Internal Validation of the Life Technologies® Yfiler™ PCR Amplification Kit

Katie Jacque, BS[1], Lynnett Redhead, MS[2], Sarah Bowen, MS[1], Pamela Staton, PhD[1]

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

[2]Prince George's County Police Department Serology/DNA Laboratory, 7600 Barlowe Road, Palmer Park, MD 20785

Abstract

Standard 8 of the National Quality Assurance Standards for Forensic Science Laboratories requires that an internal validation be performed before new DNA technology can be introduced into casework analysis. The Life Technologies™ AmpF*l*STR® Yfiler™ PCR Amplification Kit is a multiplex assay that amplifies 17 loci located on the Y chromosome. Y-STR amplification is of interest at Prince George's County Police Department Serology/DNA Laboratory due to the overwhelming number of cases with complex mixtures encountered annually. Eight studies were performed in the validation: analytical threshold, precision, contamination, sensitivity, reproducibility, concordance, mixtures, and stutter. The results demonstrated that the Yfiler™ kit successfully amplified evidence samples, is male specific, and is precise.

Introduction

Y chromosomal short tandem repeat (Y-STR) amplification targets the male component, the Y chromosome, and can be utilized in forensic casework where the male component is of interest, such as complex mixtures. The majority of complex mixtures are composed of a high female component and a low male component concentration, which can be isolated by Y-STR analysis.

Materials and Methods

Kits and Instrumentation

- EZ1® DNA Investigator Kit* and BioRobot EZ1® Workstation*
- Quanitfilier® Human Kit^ and 7500 Real-Time PCR System^
- AmpF*l*STR® Yfiler™ PCR Amplification Kit^ and GeneAmp® PCR System 9700^
- Hi-Di™ Formamide, GeneScan™ 500 LIZ™ Size Standard, Performance Optimized Polymer (POP) 4, and 10X Run Buffer^ and a 3130 Genetic Analyzer^
- GeneMapper® *ID* software version 3.2.1^

Threshold – 5s, 10s, 15s

- Each dye channel was analyzed for the three highest artifact peaks: the highest peak before, after, and between alleles

Reproducibility and Concordance – 5s

- Amplification and run duplicated, with the run on a separate day
- Amplified and run on the 3130 Genetic Analyzer (B) and then re-set up and run on the 3130 Genetic Analyzer (A)

Sensitivity – 5s, 10s, 15s

- 2.0-0.0625ng in a serially diluted fashion

Precision and Contamination – 5s

- Allelic Ladders and run negatives in a checkerboard pattern
- Average and standard deviation for each allele and marker
- Run negatives were inspected for allele calls

Mixtures – 5s

- Male-male mixtures 1:1-1:20 and 1:1:1
- Male-female mixtures 1:-1:10,000 and 1m:1f:1f

Stutter – 5s

- 20 RFU threshold and no marker specific stutter ratio
- Used peaks in stutter position for each locus
- Minimum stutter ratio, maximum stutter ratio, average stutter ratio, standard deviation, and average + 3 σ

Results

Threshold – 5, 10s, 15s

- Three highest peaks per dye channel were recorded (Table 1) for 51 total peaks per channel
- Average and standard deviation calculated (Table 2), threshold an average of the orange boxes
- Threshold set at 150 RFU for 5s and 10s injection time, and 200 RFU for a 15s injection time

Table 1: Peak height in RFU for reagent blank (first column) & DNA sample (second column) – 5s

Dye Channel	Peak Before First Allele (RFU)		Peak Between (RFU)		Peak After Last Allele (RFU)	
BLUE	13	27	11	25	4	10
GREEN	46	80	11	40	4	5
YELLOW	16	49	11	38	9	11
RED	10	39	25	22	8	11

Table 2: 5s injection time threshold study results, RFU set at the average of the two orange boxes (150 RFU)

Dye	Ave	STDEV	AVE+3σ	AVE+5σ	AVE+7σ	AVE+10σ
BLUE	16.69	11.56	51.37	74.50	97.63	132.31
GREEN	32.82	31.42	127.07	189.91	252.74	346.99
YELLOW	19.75	13.00	58.74	84.74	110.73	149.73
RED	18.73	11.12	52.09	74.34	96.59	129.96
KIT	23.56	19.71	82.70	122.13	161.56	220.70

Reproducibility and Concordance – 5s

- Allele calls matched and RFU values were similar

Sensitivity – 5s, 10s, 15s

- Optimal input DNA concentration for 5s is 2.0-0.250ng, 10s is 1.0-0.250ng, and for 15s is 0.50-0.125ng (Figure 1)

