Ongoing Validation of the 3500XL Genetic Analyzer using the AmpF*l*STR Identifiler Plus® Kit

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Abstract

An internal validation of the Applied Biosystems' 3500XL Genetic Analyzer was started for the DNA Forensic Biology Laboratory of the New York City Office of Chief Medical Examiner in New York City, NY. The Applied Biosystems' AmpF*l*STR® Identifiler® Plus Amplification Kit was chosen to validate the instrument since the lab is currently validating this kit for low copy samples at twenty-nine and thirty-one cycles at half reactions. This amplification kit has shown higher sensitivity as opposed to the Applied Biosystems' AmpF*l*STR® Identifiler® Amplification Kit given that the master mix was developed to overcome inhibition.

Using exemplar samples, a sensitivity, threshold, and concordance study were performed using the Identifiler® Plus Kit on the Applied Biosystems' 3500XL Genetic Analyzer. The samples were run on an Applied Biosystems' 3130XL Genetic Analyzer as well in order to gauge the performance of the new instrument. The studies performed determined that the 3500XL Genetic Analyzer had higher sensitivity than the 3130XL Genetic Analyzer regarding peak heights and the amount of DNA needed to obtain a full profile. The threshold study showed that individual dye channel thresholds should be selected. When the same samples were ran on the 3130XL Genetic Analyzer and the 3500XL Genetic Analyzer, a concordance study verified that the correct profile was obtained for all the samples.

Introduction

The 3500XL Genetic Analyzer is a new series of genetic analyzer that has been released by Applied Biosystems for the use of generating DNA profiles from processed case samples. The previous model, the 3100 series has been discontinued by Applied Biosystems since June 2011, but will officially continue support until 2016.[1] Therefore, parts will be scarce and trying to get an Applied Biosystems representative to perform necessary maintenance will be hard to accomplish. Therefore, as with all new instruments or techniques used in the forensic community, the 3500XL Genetic Analyzer needs to be internally validated by individual laboratories transitioning to the new instrument before it can be used in casework.

3500XL vs. 3130XL

There are many differences between the 3500XL Genetic Analyzer and the 3130XL Genetic Analyzer being compared in this validation study. The 3500XL Genetic Analyzer has increased the number of capillaries used, allowing one to run more samples per injection while reducing the time of a run.[2] The energy being used by the instrument has also been reduced by decreasing the power supply necessary to run the instrument. The 3500XL Genetic Analyzer also makes use of consumables containing RFID (Radio Frequency Identification) tagged labels which allows for automated tracking of lot numbers and expiration dates.[4] The GS600 LIZ size standard with the option of normalization will allow the user to correct signal variations between instruments and capillaries.[3] The 3500XL instrument has a much higher off-scale limit than the 3130XL Genetic Analyzer, making it more sensitive and will likely reduce the number of re-injections a laboratory must run to obtain a full profile. The 3130XL Genetic Analyzer can reach peaks as high as 30,000 Rfus (relative frequency units) with an optimum around 10,000 Rfus, whereas the 3500XL instrument has a height cap of about 60,000 Rfus with optimal peak heights reaching about 30,000 Rfus.

GeneMapper ID-X Software ve. GeneMapper ID Software

The 3500XL uses a remodeled software, GeneMapper ID-X version 1.2 rather than GeneMapper ID version 3.2.1, to analyze the data which has been developed to be more user friendly. The GeneMapper ID-X version 1.2 Software possesses a layout that is very similar to that of the GeneMapper ID version 3.2.1 Software so that the transition to the ID-X version will be a smooth process. After selecting an analysis method, panels, and hit the play button, there will be an "Analysis Requirement Check" which states if one has forgotten to assign any parameters to a sample before analysis of the run is completed.[6] The quality assessment of the allelic ladders is also done before the analysis of the injection and will exclude low quality "AL" (allelic ladder) samples.[6] Once analysis has completed, the "Analysis Summary" window will be displayed which will notify the analyst of any quality flags.[6] The program also allows one to take advantage of tools like the "Label Edit Viewer" tool for tech reviews, a "Concordance Evaluation" tool so you can see the percent match between samples that have been analyzed, and a "Report Manager" tool which will display the allele calls for the samples.[6] It has the capabilities of an expert system, but the user has to import the parameters needed for the program to analyze the system. Although it can be used as an expert system, it is not the common practice for the forensic community.

http://img.ugent.be/images/pictures/3130xl%20Genetic%20Analyzer.jpg

http://www.dylanfraser.com/wp-content/uploads/2012/03/hitachi3500.jpg

Quality Control Test

Before conducting the validation studies, the instrument first has to be tested to make sure that the instrument was running correctly. In order to achieve this, an injection of size standard is ran. If the electropherograms produced are clean and the size standard is called for all samples the instrument has passed. An injection of master mix, 0.3µL of LIZ and 8.7µL of Hi-Di for each sample, was plated, dispensing 9µL of the master mix into the 24 wells that make up an injection. Once the master mix was dispensed, the plate was covered with a septa, centrifuged, and put through the denaturation/cooling protocol on the thermocycler. The plate was placed on the 3500XL Genetic Analyzer and ran at I.

