Internal Validation of the Applied Biosystems® GlobalFiler™ Express PCR Amplification Kit

FORENSIC SCIENCE

Shanna Saunders[1], Kyra Groeblinghoff[2], Season Seferyn[1], Dr. Pamela Staton[1]

[1]Marshall University Forensic Science Center, 1401 Forensic Science Drive, Huntington, WV 25701

[2]St. Louis County Police Crime Laboratory, 111 South Meramec, Clayton, MO 63105

Abstract

The conventional process for obtaining DNA profiles from reference standards includes extraction and quantitation steps which add cost and time to the workflow. Advancements in buffers and amplification kits allow these steps to be modified or removed which can decrease costs and increase time savings allowing these resources to be redirected to casework. The Applied Biosystems® GlobalFiler™ Express DNA Amplification Kit utilizes these advancements and is a novel kit to the St. Louis County Police Crime Laboratory. Per FBI Quality Assurance Standards (Sept. 2011), any novel PCR amplification chemistry needs to be validated in order to ensure that reliable data is being produced. The St. Louis County Police Crime Laboratory conducted an internal validation of the Applied Biosystems® GlobalFiler™ Express DNA Amplification Kit on the Applied Biosystems® 3500 Genetic Analyzer. All studies were analyzed using Applied Biosystems'® GeneMapper ® *ID-X* v1.4. Since the National DNA Index System (NDIS) has approved the kit, the Applied Biosystems® GlobalFiler™ Express Kit will be put into use by the St. Louis County Police Crime Laboratory.

Introduction

Through the National Institute of Justice's Technical Assistantship Program, the St. Louis County Police Crime Laboratory requested an internal validation of the Applied Biosystems® GlobalFiler™ Express Amplification Kit be performed on the Applied Biosystems® 3500 Genetic Analyzer. Through these validation studies, optimal settings were determined so that the Applied Biosystems® GlobalFiler™ Express Amplification Kit would produce optimal results from common types of reference samples while being cost and time effective.

Materials & Method

The studies performed in this validation used FTA® blood card and buccal swab (q-tip and Whatman® comb style) samples that were previously gathered. The following kits, instruments, and software were used:

- Applied Biosystems® GlobalFiler™ Express Amplification Kit
- AmpFLSTR® Identifiler® PCR Amplification Kit
- Applied Biosystems® GeneAmp® PCR System 9700
- Applied Biosystems® 3500 Genetic Analyzer
- GeneMapper® *ID-X* version 1.4

Figure 1: Applied Biosystems® GlobalFiler™ Express Amplification Kit
Applied Biosystems. GlobalFiler™ Express PCR Amplification Kit User Guide. Rev 10/2012.

Validation Studies Performed

Sensitivity/Non-Probative Sample Type	Reproducibility
Injection Time	Concordance*
Analytical Threshold	Population*
Stochastic Threshold	Internal Stutter
Contamination*	Mixture*
Precision	Pass Rate*

** Indicates studies that are not discussed in this poster*

Sensitivity/Non-Probative Sample Type

- Determine the cutting size and dilution to produce the ideal DNA target load to produce reliable results
- FTA® Card samples were prepared in 20, 50, 100, and 200 µL of PrepNGo™ Buffer; buccal swab samples were prepared in 200 µL of PrepNGo™ Buffer; all were incubated at 37°C for 20 minutes
- A range of volume of PrepNGo™ Buffer was used to determine the best dilution to allow the ideal DNA target load to be amplified

Injection Time

- Determine the injection time to produce reliable results
- 5, 10, 15, and 20 second injection times were studied throughout the validation
- A range of injection times were used to determine the injection parameters for the Applied Biosystems® GlobalFiler™ Express DNA Amplification Kit on the Applied Biosystems® 3500 Genetic Analyzer

Table 1: Injection Times and Related Peak Heights.

