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

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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 Cycler as recommended by the manufacturer. The Applied Biosystems 3130 Genetic analyzer and Applied Biosystems GeneMapper® ID v2.2.1 were used for fragment analysis and interpretation, respectively.

Reaction Volumes: 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 solution stock of indigo was prepared by dissolving indigo powder in 0.2% Triton X-100 in H2O. Similarly, a humic acid dilution was prepared by creating a 1000 ng/mL stock solution in deionized water. 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/mL, 50pg/mL, 30pg/mL, 20pg/mL, 10pg/mL, 5pg/mL, 2.5pg/mL and 1.25pg/mL 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 programmed into the GeneAmp® PCR 9700™ Thermal Cycler 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 7 kV for 20 seconds.

Results

| Reaction Volume: Amplify using non-variable binning, the default method for STR fragment collection, and variable binning, the alternative collection spectrums, 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. |

Discussion

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 NYO OCME.

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References