version 1.4 (Life Technologies) with manufacturers validated thresholds.

Results and discussions

Buccal swabs samples using microFLOQ® with different volumes of GFE and different PCR cycles

Buccal swabs samples were amplified with 25, 26 and 27 cycles. Each cycles hold 3 different volumes of GFEmastermix which were 14.5µl, 10µl and 8µl. The bar chart was plotted for each cycle as well as the peak height ratio of each signal for heterozygous alleles. In order to determine the optimum volume of mastermix, the chart was analysed with T-Test analysis (2-tailed, n=3) and significant p-value < 0.05 was determined.

Figure 1 showed that at 25 cycles, all microFLOQ® swabs showed the capability to capture sufficient DNA to gain full profiles. The optimum volume of GFE for this cycle was 8µl and the peak height ratio obtained were 85% in minimum. This result also evaluated that the minimum peak height ratio of all the genetic markers were accepted within laboratories analytical protocols. 4N6FLOQ®Swabs reference collectionalso proved that quality DNA could be collected for profiling and this will be resulted in saving cost and time for amplification process with GFE. Each genetic marker in GFE showed consistency in providing highest average peak height with optimum volume.

With these consistencies, microFLOQ® has shown the capability in evenly distribution of DNA at theirs' tips.

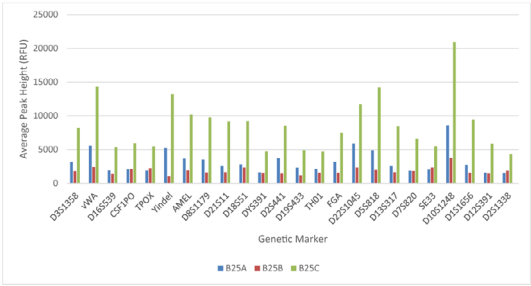

Figure 1: Average Peak Height (RFU) and Peak Height Ratio (%) of Genetic Marker of microFLOQ®from Buccal Swabs with Different Volume of GFEMastermix for 25 Cycles

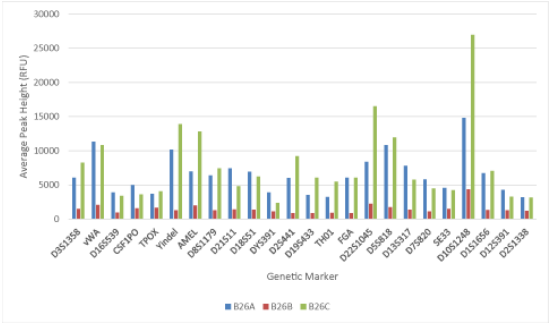

Figure 2: Average Peak Height (RFU) and Peak Height Ratio (%) of Genetic Marker of microFLOQ®from Buccal Swabs with Different Volumes of GFEMastermix for 26 Cyclesent Volume of GFEMastermix for 25 Cycles

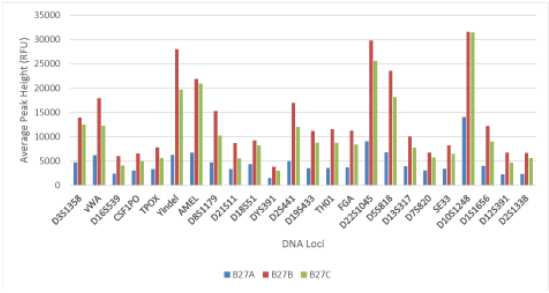

Figure 3: Average Peak Height (RFU) and Peak Height Ratio (%) of Genetic Marker of microFLOQ®from Buccal Swabs with Different Volumes of GFE Mastermix for 27 Cycles

Table 1: Percentages of profile generated with different volumes of GFE mastermix and different

PCR Cycles	Volumes of Mastermix (µl)	Percentage of profile generated (%)
25	14.5	87.5
		92
	8	100
26	14.5	92
	10	100
	8	96
27	All volumes	100

When the cycle increased to 26 and 27 (Figure 2 &3), the pattern of average peak height still equivalent with 25 cycles.

