Internal Validation of the AmpFℓSTR® Identifiler® Plus PCR Amplification Kit and Comparison to Identifiler® for the Boston Police Department Crime Laboratory

Allison Gapinski, B.S.[1], Julie K. James, M.S.[2], Misty Marra, M.S.F.S.[1], and Pamela Staton, Ph.D.[1]

[1]Marshall University Forensic Science Center, 1401 Forensic Science Dr., Huntington, WV 25701
[2]Boston Police Department Crime Laboratory, One Schroeder Plaza, Boston, MA 02120

Abstract

An internal validation of the AmpFℓSTR® Identifiler® Plus PCR amplification kit was performed at the Boston Police Department (BPD) Crime Laboratory. The kit produced reliable, reproducible, and robust results throughout various validation studies. Identifiler® Plus also demonstrated an overall greater sensitivity and an improved performance on degraded samples as compared to the Identifiler® amplification kit currently in use.

Introduction

Amplification is an important step in forensic DNA analysis, and the amplification kit used can affect the quantity and quality of results obtained. Numerous validation studies were performed on the Identifiler® Plus amplification kit, including:

Sensitivity Study: To determine an ideal range of DNA input to produce a reliable profile with limited stochastic effects

Mixture Study: To demonstrate the kit's ability to resolve mixtures

Precision Study: To show reliable and precise typing based on allele size deviation over different runs

Concordance Study: To show accurate typing of the kit

Reproducibility Study: To demonstrate reproducible genotyping over time

Contamination Study: To examine all negative controls to ensure no contamination was present

Comparison studies between the Identifiler® Plus kit and the Identifiler® kit, currently in use at the BPD, were performed to determine if the Identifiler® Plus kit should be implemented for forensic casework. The following studies were performed:

Touch DNA Study: To compare kit performance on low level samples

Degraded Sample Study: To compare kit performance on degraded samples

Materials and Methods

- Known control blood sample extracts provided by the BPD
- Touch DNA samples collected by analysts at the BPD
- AmpFℓSTR® Identifiler® PCR amplification kit
- AmpFℓSTR® Identifiler® Plus PCR amplification kit
- GeneAmp® PCR System 9700 Thermal Cycler- 28 Cycle Amplification
- Applied Biosystems 3130*xl* Genetic Analyzer
- Applied Biosystems GeneMapper® ID v3.2.1

Results and Discussion

Validation Studies

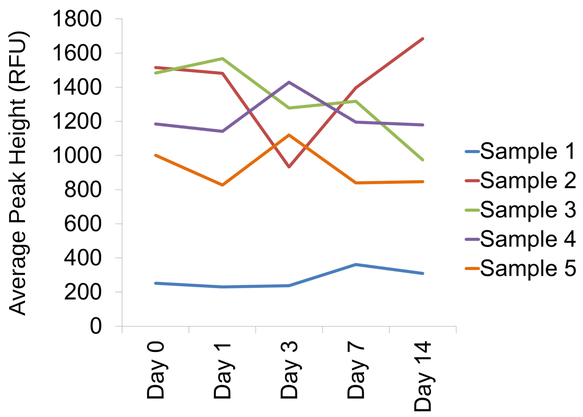

Figure 1. Reproducibility Study: No Trend in Peak Height Averages per Sample Over 14 Days

- Sensitivity Study: A range of 0.125-1.0 ng of DNA input was established, with an optimum of 0.75 ng.
- Mixture Study: Mixtures were detected when the minor component comprised 5% or more of the mixture. Samples with minor components comprising 25% or less of the total DNA input rarely crossed the stochastic threshold.
- Precision Study: Precise typing can be performed due to the low standard deviation (less than 0.15 basepairs) of the alleles of the ladder over several runs.
- Concordance Study: Allele calls were concordant with the Identifiler® kit allele calls. The Identifiler® Plus amplification kit is accurate.
- Reproduciblity Study: See Figure 1. The kit was shown to be reproducible in allele calls and peak heights up to 2 weeks after amplification
- Contamination Study: All negative controls were clean. No contamination was present in the kit, capillaries, or pipettes.

Comparison Studies

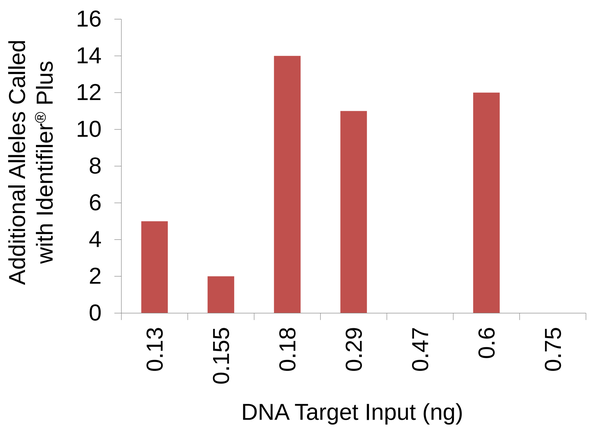

Figure 2. Touch DNA Study: Increased Performance with Identifiler® Plus over Identifiler® by DNA Target Input