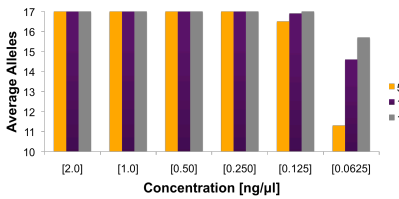

Figure 1: Average alleles from 10 samples for 5s,10s, and 15s injection time

Precision and Contamination – 5s

- No contamination was found
- 3288 concordant allele calls for the Allelic Ladders
- The average standard deviation ranged from 0.045–0.152
- All were below 0.15, except DYS385. DYS385 had all alleles within the ±0.5 base pair window (Figure 2)

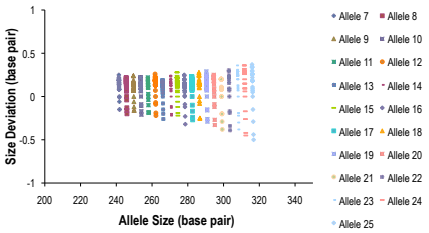

Figure 2: Size deviation of the 19 alleles of locus DYS385 from 24 samples

Male-Male Mixtures – 5s

- Full minor profiles for the 1:1-5:1 and 1:1:1 ratios
- The 1:5 ratio showed allelic dropout at 5 loci for one run and four loci for the other run
- The major contributor RFU values were higher than the minor contributor values

Male-Female Mixtures – 5s

- Full male profiles obtained from the 1:1 and 1:1:1 ratio
- Partial profiles obtained from the 1:100, average of 6 loci

Stutter – 5s

- 727 data points were used, with 512 for minus stutter and 215 for plus stutter
- 10 of 18 calculated values greater than published values
- Concluded to go with the published values because they are more conservative

Conclusion

- Threshold set at 150 RFU for 5s and 10s injection time and 200 RFU for a 15s injection time
- Optimal input DNA concentration for 5s is 2.0-0.250ng, for 10s is 1.0-0.250ng, and for 15s is 0.50-0.125ng
- Results are reproducible and concordant
- No contamination and is precise
- Full minor male profiles obtained for 1:1-5:1 and 1:1:1 male-male ratios with partial profiles for 1:5
- Full male profiles for the 1:1 and 1m:1f:1f ratios with partial profiles for 1:100
- Use published stutter values from Life Technologies™
- Successfully validated for the Prince George's County Police Department Serology/DNA Laboratory

References

1. Applied Biosystems. AmpF*l*STR® Yfiler™ PCR amplification kit user's manual. USA; 2006. 230p.
2. Decker AE, Kline MC, Vallone PM, Butler JM. The impact of additional Y-STR loci on resolving common haplotypes and closely 2010; Available from: http://www.fbi.gov/about-us/lab/codis/swgdam-interpretation-guidelines.
3. Scientific Working Group on DNA Analysis Methods (SWGDAM). Revised validation guidelines. Forensic Science Communications [Internet]. July 2004; 6(3) Available from: fbi.gov. related individuals. Forensic Science International: Genetics [Internet]. 2007; 1(2); 215-217. Available from: www.sciencedirect.com.
4. Djelloul S, Sarafian V. Validation of a 17-locus Y-STR multiplex system. Forensic Science International: Genetics Supplement Series [Internet]. 2008 Aug; 1(1): 198-199. Available from: www.sciencedirect.com.
5. Federal Bureau of Investigation. Quality assurance standards for forensic DNA testing laboratories, Forensic Science Communications [Internet]. 2000; 2(3) Available from: fbi.gov.
6. Gross AM, Liberty AA, Ulland MM, Kuriger JK. Internal validation of the AmpF*l*STR® Yfiler™ amplification kit for use in forensic casework. Journal of Forensic Science [Internet]. 2008; 53(1): 125-134. Available from: doi: 10.1111/j.1556-4029.2008.00591.x.
7. Joachimsson, CO. Validation of AmpF*l*STR® Yfiler™ and evaluation of the use of Y-STR in forensic casework in Europe. Uppsala University [Internet]. 2007:1-50. Available from: http://www.fbi.gov/about-us/lab/codis/swgdam-interpretation-guidelines.
8. Scientific Working Group on DNA Analysis Methods (SWGDAM). Interpretation guidelines for autosomal STR typing by forensic DNA testing laboratories. [Internet]. Jan 2010; Available from: http://www.fbi.gov/about-us/lab/codis/swgdam-interpretation-guidelines.

Acknowledgements

*Qiagen®, Valencia, CA; ^Life Technologies™, Foster City, CA

I thank Lynnett Redhead, Kristen Lease, Jessica Charak, Tyiesha Moore, Mary Sanchez, and Christina Tran from Prince George's County Police Department Serology/DNA Laboratory for their support, encouragement, and the experience. I also thank Justin Godby, Jenn Hayden, and Sarah Bowen at Marshall University Forensic Science Center for their help and direction. This project was supported by Cooperative Agreement Number 2009-IJ-CX-K111 awarded by the National Institute of Justice, Office of Justice Programs, US Department of Justice. The opinions, findings and conclusions or recommendations expressed in this publication/exhibition are those of the author(s) and do not necessarily reflect the views of the Department of Justice.