Internal Validation of Identifiler® Plus Amplification System

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ABSTRACT

An internal validation of Applied Biosystems AmpFISTR® Identifiler® Plus amplification kit was conducted to assist the Rhode Island Department of Health in improving turn around time and decreasing consumable usage. A major advantage was the replacement of their current two kit amplification system, AmpFISTR® Profiler Plus®/COfiler®, to the single kit amplification system of Identifiler® Plus.

INTRODUCTION

Nine validation studies were performed:

Sensitivity Study: Purpose was to determine ideal range of amplifiable DNA to produce a reliable profile with limited stochastic effects.

Precision/Reproducibility: Purpose was to permit accurate and reliable genotypes to be generated for analysis.

Concordance: Purpose was to determine concordance of Identifiler® Plus with NIST SRM 2391b reference samples.

Mixture: Purpose to reveal a sample's behavior containing two contributors.

Known and Nonprobative Evidence Samples: Purpose to analyze how Identifiler® Plus amplifies known evidence samples.

Match Criteria: Purpose was to examine all positive samples for expected profiles.

Contamination: Purpose was to examine all negative samples for contamination.

Denature: Purpose was to establish if denaturing and snap cooling of samples prior to capillary electrophoresis produced lower peak height.

Intra-Laboratory Stutter: Purpose was to determine stutter percentage and occurrence.

MATERIALS AND METHODS

- Qiagen BioRobot EZ1® Trace Protocol
  - Elution volume of 50µL
- Corbett CAS-1200™ Precision Liquid Handling System
- Applied Biosystems Quantifiler® Human DNA Quantification kit
- Applied Biosystems 7500 Real Time PCR System
- GeneAmp® PCR System 9700 Thermal Cycler
- Applied Biosystems PRISM® 310 Genetic Analyzer
  - GeneMapper® *ID* v3.1.0 software
- Known positive control, 9947A
  - 2.5ng, 1.0ng, 0.5ng, 0.25ng, 0.125ng, 0.075ng, 0.025ng, and 0.015ng
- Five single source, convicted offender samples
  - Provided by Rhode Island Department of Health
- Ten Applied Biosystems® Identifiler® allelic ladders
- Ten NIST Standard Reference Material 2391b samples
- Three female and three male convicted offender samples
  - Provided by Rhode Island Department of Health
  - Mixed at ratios of 10:1, 3:1, 2:1, 1:1, 1:2, 1:3, and 1:10
- Twenty-two non-probative samples
  - Provided by Rhode Island Department of Health

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RESULTS

Genetic Analyzer	Average Peak Height	Minimum PHR	Analytical Threshold	Target Range
ABI 2	3069 RFU	72.3% at D3S1358	75	0.25ng - 1.0ng
ABI 3	1711 RFU	69.4% at D3S1358	50	0.25ng - 1.0ng

Table 1: Data generated for the ABI 2 and ABI 3 Sensitivity Study. 0.25ng concentration had various markers with peak height ratios below the recommended 65%.

Genetic Analyzer	Sample Name	Average STDEV	Avg STDEV Range	Avg 250bp Migration
ABI 2	Ladder	0.085	±0.038 – 0.21	0.61
ABI 3	Ladder	0.10	±0.05 – 0.21	0.30
ABI 2	CO	0.058	±0.00 – 0.24	0.61
ABI 3	CO	0.11	±0.01 – 0.25	0.49

Table 2: ABI 2 and ABI 3 Precision/Reproducibility Study data showing average standard deviation, standard deviation range, and 250 bp size standard peak migration.

Genetic Analyzer	# of Samples	# of Expected Profiles	Amp Negative Controls	Run Negative Controls
ABI 2	10	10	Clean	Clean
ABI 3	10	10	Clean	Clean

Table 3: Concordance Study for both Genetic Analyzers ABI 2 and ABI 3 produced expected profiles with clean amplification and electrophoresis run negatives.

Table 4: ABI 2 Major Profiles per Marker

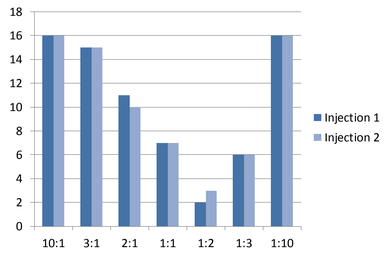
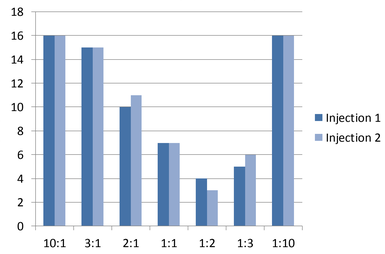


Table 5: ABI 3 Major Profiles per Marker



Charts 1 & 2: Mixture Study comparison showing the number of major profiles per marker for injections one and two. ABI 2 and ABI 3 present around the same number of major profiles per marker.

