



Comparison of Direct Amplification vs. Extraction, Quantification, and Amplification using GlobalFiler™ or Fusion™ Kits



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Abstract

With the recent purchase of Applied Biosystems® 3500 Genetic Analyzers, the Tennessee Bureau of Investigation (TBI) was looking to optimize these instruments for their CODIS division. The purpose of this comparison study was to compare two different kits and also two different methods for obtaining the profiles for input into CODIS. The first method tested was to perform the traditional, and more time consuming, method of extraction, quantitation, and amplification of buccal swabs before performing capillary electrophoresis. This was done using either the Applied Biosystems® GlobalFiler™ DNA Amplification kit or the Promega PowerPlex® Fusion™ kit using 7 different reference samples for multiple runs. The second method was to perform direct amplification of buccal swabs before performing capillary electrophoresis using either the GlobalFiler™ Express Kit or the Fusion™ Direct kit using the same 7 reference samples.

When comparing the kits of GlobalFiler™ Express and Fusion™ Direct, it was found that Fusion™ Direct produced higher quality results especially in terms of peak height ratio. It was suggested that future studies, and eventually validation, be performed with the Fusion™ kit for use in the CODIS unit at TBI.

Introduction

At the beginning of this study, TBI was using the Applied Biosystems® AmpFLSTR® Identifier® Plus PCR Amplification Kit (Life Technologies™, Foster City, CA). TBI was hoping that with the purchase of new instruments and new kits, direct amplification would be possible with the same level of results as the traditional analysis method. With the number of backlogs that every lab has today, a more cost effective method with a faster turnaround time is needed. Single-source samples, which were the only sample type used for this study, are usually of good quality and do not need to be reamplified or rerun to produce a full profile. With the abundance of these types of samples and the lack of need to quantitate them, per Standard 9.4 of the FBI's Quality Assurance Standards, the decrease in time and cost for the GlobalFiler™ Express Amplification Kit or Fusion™ Direct Kit is optimal for these types of samples.

Materials & Methods

The studies performed in this comparison study were cotton tip buccal swab samples from 7 individuals. The following kits, instruments, and software were used:

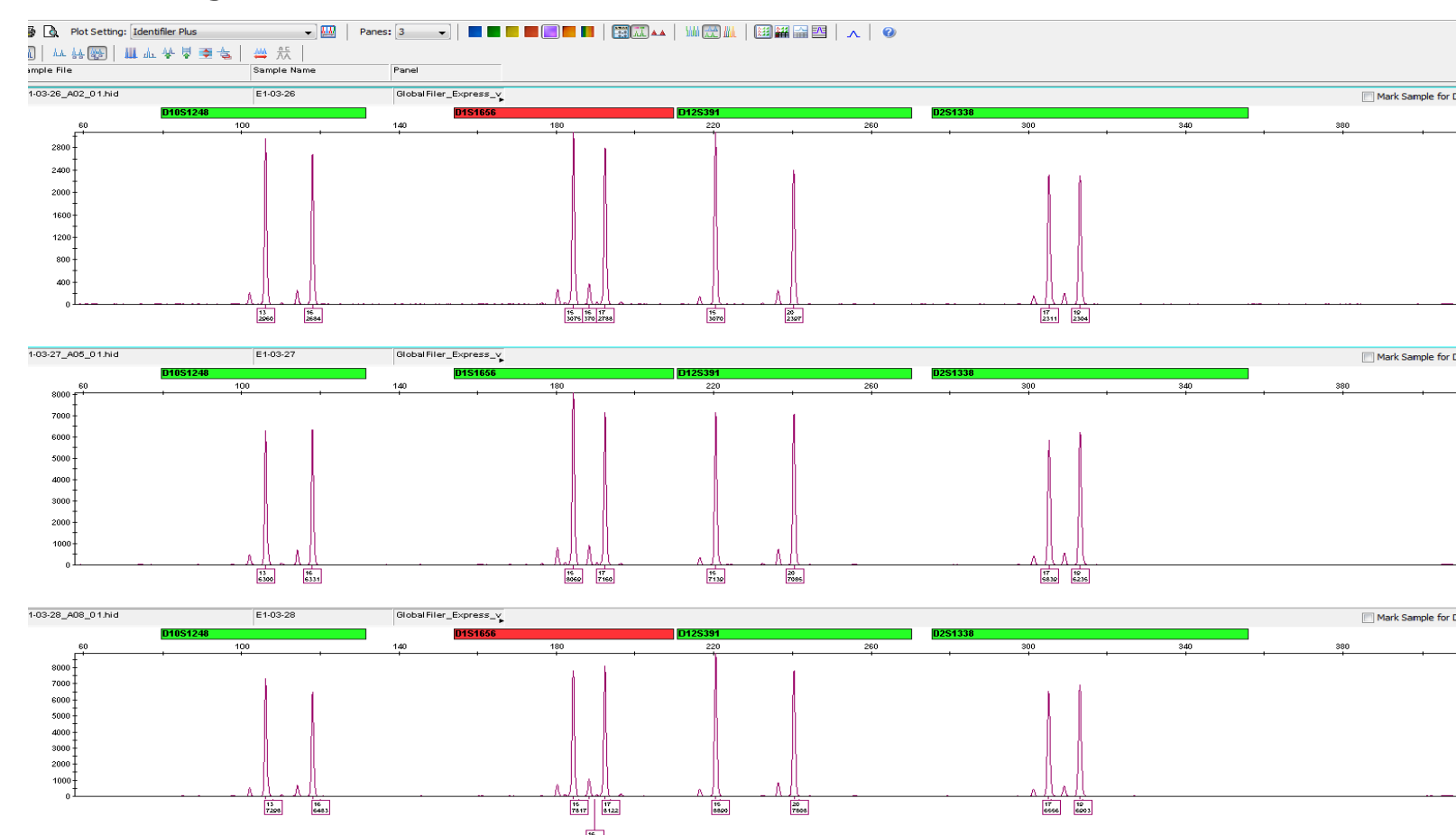
- Applied Biosystems® GlobalFiler™ Amplification Kit
- Applied Biosystems® GlobalFiler™ Express Amplification Kit
- PowerPlex® Fusion™ Amplification Kit
- PowerPlex® Fusion™ Direct Amplification Kit
- Applied Biosystems® GeneAmp® PCR System 9700
- Applied Biosystems® 3500 Genetic Analyzer
- GeneMapper® ID-X version 1.4