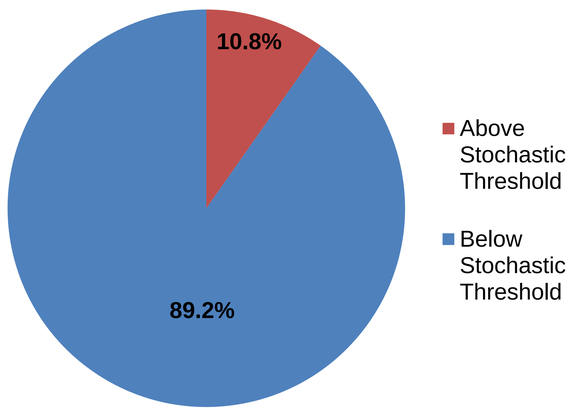

Figure 3. Touch DNA Study: Additional alleles called with Identifiler® Plus by Peak Height

- Touch DNA Study: See Figures 2 & 3. More alleles were called with the Identifiler® Plus kit than Identifiler® when analyzed at the same threshold, even though the target DNA input was lower with the Identifiler® Plus kit. This was especially seen at low DNA input levels. However, most of the additional alleles called fell below the stochastic threshold.
- Degraded Sample Study: A total of fourteen additional alleles were called in the eight degraded samples amplified with the Identifiler® Plus kit when compared with Identifiler® results from 3 years ago. The Identifiler® Plus samples in this study used half the target of the Identifiler® samples.

Conclusions

Table 1. Overall Comparison of Identifiler® Plus vs. Identifiler®

	Identifiler® Plus	Identifiler®
DNA Target Input	0.75 ng	1.0 ng
Full Profiles To	0.125 ng	0.25 ng
Peak Height Ratios	>70%	>50%
Amplification Times	2.5 hours	3.5 hours

- Identifiler® Plus was successfully validated for use at the BPD, as it was found to be reliable, reproducible, and robust.
- Identifiler® Plus was more sensitive and demonstrated improved performance on degraded samples compared to Identifiler®.
- Identifiler® Plus will be implemented for use at the BPD Crime Laboratory for forensic casework.

References

- AmpFℓSTR® Identifiler® Plus PCR Amplification Kit Product Bulletin; 2010 [cited 2012 Aug 3]. Available from: https://products.appliedbiosystems.com.
- AmpFℓSTR® Identifiler® Plus PCR Amplification Kit User's Guide; 2012 [cited 2012 Aug 3]. Available from: https://products.appliedbiosystems.com.
- Butler JM. 2010. SWGDAM STR interpretation guidelines. ISHI/Promega 2010 Technical Leaders Meeting; 2010 Oct 14; San Antonio, Texas. http://www.cstl.nist.gov/strbase/pub_pres_Butler-SWGDAMGuidelines-Promega2010-TL.pdf [Available 2011 March 10].
- Coble MD, PhD. 2011. Design and execution of validation studies for establishing DNA mixture interpretation procedures. NFSTC DNA Mixture Interpretation Workshop; 2011 March 15-17; Largo, Florida.
- Romanini C, Ferrer MR, Catelli ML, Vullo C. 2011. A comparison of AmpFℓSTR Identifiler™ Kit versus AmpFℓSTR Identifiler Plus™ Kit in challenging bone samples by using normal and increased PCR cycle number. Forensic Science International: Genetics Supplement Series 3:e514-e515.
- Sgueglia JB. 2011. Developing thresholds, protocols and validation studies using the new SWGDAM guidelines. American Academy of Forensic Science 2011 Conference DNA Mixture Analysis Workshop; 2011 Feb 22; Chicago, Illinois.
- Terrill M, Butler J. 2006. Applied Biosystems validation project. Personal email correspondence; 2006 Sept 6.
- Wang DY, Chang C, Lagacé RE, Calandro LM, Hennessy LK. 2012. Developmental validation of the AmpFℓSTR® Identifiler® Plus PCR amplification kit: an established multiplex assay with improved performance. J Forensic Sci 57(2):453-465.
- Westen AA, Nagel JHA, Benschop CCG, Weiler NEC, De Jong BJ, Sijen T. 2009. Higher capillary electrophoresis injection settings as an efficient approach to increase the sensitivity of STR typing. J Forensic Sci 54(3):591-598.

Acknowledgments

The authors thank everyone at the Boston Police Department Crime Laboratory and Marshall University Forensic Science Center for their help and support. This project was funded by the NIJ Cooperative Agreement 2009-IJ-CX-K111. The opinions, findings, and conclusions expressed in this poster are those of the authors and do not reflect the views of the Department of Justice.