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Comparison of the Efficiency of Globalfiler™ IQC PCR Amplification Kit and Powerplex® Fusion 6C System with Half-Volume Reactions for Forensic Trace DNA Testing

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Abstract

Two major challenges in daily and routinely forensic genetics test are samples with low or very low quantities of DNA and high cost of processing. To overcome these obstacles, a reduction in reaction volume to half would offer a substantial benefit, but even so it should provide complete genetic profiles without conceding the quality of the results. The aim of this study is to test the robustness of two commercially available kits, GlobalFiler™ IQC PCR Amplification Kit (GF-IQC) and PowerPlex® Fusion 6C System (P-6C), with half volume reaction in accordance with SGWDAM guidelines. GF-IQC showed minimum and stochastic threshold of 45 and 610 RFU, while P-6C demonstrated 60 and 670 RFU of those values respectively. The P-6C can tolerate to many inhibitors including melanin, hematin, and humic acid. Both kits showed the same performance on case work profiling and the sensitivity of allele detection of P-6C was not significantly different from that of GF-IQC (p=0.144). The results of this study demonstrated that GF-IQC and P-6C with half volume reaction can produce good quality profiles and could be applied for forensic DNA examination.

Keywords: Forensic genetic; DNA typing; short tandem repeats; human identification; GlobalFiler™ IQC PCR Amplification Kit; PowerPlex® Fusion 6C System.

Introduction

The gold standard for human-based DNA profiling is Short Tandem Repeat (STR) fragment analysis, using multiplex PCR and capillary electrophoresis (CE).[1] In 2017, the Combined DNA Index System (CODIS) reported original 13 STR loci and expanded with another 7 STR loci for human identification. Hence, at present commercial PCR kits consist of up to 20 loci for forensic DNA profiling.[2] It is necessary for a forensic laboratory to validate a commercial PCR kit before using it with casework, and do this following the SGWDAM Guidelines.[3]

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Many parameters such as baseline noise, sensitivity, inhibitor tolerance, accuracy and precision are used in the internal validation.[4] Also, these parameters can be used to evaluate and compare commercially available kits.[5-6]

GlobalFiler™ IQC PCR Amplification Kit (GF-IQC) consists of 21 autosomal loci, amelogenin and 2 Y-chromosome markers with an additional IQC (internal quality control system) marker, whereas PowerPlex® Fusion 6C System (P-6C) consists of 23 autosomal loci, amelogenin and 4 Y-chromosome markers. Both kits include autosomal STRs from the CODIS core STRs.[7,8] For successful DNA profiling, use of a high-performance kit is important because the trace DNA in forensic samples is often of low quantity and contains PCR inhibitors.[9] The manufacturer's recommended PCR volume for most available kits is 25 µL, however several studies were successful in obtaining solutions with reduction in reaction volume to half.[10,11] A reduction in reaction volume to half would provide many considerable advantages, such as complete genetic profiles even in low DNA samples or the presence of PCR inhibitors, and would be cost-saving in the daily forensic genetics routine. But optimization and comparison studies should be carried out to confirm the amplification efficiency and the quality of DNA profiles.

This study aimed to evaluate and compare the performance of two commercial PCR kits, GF-IQC and P-6C, with half-volume reactions. Multiple forensic parameters, including baseline noise, sensitivity and stochastic threshold, inhibitor tolerance and heterozygous balance, were investigated.

Materials and Methods

DNA samples

The samples used in this study were comprised of control DNA 1224, 1230 (InnoGenomics), 007 (Applied Biosystems), 2008M (Promega) and DNA left over from 31 real-casework samples (Table 1). Consent for their use was given by the Subdivision of Biology and DNA, Central Police Forensic Science Division. The DNA quantity was determined by Quantifiler™ HP DNA Quantification Kit (Applied Biosystems).

Table 1: Type of samples

Type of sample	Number of samples
Buccal swab	6
Blood	6
Tissue	1
Semen	2
Hair	1
Fingernails	2
Cigarettes	2
Vagina swab	2
Toothbrush	1
Touched DNA	8
Total	31

Amplification

For GF-IQC (Applied Biosystems), the components for a reaction consisted of 3.75 µL master mix, 1.25 µL primer set and 7.5 µL DNA template (total 12.5 µL; half reaction) with 29 cycles of 95°C for 1 min, 94°C for 10 sec, 59°C for 90 sec and 60°C for 5 min. As for P-6C (Promega), the components for a reaction consisted of 2.5 µL master mix, 2.5 µL primer set and 7.5 µL DNA template (total 12.5 µL; half reaction) with 29 cycles of 96°C for 1 min, 96°C for 5 sec, 60°C for 60 sec and 60°C for 10 min.