Cycle Time Study

26, 27, 28 cycles were tested for amplification for both GlobalFiler™ Express and for Fusion™ Direct. All 3 cycle times were tested first and then the two best cycle times between the three were tested for final comparison using a different sample set.

GlobalFiler™ Express Cycle Test

27 cycles were chosen due to fewer artifacts present and the peak height ratios calculated



Fusion™ Direct Cycle Test

26 cycles were chosen due to lower artifacts, good peak heights and peak height ratios produced



Results

Individual kits were averaged along with standard deviations for comparison

	Peak Height average	Peak Height standard deviation	Peak Height Ratio Average	Peak Height Ratio Standard Deviation
GlobalFiler™	6465.157	2845.86	0.8715	0.04907
GlobalFiler™ Express	7048.39	3722.612	0.8655	0.052139
Fusion™	10077	5028.165	0.82326	0.05362
Fusion™ Direct	8435.407	5687.885	0.925695	0.023819

GlobalFiler™ kit Comparison

Out of the 159 loci calculated, 123 were heterozygotes with 51.2% of the average peak height ratio being better for GlobalFiler™ at an average of 0.05433 difference in the peak height ratios between the two methods. There was an 0.045981 average difference in the peak height ratios of the two methods when GlobalFiler™ Express had a better peak height ratio at a particular loci and sample. For the remaining 36 homozygote loci, GlobalFiler™ had a greater peak height average than GlobalFiler™ Express 55.56% of the time with an average difference between the two methods of 1782.674. While fewer loci had a greater peak height average for Express, the average for the difference was almost double GlobalFiler™ at 2756.924.

Fusion™ kit Comparison

The average of the difference between the peak height or peak height ratio between the two methods was also calculated across all loci. Out of the 164 loci calculated for, 131 were heterozygotes. Only two of those peak height ratios produced a better balance for Fusion™ at an average of 0.030325 difference in the ratios between the two kits. Fusion™ Direct produced a better balance in the other 129 loci at an average of 0.104852 average difference in the two methods at a particular loci and sample.

For the remaining 33 homozygote loci, Fusion™ produced a greater peak height average than Fusion™ Direct 63.6% of the time with an average difference between the two kits of 3826.728. For the other 36.4% of the time, Direct was greater at an average of 3213.429.

Traditional Method Comparison

148 loci were calculated for the GlobalFiler™ and Fusion™ kits with 117 being heterozygotes and the remaining 31 being homozygotes. GlobalFiler™ had a better peak height ratio for 90 of the 117 heterozygote loci with an average difference of 0.074718. Fusion™ showed a better peak height ratio for 27 out of the 117 heterozygote samples with an average of 0.042894.

For the 31 homozygotes, Fusion™ produced a higher peak height at 24 of those with an average of 5123.586 between the two kits. For the remaining 7 homozygotes where GlobalFiler™ was better, there was an average difference of 2338.698.

Direct Amplification Comparison

149 loci were calculated for GlobalFiler™ Express and Fusion™ Direct with 118 being heterozygotes and the remaining 31 being homozygotes. For the heterozygotes, Fusion™ Direct had a more balanced peak height ratio at 109 of the 118 heterozygote samples with an average difference of 0.062838. For the remaining 9 loci where GlobalFiler™ Express produced a better peak height ratio, the average difference was 0.013414.

For the homozygotes, there was more of a balance between the two kits and their peak heights. GlobalFiler™ Express exhibited higher peak heights at 16 of the 31 with an average of 2992.86. Fusion™ Direct exhibited a much higher difference of 6782.725 for the remaining 15 homozygote samples.

Conclusions

With GlobalFiler™, almost equal results were obtained for it and GlobalFiler™ Express. With PowerPlex® Fusion™ and Fusion™ Direct, Direct was shown to have preferred results over the traditional method using extraction. Comparing the two kits of the direct amplification method, PowerPlex® Fusion™ Direct was shown to be a choice kit in terms of allele calling when compared to GlobalFiler™ Express due to the peak height ratios and peak heights obtained when using the Fusion™ Direct kit. Fewer artifacts were also shown to be present with the Fusion™ Direct kit.

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