Validation of the Applied Biosystems® 3500 Genetic Analyzer with a comparison of the Identifiler® Plus and PowerPlex® 16 HS amplification kits

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Abstract

The Anne Arundel County Crime Laboratory upgraded from an Applied Biosystems® (AB) 310 genetic analyzer to an AB 3500 genetic analyzer. The internal validation of this AB 3500 included a comparison of the PowerPlex® 16 HS (PP16HS) and Identifiler® Plus (ID+) amplification kits using the manufacturer's recommended protocols to determine if one kit had any benefit to forensic casework analysis over the other, when used in conjunction with the AB 3500.

Introduction

Based on a previous validation of the AB 3500 by the Mansfield Police Laboratory, injection times of 7s and 15s at 1.2kV were validated at the Anne Arundel County Crime Laboratory. Forensic casework samples were used to perform the following studies:

- Analytical Thresholds*
- Stochastic Thresholds
- Denaturation-Snap Cooling*
- Sensitivity*
- Precision*
- Contamination*
- Concordance
- Reproducibility*
- Stutter
- Consumables Study
- PP16HS vs. ID+*

Duplicate amplifications were performed with PP16HS and ID+ for the studies listed with an (*) for amplification kit comparison.

Materials and Methods

Instrumentation:

- Qiagen BioRobot EZ1 Advanced XL
  - Trace TD protocol, elution in water
- AB 7500 Real-Time PCR System
- PowerPlex® 16 HS Amplification System
- AmpFlSTR® Identifiler® Plus Amplification Kit
- AB GeneAmp® PCR System 9700
- AB 3500 Genetic Analyzer

Samples:

- NIST Standard Reference Materials 11 and 12
- 9947A Dilution Series
  - 0.1ng, 0.25ng, 0.5ng, 0.75ng, 1.0ng, 1.5ng, 2.5ng
- Known male and known female 1:1 mixtures
  - 0.2ng, 0.6ng, 1.0ng
- Known heterozygous individual's DNA extract
- 96-well checkerboards of allelic ladders and run negatives
- 24 ID+ re-amplifications of PP16HS casework samples
- 57 PP16HS casework samples at 0.6ng or 0.7ng
- 18 PP16HS casework samples re-amplified at 1ng

Analysis Software:

- GeneMapper® ID-X version 1.2

Results

Analytical Threshold Study

Table 1: Dye specific analytical thresholds were calculated, but universal thresholds were chosen for each chemistry at each injection time to give a more conservative value.

PP16HS 7s Analytical Thresholds

Dye Channel	Analytical Threshold
Blue	45 RFU
Green	50 RFU
Yellow	55 RFU
Universal	75 RFU

PP16HS 15s Analytical Thresholds

Dye Channel	Analytical Threshold
Blue	55 RFU
Green	85 RFU
Yellow	100 RFU
Universal	100 RFU

ID+ 7s Analytical Thresholds

Dye Channel	Analytical Threshold
Blue	25 RFU
Green	40 RFU
Yellow	70 RFU
Red	100 RFU
Universal	100 RFU

ID+ 15s Analytical Thresholds

Dye Channel	Analytical Threshold
Blue	25 RFU
Green	45 RFU
Yellow	80 RFU
Red	110 RFU
Universal	125 RFU

PP16HS and ID+ Comparison Study

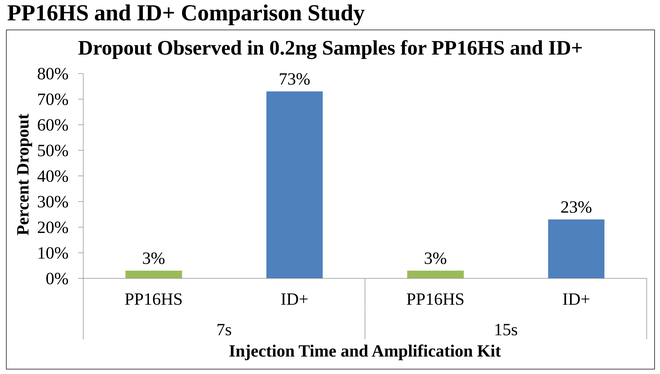

Figure 1: Less dropout is observed with PP16HS compared to ID+ when using manufacturer's recommended protocols. Mixtures show the same results.