DNA electrophoresis

Prior to analysis, the samples were prepared by mixing 9.5 µL HiDi™ formamide, 0.5 µL size standard reagent and 1 µL PCR product. The samples were then denatured for 3 min at 95°C and applied to ABI 3500 Genetic Analyzer using POP-4 polymer and 36 cm capillaries with 1.2 kV 15 sec injection condition. The results were analyzed by GeneMapper® IDX v.1.4 (Thermo Scientific).

Baseline study

Nine samples of Low TE^{-4} were amplified with each kit with half-volume reactions. Then, the products were electrophoresed and examined at 1 RFU. The total results were computed to obtain an average of baseline peak height and a standard deviation (SD) of each fluorescent channel. These values were used to calculate the minimum threshold as average + 3*SD (Limit of Detection; LOD) and average + 10*SD (Limit of Quantification; LOQ) of each kit.[12]

Sensitivity and stochastic study

Serial amounts of input DNA, including 0.008, 0.016, 0.031, 0.063, 0.125, 0.25, 0.5, 1 and 2 ng per reaction of Control DNA 1224 were triply amplified with each kit. Then, the products were electrophoresed and examined at the minimum

threshold which was obtained from the base line study. Data were computed as percentage of allele detection, average heterozygous peak height, and peak height ratio (PHR). These results were used to assess the optimal input DNA, heterozygous peak balance and stochastic threshold for each kit.

Inhibitor study

Melanin, hematin and humic acid were dissolved in an appropriate solvent (melanin: 0.5 M NH_4OH; hematin: 0.1 M NaOH; humic acid: nuclease-free water).[13] Each inhibitor, along with an optimal amount of input control DNA 1224, was used as half-volume for amplification. Then, the samples were tested with an ABI 3500 Genetic Analyzer and examined at 175 RFU. The percent of allele detection and average heterozygous peak height were calculated and used to assess each kit's tolerance to inhibitors.

Casework study

DNA samples from Table 1 were amplified and analyzed. For interpretation, results were determined at 175 RFU analytical threshold and the stochastic threshold from the above sensitivity study to assess kits' performance; percent allele detection was used to compare the results of the two kits.

Calculation and statistical methods

Results were organized and calculated using Microsoft Excel. Parameter calculations were as follows.

(1) Percent allele detection

% allele detection = (allele count)/ (total alleles) × 100

(2) Peak height ratio

Peak height ratio (PHR) = RFUmin/ RFUmax × 100

(3) Stochastic threshold

The highest surviving sister allele was defined as the stochastic threshold obtained from the results of serial input DNAs.[14]

(4) Wilcoxon rank test

Wilcoxon rank test was used for statistical comparison of percent allele detection and average peak height.[15]

Results

Baseline study

Limit of detection (LOD)and limit of Quantification (LOQ) that can be calculated from the average peak height of background noise of the GF-IQC and P-6C are shown in Table 2. GF-IQC gave its highest LOQ of 44.53 RFU in the green (VIC™) channel. Yet, P-6C's highest LOQ (55.16 RFU) was also in the green (JOE-6C) channel. The minimum threshold can be determined using the LOQs of each kit. GF-IQC and P-6C, with half-volume specimens, had 45 and 60 RFU as the minimum thresholds, respectively.

Table 2: LOD, LOQ and minimum threshold of GF-IQC and P-6C with half-volume reaction.

Dye channel	GF-IQC				P-6C			
	Dye	LOD	LOQ	Minimum threshold	Dye	LOD	LOQ	Minimum threshold
Blue	6-FAM™	14.27	33.56	35	FL-6C	18.79	42.20	45
Green	VIC™	20.14	44.53	45	JOE-6C	25.17	55.16	60
Yellow	NED™	11.94	29.37	30	TMR-6C	12.94	29.30	30
Red	TAZ™	18.36	41.23	45	CXR-6C	19.60	42.62	45
purple	SID™	19.24	43.57	45	TOM-6C	16.75	37.19	40

Sensitivity and stochastic study

The relationship between percentage allele detection and DNA input is shown in Figure 1. GF-IQC produced complete profiles when the range of DNA input was 125 pg - 2 ng. The allelic

dropout occurred when input DNA amount was 8 - 63 pg. For P-6C, the sensitivity was not found significantly different from that of GF-IQC (p= 0.144). Nevertheless, average heterozygous peak height of GF-IQC was significantly higher than that of P-6C (p= 0.008).

