Internal Validation of the Promega PowerPlex® Fusion System using the Applied Biosystems® 3130*xl* Genetic Analyzer

Roy Al Ahmar, B.S., Joshua Stewart, M.S.F.S., Jason Chute, M.S.F.S., Pamela Staton, Ph.D.*

Marshall University Forensic Science Center, 1401 Forensic Science Drive, Huntington, WV 25701

Abstract

•The Promega ® PowerPlex® Fusion System is a 24-locus multiplex used for human identification applications., and uses a 5-dye chemistry .

•Autosomal STR loci include the 13 core CODIS (U.S.A.) loci as well as the 12 European Standard Set (ESS) core loci in addition to D2S1338, D19S433, Penta D, Penta E, Amelogenin, and DYS391.

•Validation studies were performed to ensure the reliable functionality of the kit chemistry.

•The results of this validation showed that the PowerPlex® Fusion System produces accurate and reproducible STR profiles.

•Future studies will include a non-human DNA study, and enhancement to the mixture study including mixtures of relatives.

Introduction

•Optimal parameters for amplification and capillary electrophoresis were determined to be 0.5ng to 1.0ng template input with 30 cycles of PCR and injection for 5 seconds.

•Analytical thresholds were variable between dye channels with 35 RFU for the blue channel, 40 RFU for green, 55 RFU for yellow and 70 RFU for red.

•All 35 samples previously typed with PowerPlex® 16 were concordant with PowerPlex® Fusion typing results at the loci common to both kits.

•Of all peaks evaluated for the precision study, the largest 3X standard deviation of base-pair sizes was 0.39bp, which falls within acceptable limits.

•Calculated mixture proportions obtained from electrophoresis data was generally comparable to the known donor-ratios of mixed samples. Contamination risk is low, with the note that lab personnel must exercise care when setting up laboratory procedures due to the high sensitivity of the kit.

Methods

Performed Studies:
•Threshold Studies(Analytical Threshold/Stochastic Threshold)
•Sensitivity Studies
•Contamination Study
•Concordance Study
•Inhibition Study
•Mixture Studies
•Cycle Number, DNA Target, Injection Time Study
•Precision Study
•Peak Height Ratio Study

Materials

•*Samples:* in-house NIST traceable FTA blood card (TF) and 35 blood and saliva samples previously tested using the Promega® PowerPlex 16 System.

•*Extraction*: using the Qiagen EZ1 DNA Investigator Kit® and the Qiagen EZ1® Advanced XL

•*Quantitation*: using the Qiagen Investigator Quantiplex HYres® quantification kit using the ABI® SDS 7500

•*Instrumentation:* validation of the PowerPlex Fusion ® System amplification kit was performed using a Applied Biosystems® 3130*xl* Genetic Analyzer.

•*Analysis:* Data was analyzed using GeneMapper® ID v3.2.1.

Stutter Study

The following table shows the stutter percentages calculated from 35 samples and the stutter percentage chosen by comparing the stutter calculated with the maximum observed stutter.

Locus	Min	Max	Avg PHR	STD PHR	Stutter*
D10S1248	5.556	17.582	8.930	2.620	12.4%
D12S391	4.178	20.619	10.120	3.455	15.8%
D13S317	1.573	28.125	6.431	4.045	9.8%
D16S539	3.287	14.925	5.986	1.950	10.2%
D18S51	3.752	22.472	9.281	3.408	14.6%
D19S433	3.241	13.359	7.479	2.171	11%
D1S1656	4.247	19.802	9.093	2.813	14.2%
D21S11	5.395	22.115	9.191	2.533	11.6%
D22S1045	2.581	17.857	10.922	2.992	16.4%
D2S1338	5.040	14.043	8.781	2.061	13.9%
D2S441	2.164	10.313	5.514	1.661	9.2%
D3S1358	5.726	13.043	8.681	2.007	11.9%
D5S818	2.257	21.359	7.176	2.971	9.5%
D7S820	2.379	24.528	6.600	3.578	11%
D8S1179	3.414	13.873	7.656	2.020	10.9%
DYS391	5.157	15.302	8.069	2.056	8.7%
FGA	3.994	17.021	8.017	2.473	12.1%
TH01	1.266	7.014	2.851	1.519	4.6%
CSF1PO	2.558	11.607	7.018	1.747	9.5%
TPOX	1.808	7.962	3.570	1.353	5.5%
vWA	4.895	28.986	9.306	4.040	11.2%

Table 1: Stutter Percentage

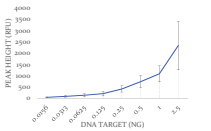
* Promega® Stutter Percentage

Cycle Number, Sensitivity, Injection Time Study

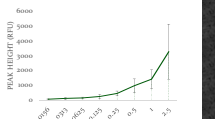
Amplification Cycle Numbers tested:
- 29 cycles
- **30 cycles***
- 31 cycles

