Internal Validation of the Applied Biosystems® 3500xL Genetic Analyzer using AmpF/STR® Identifiler® Direct

Carrie Schmittgen BS[1], Amy Barber MS[2], Joshua Stewart MSFS[1], Pamela Staton PhD[1]

[1] Marshall University Forensic Science Center – 1401 Forensic Science Drive, Huntington, WV 25701; [2] Massachusetts State Police Forensic and Technology Center – 124 Acton St, Maynard, MA 01754

Abstract

Validations are essential to demonstrate the capabilities and limitations of new technology. In accredited forensic laboratories, it is required by Standard 8 of the FBI Quality Assurance Standards (2011) that internal validations be performed on new procedures, including instrumentation and dye chemistries, prior to their implementation into casework. Specific studies are completed to gain the appropriate knowledge that the method is efficient, performing as expected, and producing reliable and reproducible results. At Massachusetts State Police Forensic and Technology Center (MSPFTC), the internal validation of the Applied Biosystems® 3500xL Genetic Analyzer was conducted in the DNA unit.

Eleven studies were conducted in this internal validation to show the abilities of the 3500xL based on the Scientific Working Group for DNA Analysis Methods (SWGDAM) guidelines. These studies included: LIZ comparison, LIZ optimization, analytical threshold, injection time, sensitivity, precision, stutter, heterozygote balance, contamination, concordance, and reproducibility. Based on the results of these studies, certain parameters and settings were recommended to MSPFTC to be included in the standard operating procedure for the 3500xL. The combination of these studies showed the 3500xL performed as expected giving reliable, reproducible, and robust results with Identifiler® Direct. Future studies, such as non-probative and cycle number, should be conducted to optimize the setting parameters for blood and saliva samples.

Introduction

The 3500xL Genetic Analyzer is an automated 24 capillary instrument that uses fluorescence-based detection for human identification applications. The instrument has numerous enhanced capabilities over the older platforms that perform capillary electrophoresis (e.g. the 3100 Genetic Analyzer series). Some capabilities include having only one pump block to save polymer, prepackaged consumables to minimize laboratory variability and analyst hands-on time, and an increased number of capillaries for higher throughput.

Materials and Methods

Kits and Instrumentation

- Applied Biosystems® 3500xL Genetic Analyzer
- BSD600® Duet Series II Semi-automated Punch System
- Janus™ Automated workstations
- GeneAmp® PCR System 9700
- AmpFlSTR® Identifiler® Direct amplification kit
- GeneScan™ LIZ 500 and LIZ 600 v2.0
- GeneMapper® ID-X (GMIDX) version 1.3
- Hi-Di Formamide
- Performance Optimized polymer (POP) 4

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Results

Based on all results obtained from this validation, the following parameters and results will be used in the future in Massachusetts State Police Forensic and Technology Center's DNA unit.

LIZ Comparison

- LIZ 600 v2.0 showed a lower, more consistent standard deviation of allele base pair size (IDD ladders) than with LIZ 500.

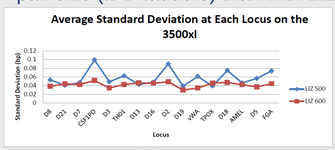

Figure 1: Comparison of LIZ 500 versus 600 v.2.0

LIZ Optimization

- 0.2µL gave consistent base pair sizing at each allele in each locus and no extraneous peaks or artifacts were called in any of the dyes with a threshold of 50 RFU.

Thermal Cycling & Injection Parameters

- 24 seconds/1.2kV on 26 cycles amp

Analytical Threshold

- 65 RFU Threshold for 24 sec injection
- The red dye channel was used because it had to highest background noise.