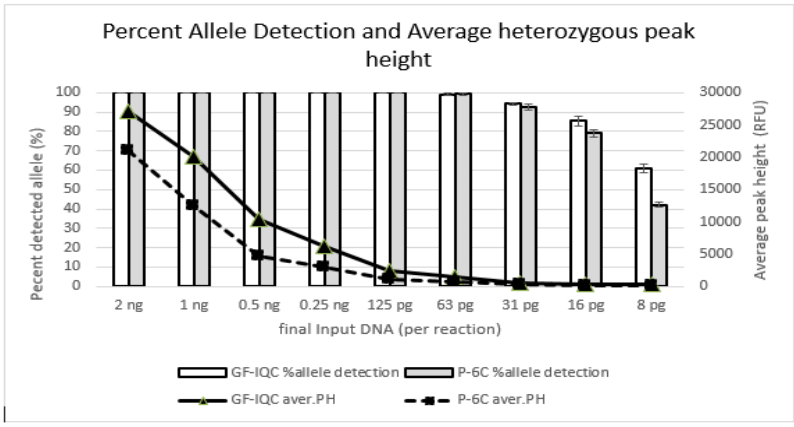

Figure 1: Percent allele detection (% allele detection) and Average heterozygous peak height (aver.PH) of serial input DNA, amplified by GF-IQC and P-6C.

Using half-volume reaction, GF-IQC performed up to 56% PHR in 0.5 - 2 ng input DNA (Figure 2). The lowest PHR was found at 10% in 31 pg input DNA. Whereas the P-6C had similar performance to GF-IQC (Figure 3). In range of 0.5 - 2 ng, more than or equal 60% PHR was found. The lowest PHR was found at 8% in 31 pg input DNA.

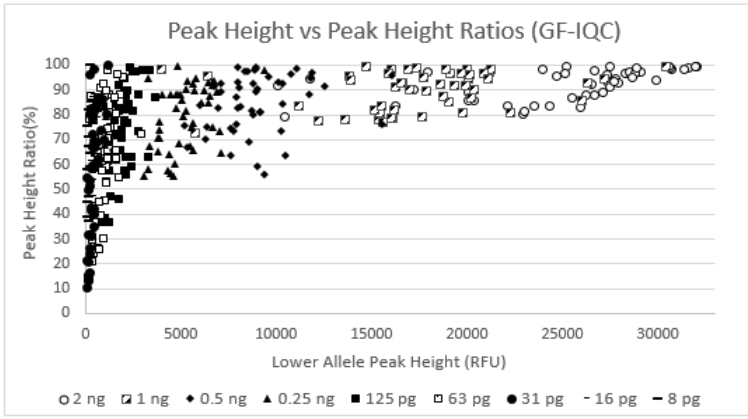

Figure 2: Average heterozygous peak of serial input DNA amplified by GF-IQC.

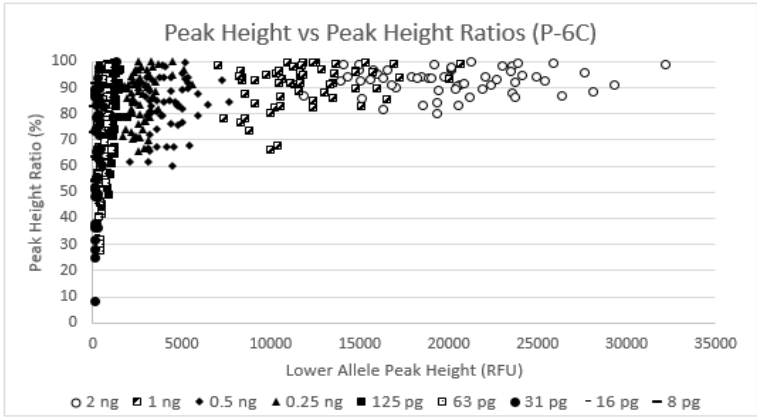

Figure 3: Average heterozygous peak of serial input DNA amplified by P-6C.

The evaluation of half-reaction performance of both kits showed that the optimal input DNA of both kits was 0.5 ng. This conclusion used three criteria: 1) revealing optimal peak height, 2) disappearing the pull up or off-scale peaks, and 3) providing a complete profile or lacking allele drop out [16]. Moreover, the stochastic thresholds of GF-IQC and P-6C were at 610 and 970 RFU, respectively. The highest surviving sister allele of GF-IQC was found at D19S433 (16 pg input DNA), while it was found at Amelogenin (63 pg input DNA) of the P-6C.