Injection Time Tested : (at 3kV Injection Voltage)
- 3 seconds
- **5 seconds***
- 10 seconds
- 15 seconds

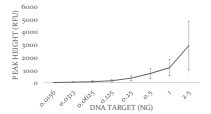
** Promega® Recommended*



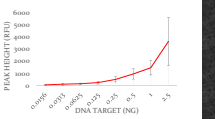
Graph 1: **Average Peak Height and Peak Height SD vs. DNA Target** *(Blue Channel)*



Graph 2: **Average Peak Height and Peak Height SD vs. DNA Target** *(Green Channel)*



Graph 3: **Average Peak Height and Peak Height SD vs. DNA Target** *(Yellow Channel)*



Graph 4: **Average Peak Height and Peak Height SD vs. DNA Target** *(Red Channel)*

Analytical Threshold

Method 1: The analytical threshold was then calculated using two different methods. According to the IUPAC (International Union for Pure and applied Chemistry) that utilizes the following formula:

$$AT = Y_{bl} + ks_{bl}$$

AT= Analytical Threshold
Y_{bl}= Average Reagent Blank RFU signal
k= 3
S_{bl}= Standard Deviation of the blank signal

Dye	Average Height	Standard Deviation Height	Minimum Height	Maximum Height	Analytical Threshold
Blue	4.63	1.56	1	18	9.31
Green	6.03	1.87	1	20	11.63
Yellow	8.37	2.46	2	27	15.75
Red	6.34	1.86	2	35	11.93

Table 2: Analytical Threshold Method 1

Method 2: Section 1.1 of the SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories provided an example of a means to calculate the analytical threshold.

$$AT = 2(Y_{max} - Y_{min})$$

AT= Analytical Threshold
Y_{max}= Highest peak within instrumental noise
Y_{min}= Signal of the lowest trough

Dye	Average Height	Standard Deviation Height	Minimum Height	Maximum Height	Analytical Threshold
Blue	4.63	1.56	1	18	34
Green	6.03	1.87	1	20	38
Yellow	8.37	2.46	2	27	52
Red	6.34	1.86	2	35	68

Table 3: Analytical Threshold Method 2

Stochastic Threshold

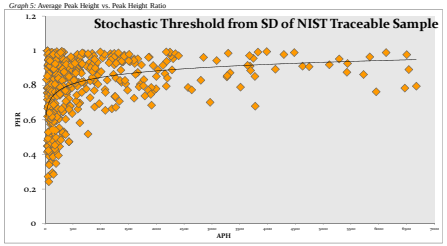
The stochastic threshold is the limit at which a homozygote peak can be called without the consideration of drop-out occurring. The stochastic threshold was calculated using the following formula:

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

Dye	AVG PHR	STD PHR	AT-M1	AT-M2	ST-M1	ST-M2
Blue	0.8191	0.0883	9.31	34	16.80	61.37
Green	0.8023	0.0666	11.63	38	19.31	63.09
Yellow	0.7874	0.1107	15.75	52	34.60	114.24
Red	0.7611	0.1234	11.93	68	30.51	173.94

*Table 4:*Stochastic Threshold

Graph 5: Average Peak Height vs. Peak Height Ratio



LOD/LOQ

Dye	Average Height	Standard Deviation Height	Minimum Height	Maximum Height	LOD	LOQ
Blue	4.63	1.56	1	18	9.31	20.21
Green	6.03	1.87	1	20	11.63	24.72
Yellow	8.37	2.46	2	27	15.75	32.96
Red	6.34	1.86	2	35	11.93	24.96

Table 5: LOD/LOQ

Limit of Detection- minimum peak height detected by the chemistry- and Limit of Quantification- minimum peak height that the chemistry can quantify- were also calculated using the following formulas:
LOD= Average noise signal + 3 * Standard Deviation
LOQ= Average noise signal + 10 * Standard Deviation

Conclusion

•The amplification chemistry of PowerPlex® Fusion provided accurate profiles and a wide range of input target DNA with low amount of artifacts.

•The results of the performed validation studies demonstrated the robustness and reliability of the kit, comparable to other STR kits employed by MUFSC.

•The optimal DNA target load was determined, a laboratory specific stutter percentage table per loci, and a specific mixture interpretation guideline produced.

•Future studies will be conducted on this amplification chemistry to test the human specific amplification relative to human normal flora of the mouth and the genital and anal region, as well as a further detailed guideline for mixture interpretation.

•The use of the Promega® PowerPlex Fusion amplification kit is recommended for the use in future casework samples based on the validation studies .

Disclaimer

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Contact Information

Roy Al Ahmar
alahmar@live.marshall.edu
(304) 710-8504