	Injection Times (s)	Average PH (RFU)	Max PH (RFU)
Ladders	20	9179	11505
	15	7930	9871
	10	3607	4439

Analytical & Stochastic Threshold

- Analytical Threshold (AT) is the peak height at which a true peak can be detected
- Calculated by analyzing the instrumental noise of eight amplification negatives at three different injection times
- Separate thresholds were calculated per injection time and per dye color using the following two equations:

Equation 1: $AT = 2(Maximum\ peak\ height - Minimum\ peak\ height)$

Equation 2: $\text{AT} = Average\ peak\ height + (3 \times Standard\ Deviation\ peak\ height)$

- Rounded up to the nearest ten and the maximum calculated AT between the two equations was taken
- Stochastic Threshold (ST) is the peak height at which a homozygous peak can be called without concern of heterozygous peak dropout
- Calculated by analyzing eighty five samples at the determined Analytical Threshold
- Separate thresholds were calculated per dye color using the following equation:

$$ST = Analytical\ Threshold\left(\frac{1}{Average\ PHR - (3 \times Standard\ Deviation\ PHR)}\right)$$

- Highest surviving false homozygote peak height was then taken into consideration (270 RFU)

Table 2: Calculations of AT and ST for Each Dye Color.

	Average PHR	Std. Dev. PHR	Analytical Threshold (RFU)	Stochastic Threshold (RFU)
Blue	0.8778	0.0884	50	81.62
Green	0.8594	0.0985	70	124.13
Purple	0.8792	0.0939	60	100.44
Red	0.8577	0.1234	70	143.59
Yellow	0.8990	0.0843	80	123.82

Precision & Reproducibility

- Precision Study was completed to ensure that sizing within an injection was consistent
- Twenty four allelic ladders were run and base pair size was analyzed for precision
- Reproducibility Study was performed to ensure that sizing between injection was consistent
- Twenty four allelic ladders were run on three different days and base pair size was analyzed for reproducibility

Internal Stutter

- Determine the appropriate stutter ratios (SR)
- Eighty samples were analyzed with a stutter filter set to 0
- All artifacts were removed besides the stutter artifacts Minus 8, Minus 4, Plus 4, and Minus 2 (D22S1045 is a trinucleotide meaning stutter artifacts are Minus/Plus 3)
- Stutter artifacts were separated according to locus and the following equation was used:

$$SR = Average\ Stutter\ Ratio + (\,3\ \times Standard\ Deviation\ of\ the\ Stutter\ Ratio)$$

Table 3: Comparison of Internal and Life Technologies™ Stutter Ratios.

	Internal			Life Technologies™
Marker	Stutter %	Std. Dev.	Calculated %	Stutter %
D3S1358	8.19	1.78	13.53	12.45
vWA	6.99	1.94	12.82	12.33
D16S539	5.58	1.63	10.48	10.17
CSF1PO	5.21	1.25	8.97	11.4
TPOX	2.76	1.03	5.84	5.43
D8S1179	6.03	2.36	13.11	10.22
D21S11	6.84	1.38	10.99	11.42
D18S51	7.56	2.04	13.68	13.47
D2S441	5.10	1.25	8.85	8.75
D19S433	7.03	1.50	11.54	10.58

	Internal			Life Technologies™
Marker	Stutter %	Std. Dev.	Calculated %	Stutter %
TH01	2.11	0.60	3.91	5.24
FGA	7.28	1.43	11.57	11.96
D22S1045	9.06	2.87	17.67	8.27
D5S818	6.32	1.30	10.22	10.84
D13S317	4.98	1.20	8.58	9.98
D7S820	4.74	1.65	9.71	10.21
SE33	8.82	2.22	15.47	14.42
D10S1248	7.81	1.46	12.21	12.5
D1S1656	7.60	1.95	13.44	13.08
D12S391	8.80	2.61	16.62	15.08
D2S1338	8.17	1.63	13.07	12.45

Conclusions

The GlobalFiler™ Express PCR Amplification Kit on the 3500 Genetic Analyzer is recommended for use with future single-source samples to increase throughput for the St. Louis County Police Crime Laboratory. The reliability and reproducibility of the incorporation of this chemistry and platform are based on the settings and recommendations determined from the validation studies.

- For FTA® card samples, two punches per sample in 20 µL of PrepNGo™ Buffer gave the ideal target DNA concentration ; A one-half cutting of a swab sample in 200 µL of PrepNGo™ Buffer produced the ideal target DNA concentration
- A combination of 10 and 20 second injection times will account for high and low yield samples
- The Analytical Threshold was determined to be 80 RFU and the Stochastic Threshold was determined to be 300 RFU
- Size calling was precise within injections and reproducible between injections
- Applied Biosystems® recommended minus 4 and minus 2 stutter ratios will be used while calculated minus 8 and plus 4 stutter ratios will be used

Future Studies will include a comparison of the Identifiler® and GlobalFiler™ Kits to the GlobalFiler™ Express Amplification Kit on the 3500 Genetic Analyzer.

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