Sensitivity Study

Once the samples were quanted a minimum of three times, the average neat value was taken and dilutions of the extracts were made to achieve target concentrations of 100pg, 250pg, and 500pg while the positive control was held at 200pg. The samples were amped using half reactions of the AmpF/STR Identifiler Plus® Kit at 29 cycles with a 60 minute soak. The amplified product was then ran on the 3130XL and 3500XL instruments to compare the peak heights and peak height ratios observed in the profiles generated. The 100pg samples showed drop-out so they had to be run at I and IR parameters. The 250pg and 500pg samples had 1µL and 3µL of sample injected onto both instruments.

Analytical Threshold Study

Thirty-six negative samples were amped using half reactions of the AmpF/STR Identifiler Plus® Kit at 29 cycles with a 60 minute soak. The samples were run at I and IR parameters on the 3500XL and analyzed with the dye channel threshold being held at 1Rfu. The size standard basepairs were then used to eliminate peaks present that were outside a ±2 base pair range of the base pairs found in the size standard. The IUPAC and SWGDAM methods for calculating the analytical threshold were then used to find the analytical threshold for the 3500XL.

Concordance Study

The samples analyzed in the sensitivity study were used to compare the generated profiles from the 3130XL and 3500XL to one another and to the known profile of the reference samples to check that the alleles called for each marker match for each sample.

Results

The sensitivity study proved that the 3500XL generates profiles with higher peak heights than those found in the produced 3130XL profiles for the same target concentration. It was also observed that as the target concentration increases so does the peak heights present in the profile. If the sample is run at a higher parameter on the genetic analyzer the peak heights will also be increased. All of these conditions showed a significant difference in the peak height, but when the peak height ratios were compared there was no significant difference noted. The results for the sensitivity study can be seen in Tables 1-4.

Sensitivity Study Results for the 3130XL ran at I with 1µL of Sample

DNA input	Maximum Pk Ht	Minimum Pk Ht	Average Pk Ht	Maximum PHR	Minimum PHR	Average PHR	% PHR Standard Deviation	Range between Min & Max	# of loci used for PHR Calculation
100pg	1991	80	362	99.87%	16.10%	71.09%	19.17%	83.78%	180
250pg	3872	206	1006	99.45%	32.76%	76.31%	15.15%	66.69%	192
500pg	7632	465	2022	99.93%	50.37%	84.02%	11.42%	49.56%	192

Table 1. The peak height and peak height ratios for the samples ran on the 3130XL at I .

Sensitivity Study Results for the 3500XL ran at I with 1µL of Sample

DNA input	Maximum Pk Ht	Minimum Pk Ht	Average Pk Ht	Maximum PHR	Minimum PHR	Average PHR	% PHR Standard Deviation	Range between Min & Max	# of loci used for PHR Calculation
100pg	7634	124	1476	99.26%	14.39%	68.97%	20.95%	84.88%	191
250pg	12126	943	3575	99.81%	33.35%	76.12%	15.16%	66.46%	192
500pg	34541	2104	8985	99.91%	47.22%	83.91%	11.41%	52.69%	192

Table 2. The peak height and peak height ratios for the samples ran on the 3500XL at I.

Sensitivity Study Results for 100pg ran on the 3500XL at I and IR for the 1µL and 3µL Injected

DNA input	Maximum Pk Ht	Minimum Pk Ht	Average Pk Ht	Maximum PHR	Minimum PHR	Average PHR	% PHR Standard Deviation	Range between Min & Max	# of loci used for PHR Calculation
100pg (1µL)	7634	124	1476	99.26%	14.39%	68.97%	20.95%	84.88%	191
100pg (1µL)	9505	137	1698	99.64%	14.12%	68.78%	20.93%	85.52%	191
100pg (3µL)	16535	219	3101	99.78%	14.54%	68.90%	21.09%	85.24%	191
100pg (3µL)	19922	8891	3745	99.91%	14.74%	68.51%	20.83%	85.16%	191

Table 3. The peak height and peak height ratios for the 100pg samples ran on the 3500XL at I and IR for 1µL and 3µL sample injections.