Red	All	12 sec	18 sec	24 sec	30 sec
Average	31.09	30.28	30.85	31.22	31.99
Standard Deviation	10.41	9.29	9.33	10.92	11.76
Maximum	91	89	89	87	91
Minimum	7	11	7	9	10
AT= $2(Y_{max}-Y_{min})$	168	156	164	156	162
AT= Avg+3(std)	62.31	58.14	58.86	63.99	67.27

Figure 2: Red channel Analytical Threshold results

Sensitivity

- Optimal range: 5.0ng/µL – 0.31ng/µL
- Dropout occurred at 0.15ng of DNA

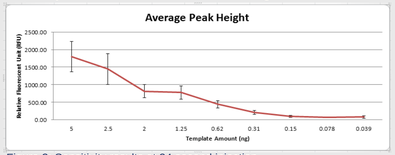

Figure 3: Sensitivity results at 24 second injection

Stutter

- Calculated negative stutter values were consistent with the recommended Applied Biosystems® stutter percentages.

	Data Points	Average	Min	Max	S.D.	(+3) S.D.	ABI Stutter ratios
D8S1179	263	6.69%	3.28%	11.63%	1.48%	11.13%	9.54%
D21S11	240	7.30%	5.12%	10.40%	1.03%	10.40%	10.42%
D7S820	175	4.50%	2.20%	6.99%	1.23%	8.19%	8.60%
CSF1PO	176	5.58%	3.85%	9.60%	1.11%	8.90%	8.48%
D3S1358	209	8.26%	5.20%	12.71%	1.56%	12.96%	11.45%
TH01	186	1.92%	1.01%	3.60%	0.74%	4.14%	4.76%
D13S317	198	4.83%	1.64%	8.47%	1.44%	9.14%	9.39%
D16S539	220	5.91%	2.38%	12.15%	1.78%	11.26%	9.42%
D2S1338	283	8.32%	5.43%	12.37%	1.72%	13.48%	11.77%
D19S433	234	7.32%	3.53%	15.54%	1.47%	11.72%	11.15%
vWA	225	7.21%	2.52%	11.70%	1.69%	12.29%	11.99%
TPOX	200	2.89%	0.93%	5.60%	0.99%	5.87%	5.27%
D18S51	258	7.82%	3.97%	14.20%	1.93%	13.62%	12.89%
D5S818	228	6.34%	2.88%	11.20%	1.54%	10.95%	9.89%
FGA	222	6.88%	3.80%	15.79%	2.05%	13.05%	11.62%

Figure 4: Calculated Stutter results

Contamination

- None occurred in wells, across sample wells, or in wells in a sequential injection.

Precision

- All loci, alleles, and dye channels tested in the precision study had less variation than the recommended 0.15bp for each study.

Locus	Standard deviation
D8	0.00417
D21	0.00422
D7	0.00608
CSF1PO	0.00862
D3	0.00082
TH01	0.00473
D13	0.00727
D16	0.00901
D2	0.00765
D19	0.00254
vWA	0.00278
TPOX	0.00880
D18	0.00425
AMEL	0.00522
D5	0.00191
FGA	0.00552

Figure 5: Standard deviation at each locus

Concordance and Reproducibility

- Identical and concordant genotypic results were obtained when comparing the 3130xL to the 3500xL genetic analyzer in 36 samples. Due to oversaturation of the camera, 9 samples failed analysis.
- Reproducibility of peak heights were assessed (n=3 injections). On average, there was a 1.4 fold difference between the tallest & shortest peaks. Variability tended to decrease with increased peak height.

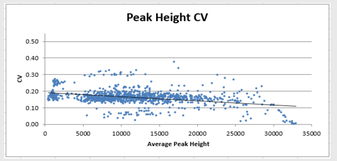

Figure 6: Average Peak Height versus CV

Heterozygous Balance

- All samples consistently produced balanced sister alleles greater than the expected 70% sister allele balance minimum.

