Internal Validation of PowerPlex® 16 HS with the Applied Biosystems® 3500*xL* Genetic Analyzer

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Abstract

An internal validation is performed in order to demonstrate reliability and reproducibility of new instruments and chemistries within a specific laboratory. These studies show that results are accurate and consistent with previous methods. The validation study performed at the Philadelphia Police Department Forensic Science Bureau (PPD) demonstrated the reliability of the PowerPlex® 16 HS amplification chemistry and the use of the Applied Biosystems® 3500*xL* to provide accurate and reliable data. The chemistry and instrument are both novel technologies and platforms to the PPD DNA laboratory, thus requiring an internal validation to be performed prior to use with casework samples. All studies were analyzed using Applied Biosystems' GeneMapper® *ID-X* v1.2. Each of these studies demonstrated the proper settings to optimize the use of the amplification chemistry on the genetic analyzer. Overall, this validation demonstrated that consistent and reliable results could be obtained through the use of PowerPlex® 16 HS with the AppliedBiosystems® 3500*xL* Genetic Analyzer.

Introduction

An internal validation was requested for the Philadelphia Police Department Forensic Science Bureau through the National Institute of Justice's Technical Assistance Program on the use of the PowerPlex® 16 HS amplification chemistry (Promega Corporation, Madison, Wisconsin) with the Applied Biosystems® (Foster City, California) 3500*xL* Genetic Analyzer. The results of the performed validation studies demonstrated the robustness and reliability of the kit and instrument. Based on the findings of these studies, specific settings were recommended to be incorporated into the standard operating procedure of the Philadelphia Police Department Forensic Science Bureau DNA Laboratory. The use of this amplification chemistry and instrument is recommended for use with future casework samples to increase both sensitivity and throughput.

Materials and Methods

The studies performed during the validation of the Applied Biosystems® 3500*xL* Genetic Analyzer using PowerPlex 16® HS used extracts previously obtained from reference samples as well as previously analyzed casework samples. All data was analyzed using GeneMapper® *ID-X* version 1.2.

- Applied Biosystems®**3500*xL* Genetic Analyzer
- Applied Biosystems®** 3130*xL* Genetic Analyzer
- PowerPlex®^ 16 HS
- PowerPlex®^ 16
- Promega®^ Plexor®^ HY
- Applied Biosystems ®** 7500

Internal Validation Studies

- **Target**
- **Injection voltage and time**
- **Analytical threshold**
- **Stochastic threshold**
- **Precision**
- **Sensitivity**
- Reproducibility and Concordance
- Contamination
- **Stutter**
- Mixture interpretation
- Non-Probative samples

Studies not in bold are not discussed in this poster

Target and Injection Time/Voltage Studies

- Determine the ideal DNA target load to inject at a specific injection voltage and time to produce reliable results which limit artifacts and stochastic events
- Dilution series was created from a previously analyzed non-probative reference sample, ranging between 10 ng. and 0.0156 ng.
- Each sample was setup in triplicate and injected under the following conditions: injection of 24 seconds and 1.2 kV (instrument default setting), 12 seconds and 1.2 kV, 10 seconds and 3 kV, and 5 seconds and 3 kV.
- Range of injection voltages and injection times were used to determine the ideal injection parameters for the 3500*xL* and the ideal DNA target load to inject at the decided voltage and time

Table 1: Average peak heights, peak height standard deviations, average peak height ratios, and peak height ratio standard deviations for the DNA target and injection time/voltage study.

