Optimization of AmpFLSTR® Identifiler® Plus for High/Low Copy DNA Analysis

Alyssa Ströhbusch B.S.[1], Deputy Director Theresa Caragine Ph.D.[2], Jennifer Hayden M.S.F.S.[1], Justin Godby M.S.F.S.[1]

[1]*Marshall University Forensic Science Program* [2]*New York City Office of Chief Medical Examiner Forensic Biology Laboratory*

Abstract

Processing over 9,000 cases in 2010 alone, The New York City Office of Chief Medical Examiner (NYC OCME) completes more DNA analyses than any other public laboratory in the United States. With such a high case load, it is imperative that the facility be as efficient as possible in an attempt to prevent excessive backlogs and maintain productivity. In order to improve performance, data quality, and procedural efficiency, the NYC OCME engaged in the validation of the next generation Identifiler® Plus (ID+) amplification chemistry. Optimization studies including reaction volume, cycle number, inhibition and extended PCR conditions were manipulated using current NYC OCME protocols to determine the optimal parameters for this new system.

Introduction

The NYC OCME has extensively optimized their protocols and interpretation parameters to ensure reliability, robustness, and reproducibility of the short tandem repeat (STR) profiles they generate in both their high copy and high sensitivity units. Utilizing the ID amplification chemistry for analysis of length polymorphisms since 2006, the NYC OCME recently identified the intrinsic benefits of AB's next generation kit, ID+. This amplification kit allows for a more streamlined reaction setup, greater capability to overcome inhibition, and boasts an improved heterozygote balance for an overall decrease in analysis time and increase in profile confidence.

Materials & Methods

The ID+ kit was utilized at half reaction volumes in combination with Applied Biosystems GeneAmp® PCR 9700™ Thermal Cyclers as recommended by the manufacturer. The Applied Biosystems 3130xl genetic analyzer and Applied Biosystems GeneMapper® ID v3.2.1 were used for fragment analysis and interpretation, respectively.

Reaction Volume: The AB recommended full reaction was compared against half and half + Taq parameters in terms of fragment performance. Ten replicate samples containing 50pg of Control DNA were amplified for each parameter manipulation following the AB 28 cycle protocol. Samples were injected at 1 kV for 22 seconds with non-variable binning and were subsequently re-injected using the variable binning method.

Inhibition Study: Sample extract was treated with two known Polymerase Chain Reaction (PCR) inhibitors in triplicate. A 100 mM stock solution of indigo was prepared by dissolving indigo powder in 0.2% Triton X-100 in ddH_2O. Similarly, a humic acid dilution was prepared by creating a 1000 ng/µL stock solution in ddH_20. A total input of 100pg Control DNA was added to half reaction volumes with known concentrations of inhibitor for both ID and ID+ amplification chemistries and amplified using 28 cycles. All samples were subsequently injected at 5 kV for 20 seconds.

Cycle Number Study: A serial dilution of 1ng ID+ Control DNA 9947A was created to yield concentrations of 100pg/µL, 50pg/µL, 30pg/µL, 20pg/µL, 10pg/µL, 5pg/µL, 2.5pg/µL and 1.25pg/µL for testing. Five microliters of each dilution were added to a half reaction amplification setup for each cycle number parameter to achieve the desired input amount. All samples were created in triplicate per total amount of DNA amplified for each cycle number.

Annealing Time Study: Three *5:1* 100pg and three *4:1* 25pg mixtures were created using buccal swab samples extracted with the Qiagen BioRobot M48 for amplification of 29 and 32 cycles, respectively. Changes to the ID+ thermal protocol were programed within the GeneAmp® PCR 9700™ Thermal Cyclers to accompany annealing time parameters of 3, 12, and 20 minutes. Samples were created in triplicate for each annealing time parameter. The 29 cycle amplification products were injected at 5 kV for 20 seconds and samples having undergone 32 cycles were injected at 3 kV for 20 seconds.