However, at 27 cycles, the optimum volume was 10µl which is higher than the optimum volume at 25 cycles. This might due to the increasing of DNA templates during increased cycles of PCR. Still, the average peak height of 8 µl and 10 µl of GFE were comparable, thus either of this volume could generate a good DNA profile.

These results indicated that, the buccal swabs taken with 4N6FLOQ®Swabs reference collection and tipped with microFLOQ® could be used for references DNA profiling method and skipping the extraction and quantification process which could save more time, cost and less in human labour and error. The minimum volume of master mix was also indicated that lower cost for profiling purposes could be achieved with several modifications in the laboratory standard operating procedure.

Blood swabs samples using microFLOQ® with different volumes of GFE and different PCR cycles

Blood swabs were analysed with three different PCR cycles and different volumes which were 14.5µl, 10µl and 8µl. At 25 cycles, only one volume could generate full DNA profiles which is 8µl while at 26 cycles, only 10µl of master mix could generate full DNA profile. Full DNA profiles could be generated with 27 cycles for all the volumes tested. These results were simplified in Table 1.

Based on the results obtained, the best cycle number for amplification of blood samples would be 27 while for 26 and 25 cycles, only one amount would fit each other; 25 cycles with 8µl and 26 cycles with 10µl. This protocol is slightly deviated from the manufacturer's recommendation which is 28 to 30 cycles for bloodstained samples, which means that the time needed for amplification process could be reduced and resulting in saving more time on gaining DNA profile.

Factors contributing to the results obtained for direct amplification of buccal swabs and blood swabs

The results obtained from both experiments of buccal and blood swabs showed significant differences in providing full profiles as well as the PHR for each condition tested. In brief, buccal swabs showed, the generations of full profile were able to achieve with either 25, 26 or 27 cycles. This might due to the samples of the buccal itself which the cells from saliva and cheeks consisted less inhibitors or foreign contaminants compared to blood. Blood on the other hand, showed only at 27 cycles could developed a full profile. Raw blood has been acknowledged to contain PCR inhibitors like heme [3-8] therefore, the cycle number must be larger due to its complexity and appropriate copy numbers for full profile development.

For buccal swabs, the sampling techniques are crucial since it involved the transfers of DNA from individual to swabs. The effect of pressure plays an important role for DNA deposition [5,6]. Same goes when the DNA was transferred from swabs to microFloq®. The microFloq®

tips are very small and sensitive, however it is depended on the sample that is needed to be captured. For example, if the buccal swabs were still wet, they needed to be partially dried before using microFLOQ® or the DNA would be diluted and deteriorate in the presence of water. The wetting procedure of microFloq® were applied only when the samples were dried in order to increase the DNA captures on the tips.

Conclusion and recommendation

In conclusion, microFloq® has shown the capability to capture DNA from 4NFLOQ® swabs either for buccal or blood swabs but different cycles of PCR generates different output profiles. The best cycle for amplification process of buccal swabs is 25 cycles with 8µl of Globalfiler™ Express mastermix while 27 cyles with 8µl of mastermix. These results indicate that the laboratory could reduce a lot on its expenses on reagents and consumables per year. Plus, by using these simple methods for database purposes could reduce in time and man power.

However, microFloq should be tested further since the study only focused on the volume of mastermix and PCR cycle numbers. In fact, angle of swabbing is still need to be studied further for optimum DNA capturing. Furthermore, there were other kits could be tested with microFloq® which is much more sensitive, equipped with quality sensors, as being reported by Habib et al 2017 (7), that is believed to generate different outcomes to this study, so that in future, microFLOQ® could fit the purposes of capturing DNA at the crime scene prior analysis to save cost on analysis and increase the rate of crime solving cases.

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