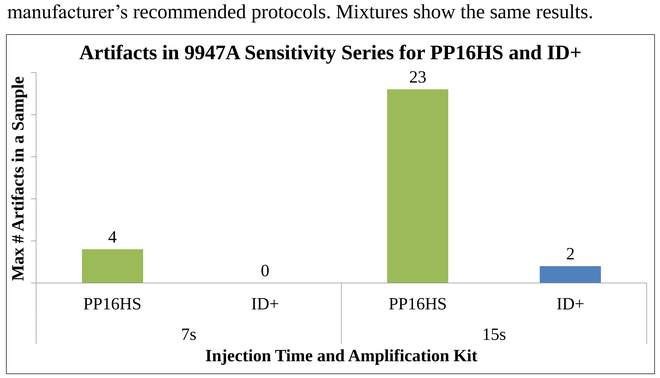

Figure 2: PP16HS shows increased artifacts compared to ID+.

Sensitivity Study

Table 2: Lowest amount of DNA (9947A) to yield a full profile on the AB 3500.

Injection Time	Amplification Kit	1st Complete DNA Profile
7s	PP16HS	≤50pg / 50pg
	ID+	50pg
15s	PP16HS	≤50pg / ≤50pg
	ID+	50pg

Table 3: Lowest amount of DNA (9947A) to show good peak height ratios (>50%) on the AB 3500. 7 randomly chosen single source samples showed an average peak height ratio of 0.83 for both PP16HS and ID+.

Injection Time	Amplification Kit	Amplified DNA	Lowest PHR
7s	PP16HS	≤50pg	55%
	ID+	90pg	53%
15s	PP16HS	50pg	60%
	ID+	90pg	54%

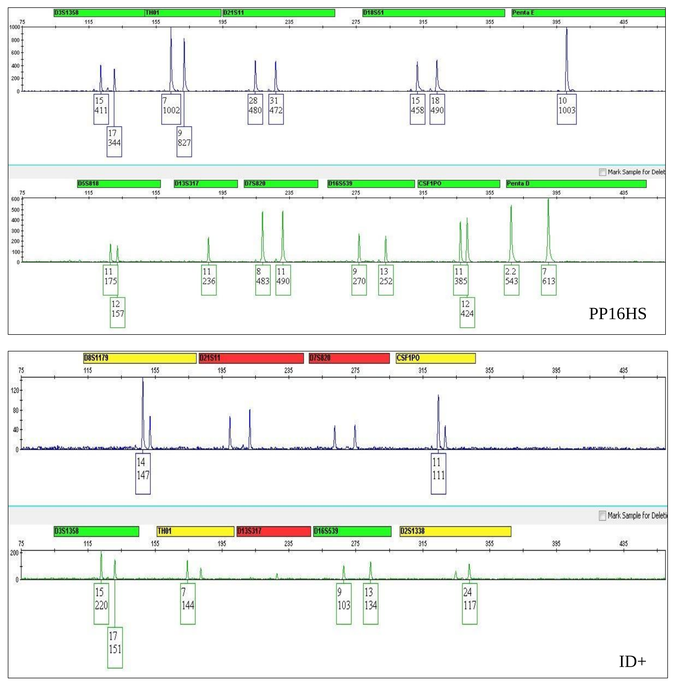

Figure 3: Non-probative casework sample CS-1-A amplified at 0.7ng with PP16HS (top) and ID+ (bottom). PP16HS recovered a full profile and ID+ recovered a partial profile using the 7s injection time on the AB 3500. Blue and green dye channels are shown for each amplification kit.

Discussion and Conclusions

- Amplification target: 0.6ng to 1.0ng DNA
- Stochastic Thresholds for PP16HS:
  7s – 425 RFU 15s – 600 RFU
- Precision:
  PP16HS kit standard deviation – 0.046
  ID+ kit standard deviation – 0.058
- Concordance: average decrease of ~210 RFU from AB 310 to AB 3500. All allele calls remained constant.
- Denaturation and snap cooling prior to an instrument run does not improve data produced by the AB 3500.
- POP 4 has a recommended usage time of 7 days after installation, but remains usable for an extended period if kept in the fridge when not on the instrument.
- PP16HS is slightly more sensitive than ID +, but shows increased artifacts.
- No difference in performance was observed on the AB 3500 between PP16HS and ID+ during the denaturation and snap cooling, precision, contamination and reproducibility studies.
- No contamination issues were observed during this validation.
- The Anne Arundel County Crime Laboratory will continue to use PP16HS with the AB 3500 for casework analysis.
- SOP will not include denaturation and snap cooling prior to an instrument run.

References

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