Results

Reaction Volume: Allele recovery using non-variable binning, the default method for STR fragment collection, and variable binning, the alternative collection spectra, per reaction volume can be referenced in Table 1. Further investigation of allele recovery within each dye channel showed the non-variable binning method recovered 32% more alleles in the blue channel, 20% more alleles in the green channel and 5% more alleles in the yellow channel while the alternative, variable binning, recovered 15% more alleles in the red channel.

Table 1: *Reaction Volume Study Data*

	Parameter	Average # of Alleles Called Per Sample (/26)	Total # of Alleles Called (/260)	% of Total Alleles Called
Non-Variable Binning	Full Reaction	10.6	106	40.8
	Half Reaction	19.4	194	74.6
	Half Reaction + Taq	18.2	182	70.0
Variable Binning	Full Reaction	6.1	61	23.5
	Half Reaction	16.3	163	62.7
	Half Reaction + Taq	15.3	153	58.8

Inhibition Study: Complete inhibition occurred within the current NYC OCME validated ID protocol when subjected to humic acid and indigo dye at concentrations of 30 ng/µL and 50 nmol/µL, respectively, whereas the ID+ chemistry required the addition of 300 ng/µL and 150 nmol/µL, respectively, to produce fully inhibited results, an increase of ten and three fold respectively as shown in Figures 1 and 2 below.

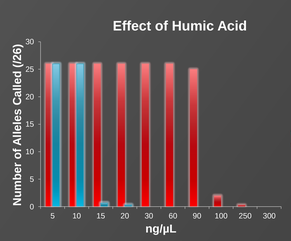

Figure 1: *Identifiler® and Identifiler® Plus Resistance to Humic Acid*

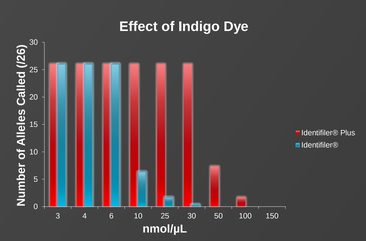

Figure 2: *Identifiler® and Identifiler® Plus Resistance to Indigo*

Cycle Number Study: Full profiles were exhibited for 29, 31 and 32 cycles at the 25pg input DNA and at 50pg for the 28 cycle amplification as shown in Figure 3. Peak height ratio data for each cycling parameter within this study is displayed within Figure 4 below.

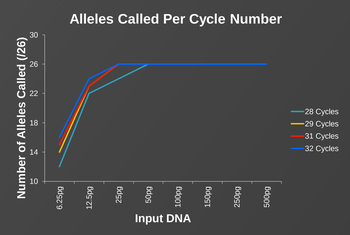

Figure 3: *Average Number of Alleles Called per Initial DNA Input*

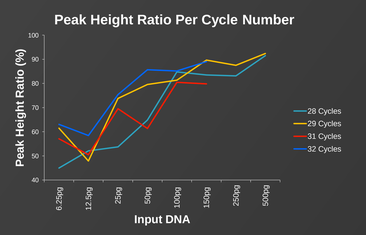

Figure 4: *Peak Height Ratio Results of the Cycle Number Study*

Annealing Time Study: Averaged across all 29 cycle amplification samples the 12 minute annealing time provided an 8.0% total increase in peak height ratio of the minor. Likewise, when amplified with 32 cycles this parameter provided a 14.4% total increase as compared to the next best parameter. These results are shown by Figure 5 below.

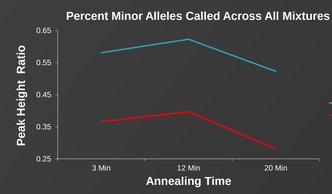

Figure 4: *Peak Height Ratio Results of the Cycle Number Study*

Discussion

Reaction Volume: Although variable binning recovered 15% more alleles in the red channel, on average, 4.1 alleles were lost across the remaining three color channels rendering this binning method subpar. In terms of reaction volume, the half reaction protocol recovered 4.6% more alleles than the half reaction + Taq parameter and 30.8% more alleles than the full reaction when utilized in combination with the preferred non-variable binning method. The half reaction volume using non-variable binning was determined to be the optimal setup parameter for future validation of the ID+ amplification chemistry for use at the NYC OCME.