1 ng/µL	sec / kV	Avg. Ht.	Pk. Ht. Std. Dev.	Avg. PHR	PHR Std. Dev.
	24 sec / 1.2 kV	4144.936	1239.278	0.792	0.078
	12 sec / 1.2 kV	1946.500	584.562	0.766	0.147
	10 sec / 3 kV	4134.064	1218.391	0.792	0.217
	5 sec / 3 kV	2083.256	615.286	0.797	0.078
0.5 ng/µL	**sec / kV**	**Avg. Ht.**	**Pk. Ht. Std. Dev.**	**Avg. PHR**	**PHR Std. Dev.**
	24 sec / 1.2 kV	1688.808	510.221	0.753	0.088
	12 sec / 1.2 kV	792.654	248.531	0.754	0.090
	10 sec / 3 kV	1598.590	525.729	0.700	0.212
	5 sec / 3 kV	834.295	257.651	0.754	0.089
0.25 ng/µL	**sec / kV**	**Avg. Ht.**	**Pk. Ht. Std. Dev.**	**Avg. PHR**	**PHR Std. Dev.**
	24 sec / 1.2 kV	920.974	342.673	0.748	0.156
	12 sec / 1.2 kV	435.038	176.043	0.749	0.156
	10 sec / 3 kV	961.449	369.443	0.695	0.244
	5 sec / 3 kV	460.423	198.198	0.730	0.192

Analytical and Stochastic Threshold Studies

- Analytical threshold is necessary to determine at what level a true peak can be differentiated from background noise
- Calculated by analyzing a run of 24 amplification negative controls (3 columns), generated from each of the three amplification negative controls
- Prior to analysis, an analytical threshold of 1 RFU was selected for all dyes to allow for the calling of "background noise" above this threshold
- Exported to an Excel spreadsheet, peaks below a base pair size of 90 were removed to prevent interference from the primer peak as well as all peaks +/- 2 base pairs from the size standard peaks

$$AT_{M3} = 2(Y_{max} - Y_{min})$$

Y_{max} *is the highest peak within instrumental noise data*

Y_{min} *is the signal of the lowest trough*

AT_{M3} *the analytical threshold calculated using Method 3*

Table 2: Calculated analytical threshold for each dye channel.

DYE	AVERAGE	STDEV	MIN	MAX	Lowest Trough	AT
BLUE	5.07	3.65	1	53	1	104
GREEN	5.58	2.13	1	43	1	84
YELLOW	4.40	1.76	1	39	1	76

- Stochastic threshold level is calculated to determine above what RFU level a homozygote peak can be identified without the consideration of dropout of a heterozygote sister allele
- Target study dilution series data at 24 sec / 1.2 kV (the selected injection voltage and time)
- Average peak height ratio for all dye channels and each individual channel

ST= [1/ (Average PHR- 3x STD)] x AT

Table 3: Calculated stochastic threshold for combined dyes and individual dyes

	Stochastic Threshold
All Dyes	323.82
Blue	277.70
Green	352.17
Yellow	349.72

Precision Study

- Precision study performed to demonstrate the reliability and degree of precision of the allele size calling
- Three consecutive days with a new plate prepared each day containing three rows of allelic ladders
- Plate from day one reinjected each day to ensure precise size calling when a plate is stored for multiple days

Sensitivity Study

- A sensitivity study was performed to determine the ideal target DNA load within the recommended range determined by the Target study
- Target dilution series between 0.125 ng. and 1.5 ng.
- The sensitivity study consisted of analyzing a dilution series between 0.30 ng/µl and 0.025 ng/µl. These dilutions were created to target 0.125 ng, 0.25 ng, 0.5 ng, 0.75 ng, 1.0 ng, 1.25 ng, and 1.5 ng.

Stutter Study

- Stutter study was performed to indicate the appropriate stutter ratios
- 53 single source reference profiles, each at a target of 0.5 ng.
- Samples were analyzed using GeneMapper® *ID-X* with all artifacts removed except for stutter peaks
- Positive and negative stutter peaks were labeled as stutter as well as an indication of the allele from which they originated.
- Separated according to marker
- The peak height ratio, peak height ratio standard deviation, maximum peak height ratio, and minimum peak height ratio were then calculated within each marker

Stutter = Average PHR + 3STDev

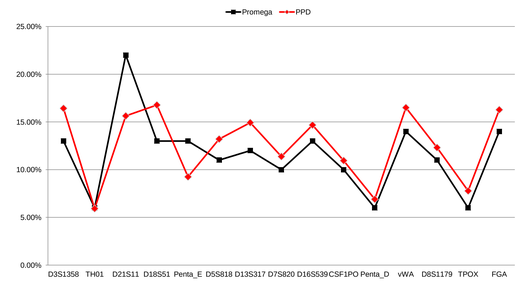

Figure 1: Comparison of calculated stutter values generated for the use of PowerPlex® 16 HS to the Promega Corporation's recommended stutter values