Sensitivity Study Results for 1µL and 3µL Injected on the 3500XL at I

DNA input	Maximum Pk Ht	Minimum Pk Ht	Average Pk Ht	Maximum PHR	Minimum PHR	Average PHR	% PHR Standard Deviation	Range between Min & Max	# of loci used for PHR Calculation
100pg (1µl)	7634	124	1476	99.26%	14.39%	68.97%	20.95%	84.88%	191
100pg (3µl)	16535	219	3101	99.78%	14.54%	68.90%	21.09%	85.24%	191
500pg (1µl)	34541	2104	8985	99.91%	47.22%	83.91%	11.41%	52.69%	192
500pg (3µl)	46587	25599	13288	99.83%	49.88%	83.98%	11.52%	49.95%	192

Table 4. The peak height and peak height ratios for the 100pg samples ran on the 3500XL at I for 1µL and 3µL sample injections.

The analytical threshold calculations done using the IUPAC and SWGDAM methods proved that the dye channels will each have a different analytical threshold. Therefore, specific dye channel thresholds should be used instead of one threshold for all dye channels. The calculations can be seen in Tables 5-8.

Results Continued

IUPAC Method for 3500XL Samples Ran at I

n=36

DYE	AVERAGE	STDEV	MIN	MAX	AT	SQRT(36)
BLUE	6.23	2.66	1	25	7.31	6
GREEN	11.87	4.10	3	38	13.53	6
YELLOW	22.67	6.74	5	47	25.40	6
RED	33.67	9.64	10	74	37.58	6

Table 5. IUPAC calculations for the 3500XL run at I analytical thresholds.

SWGDAM Method for 3500XL Samples Ran at I

DYE	AVERAGE	STDEV	MIN	MAX	Lowest Trough	AT
BLUE	6.23	2.66	1	25	1	48
GREEN	11.87	4.10	3	38	3	70
YELLOW	22.67	6.74	5	47	5	80
RED	33.67	9.64	10	74	10	128

Table 6. SWGDAM calculations for the 3500XL run at I analytical thresholds.

IUPAC Method for 3500XL Samples Ran at IR

n=36

DYE	AVERAGE	STDEV	MIN	MAX	AT	SQRT(36)
BLUE	3.48	2.38	1	24	4.44	6
GREEN	4.00	4.60	1	65	5.87	6
YELLOW	5.95	2.90	1	40	7.13	6
RED	9.18	3.75	2	34	10.70	6

Table 7. IUPAC calculations for the 3500XL run at IR analytical thresholds.

SWGDAM Method for 3500XL Samples Ran at IR

DYE	AVERAGE	STDEV	MIN	MAX	Lowest Trough	AT
BLUE	3.48	2.38	1	24	1	46
GREEN	4.00	4.60	1	65	1	128
YELLOW	5.95	2.90	1	40	1	78
RED	9.18	3.75	2	34	2	64

Table 8. SWGDAM calculations for the 3500XL run at IR analytical thresholds.

The concordance study showed that the 3500XL yielded profiles with a 100% concordance with the profiles produced by the 3130XL . This means that when a lab transitions to a 3500XL one does not need to worry that there will be adverse affects to the profiles generated by the new instrument.

Conclusion and Future Work

The validation studies that have been performed have proven thus far that the 3500XL is more sensitive than the 3130XL, that the dye channels need dye specific thresholds, and that there is 100% concordance between the two genetic analyzers.

To complete the validation of the 3500XL, more validation studies need to be performed. A sensitivity study with low dilutions needs to performed to discover the limit of the 3500XL that will still produce a full profile. The threshold study needs more samples to get a more accurate threshold value for the dye channels. A concordance sample can be conducted on the 3500XL using different amplification kits. An injection and cycle study can be performed to obtain the optimal run parameters for the 3500XL. A mixture study can be done to see which ratio will allow one to distinguish between the minor and major components. A non-probative study, contamination study, a stutter study, and a heterozygosity study can also be done.

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Acknowledgments

I thank everyone at the NYC OCME. A special thanks to Dr. Caragine for her advice regarding the project, Grace Axler-Diperete for being a wonderful supervisor, and Cindy Rodriquez and Desarae Harmon for training me. I thank Juliette Smith for getting the project off the ground. A special thanks to the awesome interns at the OCME.

I thank Dr. Staton for her substantial help and Valerie Bostwick for her great review skills. Also, Ashleigh Zeile for being a great partner in crime.