Inhibition Study: This study proved the optimization implemented within the next generation ID kit, ID+, drastically increased its resistance to inhibition. ID+, which has yet to undergo full optimization and validation at NYC OCME, outperformed the current online amplification chemistry, ID, in the presence of both tested inhibitors.

Cycle Number Study: For implementation in HCN testing, when utilizing the 29 cycle amplification, peak heights increased 48.47 ± 14.39 % compared to 28 cycle amplification. The 32 cycle amplification yielded peak heights 28.13 ± 9.09 % greater than the 31 cycle amplification for LCN parameters. In terms of peak height balance, the 29 cycle amplification produced greater balance in six of the eight input values, as compared to the 28 cycle amplification. Similarly, the 32 cycle amplification produced greater average peak height ratios throughout all samples as compared to the 31 cycle thermal cycling protocol. In order to determine the optimal thermal cycling parameters for both HCN and LCN testing, injection parameter studies must be performed prior to the completion of this study.

Annealing Time Study: Optimized results of peak height ratio as well as allele recovery for major and minor contributors were produced by the 12 minute annealing time exclusively for both 29 and 32 cycle amplifications. When averaged across all mixture samples, the 12 minute annealing parameter also averaged a 4.2% total increase in the number of alleles identified with 29 cycles of amplification and provided a 2.0% increase for 32 cycle products. The benefits of extending the annealing time could prove extremely beneficial for the detection and identification of sister alleles within minor contributor samples.

Conclusion

The combination of studies performed within this optimization of the ID+ amplification chemistry has proved to maximize the performance and efficiency of this multiplex for use in both HCN and LCN testing. The optimum parameters identified will be utilized for future validation studies and ultimately the implementation of ID+ amplification chemistry at the NYC OCME.

Acknowledgements

This project was supported by Award No. 2008-DN-BX-K219 awarded by the National Institute of Justice, Office of Justice Programs, U.S. 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.

References

- Applied Biosystems, Human Identification Department. AmpFISTR® Identifiler™ Plus PCR Amplification KitUser's Manual. Foster City (CA): Applied Biosystems; 2009.
- Forster L, Thomson J, Kutranov S. Direct Comparison of Post-28-Cycle PCR Purification and Modified Capillary Electrophoresis Methods with the 34-Cycle "Low Copy Number" (LCN) Method for Analysis of Trace Forensic DNA Samples. *Forensic Sci Int; Genet.* 2008;2:318–28.
- Gill P, Whitaker J, Flaxman C, Brown N, Buckleton J. An Investigation of the Rigor of Interpretation Rules for STRs Derived from Less Than 100 pg of DNA. *Forensic Sci Int.* 2000;112:17–40.
- Kloosterman AD, Kersbergen P. Efficacy and Limits of Genotyping Low Copy Number (LCN) DNA Samples by Multiplex PCR of STR Loci. *J Soc Biol.* 2003;197:351–59.
- Roeder AD, Elsmore P, Greenhalgh M, McDonald A. Maximizing DNA Profiling Success From Sub-Optimal Quantities of DNA: a Staged Approach. *Forensic Sci Int; Genet.* 2009;3:128–37.
- Smith PJ, Ballantyne MS, Ballantyne J. Simplified Low-Copy-Number DNA Analysis. *J. Forensic Sci.* 2007;52:820–829.
- Taberlet P, Griffin S, Goossens B, Questiau S, Manceau V, Escaravage N, et al. Reliable Genotyping of Samples with Very Low DNA Quantities Using PCR. *Nucleic Acids Res.* 1996;24:3189–3194.
- Weiler, Natalie E C & Matai, Anuska S & Sijen, Titia. Extended PCR Conditions to Reduce Drop-out Frequencies in Low Template STR Typing Including Unequal Mixtures. *Forensic Science International.* Genetics. 2011. n.p. <http://www.biomedsearch.com/nih/Extended-PCR-conditions-to-reduce/21454145.html>.