Inhibitor study

Overall, the peak height of DNA profiles was decreased when the concentration of inhibitors was high, and amplifying without inhibitors always produced complete DNA profiles. As shown in Figure 4, melanin started to inhibit the activity of GF-IQC and P-6C at 60 and 80 ng/μL, respectively. Complete inhibition was found with melanin concentrations of 80 and 100 ng/μL. However, these melanin concentrations did not cause complete inhibition of the P-6C even at the higher concentration of 100 ng/μL. The hematin caused partial inhibition of amplification by GF-IQC at 750 μM, and complete inhibition at 1000 and 1250 μM (Figure 5). However, even the highest concentration of hematin did not inhibit the amplification of P-6C. Humic acid, at concentrations of 200 and 300 ng/μL, inhibited amplification activity of both GF-IQC and P-6C (Figure 6).

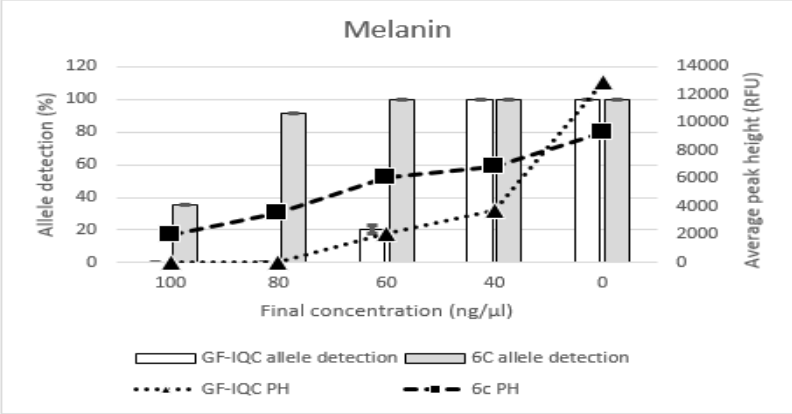

Figure 4: Percent allele detection and average heterozygous peak height with 5 concentrations of melanin in amplification using GF-IQC and P-6C. Bars and lines represent percent allele detection and the average heterozygous peak height, respectively.

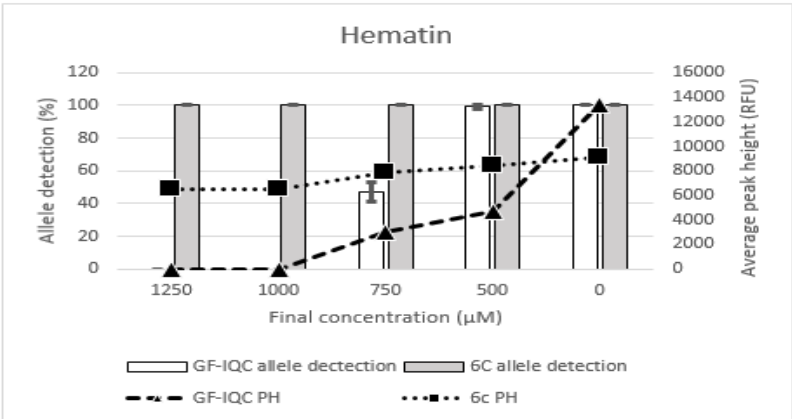

Figure 5: Percent allele detection and average heterozygous peak height with 5 concentrations of hematin in amplification using GF-IQC and P-6C. Bars and lines represent percent allele detection and the average heterozygous peak height, respectively.

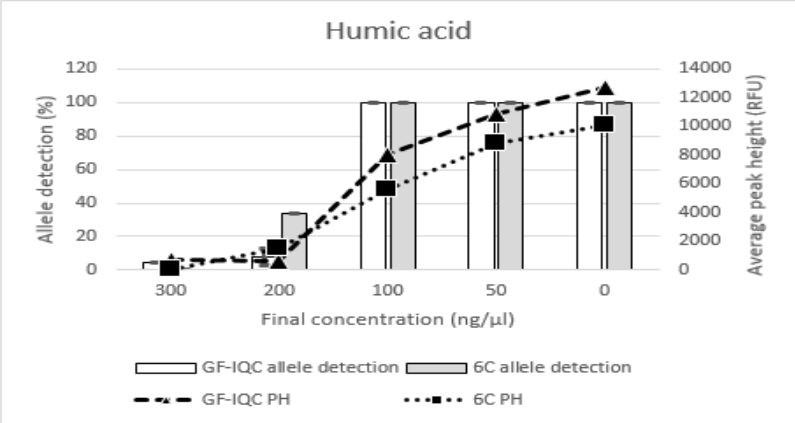

Figure 6: Percent allele detection and average heterozygous peak height with 5 concentrations of humic acid in amplification using GF-IQC and P-6C System. Bars and lines represent percent allele detection and the average heterozygous peak height, respectively.

