

Internal Validation of Yfiler® for Casework at the St. Louis Metropolitan Police Department (SLMPD)



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Abstract

The Applied Biosystems®'s (AB) Yfiler® DNA amplification kit was internally validated at the St. Louis Metropolitan Police Department (SLMPD) for the detection of male DNA. For the internal validation of Yfiler®, sensitivity, precision, mixture, probative/non-probative, and species cross reactivity studies were performed. The Yfiler® kit when a 0.8ng amplification target was used showed to be precise and sensitive. The mixture study demonstrated male DNA can be detected in ratios of 1:5000 parts female DNA and confirmed the kit's ability to detect multiple male profiles. The samples used in the probative/non-probative study successfully proved the kit's ability to amplify forensic type samples. The species cross reactivity study proved human specificity.

Introduction

The AB AmpFℓSTR® Yfiler® kit was validated for the SLMPD based upon the guidelines produced by the Scientific Working Group on DNA Analysis Methods (SWGAM). These studies included: sensitivity, precision, probative/non-probative, mixture and species cross-reactivity studies. These studies were used to determine the practical working conditions of Yfiler® for use in casework at the SLMPD.

The Yfiler® amplification kit is a Short Tandem Repeat (STR) kit specific to the sex determining region on the Y-chromosome. Yfiler® kits can be valuable when there is a high female to male ratio of DNA in a sample, if there is an absence or lack of sperm preventing a differential extraction and in determining the minimum number of males in a multiple assailant crime.

Materials and Methods

Samples were subjected to the following:

Extraction: Qiagen® EZ1® DNA Investigator kit (Sexual assault samples were differentially extracted before placement on EZ1®)

Quantitation: AB's 7500 Real-Time PCR System and Quantifiler® Duo

Amplification: AB's AmpFℓSTR Yfiler® PCR kit on AB's GeneAmp® PCR System 9700

CE and Analysis: AB Prism® 3130 Genetic Analyzer and Genemapper® ID v. 3.2

Samples were developed for the following studies:

Sensitivity Study

- One known male sample was amplified in a range of 3.0 ng to 0.05 ng.

Precision Study

- Allelic Ladder (9 wells; injected 5 times)

Probative/Non-Probative Study

- Swabs of: a newspaper vending machine, a cell phone, 5 different door handles, a handrail, a drinking cup, a keyboard, a laptop and a pen
- 44 Casework Samples (unknown genders)
- 8 mock sexual assault samples (created from a semen dilution series being added to different vaginal swabs); EF and MF used

Species Cross-Reactivity Study

- 7 separate DNA extracts of: cow, dog, chicken, baboon, mouse, orangutan, and deer (Unknown genders)

Mixture Study

- Male/Female, Male/Male, and Male/Male/Male mixtures were prepared as shown in Table 1.

Table 1: Prepared Ratios for the Mixture Study

Male to Female		Male to Male		Male to Male to Male	
Sample	Males : Female	Sample	Male A : Male B	Sample	Male A : Male B : Male C
MF1	1 : 0	MM1	1 : 0	MMM1	1 : 1 : 1
MF2	1 : 1	MM2	19 : 1	MMM2	1 : 1 : 2
MF3	1 : 10	MM3	9 : 1	MMM3	1 : 2 : 1
MF4	1 : 100	MM4	4 : 1	MMM4	2 : 1 : 1
MF5	1 : 500	MM5	2 : 1		
MF6	1 : 1000	MM6	1 : 1		
MF7	1 : 600	MM7	1 : 2		
MF8	1 : 1200	MM8	1 : 4		
MF9	1 : 2400	MM9	1 : 9		
MF10	1 : 5000	MM10	1 : 19		
MF11	1 : 10000	MM11	0 : 1		

Results

The results from the validation of Yfiler® as follows:

Sensitivity Study

Table 2: Results of the Sensitivity Study

Male DNA- Known Profile	Alleles Detected (%)	Artifacts Present
3.00 ng	100	Pull-up, Stutter
2.00 ng	100	Pull-up, Stutter
1.00 ng	100	None
0.80 ng	100	None
0.60 ng	100	None
0.47 ng	100	None
0.30 ng	100	None
0.10 ng	100	None
0.08 ng	25	None
0.05 ng	6.25	None