Sample	Average PHR	Sample	Average PHR
9947A	91.4%	25	94.7%
1	91.8%	26	94.0%
2	94.6%	28	91.4%
3	94.2%	29	92.7%
4	92.4%	30	90.2%
6	92.0%	31	92.8%
7	93.6%	32	94.8%
8	89.5%	33	90.7%
9	92.3%	34	88.9%
10	93.8%	35	93.4%
11	92.2%	36	95.6%
12	91.7%	37	92.7%
13	94.8%	1S	91.2%
14	93.1%	2S	88.8%
15	93.6%	3S	93.3%
16	90.2%	4S	93.1%
18	90.2%	6S	95.5%
21	93.4%	7S	89.4%
23	91.6%	8S	88.8%
24	93.4%		

Figure 7: Heterozygote peak height balance

Conclusions and Future Needs

Based on the results of the studies completed, the 3500xL performed as expected giving reliable, reproducible, and robust results with Identifiler® Direct and is thus validated for MSPFTC.

Although this is a fully validated instrument, the completion of these additional studies would further add supporting evidence to this validation. Another sensitivity study of blood samples on FTA® card based on direct amplification. This could be conducted by creating different dilutions of blood, pipetting those onto the FTA® cards, punching the cards, and continuing the process of direct amplification. Also, a cycle number study should be conducted to overcome the camera's oversaturation observed with the concordance and reproducibility studies. The cycle number for blood card samples may need to be decreased so oversaturation does not affect the LIZ sizing. Another LIZ optimization may need to be conducted on the Janus™ if the cycle number changes for blood card samples in this case.

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Works Cited

Applied Biosystems® by Life Technologies™. AmpFℓSTR® Identifiler® Direct PCR Amplification Kit User Guide. Carlsbad, CA: Life TechnologiesTM Corporation 2012.

Applied Biosystems®. Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide. Foster City, CA: Life TechnologiesTM Corporation. 2010.

Applied Biosystems®. Applied Biosystems® 3500/3500xL Genetic Analyzer User Bulletin. Foster City, CA: Life TechnologiesTM Corporation 2010.

"CODIS and NDIS Fact Sheet". *FBI*. FBI, 30 Aug. 2010. <http://www.fbi.gov/about-us/lab/biometric-analysis/codis/codis-and-ndis-fact-sheet>.

Grgicak, Catherine M. "Analytical Thresholds: Determination of Minimum Distinguishable Signals." 21st International Symposium of Human Identification. Mixture Interpretation Workshop: Principles, Protocols and Practice. San Antonio, TX. 11 Oct. 2010. http://www.cstl.nist.gov/biotech/strbasse/training.htm [Available Feb. 7, 2011].

Park SJ, Kim JY, Yang YG, Lee SH. Direct STR amplification from whole blood and blood- or saliva-spotted FTA® without DNA purification. *Journal of Forensic Science* 2009; 53(2): 335–41.

"Quality Assurance Standards for DNA Databasing Laboratories." *FBI*. FBI, 10 June 2011. <http://www.fbi.gov/about-us/lab/biometric-analysis/codis/qas-standards-for-dna-databasing-laboratories-effective-9-1-2011>.

Rosenblum, Bernett B., Frank Oaks, Steve Menchen, and Ben Johnson. "Improved Single-strand DNA Sizing Accuracy in Capillary Electrophoresis." *Nucleic Acids Research* 25.19 (1997): 3925-3929.

Scientific Working Group on DNA Analysis Methods (SWGDAM). Interpretation guidelines for autosomal STR typing by forensic DNA testing laboratories. Jan 2010, <http://www.fbi.gov/about-us/lab/codis/swgdam-interpretation-guidelines>.

Scientific Working Group on DNA Analysis Methods (SWGDAM). Revised validation guidelines. Forensic Science Communications. December 2012; 6(3) <http://swgdam.org/SWGDAM_Validation_Guidelines_APPROVED_Dec_2012.pdf>.

Wang DY, Chang C, Oldroyd N, Hennessy LK. Direct amplification of STRs from blood or buccal cell samples. Forensic Science International: Genetics Supplement Series 2009; 2:113-4.

Wang, Dennis Y., Chien-Wei Chang, Robert E. Lagace, Nicola J. Oldroyd, and Lori K. Hennessy. "Development and Validation of the AmpFlSTR® Identifiler® Direct PCR Amplification Kit: A Multiplex Assay for the Direct Amplification of Single-Source Samples." *Journal of Forensic Sciences* 56.4 (2011): 835-45.