Casework study

Among thirty-one samples, both kits detected 19 complete profiles, 4 partial profiles and 6 mixture profiles (data not shown). The kits' results were concordant except for sample S24 which originated from a fingernail. The GF-IQC gave a partial profile with 99% percent allele detection (SD=2), while the other kit gave a complete profile. Yet, both kits remained amplifying a concordant profile for this sample. Lastly, neither kit detected the trace DNA on a swab of a keyboard (code S20).

Discussion

This study is the first to evaluate and compare the performance of GF-IQC and P-6C, with half-volume reactions. P-6C generated higher baselines than did GF-IQC with these half-volume reactions, which is consistent with the results when full volume reactions are used.[12,18] The GF-IQC with half-volumes showed minimum thresholds similar to previously published data with full volume reactions.[5,17]

GF-IQC with half-volume reactions produced complete profiles with 0.125 ng of input DNA and there was no difference in sensitivity from that with full volume reactions.[5,13,18] P-6C with half-volume reactions also produced complete profiles with 0.125 ng input DNA. This was consistent with full volume reactions reported by Cisana et al.[19] but differed from the report by Feng et al.[20] the groups reported complete profiles with DNA amounts of 0.125 and up to 62.5 ng, respectively. GF-IQC and P-6C demonstrated similar results of percent allele detection, although the heterozygous peaks were significantly different. As described earlier, half-volume reactions of both kits amplified as well as full volume reactions. As a result, 0.5 ng was suggested as the optimal input DNA amount for both kits. The stochastic threshold is crucial for DNA interpretation. The highest false-homologous peak which can determine stochastic threshold was observed in both kits with low input DNA. The thresholds with half-volume reactions were suggested at 610 RFU for the GF-IQC and 970 for the P-6C.

Performance in terms of generating balanced heterozygous peaks by the P-6C was slightly better than that of the GF-IQC. With optimal quantity of DNA (0.5 ng per reaction), Short Tandem Repeat amplification kits should perform more than 60% PHR.[21] P-6C with half-volume reactions met this parameter adequately at 60%, whereas GF-IQC gave a slightly poorer PHR at 56%. Moreover, low input DNAs are likely to cause a decrease in PHR. In this study, the lowest PHRs were 10% and 8% for GF-IQC and P-6C, respectively (obtained from 31 pg input DNA).

Melanin, hematin and humic acid usually originate from hair, blood and soil, respectively, and are commonly found in evidence. In this study, P-6C was more tolerant to these three inhibitors (especially hematin) than was GF-IQC. Surprisingly, P-6C still showed complete profiles even when 1250 µM hematin was added into the reaction. Tan et al. reported that 2000 µM hematin cannot inhibit full volume reactions of P-6C.[17] Thus P-6C with half-volume reactions can be used effectively with blood-related evidence. Moreover, both kits with half-volume reactions successfully amplified various types of samples from real casework, as shown by the results of 31 casework samples, and gave concordant results.

Conclusions

We evaluated and compared the performance of GF-IQC and P-6C with half-volume reactions. The optimal amount of input DNA was 0.5 ng for both kits. We suggest a 45 RFU minimum threshold and

610 RFU stochastic threshold for GF-IQC. We also suggest a 60 RFU minimum threshold and 970 RFU stochastic threshold for the P-6C.

In contrast, the GF-IQC was less tolerant to 3 inhibitors and produced imbalanced peaks more easily than did the P-6C. Yet, the GF-IQC produced significantly higher average peak heights however the kits had no significant differences in sensitivity, and performed with high robustness. Our overall assessment was that the GF-IQC and P-6C, with half-volume reactions, could both be applied productively for forensic purposes.

Ethical approval statement: The experimental protocol was approved by the Ethics Committee of Thammasat University (COA No. 024/2564).

Conflicts of interest: Nil.

Source of funding: Self.

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