Table 6: ABI 2 Known/Nonprobative Samples

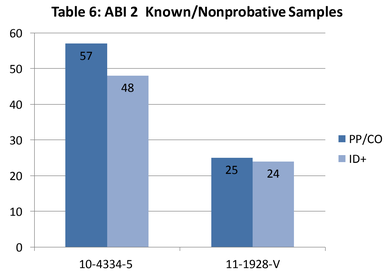
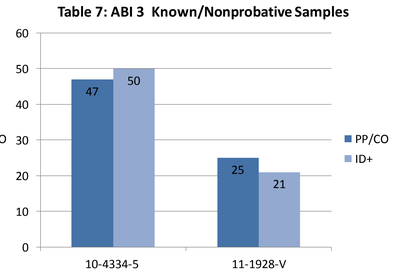


Table 7: ABI 3 Known/Nonprobative Samples



Charts 3 & 4: Known and Nonprobative evidence samples containing single profiles with varying allele calls per kit and genetic analyzer.

Genetic Analyzer	Match Criteria	Contamination
ABI 2	Clean	Clean
ABI 3	Clean	Clean

Table 4: The Match Criteria Study's extraction and amplification positives were concordant and the Contamination Study's negative controls were clean for both ABI 2 and ABI 3.

Total Alleles	Non-Denatured	Non-Denatured Percentage	# of Alleles: Denatured	Denatured Percentage
167	106	63.47%	61	36.53%

Table 5: Comparison of Non-Denaturing and Denaturing prior to Capillary Electrophoresis Separation concluded non-denaturing produced higher peak heights.

Genetic Analyzer	Greatest Peak Height	Greatest bp Size	Lowest Stutter %	Highest Stutter %
ABI 2	682	242.76	3.89%	14.47%
ABI 3	112	171.12	5.85%	11.95%

Table 6: Intra-Laboratory Stutter Study determined that the data peaks identified as stutter in these validation studies fell below this recommended range of 15-20%.

DISCUSSION

- The Sensitivity Study revealed low baseline noise and extraneous peaks with an amplification target 0.5ng or less.
- The Precision/Reproducibility Study determined characteristic errors inherent to sizing method.
- The Concordance Study published the expected profiles with Identifiler® Plus.
- The Mixture Study resulted in higher peak heights when coupled with 29 PCR cycles.
- The Known and Nonprobative Evidence Samples Study had profiles generated with Identifiler® Plus and compared to the previous PP/CO profiles provided.
- The Match Criteria and Contamination Studies assessed migration and contamination issues.
- The Denature Study resulted in higher peaks heights when samples were not denatured.
- The Intra-Laboratory Study determined if average stutter was below the 15-20% range.

CONCLUSIONS

- **Sensitivity Study**: The 0.25ng – 0.5ng range is ideal for questioned and known samples. A target of 0.3ng at 29 PCR cycles is optimal.
- **Precision/Reproducibility and Concordance Studies**: Results were concordant with those reported in the NIST reference sample documentation, demonstrating inter-laboratory concordance with both ABI 2 and ABI 3.
- **Mixture Study**: Results show a major contributor may be extracted at every marker in samples containing mixtures at ratios of 10:1 and 1:10.
- **Known and Nonprobative Evidence Samples Study**: All Identifiler® Plus profiles generated were concordant to the previous PP/CO profiles provided. Any differences were noted.
- **Match Criteria Study**: The results showed that with an appropriate DNA target amount, extraction positives and amplification positives produce the correct profile with few extraneous peaks.
- **Contamination Study**: The results showed that with proper lab technique, contamination of reagent blanks, amplification blanks, or run negatives should not occur.
- **Denature Study**: Results obtained showed that denaturing and snap-cooling of the sample prior to capillary electrophoresis caused the peak heights to diminish in comparison to samples that were not denatured and chilled.
- **Intra-Laboratory Study**: Stutter percentages were under the recommended 15- 20% range.

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