Precision Study

Table 3: Precision Study- Average Standard Deviation for All Alleles at Each Locus

Locus	Avg. St. Dev. for Alleles	Locus	Avg. St. Dev. for Alleles
DYS437	0.046	DYS392	0.0647
DYS458	0.0379	DYS635	0.0456
DYS438	0.0462	DYS389I	0.0467
DYS448	0.0556	DYS390	0.0452
Y GATA H4	0.0429	DYS456	0.0445
DYS391	0.0447	DYS19	0.0518
DYS439	0.0469	DYS385	0.0688
DYS393	0.0418	DYS389II	0.0578

Probative/Non-Probative Study

- Male profiles were detected on: two door handles, cell phone, drinking cup, keyboard, and laptop samples.
- A partial male profile was detected on three door handles, newspaper vending machine, handrail, and pen samples.
- Possible mixtures were noted on four door handles, pen, handrail and keyboard samples.
- 14 Full and 12 Partial Yfiler® profiles were produced out of the 44 casework samples.
- 8 out of the 16 mock sexual assault samples produced a male profile.
- 4 out of the 16 mock sexual assault samples produced a partial male profile.

Mixture Study

- Male : Female – a full male profile was detected until the 1:5000 ratio (MF10) where dropout occurred.
- Male : Male – a mixture of at least two profiles was present; major/minor contributors could be deconvoluted (DYS458, DYS385 and DYS 393 were not included in this determination)
- Male : Male : Male – all contributors were present

Species Cross-Reactivity Study

- No reactivity was noted in any of the samples

Discussion/Conclusions

Sensitivity Study

Full profiles were obtained for all samples in the 1ng to 0.1ng range. The targeted RFU was set at 1000 to maximize resolution. After calculating the RFUs for each sample, the 0.8ng sample was the closest to the target, averaging 952.7059 RFUs. At 0.8ng there were no unexpected artifacts and acceptable baseline.

Precision Study

Nine ladders were injected five times for this study. Table 3 represents the average standard deviation of the alleles at each locus for the Yfiler® ladder. All standard deviations were below the recommended standard deviation of 0.15 bp. Therefore, Yfiler® allele sizes are precise when run on the AB Prism® 3130 Genetic Analyzer.

Probative/Non-Probative

Yfiler® was able to establish results on casework samples. Mock sexual assault samples were able to produce profiles using the Yfiler® kit. This may be valuable for casework involving sexual assault samples and other samples in which the female DNA is at a much higher level than the male DNA present. A future study to investigate autosomal STR results versus results obtained using Yfiler® should be completed.

Mixture Study

In this study, it was noted that mixtures of male DNA can be detected including mixtures of multiple men. In a 19:1 male: male mixture major and minor contributors could be determined. For the male: female mixtures, full male profiles were detected until a ratio of 1:5000. The greater presence of female DNA reduced the amplification of the male DNA resulting in partial profiles. To enhance this study, more male mixtures could be analyzed.

Species Cross-Reactivity Study

None of the animal samples reacted with the Yfiler® kit. To complete a specificity study, animal samples of known gender should be used.

References

- Applied Biosystems. AmpFℓSTR® Yfiler™ PCR Amplification Kit. Foster City: Applied Biosystems, 2006. Print
- Butler, J. M. 2005. Forensic DNA typing, 2nd Edition. Elsevier Press; New York, NY. 201-222.
- Federal Bureau of Investigation. "Revised Validation Guidelines Scientific Working Group on DNA Analysis Methods (SWGAM)." Federal Bureau of Investigation, July 2004. Web. Spring 2010.
- Gross, A. M., et al. 2008. Internal Validation of the AmpFℓSTR Yfiler Amplification Kit for Use in Forensic Casework. Journal of Forensic Sciences. 53: 1.
- Godby, Justin. "Technical Assistance Program." Class. Marshall University, Huntington. Spring 2010. Lecture Marshall University Forensic Science Center. 2007. Yfiler Validation Studies. Marshall University.
- Mulero, J. J. et al. 2006. Development and Validation of the AmpFℓSTR Yfiler PCR Amplification Kit: A male specific, Single Amplification 17 Y-STR Multiplex System. Journal of Forensic Sciences. 51: 1.

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