Table 4: Calculated stutter values and Promega recommended stutter values for minus four stutter in PowerPlex® 16 HS

Locus	Average	Std. Dev.	Min	Max	Stutter Ratio	Promega Stutter Ratio
D3S1358	0.10	0.02	0.02	0.16	16%	13%
TH01	0.03	0.01	0.00	0.05	6%	6%
D21S11	0.09	0.02	0.03	0.17	16%	22%
D18S51	0.09	0.03	0.04	0.18	17%	13%
Penta E	0.04	0.02	0.01	0.11	9%	13%
D5S818	0.07	0.02	0.03	0.12	13%	11%
D13S317	0.07	0.03	0.02	0.15	15%	12%
D7S820	0.06	0.02	0.02	0.11	11%	10%
D16S539	0.08	0.02	0.03	0.14	15%	13%
CSF1PO	0.06	0.02	0.02	0.09	11%	10%
Penta D	0.02	0.02	0.01	0.10	7%	6%
vWA	0.09	0.03	0.01	0.16	16%	14%
D8S1179	0.07	0.02	0.03	0.12	12%	11%
TPOX	0.03	0.01	0.01	0.10	8%	6%
FGA	0.09	0.02	0.05	0.17	16%	14%

Table 5: Calculated stutter values for plus four stutter in PowerPlex® 16 HS

Locus	Average	Std. Dev.	Min	Max	Stutter Ratio
D3S1358	0.02	0.00	0.01	0.03	3%
TH01	0.00	0.00	0.00	0.01	1%
D21S11	0.02	0.01	0.01	0.07	5%
D18S51	0.01	0.01	0.01	0.06	5%
Penta E	0.00	0.00	0.00	0.00	0%
D5S818	0.02	0.01	0.01	0.06	4%
D13S317	0.03	0.02	0.01	0.12	8%
D7S820	0.02	0.01	0.01	0.04	3%
D16S539	0.02	0.01	0.01	0.04	4%
CSF1PO	0.02	0.01	0.01	0.05	6%
Penta D	0.02	0.02	0.01	0.05	8%
vWA	0.01	0.02	0.00	0.11	6%
D8S1179	0.01	0.00	0.01	0.02	2%
TPOX	0.01	0.00	0.01	0.02	2%
FGA	0.03	0.02	0.01	0.09	9%

Conclusion

The PowerPlex® 16 HS amplification chemistry was found to produce reliable and reproducible results with the use of the Applied Biosystems® 3500*xL* Genetic Analyzer. The reliability and reproducibility of the incorporation of this platform and chemistry are based on the settings and recommendations set forth by the validation studies.

- Ideal injection load of 1.0 ng, 0.5 ng, and 0.25 ng. corresponds with an injection voltage and time of 1.2 kV and 24 seconds
- Size callings were precise and within the +/- 0.5 basepair window
- Ideal target range to be between 0.5 ng. and 1.0 ng.
- Found to produce peak height ratios above the required 50% peak height ratio cut-off for a single source profile
- Analytical threshold of 125 RFU and a stochastic threshold of 360 RFU
- Stutter ratios compared to the values recommended by the Promega Corporation. Promega's recommended stutter values were used for the remaining studies

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Acknowledgments

**Life Technologies™, Foster City CA
^Promega Corporation®, Madison WI

I thank Danielle Imes, Joshua Tyson, and the laboratory staff at the Philadelphia Police Forensic Science Bureau for the opportunity and assistance to complete this project.

This project was supported by Cooperative Agreement Number 2009-1J-CX-K111 awarded by the National Institute of Justice, Office of Justice Programs, US Department of Justice. The opinions, findings and conclusions or recommendations expressed in this exhibition are those of the authors and do not necessarily reflect the views of the Department of Justice.